PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTI-ATHEROGENIC & ANTIOXIDANT ACTIVITIES OF *CORDIA OBLIQUA* IN WISTAR RAT FED WITH HIGH FAT DIET

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by

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Under the guidance of

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APRIL 2020

CERTIFICATE

This is to certify that the investigation described in this dissertation entitled **"Phytochemical investigation and evaluation of anti-atherogenic & antioxidant activities of *Cordia obliqua* in wistar rat fed with high fat diet"** submitted by **Reg. No: 261815353** to The Tamil Nadu Dr. M.G.R. Medical University, Chennai for the partial fulfillment of the requirement for the Degree of Master of Pharmacy in Pharmaceutical Chemistry. This research work was carried out in the Department of Pharmaceutical Chemistry under the guidance and supervision of **Dr. J. Amutha Iswarya Devi, M.Pharm., Ph.D.,** Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil - 626126.

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Your
Welcome

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Introduction

CHAPTER I

INTRODUCTION

Herbal medicine (also Herbalism) is the study of the botany and use of medicinal plants. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. Herbal medicine is also called phytomedicine or phytotherapy. Paraherbalism is the pseudoscientific use of extracts of plant or animal origin as supposed medicines or health-promoting agents.

Herbs and plants can be processed and can be taken in different ways and forms, and they include the whole herb, teas, syrup, essential oils, ointments, salves, rubs, capsules, and tablets that contain a ground or powdered form of a raw herb or its dried extract. Plants and herbs extract vary in the solvent used for extraction, temperature, and extraction time, and include alcoholic extracts (tinctures), vinegars (acetic acid extracts), hot water extract (tisanes), long term boiled extract, usually roots or bark (decoctions), and cold infusion of plants (macerates). The expanding herbal product market could drive over harvesting of plants and threaten biodiversity.

HERBAL MEDICINE

The world health organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The traditional medicine is the synthesis of therapeutic experience of generations of practising physicians of indigenous system of medicine.

The role of herbal medicines in traditional healing

The pharmacological treatment of diseases began long ago with the use of herbs. Methods of folk healing throughout the world commonly use herbs as part of their tradition. Some of these traditions are providing some examples of the array of important healing practices around the world that use herbs for this purpose.

Natural products for modern medicine

Plants are being used in medicine from time immemorial, because they have fitted the immediate personal need, they are accessible and inexpensive, the practitioners speak to those who have used them in their own language and they are not provided from a remote professional or Government apparatus.

Such activity is not completely dismissed in scientific society and plants are also appreciated in pharmaceutical research as the major resource for new medicines and a growing body of medical literature supports the clinical efficacy of herbal treatments.

Natural products will continue to be important in three areas of drug discovery

- As targets for production by biotechnology
- As a source of new lead compounds of novel chemical structure
- As the active ingredients of useful treatments, derived from traditional systems of medicine.

Safety in herbal drugs

Major differences in the assessment of quality, safety and efficacy would hinder free circulation of herbal medicinal products may represent a risk for consumers. The complexity of herbal drug preparations and the interpretation of bibliographic data on safety and efficacy reflecting the experience gathered during long- term use are best addressed by involving specific expertise and experience.

Safety and efficacy of complex biological products, such as herbal medicinal products are directly linked to pharmaceutical details such as the way of production and the specification of extracts.

Significances of medicinal plants to human being

- Many of the modern medicines are produced indirectly from medicinal plants for example, Aspirin.
- Plants are directly used as medicines by a majority of cultures around the world. For example, Chinese medicine and Indian medicine.
- Many food crops have medicinal effects. For example, Garlic and ginger.

- Medicinal plants are resources of new drugs. It is estimated that there are more than
  250,000 flower plant species.
- Studying medicinal plant helps us to understand plant toxicity and protect human and animals from natural poisons.
- Cultivation and preservation of medicinal plants protect biological diversity. For example, Metabolic engineering of plants.

Prevalence of use

The use of herbal remedies is more prevalent in patients with chronic diseases such as cancer, diabetes, asthma and end stage renal disease. According to this survey, herbal therapy, or use of natural products other than vitamins and minerals, was the most commonly used complementary and alternative medicines (CAM) therapy.

Herbal remedies are very common in Europe. In Germany, herbal medications are dispensed by apothecaries (Eg. Apotheke). Herbal remedies are seen by some as a treatment to be preferred to pure medical compounds that have been industrially produced.

In India the herbal remedy is so popular that the government of India has created a separate department AYUSH under the Ministry of Health & Family Welfare. Sick animals tend to forage plants rich in secondary metabolites, such as tannins and alkaloids. Because these phytochemicals often have antiviral, antibacterial, antifungal, and anti-helminthic properties, a plausible case can be made for self-medication by animals in the wild.

The major use of herbal medicines is for health promotion and therapy for chronic, as opposed to life-threatening, conditions. However, usage of traditional remedies increases when conventional medicine is ineffective in the treatment of disease, such as in advanced cancer and in the face of new infectious diseases.

Herbs are applied to the treatment of chronic and acute conditions and various ailments and problems such as cardiovascular disease, prostate problems, depression, inflammation, and to boost the immune system.

ATHEROGENIC ACTIVITY

Atherosclerosis is a rheumatoid heart disease in which the inside of an artery narrows are buildup of plaque. Initially, there are generally no symptoms. When severe, it can result in coronary artery disease, stroke, peripheral artery disease or kidney problems, depending on which arteries are affected. Symptoms, if they occur, generally do not begin until middle age.

The exact cause is not known. Risk factors include abnormal cholesterol levels, high blood pressure, diabetes, smoking, obesity, family history and an unhealthy diet. Plaque is made up of fat, cholesterol, calcium and other substances found in the blood. The narrowing of arteries limits the flow of oxygen-rich blood to parts of the body. Diagnosis is based upon a physical exam, electrocardiogram and exercise stress test among others.

Prevention is generally by eating a healthy diet, exercising, not smoking and maintaining a normal weight. Treatment of established disease may include medications to lower cholesterol such as statins, blood pressure medication or medications that decrease clotting, such as aspirin. A number of procedures may also be carried out such as percutaneous coronary intervention, coronary artery bypass graft or carotid end arterectomy.

Atherosclerosis generally starts when a person is young and worsens with age. Almost all people are affected to some degree by the age of 65. It is the number one cause of death and disability in the developed world. Though it was first described in 1575, there is evidence that the condition occurred in people more than 5,000 years ago.

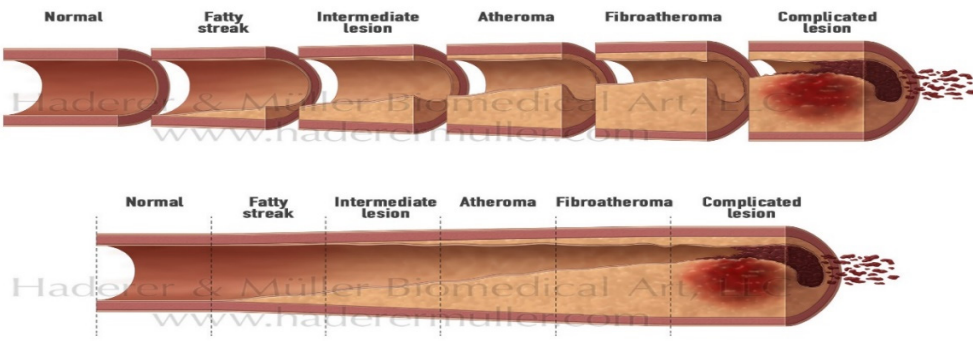


Fig. 1: Formation of atherosclerosis.

Definition of atherosclerosis

The following terms are similar and can be easily confused: arteriosclerosis, arteriolosclerosis, and atherosclerosis. Arteriosclerosis is a general term describing any hardening (and loss of elasticity) of medium or large arteries (from Greek *(arteria)*, meaning 'artery', and *(sklerosis)*, meaning 'hardening'); arteriolosclerosis is any hardening (and loss of elasticity) of arterioles (small arteries); atherosclerosis is a hardening of an artery specifically due to an atheromatous plaque. The term atherogenic is used for substances or processes that cause formation of atheroma.

Dietary of atherosclerosis

The relation between dietary fat and atherosclerosis is controversial. Writing in *Science*, *Gary taubes* detailed that political considerations played into the recommendations of Government bodies. The USDA, in its food pyramid, promotes a diet of about 64% carbohydrates from total calories.

The American Heart Association, the American Diabetes Association and the National Cholesterol Education Program make similar recommendations. In contrast, Prof. Walter Willett (Harvard School of Public Health, PI of the second Nurses' Health Study) recommends much higher levels of fat, especially of monounsaturated and polyunsaturated fat.

The role of dietary oxidized fats/lipid peroxidation (rancid fats) in humans is not clear. Laboratory animals fed rancid fats develop atherosclerosis. Rats fed DHA-containing oils experienced marked disruptions to their antioxidant systems, and accumulated significant amounts of phospholipid hydroperoxide in their blood, livers and kidneys.

Rabbits fed atherogenic diets containing various oils were found to undergo the greatest amount of oxidative susceptibility of LDL via polyunsaturated oils. In another study, rabbits fed heated soybean oil "grossly induced atherosclerosis and marked liver damage were histologically and clinically demonstrated. However, Fred Kummerow, claims that it is not dietary cholesterol, but oxysterols or oxidized cholesterols, from fried foods and smoking that are the culprit.

Rancid fats and oils taste very bad even in small amounts, so people avoid eating them. It is very difficult to measure or estimate the actual human consumption of these substances. Highly unsaturated omega-3 rich oils such as fish oil are being sold in pill form so that the taste of oxidized or rancid fat is not apparent.

The health food industry's dietary supplements are self-regulated and outside of FDA regulations. To properly protect unsaturated fats from oxidation, it is best to keep them cool and in oxygen free environments.

Risk factors of atherosclerosis

The atherosclerotic process is not well understood. Atherosclerosis is associated with inflammatory processes in the endothelial cells of the vessel wall associated with retained low-density lipoprotein (LDL) particles. This retention may be a cause, an effect, or both, of the underlying inflammatory process.

The presence of the plaque induces the muscle cells of the blood vessel to stretch, compensating for the additional bulk, and the endothelial lining thickens, increasing the separation between the plaque and lumen. This somewhat offsets the narrowing caused by the growth of the plaque, but it causes the wall to stiffen and become less compliant to stretching with each heartbeat.

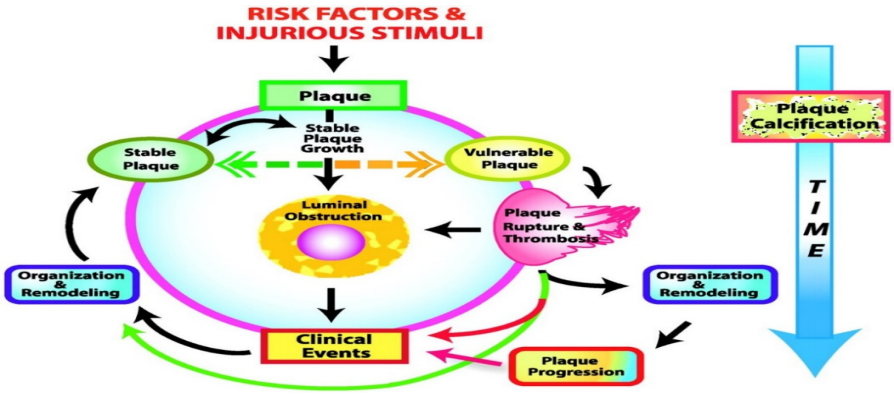


Fig. 2: Risk factors of atherosclerosis.

1. **Modifiable**
   - Diabetes
   - Dyslipidemia
   - Tobacco smoking
   - Trans fat

- Abdominal obesity
- Western pattern diet
- Insulin resistance
- Hypertension

2. **Non-Modifiable**
   - Advanced age
   - Male
   - Family history
   - Genetic abnormalities
3. **Lesser or Uncertain**
   - South Asian descent
   - Thrombophilia
   - Saturated fat
   - Excessive carbohydrates
   - Elevated triglycerides
   - Systemic inflammation
   - Hyperinsulinemia
   - Sleep deprivation
   - Air pollution
   - Sedentary lifestyle
   - Arsenic poisoning
   - Alcohol
   - Chronic stress
   - Hypothyroidism
   - Periodontal disease

Three stages of atherosclerosis

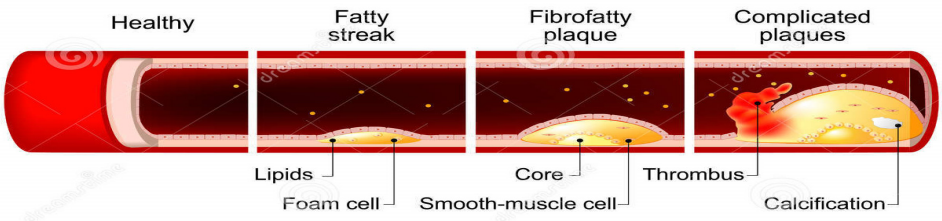


Fig. 3: Three stages of atherosclerosis.

1. The fatty streak

This first stage of atherosclerosis can sometimes be found in children as young as 10 years of age. In this stage, a yellow streak appears along major arteries, such as the aorta and carotid artery. This streak is made up of smooth muscle cells, cholesterol, and macrophages (a type of white blood cell). The fatty streak phase alone does not cause any noticeable symptoms but can progress into a more dangerous phase of atherosclerosis called a fibrous plaque.

2. Fibrous plaque

A fibrous plaque develops within the inner layer of the vessel. This plaque is made up of smooth muscle cells, macrophages, and lymphocytes (a more aggressive type of white blood cell). These cells have cholesterol inside of them. As the fibrous plaque grows, it begins to protrude into the vessel where the blood is flowing.

3. Complicated lesion

The final stage of atherosclerosis is defined when a dangerous series of events occur. When the fibrous plaque breaks apart, it exposes the cholesterol and connective tissue underneath it. This event is recognized by the body as an injury, and a team of blood clotting cells are sent to the scene.

This becomes particularly dangerous because, now the blood flow is being restricted by the initial blockage as well as the clot that has formed. The ruptured plaque in combination with the blood clot is called a complicated lesion.

Medications of atherosclerosis

1. Cholesterol medications

Aggressively lowering your low-density lipoprotein (LDL) cholesterol, the "bad" cholesterol, can slow, stop or even reverse the buildup of fatty deposits in your arteries. Boosting your high-density lipoprotein (HDL) cholesterol, the "good" cholesterol, may help, too. Your doctor can choose from a range of cholesterol medications, including drugs known as statins and fibrates. In addition to lowering cholesterol, statins have additional effects that help stabilize the lining of your heart arteries and prevent atherosclerosis.

2. Anti-platelet medications

Your doctor may prescribe anti-platelet medications, such as aspirin, to reduce the likelihood that platelets will clump in narrowed arteries, form a blood clot and cause further blockage.

3. Beta blocker medications

These medications are commonly used for coronary artery disease. They lower your heart rate and blood pressure, reducing the demand on your heart and often relieve symptoms of chest pain. Beta blockers reduce the risk of heart attacks and some heart rhythm problems.

4. Angiotensin-converting enzyme (ACE) inhibitors

These medications may help slow the progression of atherosclerosis by lowering blood pressure and producing other beneficial effects on the heart arteries. ACE inhibitors can also reduce the risk of recurrent heart attacks.

5. Calcium channel blockers

These medications lower blood pressure and are sometimes used to treat angina.

6. Water pills (diuretics)

High blood pressure is a major risk factor for atherosclerosis. Diuretics lower blood pressure.

7. Other medications

Your doctor may suggest certain medications to control specific risk factors for atherosclerosis, such as diabetes. Sometimes specific medications to treat symptoms of atherosclerosis, such as leg pain during exercise, are prescribed.

Mechanism of atherogenesis

Atherogenesis is the developmental process of atheromatous plaques. It is characterized by a remodeling of arteries leading to subendothelial accumulation of fatty substances called plaques. The buildup of an atheromatous plaque is a slow process, developed over a period of several years through a complex series of cellular events occurring within the arterial wall and in response to a variety of local vascular circulating factors.

One recent hypothesis suggests that, for unknown reasons, leukocytes, such as monocytes or basophils, begin to attack the endothelium of the artery lumen in cardiac muscle. The ensuing inflammation leads to formation of atheromatous plaques in the arterial tunica intima, a region of the vessel wall located between the endothelium and the tunica media.

The bulk of these lesions is made of excess fat, collagen, and elastin. At first, as the plaques grow, only wall thickening occurs without any narrowing. Stenosis is a late event, which may never occur and is often the result of repeated plaque rupture and healing responses, not just the atherosclerotic process by itself.

1. Cellular

Early atherogenesis is characterized by the adherence of blood circulating monocytes (a type of white blood cell) to the vascular bed lining, the endothelium, then by their migration to the sub-endothelial space, and further activation into monocyte derived macrophages. The primary documented driver of this process is oxidized lipoprotein particles within the wall, beneath the endothelial cells, though upper normal or elevated concentrations of blood glucose also plays a major role and not all factors are fully understood. Fatty streaks may appear and disappear.

Low-density lipoprotein (LDL) particles in blood plasma invade the endothelium and become oxidized, creating risk of cardiovascular disease. A complex set of biochemical reactions regulates the oxidation of LDL, involving enzymes (such as Lp-LpA2) and free radicals in the endothelium.

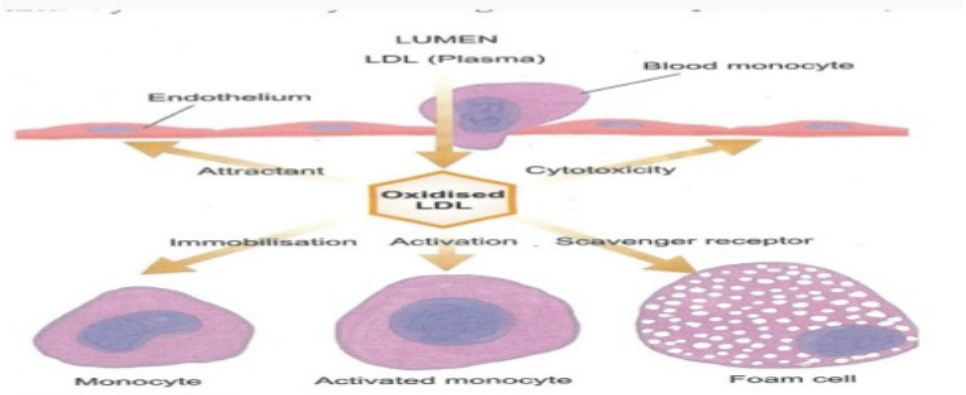


Fig. 4: Cellular mechanism of atherosclerosis.

Initial damage to the endothelium results in an inflammatory response. Monocytes enter the artery wall from the bloodstream, with platelets adhering to the area. This may be promoted by redox signaling induction of factors such as VCAM-1, which recruit circulating monocytes, and M-CSF, which is selectively required for the differentiation of monocytes to macrophages.

The monocytes differentiate into macrophages, which proliferate locally, ingest oxidized LDL, slowly turning into large "foam cells" so called because of their changed appearance resulting from the numerous internal cytoplasmic vesicles and resulting high lipid content. Under the microscope, the lesion now appears as a fatty streak. Foam cells eventually die and further propagate the inflammatory process.

In addition to these cellular activities, there is also smooth muscle proliferation and migration from the tunica media into the intima in response to cytokines secreted by damaged endothelial cells. This causes the formation of a fibrous capsule covering the fatty streak. Intact endothelium can prevent this smooth muscle proliferation by releasing nitric oxide.

2. Calcification and lipids

Calcification forms among vascular smooth muscle cells of the surrounding muscular layer, specifically in the muscle cells adjacent to atheromas and on the surface of atheroma plaques and tissue. In time, as cells die, this leads to extracellular calcium deposits between the muscular wall and outer portion of the atheromatous plaques. With the atheromatous plaque interfering with the regulation of the calcium deposition, it accumulates and crystallizes. A similar form of an intramural calcification, presenting the picture of an early phase of arteriosclerosis, appears to be induced by a number of drugs that have an anti-proliferative mechanism of action (Rainer Liedtke, 2008).

Cholesterol is delivered into the vessel wall by cholesterol-containing low-density lipoprotein (LDL) particles. To attract and stimulate macrophages, the cholesterol must be released from the LDL particles and oxidized, a key step in the ongoing inflammatory process. The process is worsened if there is insufficient high-density lipoprotein (HDL), the lipoprotein particle that removes cholesterol from tissues and carries it back to the liver.

The foam cells and platelets encourage the migration and proliferation of smooth muscle cells, which in turn ingest lipids, become replaced by collagen and transform into foam cells themselves. A protective fibrous cap normally forms between the fatty deposits and the artery lining (the intima).

These capped fatty deposits (now called 'atheromas') produce enzymes that cause the artery to enlarge over time. As long as the artery enlarges sufficiently to compensate for the extra thickness of the atheroma, then no narrowing ("stenosis") of the opening ("lumen") occurs. The artery becomes expanded with an egg-shaped cross-section, still with a circular opening. If the enlargement is beyond proportion to the atheroma thickness, then an aneurysm is created.

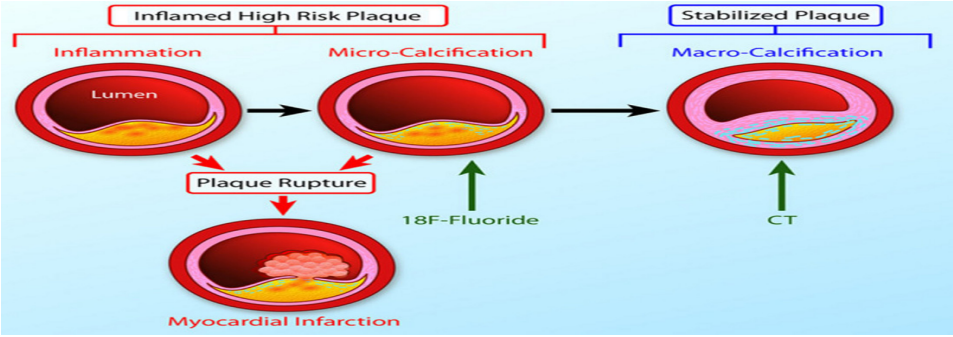


Fig. 5: Calcification and lipids mechanism of atherosclrosis.

3. Components

Apart from thromboembolism, chronically expanding atherosclerotic lesions can cause complete closure of the lumen. Chronically expanding lesions are often asymptomatic until lumen stenosis is so severe (usually over 80%) that blood supply to downstream tissue(s) is insufficient, resulting in ischemia.

These complications of advanced atherosclerosis are chronic, slowly progressive and cumulative. Most commonly, soft plaque suddenly ruptures (see vulnerable plaque), causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery in approximately five minutes. This event is called an infarction.

Diagnosis of atherosclerosis

Areas of severe narrowing, stenosis, detectable by angiography, and to a lesser extent "stress testing" have long been the focus of human diagnostic techniques for cardiovascular disease, in general. However, these methods focus on detecting only severe narrowing, not the underlying atherosclerosis disease.

As demonstrated by human clinical studies, most severe events occur in locations with heavy plaque, yet little or no lumen narrowing present before debilitating events suddenly occur. Plaque rupture can lead to artery lumen occlusion within seconds to minutes, and potential permanent debility and sometimes sudden death.

Plaques that have ruptured are called complicated plaques. The extracellular matrix of the lesion breaks, usually at the shoulder of the fibrous cap that separates the lesion from the arterial lumen, where the exposed thrombogenic components of the plaque, mainly collagen will trigger thrombus formation.

The thrombus then travels downstream to other blood vessels, where the blood clot may partially or completely block blood flow. If the blood flow is completely blocked, cell deaths occur due to the lack of oxygen supply to nearby cells, resulting in necrosis. The narrowing or obstruction of blood flow can occur in any artery within the body. Obstruction of arteries supplying the heart muscle results in a heart attack, while the obstruction of arteries supplying the brain results in an ischaemic stroke.

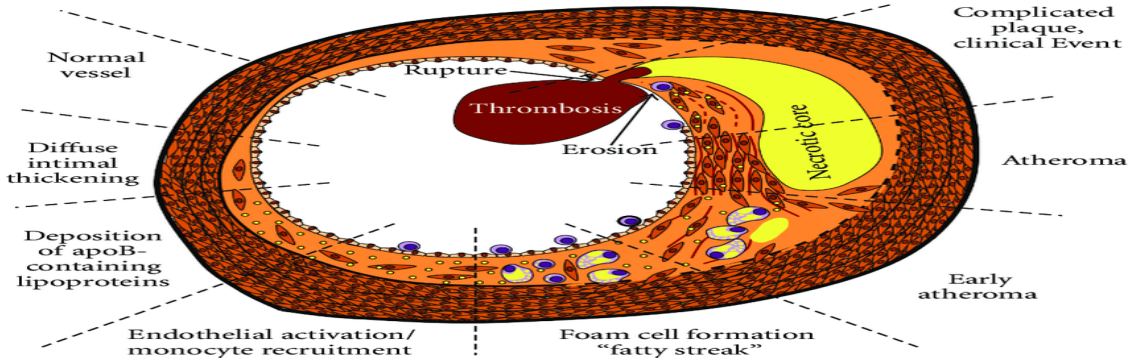


Fig. 6: Diagnosis of atherosclerosis.

Lumen stenosis that is greater than 75% was considered the hallmark of clinically significant disease in the past because, recurring episodes of angina and abnormalities in stress tests are only detectable at that particular severity of stenosis.

However, clinical trials have shown that only about 14% of clinically debilitating events occur at sites with more than 75% stenosis. The majority of cardiovascular events that involve sudden rupture of the atheroma plaque do not display any evident narrowing of the lumen. Thus, greater attention has been focused on "vulnerable plaque" from the late 1990s onwards

Besides the traditional diagnostic methods such as angiography and stress-testing, other detection techniques have been developed in the past decades for earlier detection of atherosclerotic disease. Some of the detection approaches include anatomical detection and physiologic measurement. Examples of anatomical detection methods include coronary calcium scoring by CT, carotid IMT (intimal media thickness) measurement by ultra sound, and intravascular ultra sound (IVUS).

Pathophysiology of atherosclerosis

- Atherosclerotic plaque, rupture and thrombus formation.
- Obstruction of coronary circulation.
- Necrosis of the heart tissue.
- Irreversible cardiac injury if occlusion is complete for 15-20 mins.
- Starts from endocardium and spreads towards epicardium.
- If full thickness of myocardium is involved then it is transmural infarct.

Pathogenesis of atherosclerosis

Endothelial injury

1) Initial triggering event in the development of atherosclerotic lesions.
2) Causes ascribed to endothelial injury in include mechanical trauma, hemodynamic forces, immunological and chemical mechanism, metabolic agents like chronic hyperlipidemia, homocystine, circulating toxins from systemic infections, viruses and tobacco products.

Treatment of atherosclerosis

Treatment of established disease may include medications to lower cholesterol such as statins, blood pressure medication, or medications that decrease clotting, such as aspirin.

A number of procedures may also be carried out such as percutaneous coronary intervention, coronary artery bypass graft, or carotid endarterectomy.

Medical treatments often focus on alleviating symptoms. However measures which focus on decreasing underlying atherosclerosis as opposed to simply treating symptoms are more effective.

Non-pharmaceutical means are usually the first method of treatment, such as stopping smoking and practicing regular exercise.

If these methods do not work, medicines are usually the next step in treating cardiovascular diseases and, with improvements, have increasingly become the most effective method over the long term.

The key to the more effective approaches is to combine multiple different treatment strategies. In addition, for those approaches, such as lipoprotein transport behaviors, which have been shown to produce the most success, adopting more aggressive combination treatment strategies taken on a daily basis and indefinitely has generally produced better results, both before and especially after people are symptomatic.

1. Statins

The group of medications referred to as statins are widely prescribed for treating atherosclerosis. They have shown benefit in reducing cardiovascular disease and mortality in those with high cholesterol with few side effects.

These data are primarily in middle-age men and the conclusions are less clear for women and people over the age of 70.

2. Surgery

When atherosclerosis has become severe and caused irreversible ischemia, such as tissue loss in the case of peripheral artery disease, surgery may be indicated.

Vascular bypass surgery can re-establish flow around the diseased segment of artery, and angioplasty with or without stenting can reopen narrowed arteries and improve blood flow.

Coronary artery bypass grafting without manipulation of the ascending aorta has demonstrated reduced rates of postoperative stroke and mortality compared to traditional on pump coronary revascularization.

Then the following surgical procedures.

- Angioplasty and stent placement.
- Endarterectomy.
- Fibrinolytic therapy.
- Bypass surgery.

3. Others

There is evidence that some anticoagulants, particularly warfarin, which inhibit clot formation by interfering with Vitamin K metabolism, may actually promote arterial calcification in the long term despite reducing clot formation in the short term.

Prevention of atherosclerosis

Upto 90% of cardiovascular disease may be preventable if established risk factors are avoided. Medical management of atherosclerosis first involves modification to risk factors for example, via smoking cessation and diet restrictions. Prevention then is generally by eating a healthy diet, exercising, not smoking, and maintaining a normal weight.

1. Diet

Changes in diet may help prevent the development of atherosclerosis. Tentative evidence suggests that a diet containing dairy products has no effect on or decreases the risk of cardiovascular disease.

A diet high in fruits and vegetables decreases the risk of cardiovascular disease and death. Evidence suggests that the Mediterranean diet may improve cardiovascular results. There is also evidence that a Mediterranean diet may be better than a low-fat diet in bringing about long-term changes to cardiovascular risk factors (E.g., lower cholesterol level and blood pressure).

2. Exercise

A controlled exercise program combats atherosclerosis by improving circulation and functionality of the vessels. Exercise is also used to manage weight in patients who are obese, lower blood pressure, and decrease cholesterol. Often lifestyle modification is combined with medication therapy.

For example, statins help to lower cholesterol, antiplatelet medications like aspirin help to prevent clots, and a variety of antihypertensive medications are routinely used to control blood pressure.

If the combined efforts of risk factor modification and medication therapy are not sufficient to control symptoms, or fight imminent threats of ischemic events, a physician may resort to interventional or surgical procedures to correct the obstruction.

ANTIOXIDANT ACTIVITY

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative stress, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase) produced internally, or the dietary antioxidants vitamin C and vitamin E.

Fig. 7: Natural sources of antioxidant

Definition

Antioxidant activity is defined “as a limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions” and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton’s reaction.

Oxidative stress in health and diseases

Free radical is defined as any atom (Eg. oxygen, nitrogen) have at least one unpaired electron in the outermost shell, and is accomplished of independent subsistence. Oxygen is the most significant element for life which is the major resource of free radicals.

Oxygen is used by cell for generate energy, which leads to created free radicals are as end result of ATP (adenosine triphosphate) production by the mitochondria. Free radicals occurs not only normal cellular process always occurs upon revelation to certain chemicals such as polycyclic aromatic hydrocarbon, cadmium, lead, etc., radiation, cigarette smoke and high fat diet. A balance between formations of essential for normal cellular function.

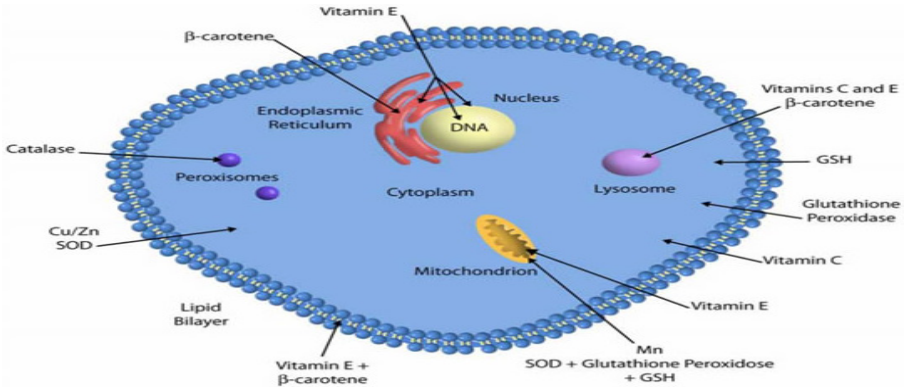


Fig. 8: Cell functions of antioxidant

Classification of antioxidants

1. Primary antioxidant

Primary antioxidants defined as the free radical chain reaction terminate by donating hydrogen or electrons which results to converting to more stable products. They are further classified into phenolic, hindered phenolic group.

The peroxy antioxidant compounds forming by the antioxidant free radical interfere with chain propagation reactions.

2. Synergistic antioxidant

Synergism defined as two or more antioxidants combine, which provide more protection than would be predictable from the sum of that provided by the individual components.

The stability of primary antioxidants will be improve by synergists which provide acidic medium. Both oxygen scavengers and chelating agents belong to this group.

- **Oxygen scavengers**

The free oxygen group react by oxygen scavengers which end of results remove oxygen in closed system.

Eg. Sulphites, ascorbic acid, ascorbyl palminate and erythobic acid.

- **Chelating agents**

Chelators are not antioxidant but establish the chelating action when the molecular structure contain unshared pair of electron. Pro-oxidant metals like iron, copper and chelators react together to form stable complexes which promote and raise the energy of activation of the initiation reactions.

Eg. Polyphosphates, EDTA, tartaric acid and citric acid.

3. Secondary antioxidant

The lipid peroxides decomposing into stable products by secondary antioxidants.They are also called as preventive antioxidants. Secondary antioxidant consists of various trivalent phosphorous and divalent sulphur containing compounds.

Eg. Thiodipropionic acid, dilauryl and distearyl esters.

4. Miscellaneous antioxidant

The miscellaneous antioxidant such as flavonoids, amino acid, zinc, β-carotene and selenium act as different role synergist, inhibits lipid peroxidation, prevent the formation of hydroperoxides, preventing the accumulation of hydrogen peroxide respectively.

Sources of antioxidants

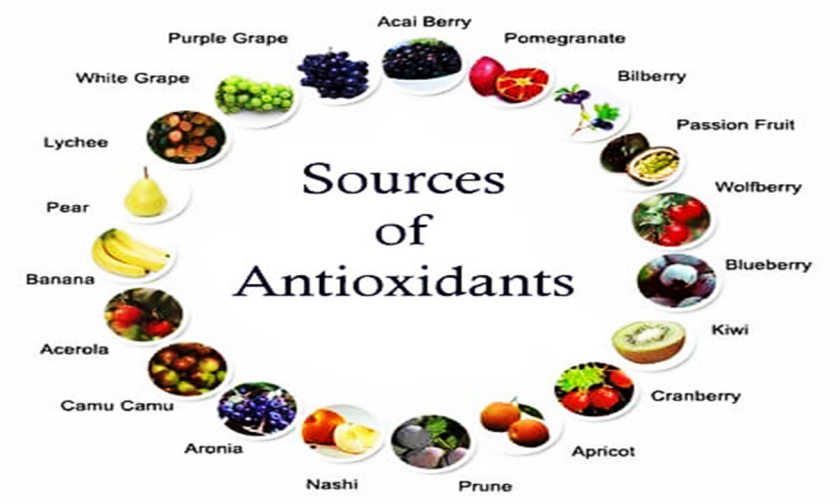


Fig. 9: Sources of antioxidants

1. Natural antioxidant

Vegetables, fruits, grain cereals, legumes and nuts etc. have varying amount of antioxidants.

Table No. 1: Natural antioxidants and their sources

S. No	Antioxidant	Food source
1	Carotenoids	Yellow / orange vegetables and fruits, dark green leafy vegetables
2	Flavonoids	Vegetables and citrus fruits
3	Isoflavones	Soyabeans
4	Phenols	Fruits, vegetables, green tea and wine
5	Sterols	Soyabeans
6	Protease inhibitor	Seeds and legumes

2. Synthetic antioxidant

Synthetic antioxidants are mainly phenolic and include butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tert-butyl hydro quinone (TBHQ), propyl, octyl and dodecyl gallates. Polymeric antioxidant such as anoxomer, lonox-30 and lonox-100, a derivative of BHT but they are not being used commercially.

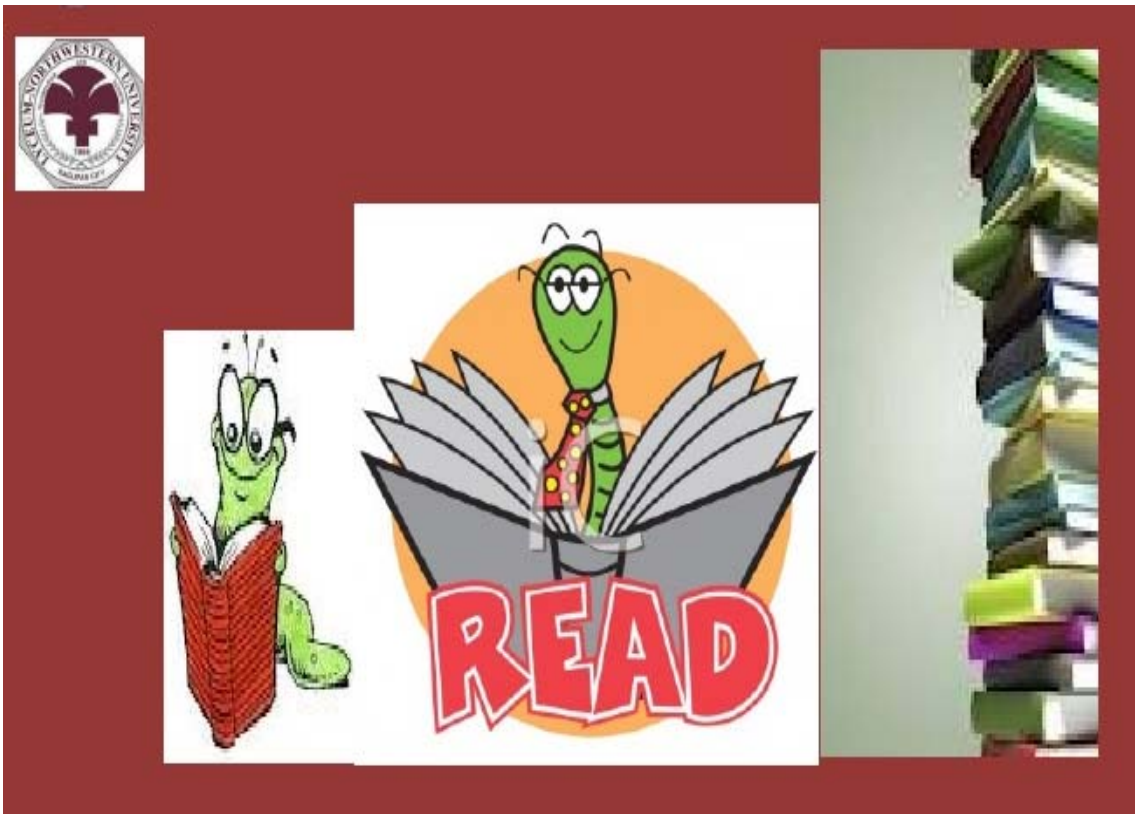
Adverse effects

High doses of some antioxidants may have harmful long term effects. The beta carotene and retinol efficacy trial (CARET) study of lung cancer patients found that smokers given supplements containing beta carotene and vitamin A had increased rates of lung cancer. Subsequent studies confirmed these adverse effects.

No health risk was seen when all the randomized controlled studies were examined together, but an increase in mortality was detected when only high quality and low bias risk were examined separately.

Uses of antioxidants

- Antioxidant used for treatments of stroke and neurodegenerative diseases such as alzheimer's disease and parkinson's disease.
- Antioxidants prevent the cell- damaging by free radicals.
- Rich source of antioxidant vegetables and fruits help to lower risk of heart disease and some neurological disease.
- Antioxidant are also widely used as an ingredients in dietary supplements in the hope of maintaining health.
- Some evidence proven antioxidant source having vegetables and fruits who consume protect against a number of cancers.
- Antioxidants are also used for preservatives in food and cosmetics materials in industry.
- Antioxidant used for preventing the degradation of rubber and gasoline.
- It is used to prevent oxidation of fuels and lubricants.



Literature Review

CHAPTER II

LITERATURE REVIEW

There are plenty of publications from all over the world on natural product chemistry, the multidisciplinary branch of science. Studies on the natural products of the various plant families and the variation of the compounds in respective members were a matter of interest for the chemists and have developed in recent years as a distinct discipline. Screening of both synthetic organic compounds and extracts of natural products have an impressive history of identifying active agents. A detailed review of past literature pertaining to the subject area is arranged under different titles such as

2.1. Review of biological properties of *Cordia* genus of *Boraginaceae* family.

2.2. Review of biological properties of various genus of *Boraginaceae* family.

2.3. Review of genus *Cordia obliqua*.

2.4. Review of *in vitro* antioxidant.

2.5. Review of GC-MS.

2.6. Review of *Insilico* molecular docking studies.

2.7. Review of anti-atherogenic activity.

2.1. Review of biological properties of *Cordia* genus of *Boraginaceae* family

Ketan Vinayakrao Hatware *et al.,* 2018 demonstrated the gastro protective effect of methanolic extract of CD leaves (MECD) obtained using Soxhlet extractor. In this study the qualitative phytochemical analysis of MECD revealed the presence of bioflavonoids and determination of quercetin was confirmed by HPLC analysis. The MECD was administered orally at doses 50 mg/kg, 100 mg/kg and 200 mg/kg against indomethacin induced gastric ulceration and stress-induced gastric ulceration in wistar rats. Omeprazole at 10 mg/kg orally was used as the reference standard. The various parameters like gastric volume, gastric pH, total acidity, ulcer

index, percent protection were estimated for assessment of anti-secretory and gastro protective effects of MECD. At the same time antioxidant parameters like superoxide dismutase (SOD), catalase (CAT) and malon-di-aldehyde (MDA) in addition to that inflammatory parameters such as tumor necrosis factor-α (TNF-α), interleukin-6 and interleukin-10 were also estimated according to their respective method of estimation using analysing kit. The MECD have reduced gastric volume, total acidity and gastric mucosal damage in both the experimental models significantly and dose dependently as compared with control group. Similarly the antioxidant enzymes like SOD and CAT were increased while MDA levels were decreased significantly, at the same time TNF-α and IL-6 levels were decreased and anti-inflammatory IL-10 levels were increased significantly in MECD treated groups. Thus the pretreatment with MECD has shown significant gastro protective potential probably due to its anti-oxidant and anti-inflammatory properties.

Kanagaraj Prabu *et al.,* 2018 investigated of phytochemical screening, anti-oxidant activity and HPLC analysis of methanol and petroleum ether extract of *Cordia diffusa* leaves. The *in vitro* anti-oxidant activity of *Cordia diffusa* leaf samples were investigated spectrophoto metrically using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Methanolic extract showed significant anti-oxidant activity compared to petroleum ether extract. Determination of phytochemical constituents like phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, anthocyanin, betacyanin and glycoside tests were carried out. HPLC chromatogram supported the presence of 1–8 phytocompounds. In *Cordia diffusa* leaves might be used as a potential antioxidant source in pharmaceutical and food industries.

Ali Esmail Al-Snafi *et al.,* 2016 investigated preliminary phytochemical screening carried out on *Cordia myxa* fruit extract revealed the presence of oil, glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, phenolic acids, coumarins, tannins, resins, gums and mucilage. Pharmacological studies revealed that *Cordia myxa* possessed analgesic, anti-inflammatory, immune modulatory, antimicrobial, anti-parasitic, insecticidal, cardiovascular, respiratory, gastrointestinal and protective effects. This review was designed to highlight the chemical constituents and pharmacological effects of *Cordia myxa*.

Edinardo Fagner Ferreira Matias *et al.,* 2015 reviewed the present species of the genus *Cordia, Boraginaceae,* are widely studied with regard to the various ethno botanical and ethno pharmacological aspects. They are found principally in tropical and subtropical regions of the American, Asian and African continents, where they occur in various countries. In the genus *Cordia*, there are many species cultivated for ornamental plants, wood and medicinal applications, where they are extensively utilized by traditional communities. In the last decades, scientific studies of *Cordia* species have intensified, demonstrating the great interest in phytochemical, biological and pharmacological studies. In this review, the principal botanical aspects, ethno pharmacological information and evaluation of the bioactive and pharmacological properties of *Cordia,* its phytochemical constituents and the most common classes of secondary metabolites identified. The information reported in this work contributes scientifically to recognizing the importance of the genus *Cordia* as a target in the search for new biotechnological investments.

Abd. Malik *et al.,* 2014 studied *Cordia myxa L. (C. myxa*) leaves is included in family *Boraginaceae* which has been used as traditional medicine in Indonesia. This study to determine of total phenolic and flavonoid content of Kanunang leaves extract (*C. myxa*). They were extracted by maceration method with ethanol 70%. The level of phenolic and flavonoid content were determined by spectrophotometer UV-Vis with gallic acid and rutin as standard. It were obtained 25 gram extract from extracting 1380,000 gram powdered leaves, the rend ament shows 7.14%. Determination of phenolic content by Folin-Ciocalteau method shows 8.45% GAE (Gallic acid equivalent), while flavonoid content determined by colorimetric method AlCl3 is 1,202% RE (rutin equivalent).

Eduardo Parisotto B *et al.,* 2012 provided a comparative evaluation of the antitumor activity from *Cordia verbenacea* extracts obtained by supercritical fluid extraction (SFE) with CO at 300 bar and 50 °C and by classical organic solvent extraction (CE) with ethanol. Antitumor in vitro assays were performed for both extracts and the results demonstrate that the supercritical extract causes superior reduction in tumor cells viability and proliferation, whereas the most probable type of cell death is apoptosis. Only the extract obtained by SFE was able to reduce the expression of COX-2 in MCF-7 cells. The *in vivo* treatment using supercritical extract

decreased the tumor volume, the body weight and packed cell volume, as well as increased in 25% the mean survival time, compared to negative control. As a conclusion, the high pressure extraction method enhanced the cytotoxicity and the antitumor activity of extract from *C. verbenacea*. Additionally, the antitumor mechanism was probably caused by the inhibition of COX-2, leading to the blockage of the survival cells by apoptosis induction.

Abdallah ZA *et al.,* 2011 investigated gastric ulcer is one of the most serious diseases in the world. Although there are many drugs used for the treatment of gastric ulcer, most of these produce several adverse reactions. This study investigated the protective effects of Assyrian plum (*Cordia myxa L*.) fruit extract (CME) against indomethacin-induced gastric ulcer in rats. Gastric ulceration was induced by a single intra peritoneal injection of indomethacin (30 mg/kg -1 b.wt.). CME was administered orally at a dose of 125 mg/kg b.wt. and ranitidine (RAN), a reference drug, at a dose of 50 mg/kg b.wt. Two weeks prior to indomethacin injection. Pre-treatment with CME produced significant reduction in gastric mucosal lesions (U.I.), malon-di-aldehyde (MDA), and serum tumor necrosis factor (TNFα) associated with significant increase in gastric juice mucin content and gastric mucosal catalase (CAT), nitric oxide (NO), and prostaglandin E2 (PGE2) levels. A similar increase in mucin content, NO and PGE2 was not observed with RAN although it generated a preventive index of 75.9%. RAN significantly increased pH value and decreased pepcin activity and gastric juice free and total acidity. Histological studies of stomach mucosa confirmed these results. Stomach of rats administrated with RAN showed leukocytic infiltration in sub mucosal layer. Meanwhile, stomach of rats administrated CME either alone or with RAN showed no histo pathological changes. CME can protect indomethacin induced gastric ulceration due to its anti-oxidative and mucin enhancing properties. The protection afforded by co-administration of CME and RAN was found to be better than that of RAN alone. Results of the present study suggest that RAN should be used together with CME for better gastro protective effect as well as to reduce H2 antagonist drugs adverse effects.

2.2. Review of biological properties of various genus of *Boraginaceae* family

Jessica Ceramella *et al.,* 2019 determined the chemical composition, antioxidant effects and antitumor properties of a methanol extract of *Anchusa azurea* Mill. (*Boraginaceae*) aerial parts against four tumour cell lines (MCF-7, MDA-MB-231, RKO, and R_2C). The antioxidant effects were assessed by using β-carotene bleaching, 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2, -diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing ability power (FRAP) tests. HPLC analyses revealed chlorogenic acid, catechin, caffeic acid, and astragalin as the most abundant compounds. Interesting results were obtained in the β-carotene bleaching test with IC_{50} values of 7.6 and 27.5 μg mL^{-1} after 30 and 60 min of incubation, respectively. Furthermore, the *A. azurea* extract protects 3T3-L1 mouse cells from oxidative stress induced by menadione and exhibits good antitumor activity, with very low toxicity. Our data indicate that the antitumor properties are due to the ability to induce programmed cancer cell death through caspase 3/7 and 9 activation and interference with the cytoskeleton dynamics.

Paola Poma *et al.,* 2019 examined the biological properties of essential oils have been demonstrated in the treatment of several diseases and to enhance the bioavailability of other drugs. In natural habitats the essential oils compounds may play important roles in the protection of the plants as anti-bacterial, anti-viral, anti-fungal, insecticides and also against herbivores by reducing their appetite for such plants or by repelling undesirable others. We analysed by gas-chromatography mass spectrometry the chemical composition of the essential oil of aerial parts of *Glandora rosmarinifolia* (Ten.) D.C. Thomas obtained by hydro distillation and verified some biological activities on a panel of hepatocellular carcinoma cell lines (HA22T/VGH, HepG2, Hep3B) and triple negative breast cancer cell lines (SUM149, MDA-MB-231). In the essential oil we detected 35 compounds. The results of the biological assays indicate that essential oil of *G. rosmarinifolia* induces cell growth inhibition at concentration-dependent way in all cell line models. This oil does not seem to possess antioxidant activity, while the cytotoxicity of *G. rosmarinifolia* essential oil appeared to involve, at least in part, a pro-oxidant mechanism. Our results show for the first time the anti-tumoral and pro-oxidant activities of *G. rosmarinifolia* essential oil and suggest that it may represent a resource of pharmacologically active compounds.

Gamal Abdelhakeem Mohamed Soliman *et al.,* 2018 revealed that *Arnebia hispidissima (A. hispidissima)* a member of the family *Boraginaceae*, is dye yielding and medicinally important plant. It is widely used in the cosmetic industries. This study has been made for the preliminary standardization of *A. hispidissima* plant. The standardization evaluation comprises of powder microscopy and fluorescence analysis and TLC profiling. In addition, preliminary phytochemical screening, determination of total phenol and in vitro free radical scavenging activity (DPPH radicals scavenging assay and reducing power activity) were preformed utilizing the methanol extract. A microscopic study of powder of whole plant of *A. hispidissima* showed different types of trichomes, vessel and fibres. Fluorescence analysis showed different colours under visible light, low UV and high UV. TLC of the hexane extract developed 8, 6, 9 and 10 spots with visible light, low UV, high UV and ninhydrin-H2SO4 spray, respectively. The phytochemical analysis of the methanol extract gave a positive indication for the presence of active compounds including alkaloids, carbohydrates, glycosides, steroids, triterpenoids, saponins, phenols, tannins, flavonoids and proteins. The quantitative analysis showed the presence of a significant quantity of total phenol and the *in vitro* antioxidant activity clearly showed the terrific antioxidant property. The data generated from the present study can be utilized for the identification and quality control of *A. Hispidissima* plant.

Ruby A Ynalvez *et al.,* 2018 revealed that leaves were collected and aqueous, acetone, diethyl ether, and ethanol leaf extracts were prepared. Antimicrobial activity against bacteria and fungi were investigated via disc diffusion assay. Phytochemical screening was done to qualitatively determine secondary metabolites. The ethanol and diethyl *E. anacua* (*Boraginaceae)* extracts showed a statistically significant antimicrobial activity against *S. aureus.* Although the values, 7.4 mm and 7.5 mm for the ethanol and diethyl ether extracts could be low values for zone of inhibitions, the potential for *E. anacua* for anti-*S. aureus* activity cannot be undermined. Phytochemical analysis showed detectable presence of alkaloids, diterpenes, and phenols in the ethanol and diethyl *E. anacua* extracts. Results of this study, although preliminary, demonstrated the potential of *E. anacua* as a new source of bioactive metabolites. Further investigations are needed in order to specifically identify, quantify, and isolate the bioactive compounds that might act against *S. aureus* associated skin infections.

Luigi Menghini *et al.,* 2018 reported that the *Cynoglossum creticum* Mill (*Boraginaceae*) is used traditionally as a remedy to manage several human ailments. In this context, the present study aimed to perform multiple pharmacological investigations on the hydro alcoholic extracts prepared from *Cynoglossum* roots and aerial parts (leaves and flowers). The protective effect of the extracts on cardiomyocyte C2C12 and intestinal HCT116 cell lines challenged with hydrogen peroxide (HO) was studied. We found that the aerial parts harbored the highest amount of phenolic compounds. Generally, aerial parts showed significant antioxidant and enzyme inhibitory effects. Leaves exhibited the best lipase inhibitory activity (173.15 mg OE/g extract), followed by flowers and roots. The root and aerial extracts were equally able to blunt intracellular H O induced reactive oxygen species production from both C2C12 and HCT116 cell lines. Both cells lines could be treated with scalar concentrations of root and flower extracts in the range 50–300 µg/ml without interferences on cell viability. In conclusion, the present study showed protective effects exerted by *Cynoglossum* extracts, which could serve as a foundation for the development of pharmaceuticals and nutraceuticals derived from *Cynoglossum.*

Azizur Rahman MD *et al.,* 2016 revealed that *Cordia dichotoma* is a tall tree which grows in Sri Lanka, India and other warmer countries. Its medicinal properties are known since long time and it is traditionally used to cure several ailments. Its fruits are used as expectorant, astringent, coolant, emollient, purgative and anthelmintic. Anti-inflammatory, analgesic, hepatoprotective and several other pharmacological activities have also been reported from the plant. Aim was focused on the assessment of its present medicinal uses, phytochemistry and pharmacology in order to reveal its complete pharmacological and therapeutic potentials. Literature survey performed on electronic sources, scientific journals as well as books showed that this plant is of an enormous value because of its various potent pharmacological actions shown by it and several pharmacologically active principles like apigenin, arabinoglucan, quercetin which have been isolated from it. It will be certainly valuable to explore it for further research to be carried out on this medicinal plant.

Anupam Roy *et al.,* 2015 revealed that *Heliotropium indicum* Linn., commonly known as ‘Indian heliotrope’, is a herb with pale violet flowers belonging to

the family *Boraginaceae* and is very common in India with a long history of traditional medicinal uses in many countries in the world. It is distributed in the tropical and temperate regions of the world and found throughout India. The plant is reported to possess antibacterial, antitumor, uterine stimulant effect, antifertility, wound healing, anti-inflammatory, anti-nociceptive and diuretic activities. A few number of chemical investigations have been performed on this plant, as for example, pyrrolizidine alkaloids and other chemical compounds like Indicine-N-Oxide, tannins, saponins and heliotrine were also isolated from this plant. This review gives an update mainly on the pharmacological activities of *Heliotropium indicum* Linn.

Sohail Ahmad *et al.,* 2014 analyzed the studies of *Heliotropium bacciferum* is paramount in medicinal perspective and belongs to *Boraginaceae* family. The crude and numerous fractions of leaves, stem, and roots of the plant were investigated for phytochemical analysis and DPPH radical scavenging activity. Phytochemical analysis of crude and fractions of the plant revealed the presence of alkaloids, saponins, tannins, steroids, terpenoids, flavonoids, glycosides, and phenols. The antioxidant (free radical scavenging) activity of various extracts of the *Heliotropium bacciferum* was resolute against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical with the avail of UV spectrophotometer at 517 nm. The stock solution (1000 mg/mL) and then several dilutions (50, 100, 150, 200, and 250 mg/mL) of the crude and fractions were prepared. Ascorbic acid was used as a standard. The plant leaves (52.59 ± 0.84 to 90.74 ± 1.00), stem (50.19 ± 0.92 to 89.42 ± 1.10), and roots extracts (49.19 ± 0.52 to 90.01 ± 1.02) divulged magnificent antioxidant activities. For the ascertainment of the fatty acid constituents a gas chromatograph hyphenated to mass spectrometer was used. The essential fatty acids for growth maintenance such as linoleic acid (65.70%), eicosadienoic acid (15.12%), oleic acid (8.72%), and palmitic acid (8.14%) were found in high percentage. The infrared spectra of all extracts of the plant were recorded by IR Prestige-21 FTIR model.

Dash GK *et al.,* 2013 reported the studies of *Heliotropium indicum* Linn., commonly known as ‘Indian heliotrope’ is very common in India with a long history of traditional medicinal uses in many countries in the world. The plant is reported to possess antibacterial, antitumor, uterine stimulant effect, antifertility, wound healing, anti-inflammatory, anti-nociceptive and diuretic activities. Several pyrrolizidine

alkaloids have been isolated from this species. The active principle Indicine-N-oxide has reached Phase 1 clinical trials in advanced cancer patients. But severe toxic side-effects showed that a therapy with indicine-N-oxide was not justified. Most of the alkaloids are heap toxic and therefore internal use of *Heliotropium* species is not recommended. External application to promote wound healing and to fight infections seems to be less hazardous, but more research is needed. The comprehensive account of the chemical constituents and the biological activities are presented in this review such that the potential use of this plant in various traditional medicines can be systematically evaluated.

2.3. Review of genus *Cordia obliqua* Willd

Richa Gupta *et al.,* 2018 revealed the presence of *Cordia obliqua* Willd. (Clammy cherry) tree of family *Boraginaceae* and genus *Cordia* is a medium-sized deciduous tree and very well distributed all over India as well as other warmer regions of the world. The objective of the study was to isolate phytoconstituent from *C. obliqua* leaf methanol extract by column chromatography and characterization by various spectroscopic techniques such as ultraviolet-visible, infrared, nuclear magnetic resonance, ,and mass. The *C.obliqua* leaf methanol extract was prepared by successive solvent extraction using Soxhlet apparatus. The methanol extract was found rich in phytoconstituents with the help of chemical tests, and also it was found effective against pain, inflammation, and pyrexia in experimental animal studies. Hence, methanol extract was selected for isolation of important plant constituents by column chromatography. The column was carried out with the different solvent system used in particular ratios. A total of 50 fractions were collected and studied by thin-layer chromatography for pooling. The pooled F2 fraction was further studied by spectroscopic techniques to characterize the compound. On isolation by column chromatography, a flavonol glycoside molecule of quercetin aglycone “3’-O-Methyl Quercetin-3-glucose-6-gallic acid” was characterized by spectroscopic techniques. It justified the effect of leaf extract in the treatment of pain, swelling, and inflammation and also an antioxidant because flavonoids are a group of therapeutic active compounds due to their supreme antioxidant action.

Prakash Rama krishnan *et al.,* 2017 investigated the anti-diabetic, anti-hyper lipidemic and antioxidant activity of methanolic extract of *Cordia obliqua*

(MECO) in streptozotocin- induced diabetic rats. Acute toxicity study of MECO was carried out in rat to determine its dose for further study. Oral glucose tolerance test was performed to evaluate MECO on elevated blood glucose levels. Diabetes was induced in rats by administration of streptozotocin (STZ) (45 mg/kg) and it was confirmed 3 days after induction. The methanolic extract of *Cordia obliqua* (MECO) was orally given to the diabetic rats up to 21 days and the blood glucose levels were estimated at the end of each week. On 21st day of the experiment, rats were sacrificed after the blood collection for the biochemical, antioxidants in kidney and pancreas was isolated for histopathological observation.

Richa Gupta *et al.,* 2017 determined maximum safe dose and explore analgesic, anti-inflammatory and antipyretic activities of *Cordia obliqua* leaf methanol extract. As per our previous study report, the leaf methanol extract is rich in phytoconstituents and has good antioxidant effect; so only methanol extract was studied here. The maximum safe dose of methanol extract was found by acute oral toxicity study according to OECD guidelines 423. For analgesic effect, hot plate and tail flick method, for anti-inflammatory, Carageen an induced rat paw edema and for antipyretic study, yeast induced pyrexia method were used.

Tilak raj *et al.,* 2016 investigated antimicrobial activity of five species of traditionally used medicinal plants namely *Adhatoda vasica, Artemisia annua*, *Cordia oblique*, *Croton bonplandianum* and *Euphorbia milli* against different strains of bacteria and fungi which are known to cause various types of infectious diseases. Organic extracts of these plants leaves (dry) were prepared, and antimicrobial sensitivity of these organic extracts (Hexane, chloroform, acetone, and methanol) against selected bacterial and fungal strains were performed by disc diffusion assay method and Resazurin-based Microtitre Dilution Assay method. Among these plants, *Cordia oblique* (chloroform extract) and *Croton bonplandianum* (Hexane extract), which showed superior antimicrobial activity in the primary screening test. *Croton bonplandianum* showed the maximum yield (7.3%) and *Adhatoda vasica* showed minimum yield (0.57) of plant extract. Chloroform extracts of *Cordia oblique* and hexane extract of *Croton bonplandianum* showed very good antimicrobial activity (MIC 0.37 mg/ml) against *Staphylococcus aureus* and *Klebsiella pneumoniae*. With this *Artemisia annua* (chloroform extract) showed very remarkable antifungal activity

(MIC 0.37 mg/ml) against *Aspergillus niger*. Among the different fractions (Hexane, chloroform, acetone and methanol) tested for antimicrobial activity, the non-polar fractions were more active than the polar fractions. In this study all the tested plants *Adhatoda vasica, Artemisia annua, Cordia oblique, Croton bonplandianum* and *Euphorbia milli* showed antimicrobial activity against at least one strain of bacterium and fungus. This might justify their claimed uses in the treatment of various infectious diseases.

Gupta R *et al.,* 2016 investigated *Cordia obliqua* Willd. (Clammy Cherry) belongs to genus *Cordia* and family *Boraginaceae*. It possesses a number of traditionally mentioned medicinal activities like purgative, diuretic, antipyretic, anthelmintic, analgesic and hepato protective. The present work is related with Pharmacognostic, Phytochemical and antioxidant study of *Cordia obliqua* leaf. Under Pharmacognostic study various parameters like macroscopic, microscopic and physiochemical parameters were studied as per WHO guidelines. This work will be helpful in authentication of *Cordia obliqua* willd. After this successive soxhlet extraction was performed for leaf powder with various solvents in increasing order of polarity like Hexane, Chloroform, Methanol and water. These extracts were used to study presence of various chemical constituents as well as to determine amount of total Phenol and Flavonoids content. Further antioxidant activity study was performed with help of DPPH and H_2O_2 radical scavenging methods. The leaf methanol extract was found to have maximum amount of phenols and flavonoids. In antioxidant activity study, again the results with methanol extract were found better and comparable with standard and it may be due to presence of more amounts of phenols and flavonoids. Finally it was concluded that the leaf methanol extract is a good antioxidant and it may also be helpful in other biological activity study.

Sungkwon Park *et al.,* 2014 evaluated the free radical scavenging activity and antimicrobial activity of *Cordia obliqua* against bacteria and fungi. Results of the current study disclosed that *Cordia obliqua* exhibited antibacterial effect against gram positive *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and gram negative *Escherichia coli*. It also showed inhibition effect against *Aspergillus niger* and *Candida albicans*. Further, *Cordia obliqua* showed free radical scavenging ability against the DPPH radicals.

Jalalpure SS *et al.,* 2009 reported the presence of *Cordia obliqua* popularly known as lahsora is common remedy for various ailments leaf juice is used as antidote in snake bite, as laxative & larger dose are given in bilious affection, decoction of stem bark is given as astringent gangle along with coconut oil. It relieves colicky pairs fruit is diuretic, anthelmintic, antipyretic & anti-inflammatory. 5, 7, dimethoxytaxifolin in 3 o-α, L-rhamnopyroside and Hespertin, 7 rhamnoside have been isolated from root extract of *Cordia obliqua*. While ethanolic extract of stem bark of *Cordia obliqua* yielded allantoin and β- sitosterol 4 and the methanolic extract of fruit mucilage yielded D- galactose rhamnose sugar 5. In present study investigate the effect on carrageenan induced paw edema in rat and also establish phyto pharmacological profile in support of the traditional claim.

2.4. Review of *in vitro* antioxidant

Amutha Iswarya Devi J *et al.,* 2017 *Syzygium cumini* (L) *(S .cumini)* commonly known as jamun belongs to the *Myrtaceae* family. The aim of the present study includes phytochemical investigation and in vitro anti-oxidant capacity of various crude extracts from the bark of *Syzygium cumini* (L) by various antioxidant assays namely DPPH (2, 2-diphenyl-1-picrylhydrazyl), Nitric oxide (NO) and Hydrogen peroxide (HO). Chloroform (CH_3Cl), Ethyl acetate (EA) and Methanolic (MeOH) extracts of *S. cumini* gave positive results for steroids, alkaloids, tannins and flavonoids. The scavenging ability of chloroform, ethyl acetate and methanolic extracts along with standard (Ascorbic acid) were evaluated between the range of 20µg/ml to 300µg/ml using DPPH anti-oxidant assay and the IC_{50} values were found to 41µg/mL, 57µg/mL, 53µg/mL and 6.1µg/mL respectively. To prove further its anti-oxidant activity, they were evaluated using NO and HO antioxidant assays.

Bun Tsoi *et al*., 2015 revealed that the most commonly applied strategies for the evaluation of antioxidant capacity are the chemical- or cell-based approaches. However, the results obtained from these methods might not reflect the antioxidant ability of test samples within organisms. In this study, we propose a combination of experiments, including oxygen radical absorbance capacity (ORAC), cellular antioxidant activity assay (CAA), and the chick embryo model, as an efficient trio to evaluate antioxidant capacity of food components. Taking purine alkaloids as example, results demonstrate that chemical and cellular method might misinterpret

their true ability on anti-oxidation. In chick embryo model, caffeine and theacrine can significantly improve vessel density on chorioallantoic membrane and myocardial apoptosis. The mechanism can be involving multiple targets within the organism. We believe that the trio proposed can be widely utilized in screening massive number of antioxidant in a cost-effective way. It will also help discovering new antioxidants that are easily being omitted due to their relatively poor *in vitro* activities.

Jaffnaa Banu M *et al.,* 2015 examined the *in vitro* antioxidant activities of various extracts of aerial parts of *Premna tomentosa.* The antioxidant activity was evaluated by DPPH (α,α- diphenyl-β-picrylhydrazyl) radical scavenging activity, Iron chelating activity with reference standard rutin, EDTA and total phenolic content respectively. The methanolic extract of *Premna tomentosa* was found to more effective in the DPPH radical scavenging activity. The IC_{50} of the methanolic extract of *Premna tomentosa* and Rutin were found to be 245 µg/ml and 480 µg/ml respectively. The Iron chelating activity of the methanolic extract of *Premna tomentosa* was found to most effective than that of petroleum ether & ethyl acetate extract. An IC_{50} value of *Premna tomentosa* and EDTA were found to be 78 µg/ml and 65 µg/ml respectively. But when compare to the all the two extracts with rutin (standard), the methanolic extract of the *Premna tomentosa* showed the similar result. In addition, the methanolic and ethyl acetate extract of *Premna tomentosa* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. It is concluded that aerial parts of methanolic extract of *Premna tomentosa,* which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Amutha Iswarya Devi J *et al.,* 2014 the study was designed to examine the *in vitro* antioxidant activities of ethanolic extract of whole plant of *Lactuca runcinata*. The antioxidant activity was evaluated by DPPH radical scavenging, Nitric Oxide Scavenging and Iron chelating activity with reference standard Rutin, Ascorbic acid and EDTA respectively and total phenol content was estimated. An IC_{50} value was found that ethanolic extract of *Lactuca runcinata* is more effective in DPPH scavenging activity and nitric oxide radical scavenging activity when compared with

standard. In addition, the ethanolic extract was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. So, the *in-vitro* studies clearly showed that the ethanolic extract of *Lactuca runcinata* has a significant antioxidant activity. These *in-vitro* assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Natarajan suresh *et al.,* 2013 investigated was to examine the *in-vitro* antioxidant potential of various extracts of whole plant of *Trianthema decandra* by different *in-vitro* methods. The antioxidant activity was determined by DPPH activity, superoxide radical scavenging and iron chelating activity. An IC_{50} value was found that methanolic extract of *Trianthema decandra* is more effective in free radical scavenging activity than that of other two extracts. So, the *in-vitro* studies clearly showed that the methanolic extract of *Trianthema decandra* has a significant antioxidant activity. These *in-vitro* assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Rahmat Ali Khan *et al.,* 2012 reported to this present *Sonchus asper* (SA) is traditionally used for the treatment of various ailments associated with liver, lungs and kidneys. This study was aimed to investigate the therapeutic potential of nonpolar (hexane, SAHE; ethyl acetate, SAEE and chloroform, SACE) and polar (methanol, SAME) crude extracts of the whole plant. To achieve these goals, several parameters including free-radical (DPPH•, ABTS•+, H2O2 and •OH) scavenging, iron chelating activity, scavenging of superoxide radicals, total flavonoids and total phenolic content (TPC) were examined. The SA extracts presented a remarkable capacity to scavenge all the tested reactive species with IC_{50} values being found at the μg／ml level. The SAME was shown to have the highest TPCs while lowest IC_{50} values for the DPPH•, ABTS•+ radical scavenging capacities and iron chelating scavenging efficiency, moreover, SAME had best activities in scavenging of superoxide radicals and hydrogen peroxide as well as potently scavenged the hydroxyl radicals. These results suggest the potential of *S. asper* as a medicine against free-radical-associated oxidative damage.

Chinedu Prosper Anokwuru *et al.,* 2011 analysed the poly phenolic content and antioxidant activity of *Hibiscus sabdariffa calyx* in methanol, ethanol, acetone and water extract were studied. The Total Phenolic Content (TPC) was determined using folin Ciocalteu method while the Total Flavonoid Content (TFC) was determined using aluminium chloride method. Antioxidant activities were determined using 2, 2-diphenyl-1-picryl hydrazine (DPPH) inhibition and lipid peroxidation inhibition. Methanol extracted the highest total phenolic content (29.2 mg GAE/g DW) and was significantly different ($p<0.05$) from the water and acetone extracts only. Acetone extracted the highest flavonoid content (53.6 mg QE/g DW) and was significantly different from the other solvents used. Methanol extract gave the highest inhibition to DPPH (78%) and was only significantly different ($p<0.05$) from acetone and water extracts. Ethanol gave the highest inhibition to lipid peroxidation (26%) but was not significantly different ($p<0.05$) from the other solvent extracts. There was a stronger correlation obtained between TPC and inhibition of DPPH ($r = 0.969$) compared to TFC and DPPH ($r = 0.742$). The study showed that methanol and ethanol were better solvents for the extraction of phenols of *Hibiscus sabdariffa calyx* compared to water and acetone. It also showed that phenols contributed more to the antioxidant activity of *Hibiscus sabdariffa calyx* compared to flavonoids.

2.5. Review of GC - MS

Francesca Ieri *et al.,* 2019 revealed that essential oils are widely used as functional ingredients for potential multi-purpose functional uses. Hydrosols, co-products of the distillation of plant material, are used in food and cosmetic industries and in biological agriculture, but their volatile composition is poorly investigated. The volatile fractions of essential oils and hydrosols from four less-studied 1,8-cineol-rich *Eucalyptus* species (*E. parvula* L.A.S. Johnson & K.D. Hill, *E. cinerea* F. Muell, *E. Pulverulenta* Sims and *E. pulverulenta* baby blue Sims), cultivated in Tuscany in a system of organic farming, were characterized by solvent dilution (essential oils) or extraction (hydrosols) followed by GC-MS and by HS-SPME-GC×GC-TOFMS analysis. GC-MS analysis showed that essential oils were mainly constituted by oxygenated monoterpenes, particularly 1, 8-cineole, with monoterpenes hydrocarbons up to 10.8%. Relative differences in the abundance of minor terpenes as limonene, α-pinene, γ-terpinene, *p*-cymene, terpinen-4-ol, α-terpineol, and

alloaromandrene were pointed out and seem to be suitable for differentiation among EOs of the four different *Eucalyptus* species. Hydrosols of these species were characterized for the first time: they were mainly constituted by oxygenated monoterpenes (97.6–98.9%), with 1, 8-cineole up to 1.6 g/L, while monoterpene and sesquiterpene hydrocarbons were detected only in traces. HS-SPME-GC×GC-TOFMS analysis also allowed providing metabolic profiling of hydrosols for the direct comparison and visualization of volatile components, pointing out the potentially different uses of these products as functional ingredients in food, beverage, and cosmetic industries.

Gupta R *et al.,* 2017 reported the study of *Cordia obliqua* willd. is an important medicinal plant of family *Boraginaceae.* It is commonly known as Clammy Cherry. It is a deciduous tree and found throughout Himalayan region in India. Traditionally, it possesses a number of medicinal activities like diuretic, expectorant, antipyretic, anthelmintic and maturant. They investigate chemical constituents present in fractions of leaf methanol extract of *Cordia obliqua* plant using GC-MS analysis. Two fractions of methanol extract (CO_1 & CO_3) were studied and about 30 components were detected in each sample. Highest peak area was observed for moncyclic sesquiterpene alcohol α-Bisabolol oxide b at RT 20.84 in CO_1 and for o-methyl-dglucose at RT 16.53 in CO_3. The important constituents detected were Amino acids, Oleic acid, Oleanolic acid, Sesquiterpene lactone, trietrpenoids, various steroids, galactopyranoside, Hexadecanoic acid, Phthalic acid, Azafrin, Decatrienoic acid, Docasenamide, Morphinan and carotenoids. Among these Morphinan is an opoid alkaloid and good psychoactive drug. Other constituents are also medicinally important and many have reported biological activities. Thus GC-MS analysis revealed the existence of various types of constituents in *Cordia obliqua* leaf methanol extract fraction which confirm the application of this plant for a number of medicinal activities.

Ameera Omran Hussein *et al.,* 2016 determined the phytochemical composition from the dried galls of *Quercus infectoria*, using methanolic extraction and report the main functional components by using infrared (IR) technique. The phytochemical compound screened by gas chromatography-mass spectrometry (GC-MS) method. Twelve bioactive phytochemical compounds were identified in the

methanolic extract of *Q. infectoria*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, and molecular formula. GC-MS analysis of *Q. infectoria* revealed the existence of the Cis-p-mentha -1(7),8-dien-2-ol, 3-Nonynoic acid, Urea, N,N´-bis (2-hydroxyethyl)-, 3-Trifluoroacetoxypentadecane, Pterin -6-carboxylic acid, 2,2-Difluoroheptacosanoic acid, y-Sitosterol, Spirost-8-en-11-one, 3-hydroxy-, (3ß,5α,14ß,20ß,22ß,25R)-, Curan, 16,17-didehydro-, (20xi.)-, 17.alfa.21ß-28, 30-bisnorhopane, ethyl iso-allocholate, milbemycin B ,6,28– anhydro-15- chloro-25- isopropyl-13- dehydro-5-. The Fourier transform-infrared (FTIR) analysis of *Q. infectoria* proved the presence of alkenes, aliphatic fluoro compounds, nitro compounds, alkanes, hydrogen bonded alcohols, and phenols.

Mohanad Jawad Kadhim *et al.,* 2016 determined the chemical composition of aerial parts extract from methanol. The phytochemical compound screened by gas chromatography-mass spectrometry (GC-MS) method. Thirty one bioactive phytochemical compounds were identified in the methanolic extract of *Ocimum basilicum*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, MS fragment-ions and pharmacological actions. GC-MS and Fourier transform infrared (FT-IR) analyses of *O. basilicum* revealed the existence of paromomycin, stevioside, campesterol and ascaridole epoxide, aliphatic fluoro compounds, alcohols, ethers, carboxylic acids, esters, nitro compounds, alkanes, H-bonded H-X group, hydrogen bonded alcohols and phenols. Methanolic extract of bioactive compounds of *O. basilicum* was assayed for *in vitro* antibacterial activity against *Pseudomonas aerogenosa, Proteus mirabilis, Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* by using the diffusion method in agar. The zone of inhibition was compared with different standard antibiotics. The diameters of inhibition zones ranged from 5.70±0.10 to 0.55±0.29 mm for all treatments.

Joseph Sirdaarta *et al.,* 2015 studied this multiple sclerosis is an autoimmune disease which can be triggered in genetic susceptible individuals by *Acinetobacter spp*. and *Pseudomonas aeruginosa* infections. *Terminalia ferdinandiana* (Kakadu plum) fruit has documented therapeutic properties as a general antiseptic agent. Extracts prepared from the leaves have also been shown to

block several microbial triggers of autoimmune inflammatory diseases. This study examines the ability of Kakadu plum fruit extracts to inhibit some microbial triggers of multiple sclerosis. *T. ferdinandiana* fruit solvent extracts were investigated by disc diffusion assay against reference and clinical strains of *A.baylyi* and *P. aeruginosa*. Their MIC values were determined to quantify and compare their efficacies. Toxicity was determined using the Artemia franciscana nauplii bioassay. Active extracts were analysed by non-targeted HPLC-QTOF mass spectroscopy (with screening against 3 compound databases) and by GC-MS (with screening against 1 compound databases) for the identification and characterisation of individual components in crude plant extracts. Methanolic, aqueous and ethyl acetate *T. ferdinandiana* leaf extracts displayed potent antibacterial activity in the disc diffusion assay against the bacterial triggers of multiple sclerosis (*A.baylyi* and *P. aeruginosa*). The methanol and ethyl acetate extracts had the most potent growth inhibitory activity, with MIC values less than 1000 µg/ ml against *A. baylyi* and *P. aeruginosa* (both reference and clinical strains). In comparison, the water extract was substantially less potent. Neither the chloroform nor hexane extracts inhibited the growth of any of the bacterial strains tested. All *T. ferdinandiana* fruit extracts were nontoxic in the Artemia fransiscana bioassay. Non-biased phytochemical analysis of the ethyl acetate extract revealed only low levels of the tannins gallic acid and chebulic acid and no other tannins. The low toxicity of the *T. ferdinandiana* fruit extracts and their potent inhibitory bioactivity against the bacterial triggers of multiple sclerosis indicates their potential as medicinal agents in the treatment and prevention of this disease. Phytochemical studies indicate that this activity is likely to be due to phytochemicals other than tannins.

Amutha Iswarya Devi J *et al.,* 2014 isolated and analyzed the chemical composition in ethanolic extract from the whole plant of *Saccharum spontaneum* Linn. by gas chromatography-mass spectrometry(GC-MS). The shade dried whole plant powder was extracted with ethanol by using Soxhlet extractor and crude ethanolic extract was obtained. Qualitative analyses of ethanolic crude extract of *S. spontaneum* by using GC-MS showed that they were different types of high and low molecular weight compounds. Most of the isolated and identified compounds by GC-MS in the crude extracts are basically biologically important. Further, the *Saccharum*

spontaneum possessed certain characteristics that can be ascribed to cultivation on a domestic plantation. The crude extract was prepared from the whole plant powder of *S. spontaneum* for respective compounds can be chosen on the basis of above GC-MS analysis. Conclusion: The bioactive compounds in the ethanolic extract of *S. spontaneum* have been screened using this analysis, Isolation of individual components would however, help to find new drugs for various ailments by traditional practitioners.

Sermakkani M *et al.,* 2012 revealed that plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history it has been used as a popular folk medicine. *Cassia italica* has medicinal values; methanol leaf extract of this plant was analysed using Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of 17 compounds. In GC-MS analysis, some of the phyto components screened were phytol, squalene and n-hexadecanoic acid. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Many of them are used in industry for various applications like flavor, antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive.

Gopalakrishnan S *et al.,* 2011 revealed that *Mussaenda frondosa* is one of the medicinally important plants belonging to the family *Rubiaceae,* commonly known as ”Vellai ilai” in Tamil. Traditionally leaves are used in the treatment of jaundice, asthma, hyperacidity, ulcers, leprosy, diuretic, wound, swells, anti-microbial, diuretic activity, hypolipidemic effect, hepatoprotective activity, fever and cough. In the present study the ethanolic extract of *Mussaenda frondosa* has been subjected to GC-MS analysis. Twenty chemical constituents have been identified, the major chemical constituents are (Quinic acid (32.87 %), 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (8.30%), Naphthalene, decahydro- 2-methoxy-(7.20 %). 1, 2, 3-Benzenetriol (7.70%).

2.6. Review of *Insilico* molecular docking studies

Rajkumar Reddyrajula *et al.,* 2019 synthesized a library consisting of four sets of phenothiazine incorporated 1,2,3-triazole compounds using molecular hybridization approach. In total, 36 new hybrid molecules were synthesized and screened for in vitro growth inhibition activity against Mycobacterium tuberculosis H37Rv strain (ATCC-27294). Among the tested compounds, nineteen compounds exhibited significant activity with MIC value 1.6 μg/mL, which is twofold higher than the MIC value of standard first-line TB drug Pyrazinamide. In addition, all these compounds are proved to be non-toxic (with selective index > 40) against VERO cell lines. However, these compounds did not inhibit significantly the growth of *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* strains: the activity profile is similar to that observed for standard anti-TB drugs (isoniazid and pyrazinamide), indicating the specificity of these compounds towards the Mycobacterium tuberculosis strain. Also, we report the molecular docking studies against two target enzymes (Inh A and CYP121) to further validate the antitubercular potency of these molecules. Furthermore, prediction of in silico-ADME and pharmacokinetic parameters indicated that these compounds have good oral bioavailability. The results suggest that these phenothiazine incorporated 1,2,3-triazole compounds are a promising class of molecular entities for the development of new antitubercular leads.

Muhammad Tahir Aqeel *et al.,* 2018 reported the hyperlipidemia is a worth-mentioning risk factor in quickly expanding cardiovascular diseases, including myocardial infarction and, furthermore, in stroke. The present work describes the synthesis of phenolic derivatives **4a**–**e** and **6a**–**c** with the aim of developing antihyperlipidemic agents. The structures of the synthesized compounds were confirmed by spectroscopic data. The *in silico* docking studies were performed against human 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase enzyme (PDB ID: 1HWK), and it was observed that compounds **4a** and **6a** exhibited maximum binding affinity with target protein having binding energies −8.3 and −7.9 kcal, respectively. Compound **4a** interacts with amino acids Val805 with distance 1.89 Å and Met656, Thr558, and Glu559 with bonding distances 2.96, 2.70, and 2.20 Å, respectively. The in vivo antihyperlipidemic activity results revealed that

compound **4a** indicated minimum weight increment, ie, 20% compared with 35% weight increment with standard drug atorvastatin during 6 weeks of treatment. Moreover, increment in high-density lipoprotein cholesterol and decrease in total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels were more prominent in case of **4a** compared to atorvastatin with $P<0.05$. The synthesized compounds were nontoxic and well tolerated because none of the mice were found to suffer from any kind of morbidity and death during 6 weeks of dosing.

Amutha Iswarya Devi J *et al.,* 2017 The purpose of the study is to evaluate the α-amylase inhibitory activity of active constituents present in *Pleiospermium alatum* using *in silico* docking studies. In this perspective, active plant constituent ligands were prepared for the docking evaluation. Acarbose, a known α-amylase inhibitor was used as the standard. In silico docking studies were carried out using recentversion of GLIDE software v5.5 developed by Schrödinger. These results showed that all the active constituents showed binding energy ranging between - 3.6 to - 7.7 kcal/mol when compared with that of the standard (6.3 kcal/mol). 2-Hydroxymethyl- 5-(1-hydroxy-1-isopropyl) - 2-Cyclohexen-1-on and 1, 2-Benzene dicarboxylic acid, di iso octyl ester contributed excellent α- amylase inhibitory activity because of its structural prameters. These molecular docking analyses lead to the further development to identify the potent α-amylase inhibitors for the treatment of diabetes.

Subramanian Suganya *et al.,* 2016 reported the plant products have always been considered for many important metabolic disorders due to its abundant medicinal properties. Alarming adverse effects of overuse of statins has been reported for patients with dyslipidemia. This study was aimed to identify compounds with potent anti-dyslipidemic property from selected plants and analyze them for their efficiency in binding with HMG-CoA reductase, a key enzyme in lipid metabolism. The docking studies indicate rutin as the best compound that can inhibit HMG-CoA reductase as it had strong binding affinity to the enzyme. The molecular dynamics simulation studies confirmed the stability of the HMG-CoA reductase–rutin complex. RMSD, RMSF, Rg, H-bond results indicated that the HMG-CoA reductase–rutin complex is highly stable. Presently, statins are not preferred for individuals with pre-existing liver disease. Our study identified rutin as a promising lead compound which could be further developed into an anti-dyslipidemic molecule.

Rangachari Balamurugan *et al.,* 2015 analysed the molecular docking analysis has been carried out to examine the hypolipidemic property of γ-sitosterol against five target proteins [acetoacetyl thiolase (PDB ID: 2 F2S), 3-(HMG-CoA) reductase (1DQ8), HMG-CoA synthase (PDB ID: 2P8U), squalene synthase (PDB ID: 3 V66, Oxido squalene cyclase (PDB ID: 1W6J)] which involved in cholesterol biosynthesis. The crystallographic structures of these target proteins were retrieved from PDB data base, and their active sites were identified by CastP server. The target proteins were subjected to docking analysis using Autodock tool v 4.2 and ADT v 1.5.6 programs. The docking studies showed that γ-sitosterol is a good and significant molecule which docks well with least lowest binding energy and inhibition constant values. Hence, γ-sitosterol can be considered for developing into a potent hypolipidemic agent.

Vijay M Khedkar *et al.,* 2014 investigated into the mechanism of antihyperlipidemic action of 2-chloromethyl-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (LM-1554) was carried out through docking experiments with six different molecular targets; Niemann Pick C1 Like1 protein (NPC1L1), ATP citrate lyase (ACL), C-reactive protein (CRP), lanosterol 14α-demethylase (LDM), squalene synthase (SqS) and farnesiod X-receptor (FXR) known to be implicated in the physiology of hyperlipidemia. The interactions of LM-1554 were compared with the interactions of their respective co-crystallized native ligands at the active sites of these receptors. These comparisons are based on their docking parameters, as well as, types of interactions and vicinity with various amino acids in the active site pockets. The interaction of LM-1554 with the target, NPC1L1 has been found to be the quite favourable as compared to those with the other targets assessed in this study.

2.7. Review of anti-atherogenic activity

Nada Orsolic *et al.,* 2019 reported the study of obesity, a major health problem worldwide, is associated with increased cardiovascular risk factors, such as dyslipidemia, glucose intolerance, and hypertension. We investigated the anti-oxidative capacity of the ethanol extract of propolis (EEP) and its effect on the lipid profile, the hepatorenal function, and the atherogenic indices in mice fed with a high-fat diet (HFD). EEP (50 mg/kg) was given orally to mice for 30 days. After the

treatments, levels of the serum total triglyceride and cholesterol, the high density lipoprotein (HDL-c) and low density lipoprotein (LDL-c) cholesterols, the serum enzymes, and the metabolites were measured, and atherogenic indices [atherogenic index of plasma (AIP); cardiac risk ratio (CRR); cardioprotective index (CPI); atherogenic coefficient (AC)] were calculated and compared with the antioxidant, the reducing power, the radical-scavenging, and the chelating activity of EEP. The HFD diet with EEP significantly reduced the negative lipid profile and lowered AIP, CRR, and AC and increased CPI in animals on a HFD. In addition, EEP reduced the weight of mice and lipid accumulation in the liver, and it had significant in vitro anti-oxidative activities. The EEP possesses anti-hyperlipidemic and antioxidant activity and exhibits protective action on the cardiovascular system and hepatorenal functions. Our results contribute towards the validation of the traditional use of propolis as a food supplement in aiding hyperlipidemic disorders.

Amit Agrawal *et al*., 2019 revealed the obesity is one of the most common health problems and has become an epidemic on the global scale. Hyperlipidaemia is one of the greatest risk factors contributing to atherosclerosis and occurrence of coronary heart diseases from the first human civilization, research is going to find the drugs to treat obesity and its complications. Despite availability of many drugs in market to treat obesity, no single drug is ideal for treating all sorts of problems caused by obesity. So the research is going on finding perfect drug. In the present study evaluate antiobesity, hypolipidemic and anti-atherogenic activity of polyherbal formulations fruit of *Emblica officinalis*, rhizome of *Curcuma longa* & leaves of *Gymnema sylvestre* in high fat fed albino rats. Obesity was induced in wistar albino rats by feeding them with high fat diet for 25 days. Group-I served as normal control (1% Carboxy Methyl Cellulose (CMC)) and group-II as obese control (1% CMC) fed on high fat diet, group- III, IV were treated with various poly herbal formulations, group-V served as positive standard (Orlistat 50 mg/kg body wt.). Hyperlipidaemia was induced by feeding animals with high fat diet per orally, consisting of coconut oil and vanaspati ghee, daily *ad libitum* except normal control. The animals were treated for 14 days. At the end of the study, blood samples of the animals were sent for the estimation of the lipid profile and effects of test drug studied by comparing levels of the body weight, total cholesterol, triglycerides, HDL, LDL, and atherogenic index.

The statistical significance between groups was analysed. There was a significant reduction in food intake, body weight, TC, TG, LDL and an increase in HDL levels in high fat diet fed rats treated poly herbal formulation containing *Emblica officinalis*, rhizome of *Curcuma longa* & leaves of *Gymnema sylvestre* as compared to the positive standard and normal treated animals.

Fatima Mohamed Hussein Shediwah *et al.,* 2018 examined the presence extract of *Costus speciosus* (CSE), a herb widely used in folk medicine, was evaluated for its antioxidant, anti-hyperlipidemic and ameliorating effects on histopathological changes in atherogenic rabbits. Twenty-four male rabbits (*Oryctolagus cuniculus*) were divided into 4 groups. Three groups were fed a diet containing 3% saturated fat and 1.3% cholesterol for 40 d. One of these was sacrificed on the 40th day and was called the pathogenic (P) group; the other two groups received treatment for another 30 d as follows: one received 0.8 g/ (kg·d) of CSE and the other was given 0.01 g/ (kg·d) of simvastatin. The normal group was sacrificed on the 70th day and used as a control. CSE showed radical-scavenging ability. Administration of CSE for a 30-day period resulted in a significant decrease in total cholesterol, triacylglycerol, low-density lipoprotein and aspartate aminotransferase compared to the P group, while levels of hemoglobin, packed corpuscular volume and red blood cells were elevated. With respect to studies performed on the heart, a decrease in malondialdehyde and an increase in reduced glutathione were noted. Total protein increased in the liver, heart and aorta after treatment with CSE and also a marked improvement in histopathological parameters was demonstrated.

Chinwendu M *et al.,* 2018 investigated atherogenicity of alloxan-induced diabetic rats administered single and combinatorial herbal formulations of *acanthus montanus, asystasia gangetica, gongronema latifolium* and *solanum melongena*. A single intra-peritoneal (i.p.) injection of 90 mg/kg b.w. of alloxan monohydrate was given to the rats to induce diabetes mellitus (DM). Serum lipid profiles were measured using standard spectrophotometric methods, whereas atherogenicity, serum lipid ratios and atherogenic coefficient/indices were calculated using standard formulae. Serum total cholesterol (TC) concentrations of experimental rat groups varied between 1.59 ± 0.10 mmol/L and 2.72 ± 0.16 mmol/L ($p < 0.05$). Serum high-

density lipoprotein cholesterol (HDLC) concentration of untreated DM rat (DM-r) group was significantly lower ($p < 0.05$) than those of treated DMr groups. Atherogenic risk indices (ARIs) of treated DM-r groups were within the range of 0.74 ± 0.03 and 2.64 ± 0.21, whereas ARI of untreated DM-r was 4.04 ± 0.25. The linear regression analysis of atherognic index of plasma (AIP) versus serum low-density lipoprotein cholesterol (LDL-C) concentrations of the experimental rat groups gave a relatively close fitted regression line (R^2=0.8275). Atherogenic protection of herbal extract treated DM-r groups was within the range of 33.4–81.7%.

Geetha Kodali *et al.,* 2016 reported the study of *Saccharum spontaneum* (*Poaceae*) commonly known as Wild sugarcane is a popular folk medicine in traditional systems of medicine in India. The whole plant is used to treat mental diseases, abdominal disorders, dyspnoea, anaemia, obesity, diuretic, lithotriptic, purgative, tonic, aphrodisiac, gynecological troubles, respiratory troubles etc. The present investigation was aimed to study the effect of ethanolic extract of *Saccharum spontaneum* whole plant (ESSW) in attenuating hyperlipidemia which is associated with many cardiovascular diseases like atherosclerosis. Hyperlipidemia was induced to rats by feeding atherogenic diet (AD) for a period of 21 days. The lipid profile was taken as the major marker of hyperlipidemia and accordingly the changes in serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C), and high density lipoprotein (HDL-C) were measured (using enzymatic kits) after treatment with the ethanolic extract of *Saccharum spontaneum* whole plant (ESSW) and standard (atorvastatin). The oral administration of ESSW (100 and 200 mg/kg body weight) for 21 days significantly lowered the TC, TG and LDL-C levels while increase in HDL levels were observed in a dose dependent manner.

Jeyaraman Amutha Iswarya Devi *et al.,* 2015 the present study was designed to investigate the hypolipidemic effect of ethanolic extract from whole plant of *Lactuca runcinata* (DC.) in rats fed with atherogenic diet (AD). The acute toxicity study shows that the ethanolic extract are safe up to 2000 mg/kg, thus one tenth of this dose was consider as evaluation dose. Ethanolic extract of *Lactuca runcinata* was administered in doses of 200 and 400 mg/kg/day to rats fed with atherogenic diet to assess its possible lipid-lowering potential. There was a recognize increment in the body weight in AD fed group ($p<0.001$), which was reduced by the

administration of ethanolic extract of *L. runcinata* (400 mg/kg). The elevated levels of total cholesterol, triglycerides, phospholipids, low-density lipoprotein (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) all along with decrease in plasma HDL-C were observed in group II rats fed with atherogenic diet. After treatment of ethanolic extract of *L. runcinata,* (400 mg/kg/day) the result showed a significant ($p<0.001$) decrement in body weight, plasma and tissue total cholesterol, triglycerides, phospholipids, plasma LDL-C and VLDL-C although with an increase in plasma HDL-C when compared to group II AD rats. The ethanolic extract of *L. runcinata* could protect against atherosclerosis and decrease the atherogenic index and cardiac risk ratio. This finding provides some biochemical basis for the use of ethanolic extract of whole plant of *L. runcinata* as hypolipidemic agent having preventive and therapeutic effect against hyperlipidemia.

Ntchapda Fidele *et al*., 2014 revealed the present study of leaves of *Ficus glumosa* are used in northern Cameroon and southern Chad for the treatment of cardiovascular diseases, as food and as a stimulant for milk production in both women and animals. Atherosclerosis is a disease in which frequency increases with age. The first lesions appear at the young subject during adolescence. Atherosclerosis lesions appear very precociously and worsen with age. They interest the levels chronologically aortic, coronary then carotid. Age is a risk factor in that it reflects the exposure time of individual to the other risk factors. The frequency of the atherosclerosis increases with age because of the aging of the cells. This study was undertaken to evaluate the hypolipidemic and anti-atherosclerotic properties of aqueous extract of the leaves of *F. glumosa* in rats with hypercholesterolemia (HC). 60 male rats were fed for 4 weeks with a high-cholesterol diet (1%) and 3 doses (225, 300 and 375mg/kg) of extract of *F. glumosa* were used in these experiments. The experiments were conducted under the same conditions with atorvastatin (1mg/kg), as pharmacological reference substance. The effects of *F. glumosa* on weight gain, water and food consumption, levels of serum lipids and lipo-protein lipid oxidation and stress markers in the blood and liver were examined. The administration of *F. glumosa* extract prevented significant (P b 0.05) elevation in TC, LDL-c, VLDL-c, hepatic and aortic TG and TC. The atherogenic, triglyceride, and lipid peroxidation (TBARS) indexes were also decreased in the rats treated with the

extract. *F. glumosa* favored the performance of fecal cholesterol. It also significantly inhibited the changes and the formation of aortic atherosclerotic plaques. These results revealed the hypolipidemic and antiatherosclerotic effects of *F. glumosa* extract and support the traditional use of the extract of this plant in the treatment of hypertension and diabetes.

Gabriel A *et al.,* 2012 evaluated the antioxidant and anti-atherosclerotic activities of three *Piper* species (*Piper guineense, Piper nigrum* and *Piper umbellatum*) on atherogenic diet fed hamsters. Hamsters divided into 8 groups: normal control, atherosclerotic control and six test groups. The normal animals fed normal rodent chow, the atherosclerotic control animals fed the same rodent chow supplemented with 0.2% cholesterol and 10% coconut oil (high cholesterol diet). The 6 test groups' animals fed same diet as the atherosclerotic control group but with additional supplementation of 2 graded doses (1 and 0.25 mg/kg body weight, o.p.) of plant extracts for 12 weeks. The atherogenic diet induced a collapse of the erythrocyte antioxidant defense system (significant decrease in superoxide dismutase, catalase and glutathione peroxidase activities). Atherogenic diet also induced an increase in plasma total cholesterol, triglyceride, thiobarbituric acid reactive substances (TBARS), oxidation of low density lipoprotein cholesterol (LDL) and accumulation of foam cells in the aorta a hall mark for atherosclerosis. Administration of the Piper species prevented the collapse of the antioxidant system and the increase of plasma parameters maintaining them towards normality. The Piper species also prevented LDL oxidation by increasing the time (lag time) for its oxidation. The results suggest that these Piper species have significant antioxidant and anti-atherogenic effect against atherogenic diet intoxication.

Saravanan Subramaniam *et al.,* 2011 reported the *Terminalia arjuna* is a herb of *Combretaceae* family which contains hypolipidemic compounds and flavonoids with high anti-oxidative properties. This study was conducted to determine the effect of ethanolic fraction of *T. arjuna* on blood lipids and atherosclerosis in rabbits fed with high fat diet (HFD). Twenty New Zealand rabbits of either sex were randomly divided into five groups the first two were normal diet group and HFD (21% fat) group and the remaining three groups received high cholesterol diet supplemented with standard drug (Atorvastatin 10mg kg−1 body weight), *T. arjuna*

ethanolic fraction (100 and 200 mg kg−1 body weight), respectively. The concentration of total cholesterol (TC), low density lipoprotein (LDL) cholesterol, triglycerides (TGs), very low density lipoprotein (VLDL) cholesterol and high density lipoprotein (HDL) cholesterol was determined in rabbits at the start of the experiment, at the 14^{th}, 30^{th} days and at the end of the study. Anti-atherogenic index was calculated from the lipid profile of the rabbits before sacrifice. At the end of the experimental period, the aorta was removed for assessment of atherosclerotic plaques. Results show that *T. arjuna* significantly decreases TC, LDL and TG levels and increases HDL and lessens atherosclerotic lesion in aorta (P <0.05). Hence *T. arjuna* extract can effectively prevent the progress of atherosclerosis. This is likely due to the effect of *T. arjuna* on serum lipoproteins and its antioxidant and anti-inflammatory properties.

PLANT PROFILE

CHAPTER II

PLANT PROFILE

Cordia obliqua Willd. plant belongs to genus *Cordia* and family *Boraginaceae.* Commonly it is known as “Clammy Cherry” and “Lasora” in hindi. It is a medium sized deciduous tree and very vigorous in growth. The ripe fruits are traditionally eat by local tribes and raw fruits are used as pickle. *Cordia obliqua* is otherwise called as “Narivili” in tamil. It is one of the most traditional system of medicine in Ayurvedic and Siddha.

The leaves are useful in ulcers and in headache. The juice of the bark along with coconut oil is given in gripes. The barks and also the unripe fruit are used as a mild tonic. Fruits are used as anthelmintics, astringent, demulcent, diuretic, expectorant in bronchial affections and irritation of urinary passages. The kernals are a good remedy in ringworm. The santals use the powder of the bark as an external application in prurigo. The Japanese use the bark in fever.

The leaf is dorsiventral, xeromorphic and hypostomatic. The midrib is thick and wide and plano-convex in sectional view; the adaxial side is flat and the abaxial part is semicircular. It is 900µm thick and 850µm wide. The epidermal layer is thin and the cells are spindle shaped, thick walled and darkly stained. The ground tissue around the vascular strand is parenchymatous; the cells are circular, thick walled and compact. The trichome is 140µm long. The stomata are 20 × 30µm in sizes. The lamina is 200µm thick. The palisade cells are 80µm in height.

Fig. 10: Leaves of *Cordia obliqua* Willd.

BOTANICAL DESCRIPTION

Botanical name : *Cordia obliqua* Linn.

Family : *Boraginaceae*

Subfamily : *Cordioideae*

Kingdom : *Plantae*

Subkingdom : *Tracheobionta*

Division : *Magnoliophyta*

Subdivision : *Spermatophyta*

Order : *Boraginales*

Genus : *Cordia*

Species : *Cordia obliqua* Willd.

Domain : *Eukaryota*

Phylum : *Spermatophyta*

Subphylum : *Angiospermae*

Class : *Dicotyledonae*

Subclass : *Asteridae*

Common name : Clammy Cherry,

Mookuchalipazham.

Synonyms : *Cordia wallichii,*

Cordia myxa,

Cordia latifolia,

Cordia dichotoma.

Fig. 11: Leaf with fruit of *Cordia obliqua* Willd.

VERNACULAR NAMES

Tamil : Naruvili, Virisu.

Sanskrit : Bhukarvudara.

Kannada : Chellamara, Nakkera.

Malayalam : Pasakamaram.

Punjab : Laswara.

Telugu : Bankanakkera, Chinnabotuku.

Gujrati : Gundomoto, Lepistan.

English : Sebesten Plum.

Hindi : Lasura, Bhirala, Chhota, Laslasa, Gondi, and Rasalla.

Bengal : Bahubara, Bohari.

Marathi : Bargund, Bhokar.

Uriya : Amobhoto, Gondi.

PLANT MORPHOLOGY

Tree

Fig. 12: Tree of *Cordia obliqua*

Cordia obliqua Willd. Is a medium sized tree, 10.5m high, found scattered throughout the mid – Himalayas up to elevations of 1,470 meters. There are two forms of *Cordia obliqua* Willd. Occurring in Himachal Pradesh. The major difference between these two is the size of the fruits, which is small in one case and large in the other. The present observations were recorded only on the small – fruited type which is common.

Leaves

Fig. 13: Leaves of *Cordia obliqua*

Leaves are alternate, ovate, 10.1cm long, 5.7cm broad, entire to slightly dentate with pinnately – reticulate venation. Young leaves are tomentose from beneath and the matured leaves are glabrous, but more or less rough when full grown. Sometimes, variable in shape from elliptic lanceolate to broad ovate, often with a rounded or cordate base, basal nerves 3, rarely5, blade 3-6, petioles 2.5-5cm long.

Flower

Fig. 14: Flowers of *Cordia obliqua*

Flowers are small and average diameter of a fully open flower 6mm, very short stalked, bisexual, white in colour. Inflorescence, terminal or an axillary cyme almost resembling a biparous cyme. Flowers per cluster 14. Calyx is cup shaped and

gamosepalous, 4mm, green. Corolla, creamish white, polypetalous with 4 petals, 6 mm. Stamens 2 in number, epipetalous with a very small filament. Gynoecium has a globose ovary and bifurcate. Flowers from March – April and fruits from May – June.

Fruit

Fig. 15: Fruits of *Cordia obliqua*

Fruits is a drupe, 1.75cm in diameter, light yellow to slightly greenish in colour, with a light red tinge at the time of full maturity; epicarp, thick; mucilaginous; endocarp, hard and stony. Uses: Vegetable, Pickle.

Bark

Fig. 16: Bark of *Cordia obliqua*

Bark is dark greyish brown in colour, surface is rough with transverse and longitudinal cracks and fissures, inner surface deep greyish; fractured surface horny; taste and odour indistinct.

Parts used

Fruits, Leaves and Bark are used medicinally.

DISTRIBUTION

It is widely distributed nearly the whole of the warmer parts of India.

- **Global Distribution**
  - Pakistan, India, Srilanka, North Africa.
- **Indian Distribution**
  - State – Tamil Nadu, Kerala.
  - District – Tirunelveli, Kottayam, Kasaragode, Idukki, Malappuram, Kozhikkodi, Thrissur, Kollam, Alappuzha.
  - Place – Tenkasi.

FOLK MEDICINAL USES

- The gum obtained from mucilage is used for pasting sheets of paper and as matrix forming material in tablet formations.
- The juice of the bark is given in gripes, along with coconut oil. The bark and unripe fruit are used as a mild tonic.
- The leaves are useful as an external application to treat ulcers and headache.

PHARMACOLOGICAL USES

- Pharmacological activities confirmed *Cordia obliqua* plant as antimicrobial, hypotensive, respiratory stimulant, diuretic, and anti-inflammatory drug.
- Medicinal activities like expectorant, antipyretic, anthelmintic, and maturant.
- It is used purgative, hepatoprotective, and analgesic action.
- The fruits are edible and used as pickle.
- The raw fruits are used as vegetable.
- The mucilaginous substance of the fruits can be used as a gum for pasting sheets of paper and cardboard.


AIMS
&
OBJECTIVES

CHAPTER III

3.1 SCOPE OF THE PRESENT STUDY

Atherosclerosis generally starts when a person is young and worsens with age. Almost all people are affected to some degree by the age of 65. It is the number one cause of death and disability in the developed world. Though it was first described in 1575, there is evidence that the condition occurred in people more than 5,000 years ago.

Hyperlipidemia is a known risk factor for the advancement of cardiovascular diseases including atherosclerosis. The real risk components for the advancement of atherosclerosis are hypercholesterolemia and raised levels of low-density lipoprotein cholesterol (LDL-C). Then the per-oxidative modification of polyunsaturated fatty acids of LDL and very low-density lipoprotein (VLDL) is thought to add to the development of atherosclerotic injuries. Hypercholesterolemia contributes significantly to the occurrence and rigorousness of atherosclerosis and cardiovascular disease that could eventually affect the majority of the adult population of developed countries. **(Jeyaraman Amutha Iswarya Devi *et al.,* 2015, Pratibha K Jain *et al.,* 2017, Saravanan Subramaniam *et al.,* 2009)**

WHO has approved their safe and powerful utilize & approximately 80% of the world's populace presently utilizes herbal medicines in curative diverse diseases. India has a gold mine of very much-recorded and traditionally well-practiced information of herbal medicine. Among these herbal assets, *Cordia obliqua* Willd have been chosen for the present investigation for their evaluation of antioxidant activity and anti-atherogenic activity and its phytochemical investigations.

OBJECTIVES OF THE STUDY

1. To study the physicochemical properties of *Cordia obliqua.*

    a) Ash values

        i. Total ash value
        ii. Acid insoluble ash value
        iii. Water soluble ash value
        iv. Sulphated ash value

b) Loss on drying

c) Swelling index

d) Foaming index

e) Foreign organic matter

f) Fluorescence analysis

2. To find out the phytoconstituents present in various extracts of *Cordia obliqua.*
3. To evaluate the *in vitro* antioxidant activity of various extracts of *Cordia obliqua* the following assays have done.
    - 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical assay
    - Hydroxyl radical scavenging activity
    - Nitric oxide free radical scavenging capacity
    - Superoxide anion scavenging activity
    - Iron chelating assay
    - Ferric reducing ability power (FRAP) assay
    - Total antioxidant activity
4. To estimate the total phenolic compounds and flavonoids from various extracts of *Cordia obliqua* the following assays have done.
    - Estimation of total phenol
    - Estimation of flavonoids
5. To perform the GC-MS analysis for various extract of *Cordia obliqua.*
6. To perform the *In silico* docking study of various extracts of *Cordia obliqua.*
    - 3QNT
7. To find the effects of various extracts of *Cordia obliqua* in plasma lipid profiles in high fat diet fed rats the following parameters were estimated.
    - Total cholesterol

- Free cholesterol
- Ester cholesterol
- Triglyceride
- Phospholipid
- Free fatty acid
- Atherogenic index

8. To find the effects of various extracts of *Cordia obliqua* in plasma lipoproteins in high fat diet fed rats the following parameters were estimated.
9. To find the effects of various extracts of *Cordia obliqua* in tissue lipid levels in high fat diet fed rats the following parameters were estimated.
    - Free cholesterol
    - Ester cholesterol
    - Triglyceride
    - Phospholipid
    - Free fatty acid
10. To find the effect of tissue lipid peroxidation to verify the antioxidant roles of various extracts of *Cordia obliqua* the following parameters were estimated.
    - TBARS Level
    - Conjugated dienes
11. To assess the *in vivo* antioxidant potential of various extracts of *Cordia obliqua* in high fat diet fed rats the following parameters were estimated.

    Enzymatic

    - Superoxide dismutase (SOD) level
    - Catalase (CAT) level
    - Glutathione peroxidase (GPx) level

- Glutathione reductase (GR) level
- Glutathione -S-transferase (GST) level

Non enzymatic

- Glutathione level

12. To understand the role of various extracts of *Cordia obliqua* on the histological changes of aorta, heart and liver on feeding high fat diet rats.


MATERIALS
&
METHODS

CHAPTER IV

MATERIALS AND METHODS

4.1. MATERIALS

Table No. 1: Plant materials

S. No	Plant name	Parts used	Family	Collection period	Place of collection
1.	*Cordia obliqua*	Leaf powder	*Boraginaceae*	July 2019	Tenkasi

4.1.1. General chemicals and reagents

Unless stated, general chemicals and reagents were purchased from Sigma, Cadila. Fisher chemicals were of analytical grade or equivalent.

4.1.2. General chemicals used

Table No. 2: The general chemicals and reagents used for the present study and their source

S. No	Chemical Name	Supplier
1.	1-amino-2-naphthol-4-suphuric acid	Sd.fine chemicals, Mumbai, India.
2.	5,5'-dithio bis-2-nitro benzoic acid (AR)	Sigma Chemicals, U.S.A
3.	Acetic acid	Sd.fine chemicals, Mumbai, India.
4.	Acetic anhydride	Sd.fine chemicals, Mumbai, India.
5.	Acetone	Nice Chemicals, Kerala, India.
6.	Acetyl acetone	Sd.fine chemicals, Mumbai, India.
7.	Ammonium molybdate	Sd.fine chemicals, Mumbai, India.
8.	Ascorbic acid	Sd.fine chemicals, Mumbai, India.

9.	Catechol	Chemsworth, Surat, India.
10.	Chloroform (AR)	Sd.fine chemicals, Mumbai, India.
11.	Copper citrate	Sd.fine chemicals, Mumbai, India.
12.	Diphenyl carbazide (AR)	Sd.fine chemicals, Mumbai, India.
13.	Dipotassium monohydrogen phosphate	Sd.fine chemicals, Mumbai, India.
14.	DL-α-Tocopherol acetate	Sd.fine chemicals, Mumbai, India.
15.	DPPH	Sigma Chemicals, U.S.A
16.	EDTA	Sd.fine Chemicals, Mumbai, India
17.	Eosin	Sigma Chemicals, U.S.A
18.	Ethanol (AR)	Nice Chemicals, Kerala, India.
19.	Ethyl acetate (AR)	Sd.fine chemicals, Mumbai, India.
20.	Hydrogen peroxide	Sd.fine chemicals, Mumbai, India.
21.	Isopropanol	Sd.fine chemicals, Mumbai, India.
22.	Methanol (AR)	Sd.fine chemicals, Mumbai, India.
23.	Nitroblue terazolium	Sigma Chemicals, U.S.A
24.	Petroleum ether (AR)	Sd.fine chemicals, Mumbai, India.
25.	Phenazine methosulphate	Sigma Chemicals, U.S.A
26.	Phospho tungstic acid	Sd.fine chemicals, Mumbai, India.
27.	Potassium dichromate	Sd.fine chemicals, Mumbai, India.
28.	Potassium dihydrogen phosphate	Sd.fine chemicals, Mumbai, India.
29.	Quercetin	Sigma Chemicals, U.S.A
30.	Rutin	Sd.fine chemicals, Mumbai, India.
31.	Sodium hydroxide	Sd.fine chemicals, Mumbai, India.
32.	Sodium meta per iodate reagent	Sd.fine chemicals, Mumbai, India.

33.	Triethanolamine	Sd.fine chemicals, Mumbai, India.
34.	Tris HCl buffer	Sd.fine chemicals, Mumbai, India.

4.1.2.1. Reagents and solutions were used

4.1.2.2. Instruments used for the study

Table No. 3: Instruments used for the present study and their source

S.No	Instruments	Supplier
1.	^{13}C-NMR spectrophotometer	Bruker, USA.
2.	^{1}H-NMR spectrophotometer	Bruker, USA.
3.	Auto analyser	Shimadzu, Japan.
4.	Heating mantle	Concord Instruments Pvt. Ltd., cochin, India.
5.	Incubator	Perfit, India.
6.	IR Infra spectrophotometer	Nicolet 170SX.
7.	Mass spectrophotometer	Shimadzu, Japan.
8.	Microscope	Nickon, Japan
9.	Rotary evaporator	Perfit, India.
10.	Soxhlet apparatus	Hi Media, Ltd, Mumbai, India.
11.	Ultra centrifuge	Remi Ltd., India.
12.	UV-Visible spectrophotometer UV 1601	Shimadzu CO. Japan.
13.	Water bath	Discovery scientific, India.
14.	Weighing balance	Shimadzu, Japan.

4.2. METHODS

4.2.1. Collection and identification of *Cordia obliqua* (Willd)

The whole plant of *Cordia obliqua* was collected from Tenkasi, Tirunelveli District of Tamil Nadu, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamil Nadu. The plant of *Cordia obliqua* and were dried under shadow, segregated, crushed by a mechanical processor and went through a 40 lattice sifter. The plant powdered materials were put away in a hermetically sealed holder.

4.2.2. Physicochemical analysis of plant powder from *Cordia obliqua*

Total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, foregin organic matter, swelling index and foaming index were performed as per Indian Pharmacopoeia.

4.2.3. Fluorescence analysis of plant powder from *Cordia obliqua*

Plants powdered materials were subjected to fluorescence analysis under ultra violet light after treatment with various chemical and organic regents. Three parameters were taken into account that is observation under long UV light (365 nm), Short UV light (254 nm) and normal day light. Similarly extracts were also subjected to UV chamber and fluorescence was observed and consistency was noted as an additional character for identification (*Madhavan V et al., 2009;* Kalaskar MG *et al*., 2010 and Sama Venkatesh *et al*., 2008).

4.2.4. Extractive value analysis of plant powder from *Cordia obliqua*

Extractive values were recorded in pet.ether, ethyl acetate, chloroform, methanol, acetone, ethanol and aqueous with a view to study the distribution of various constituents of *Cordia obliqua*. Accurately weigh 0.5gm of coarsely powdered air dried material was placed in a glass stoppered conical flask and macerated with 25ml of different solvents for 6 hours. Shacking frequently and then allowed to stand for 18 hours. The mixture was filtered rapidly. Then it was transferred to a tarred flat bottomed dish and evaporate to dryness on water bath. The residue was dried at 105°C for 6 hours. Cooled in a desiccator for 30 minutes and weighed without delay.

4.2.5. Preparation of various extracts from *Cordia obliqua*

The whole plant of *Cordia obliqua* subjected to soxhlet extraction individually using different solvents with increasing order of polarity of the solvent. The solvents included petroleum ether, ethyl acetate and ethanol. About 500gm of the dry powdered plant material were packed in a muslin cloth pouch and placed in a thimble of the soxhlet extraction apparatus attached to the mouth of a round bottomed flask containing extracting solvent. Some porcelain pieces were added into the flask to avoid bumping during heating. The each solvent extraction was carried out at 40-60ºC till color change of the solvent. Each time before extracting with the next solvent of higher polarity the powdered drug (Marc) was dried in a hot air oven below 50ºC for 10 min and again repacked in the thimble (Harborne JB., 1984). Each solvent extract was collected by distilling off the solvent, which was recovered subsequently. Then each solvent extract was concentrated by evaporation to dryness under reduced pressure using a rotary vacuum evaporator. Then each solvent was removed to obtain dried extracts and weighed separately. The percentage yield of each solvent extraction was calculated in terms of initial air dried plant material packed in the thimble. Then each solvent dried extract such as petroleum ether, ethyl acetate, and ethanol on plant *viz.,* the plant extracts were stored in airtight bottles and refrigerated until use. Different phytochemical tests were conducted for the plant extract to determine the presence and absence of various phytochemical constituents.

4.2.6. Calculation of percentage yield

The percentage yield was calculated for the various extracts with reference to the crude material taken using the formula given below.

$$\text{Percentage yield with reference to crude plant material} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

4.2.7. Phytochemical analysis of various extracts of *Cordia obliqua*

The various extracts were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and providing the identity of a substance. The pharmacological

actions of crude drugs were determined by the nature of their constituents. The phytoconstituents are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used.

The various extracts of *Cordia obliqua* were subjected to the following chemical tests, separately for the identification of various active constituents (Evans, 1997).

I. Tests for Alkaloids (Evans, 1997)

a) Dragondroff's Test

A fraction of the extracts were treated with Dragondroff's reagent and observed for the formation of yellow coloured precipitate. Indicates presence of alkaloids.

b) Wagner's Test

A fraction of the extracts were treated with Wagner's reagent and observed for the formation of a reddish brown precipitate. Indicates presence of alkaloids.

c) Mayer's Test

A fraction of the extracts were treated with Mayer's reagent and observed for the formation of white precipitate or creamy coloured precipitate. Indicates presence of alkaloids.

d) Hager's Test

A fraction of the extracts were treated with Hager's reagent and observed for the formation of yellow precipitate. Indicates presence of alkaloids.

II. Test for Carbohydrates (Evans, 1997)

a) Molisch's Test

To 2 mL of the extracts, 1 mL of α-naphthol solution were added, and concentrated sulphuric acid through the sides of test tube. Purple or reddish violet colour at the junction of the two liquids revealed, the presence of carbohydrates.

b) Fehling's Test

To 1 mL of the extracts, equal quantities of Fehling's solution A and B were added, upon heating on water bath, formation of a brick red precipitate indicated, the presence of carbohydrates.

c) Benedict's test

To 5 mL of Benedict's reagent, 1 mL of extracts solution were added and boiled for 2 minutes and cooled. Formation of a red precipitate showed, the presence of carbohydrates.

III. Tests of Glycosides (Evans, 1997)

a) Legal Test

The extracts were dissolved in pyridine and sodium nitroprusside solution was added to make it alkaline. The formation of pink red to red colour showed, the presence of glycosides.

b) Baljet Test

To 1 mL of the test extracts were added with 1 mL sodium picrate solution and the yellow to orange colour revealed, the presence of glycosides.

c) Borntrager's Test

A few mL of dilute HCl was added to 1 mL of the extracts solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was then treated with 1 mL of ammonia. The formation of red colour showed, the presence of anthraquinone glycosides.

d) Keller Killani Test

The extracts were dissolved in acetic acid containing traces of ferric chloride and it was then transferred to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually became blue, confirmed the presence of glycosides.

IV. Tests for Phytosterol (Finar, 1986)

a) Liebermann Burchard's Test

Mixed 3 mL of tests solution with 3 mL of acetic acid anhydride. It was heated and then cooled. Few drops of concentrated sulphuric acid were added. A blue colour showed, presence of phytosterol.

b) Salkowski's Test

Dissolve the extracts in chloroform and equal volume of concentrate sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represented the steroid components in the tested extract.

V. Test for Flavonoids (Dey and Harborne, 1987; Evans, 1989)

a) Shinoda test

The dried powdered or extracts were treated with 5 mL of 95% ethanol. Few drops of concentrated hydrochloric acid and 0.5 g of magnesium turnings. Pink colour was observed, presence of flavonoids.

VI. Test for Tannins and Phenolic compounds (Mace, 1963)

a) Ferric chloride test

1 mL of the extracts were added with, ferric chloride and observed the formation of a dark blue or greenish black colour showed, presence of phenolic compounds.

b) Gelatin Test (Evans, 1997)

A fraction of the extracts were treated with 1% gelatin containing 10% NaCl and observed the precipitation indicates, presence of tannins.

VII. Tests for Proteins and Amino Acids (Evans, 1997)

a) Biuret Test

1 mL of the extracts were treated with 1 mL of 40% sodium hydroxide solution followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour showed the presence of proteins.

b) Xanthoproteic Test

1 mL of the extracts were treated with 1 mL of concentrated nitric acid. A white precipitate is formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia was subsequently added orange colour indicated the presence of aromatic amino acids.

c) Lead Acetate Test

A fraction of extracts were treated with 1 mL of lead acetate. Formation of a white precipitate indicated the presence of proteins.

VIII. Test for Saponins (Evans, 1997)

About 1 mL of ethanolic extracts were diluted to 20 mL with distilled water, and shaken in a graduated cylinder for 15 minutes. A1% 1 cm layer of foam indicated the presence of saponins.

IX. Test for Fixed Oils

a) SpotTest

A small quantity of extracts were pressed between two filter papers. Oil stains on the filter paper indicated the presence of fixed oils.

b) Saponification Test

1 mL of the extracts were added with few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. The formation of soap or partial neutralization indicated the presence of fixed oils.

4.2.8. *Invitro* antioxidant activity of *Cordia obliqua*

4.2.8.1. DPPH photometric assay (Mensor *et al.,* 2001)

A methanolic solution of 0.5 mL of DPPH (0.4mM) was added to 1 mL of the different concentrations of plant ethanolic extracts and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging\ activity\ (\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A518 control is the absorbance of DPPH radical with methanol; A518 sample is the absorbance of DPPH radical with sample extract/ standard.

Reagents

- 0.4 mM, DPPH.

4.2.8.2. Hydroxyl radical scavenging activity (Elizabeth and Rao, 1990)

The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-Ascorbate-EDTA-H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 mL deoxyribose (2.8mM),0.1 mL EDTA (0.1 mM), 0.1 mL H_2O_2 (1mM), 0.1 mL Ascorbate (0.1mM), 0.1 mL KH_2PO_4-KOH buffer, pH 7.4 (20mM) and various concentrations of plant ethanolic extracts in a final volume of 1 mL. The reaction mixture was incubated for 1 hour at 37°C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Reagents

- 2.8 mM, Deoxyribose.
- 0.1 mM, EDTA.
- 1 mM, Hydrogen peroxide.

4.2.8.3. Nitric oxide free radical scavenging activity (Garrat, 1964)

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions. The reaction mixture (3 mL) containing 2 mL of sodium nitroprusside (10 mM), 0.5 mL of phosphate buffer saline (1M) were incubated at 250C for 150 mins. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological

pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Ilosvay reaction at 540 nm.

Reagents

- 10 mM, Sodium nitroprusside.
- 1M, Phosphate buffered saline.
- Sulphanilic acid reagent (0.33%).
- Napthylethylene diamine dihydrochloride (1% NEDA).

4.2.8.4. Superoxide anion scavenging activity (Winterbourne *et al.,* 1975)

Superoxide radical (O_2-) was generated from the photoreduction of riboflavin and was detected by nitro blue tetrazolium dye (NBT) reduction method. The assay mixture contained sample with 0.1 mL of nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 mL of EDTA (0.1M EDTA), 0.05 mL of riboflavin (0.12 mM) and 2.55 mL of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Reagents

- 1.5 mM, NBT.
- 0.1 M, EDTA.
- 0.12 mM, Riboflavin.
- 0.067 M, Phosphate buffer.

4.2.8.5. Iron chelating assay (Benzie and Strain, 1996)

The method of Benzie and Strain (1996) was adopted for the assay. The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 mL

of 0.05% O-Phenanthroline in methanol, 2 mL ferric chloride (200µM) and 2 mL of various concentrations ranging from 125 to 1000 µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Reagents

- O-Phenanthroline (0.05%).
- 200 µM, Ferric chloride.

4.2.8.6. Ferric reducing ability power (FRAP) assay

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3$. $6H_2O$. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5mL $FeCl_3$.$6H_2O$. The temperature of the solution raised to 37ºC before using. Plant ethanolic extracts (0.15 mL) were allowed to react with 2.85 mL of FRAP solution for 30 min in the dark condition. Readings of the coloured product (Ferrous tripyridyl triazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results were expressed in µM (Fe (II) /g) dry mass and compared with that of ascorbic acid.

Reagents

- 300 mM Acetate buffer, pH 3.6.
- 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution.
- 40 mM HCl.
- 20 mM $FeCl_3.6H_2O$

4.2.8.7. Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto *et al.,* 1999). An aliquot of 0.4 mL of sample solution was combined in a vial with 4 mL of reagent

solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples are cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity expressed relative to that of ascorbic acid.

Reagents

- 0.6 M Sulphuric acid.
- 28 mM Sodium phosphate.
- 4 mM Ammonium molybate.

4.2.9. Total phenolic compounds and flavonoids from *Cordia obliqua*

4.2.9.1. Estimation of total phenol (Mallick and Singh, 1980)

2.5 mL of various concentration extracts added with 0.5 mL of Folin's phenol reagent and 2mL of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

Reagents

- 80% ethanol.
- Sodium carbonate (20%).

4.2.9.2. Estimation of flavonoids (Cameron *et al.,* 1943)

0.5 mL of various concentrations of extract added with 4 mL of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) and kept in a boiling water bath for 15 mins.

The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/mL).

Reagents

- 1% Vanillin in 70% conc. H_2SO_4.

4.2.10. Molecular Docking Analysis of *Cordia obliqua* Willd

4.2.10.1. Molecular modeling studies

Molecular docking was performed using the software Molegro Virtual Docker (MVD) v 6.0 (www.molegr.com) along with Graphical User Interface (GUI), Molegro Virtual Docker tools was utilized to generate grid, calculate dock score and evaluate conformers. Molegro virtual docker is a discriminatory programme for predicting-ligand interactions.

4.2.10.2. Ligand preparation

The ligands used in this research were prepared using the chemical structure of phytochemical compounds was obtained from PubChem compound database. It was prepared by Chemsketch and MOL SDF format of this ligand was converted to 3D structure of ligand.

4.2.10.3. Protein preparation

The three-dimensional (3D) structure of transport protein (PDB-ID: 3QNT) was transformed from the RCSB protein Data Bank. Inhibition or depletion of NPC_1L_1 reduces intestinal cholesterol absorption, resulting in reduction of plasma cholesterol levels. If the reduction of plasma cholesterol level Niemann Pick C_1 $Like_1$ protein (NPC_1L_1). This protein is an established molecular target for the cholesterol lowering drug atorvastatin.

4.2.10.4. Structure based

Structure based drug design relies on knowledge of the 3D structure of the biological target obtained through methods such as X-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may designed using interactive graphics and the intuition of a medicinal chemist. Alternatively, various automated computational procedure may be used to suggest new drug candidates.

4.2.11. Evaluation of antiatherogenic activity of various extracts of leaves of *Cordia obliqua* in atherogenic diet rats.

4.2.11.1. Experimental design

Totally 42 number of rats were utilized for this experiment. The rats were divided into seven groups of six each.

S. No	Groups	Treatment
1.	Group I	Standard chow diet (Control).
2.	Group II	High Fat Diet (HFD).
3.	Group III	HFD + Standard drug atorvastatin (1.2 mg/kg b.w.)
4.	Group IV	HFD + Ethyl acetate extract of *Cordia obliqua* (100mg/kg b.w.)
5.	Group V	HFD + Ethyl acetate extract of *Cordia obliqua* (200mg/kg b.wt)
6.	Group VI	HFD + Ethanolic extract of *Cordia obliqua* (100mg/kg b.wt)
7.	Group VII	HFD + Ethanolic extract of *Cordia obliqua* (200mg/kg b.wt)

Rats of groups VI and V were orally fed with the ethyl acetate extracts of *Cordia obliqua*, rats of group VI and VII were fed with the ethanolic extracts of *Cordia obliqua* and rats of group III were fed with standard drug atorvastatin. Both the ethyl acetate and ethanolic extracts and atorvastatin were suspended in 2% of tween 80 (Waynforth, 1980) independently and sustained to the particular rats by oral intubation. Animals were sacrificed toward the end of 30 days (4 weeks) every one by cervical dislocation after during the night fasting. Blood was gathered in heparinised tubes and plasma was isolated. Aorta, heart and liver were cleaned up adhering fat, measured precisely and utilized for the preparation of homogenate. Animals were sufficiently given consideration according to the Animal Ethical Committee's suggestions.

4.2.11.2. Animal diet

The compositions of the two diets were used as follows (Amutha Iswarya Devi *et al*., 2015)

Control diet: 22.5% of wheat flour, 60% of roasted bengal gram powder, 5% of skimmed milk powder, 4% of refined oil, 4% of casein, 4% of starch with salt mixture, 0.5% of choline mixture and vitamin.

Atherogenic diet: 20.5% of wheat flour, 52.6% of roasted bengal gram, 5% of skimmed milk powder, 4% of refined oil, 4% of casein, 4% starch with salt mixture, 9% of coconut oil, 0.5% of choline mixture and vitamin & 0.4% of cholesterol.

4.2.12. Analytical methods

4.2.12.1. Extraction of lipids from tissues and plasma (Folch *et al*., 1957)

A known measure of the tissue was homogenized with ethanol-ether mixture- 2.5 mL (3:1 v/v) and processed for around 2 hrs at 60-65ºC and the supernatant was gathered. 3 mL ethanol-ether mixture was mixed to the residue, processed further for a time of 2 hrs at 60-65ºC and the supernatant was gathered. Chloroform-methanol mixture- 1 mL (1:1 v/v) was then mixed to the residue. It was repeat processed for 60 minutes at 50-55°C and the supernatant was gathered. The upper layer was pooled and made up to a specified volume. The plasma was also treated similarly for the extraction of lipids. This lipid extract was finally used for the estimation of free and ester cholesterol, triglycerides, free fatty acids and phospholipids.

4.2.13. Determination of lipids

4.2.13.1. Estimation of free and ester cholesterol

Digitonin forms 1:1 complex with free cholesterol as cholesterol digitonide. Ester cholesterol was selectively extracted with petroleum ether (30-60°C BP). Both the ester cholesterol and free cholesterol were then subjected to Liebermann-Burchard reaction for quantitation (Varley *et al*., 1991).

Reagents

- Digitonin solution (0.5%).
- Petroleum ether (30-60°C BP).
- Acetic anhydride.
- Concentrated sulphuric acid.

- Cholesterol.
- Cholesterol stock standard solution.
- Cholesterol working standard solution.
- Liebermann-Burchard reagent.

Procedure

An aliquot of the lipid extract was taken, treated with 0.5 mL of digitonin in 95% ethanol and evaporated in a water bath. This contains ester cholesterol and digitonide of free cholesterol. About 5 mL of petroleum ether was added, brought to boil, cooled and centrifuged. The supernatant was removed and used for the estimation of ester cholesterol. The precipitate of cholesterol digitonide as well as the ester cholesterol solution were evaporated at 60-80°C and treated with 6 mL of freshly prepared Liebermann-Burchard reagent. The colour developed in dark for 30 minutes and reading was taken in spectrophotometer at 620 nm against a reagent blank. 100-500 µg of working standard solution was pipetted out and treated with 0.5 mL of digitonin solution and evaporated to dryness at 60-80°C. A blank was prepared by evaporating 0.5 mL of digitonin solution. Blank and standards were processed as for the test. The cholesterol content was expressed as mg/g wet tissue or mg/dL plasma.

4.2.13.2. Estimation of free fatty acids (Falholt *et al*., 1973)

Free unsaturated fats were removed with chloroform-heptane-methanol mixture to wipe out interference from phospholipids and the plant extracts were shaken with a high density copper reagent at pH 8.1. The copper soaps stayed in the supernatant organic layer from which an aliquot was separated and the copper content determined colorimetrically by treating with diphenyl carbazide.

Reagents

- Chloroform: Heptane: Methanol solvent (5:5:1: v/v).
- Stock copper solution.
- Triethanolamine solution (1M).
- Sodium hydroxide solution (1M).

- Copper reagent (Cu-TEA solution).
- Diphenyl carbazide solution.
- Silicic acid.

Procedure

An aliquot of the lipid extract was evaporated to dryness and dissolved in 6 mL chloroform-heptane-methanol solvent. 200 mg activated silicic acid was added and left aside for 30 minutes. The solution was then centrifuged and the supernatant transferred to tubes containing 2.5 mL triethanolamine (TEA) solution. Standards of concentration ranging from 12.8 to 64 mg were made upto a known volume with the solvent, and 2.5 mL Cu-TEA solution was added. The contents were mixed in a mechanical stirrer for 20 minutes. They were then centrifuged and two layers were separated. 3 mL of the supernatant was then treated with 0.5 mL of diphenyl carbazide reagent. The colour formation was measured at 540 nm verses a reagent blank containing 3 mL solvent and 0.5 mL diphenyl carbazide. Free fatty acids levels were expressed as mg/g wet tissue or mg/dL plasma.

4.2.13.3. Estimation of Phospholipids (Zilversmit *et al*., 1950)

The organic phospholipid phosphorus is converted to inorganic phosphorus which reacts with ammonium molybdate to form phospho molybdic acid. This on reduction with amino naphthol sulfonic acid forms a stable blue colour.

Reagents

- Concentrated sulphuric acid.
- Concentrated nitric acid.
- Ammonium molybdate, 2.5% in 3N Sulphuric acid.
- 1-amino, 2-napthol- 4-sulphonic acid (ANSA).
- Standard phosphorus solution.

Procedure

0.1 mL of lipid extract was mixed with 1.9 mL of distilled water and 1.5 mL of 10% trichloro acetic acid. The precipitated proteins were sedimented by centrifugation. The supernatant was discarded. 1 mL concentrated nitric acid and 1 mL concentrated sulphuric acid were added and digested on a sand bath until the solution became colourless. 1 mL of ammonium molybdate solution was then added, followed by 0.4 mL of ANSA reagent. The absorbance was read at 680 nm after 5 minutes. Phospholipids levels were expressed as mg/g wet tissue or mg/dL plasma.

4.2.13.4. Estimation of triglycerides

Triglycerides were estimated by the method of Foster *et al*., 1973.

Reagents

- Isopropanol.
- Alumina, (activity grade 1 for chromatography) was washed with water and dried in an oven overnight.
- Potassium hydroxide.
- Sodium metaperiodate reagent.
- Acetyl acetone reagent.
- Standard triolein solution.

Procedure

To an aliquot of dried lipid extract, 4 mL isopropanol was added, mixed well and 400 mg washed alumina was then added. This was placed in a mechanical rotor for 15 minutes and then centrifuged. To 2 mL supernatant, 0.6 mL potassium hydroxide was added and incubated at 60-70°C for 15 min, cooled and 1 mL of metaperiodate solution and 0.5 mL acetyl acetone reagent were then added. It was than mixed and incubated at 50°C for 30 minutes. A series of standards of concentration of 8 - 40µg triolein were added likewise along with a blank containing without sample. Cooled and read at 405 nm against a reagent blank. The triglyceride content was expressed as mg/g wet tissue or mg/dL plasma.

4.2.13.5. Estimation of HDL cholesterol

The apo B containing lipoprotein-chylomicron, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were precipitated by adding phosphotungstic acid and magnesium ions. The cholesterol content of the supernatant which gave the measures of high density lipoprotein cholesterol was estimated by cholesterol oxidase enzymatic method using boehringer Mannheim kit.

Reagents

- Phosphotungstic acid – 0.44 m mol/L.
- Magnesium chloride – 20 m mol/L.

Procedure

To 20 μL of sample, 500 μL precipitant were added, mixed and kept for 10 minutes. After centrifugation, 100 μL of clear supernatant was removed within two hrs for cholesterol estimation. Plasma HDL cholesterol was expressed as mg/dL.

4.2.13.6. Calculation of LDL cholesterol

LDL cholesterol was calculated using the formula.

LDL cholesterol = Total cholesterol – (HDL cholesterol + Triglyceride/5)

Plasma LDL cholesterol was expressed as mg/dL.

4.2.13.7. Calculation of VLDL cholesterol

VLDL cholesterol was calculated using the formula (Prasanna, 2000).

$$\text{VLDL cholesterol} = \frac{\text{Triglyceride}}{5}$$

VLDL cholesterol was expressed as mg/dL.

4.2.13.8. Calculation of atherogenic index

Atherogenic index was calculated using the formula (Li *et al*., 2013).

$$\text{Atherogenic Index} = \frac{\text{Log triglycerides}}{\text{HDL}}$$

4.2.13.9. Calculation of cardiac risk ratio

Cardiac risk ratio was calculated using the formula (Huang *et al*., 2013).

$$\text{Cardiac risk ratio} = \frac{\text{Total cholesterol}}{\text{HDL}}$$

4.2.13.10. Calculation of atherogenic Coefficient

Atherogenic Coefficient was calculated using the formula.

$$\text{Atherogenic Coefficient} = \frac{\text{Total cholesterol - HDL}}{\text{HDL}}$$

4.2.14. Determination of tissue lipid peroxidation

4.2.14.1. Estimation of thiobarbituric acid reactive substances (TBARS) (Nichans *et al*., 1968)

In this method malondialdehyde and other thio barbituric acid in the acidic medium generate a pink coloured chromophore, which was read at 535 nm.

Reagents

- TCA, 15% (w/v).
- HCl, 0.25 N.
- TBA, 0.375% in hot distilled water.
- TCA-TBA-HCl reagent.
- Stock standard malondialdehyde solution.
- Working standard.
- Stock solution was diluted to get a concentration of 50 nm/mL.

Procedure

0.5 mL of homogenate was diluted to 1.5 mL with double distilled water and mixed well. 2 mL of TCA-TBA-HCl reagent was then added. The reagent mixture was placed in boiling water bath for nearly 15 mints. After cooling, the tubes were centrifuged at 1000 rpm for 10 minutes and the supernatant was taken for colorimetric measurements. A series of standard solutions in the concentration of 2-10 nm were treated in a similar manner. The absorbance of chromophore was read at 535 nm against a reagent blank. The values were expressed as nM/g wet tissue.

4.2.14.2. Estimation of conjugated dienes (Beuje *et al*., 1978)

Lipid peroxidation was followed by rearrangement of double bonds in polyunsaturated fatty acids leading to the formation of conjugated dienes, which has absorbance at 233 nm. The measurement of conjugated dienes reflects the amount of lipid peroxidation taking place.

Reagents

- Chloroform.
- Methanol.
- Cyclohexane.
- Tris HCl buffer, 0.3 M, pH 7.5.

Procedure

1.0 mL tissue homogenate in 0.3 mL tris HCl buffer (pH 7.5), was mixed with 5 mL chloroform-methanol (2:1 v/v) and centrifuged for 5 minutes. 3 mL of the lower layer was evaporated to dryness. With this 1.5 mL cyclohexane was added and read the absorbance at 233 nm against a cyclohexane blank. The proper measure of hydroperoxide produced was computed utilizing a molar extinction coefficient of $2.52X10^{-4}cm^{-1}$. The concentration of conjugated dienes was expressed in mM/100g wet tissues.

4.2.15. Determination of tissue antioxidant level

4.2.15.1. Assay of superoxide dismutase (SOD, EC, 1.15.1.1) (Kakkar *et al*., 1984)

The assay of SOD was depend on the hindrance of the development of NADH-phenazine methosulphate - nitroblue tetrazolium complex. The response was started by the inclusion of NADH. After incubation for one and half mints, the response was halted by the inclusion of glacial CH_3COOH. The colour created at the end of the response was extricated into butanol layer and measured at 560 nm.

Reagents

- Sodium pyrophosphate buffer, 0.025 M, pH 8.3.
- Phenazine methosulphate, 186 μM.
- Nitroblue tetrazolium, 300 mM.
- NADH-780mM.
- Glacial acetic acid.
- n-butanol.
- Chloroform.
- Ethanol.

Procedure

0.5 mL of the sample (tissue homogenate) was diluted to 1 mL with ice cold water. 2.4 mL of ethanol and 1.5 mL chloroform (in chilled condition) were added. This mixture was shaken for 1 minute at 4°C and afterward centrifuged. The supernatant was measured for enzyme activity.

The test mixture contained 1.2 mL sodium pyrophosphate buffer, 0.3 mL nitroblue tetrazolium, and 0.1 mL phenazine methosulphate suitably diluted the enzyme substance with water make up to 3.0 mL. The response was begun by the inclusion of 0.2 mL NADH. After incubation at 30ºC for one and half mints, the response was halted by the inclusion of 1.0 mL glacial CH_3COOH. The response mixture was mixed energetically with 4.0 mL of n-butanol and shaken well. The mixture was permitted to remain for 10 minutes, and then centrifuged. The colour intensity of the chromophore in butanol layer was read at 560 nm against without sample, butanol

used as a blank and a method out of enzyme served as control. One unit of enzyme activity were defined as the enzyme reaction which gave 50% inhibition of NBT reduction in one minute under the assay conditions and the activity was expressed as units/mg protein.

4.2.15.2. Assay of catalase (CAT, EC. 1.11.1.6) (Sinha, 1972)

Dichromate in CH_3COOH was changed to perchromic acid and afterward to chromic acetate when warmed in the presence of H_2O_2. The chromic acetate derivation framed was measured at 620 nm. Catalase was permitted to divide hydrogen peroxide for distinctive intervals of time. The response was halted at distinctive time periods by the inclusion of dichromate-CH_3COOH mixture and the enduring hydrogen peroxide as chromic acetate was determined colorimetrically.

Reagents

- Phosphate buffer, 0.01 M, pH 7.0.
- Hydrogen peroxide (H_2O_2) 0.2 m.
- Potassium dichromate, 5% (w/v).
- Dichromate acetic acid reagent.
- Standard hydrogen peroxide, 2 mm.

Procedure

To 6.0 mL phosphate buffer, 0.1 mL sample and 0.4 mL hydrogen peroxide were added. The reaction was stopped at 15, 30, 45 and 60 seconds by the inclusion of 2 mL dichromate- CH_3COOH reagent. All the tubes were kept in boiling water both for 10 mints and the colour formation was measured at 620 nm. Standards in the range of 2-10 μM were taken and preceded similar to the test with blank containing reagent alone. The activities were expressed as μM of H_2O_2 consumed/ minute/ mg protein.

4.2.15.3. Assay of glutathione peroxidase (GSH-Px, EC. 1.11.1.9) (Rotruck *et al*., 1973)

A known amount of enzyme preparation was permitted to react with hydrogen peroxide in the existence of GSH for a particular time interval and the remaining GSH content was measured subsequently.

$$2GSH + H_2O_2 \xrightarrow{GSH\text{-}Px} GSSG + 2H_2O$$

Reagents

- Tris buffer, 0.4 M, pH 7.0.
- Sodium azide solution, 10 mM.
- Tricholoro acetic acid, 10% (w/v).
- EDTA, 0.4 mM.
- H_2O_2 solution, 20 mM.
- Glutathione solution, 2 mM.

Procedure

To 0.2 mL of tris buffer, 0.2 mL EDTA, 0.1 mL sodium azide, 0.5 mL sample (tissue homogenate) and 0.2 mL GSH were added followed by 0.1 mL hydrogen peroxide. The tubes containing all the chemical reagents except the sample substance were mixed well and incubated at 37°C for 10 mints. After 10 mints, the response was stopped by the inclusion of 10% TCA of 0.5 mL, then centrifuged and the upper layer was assayed for GSH.

4.2.15.4. Assay of glutathione reductase (GSH-Rx, EC. 1.6.4.2) (David *et al*., 1983)

Glutathione reductase catalyses the conversion of oxidized glutathione to reduced glutathione employing NADPH as a substrate. The amount of NADPH utilized is a direct measure of enzyme activity.

Reagents

- 0.12 M phosphate buffer, pH 7.2.
- 15 mM EDTA.
- 10 mM Sodium azide (NaN_3).
- 6.3 mM oxidized glutathione.
- 9.6 mM NADPH.

Procedure

The assay system contained 0.12 N potassium phosphate buffer 1 mL, 10 mM NaN_3 0.1 mL, 15 mM EDTA 0.1 mL, and 6.3 mM oxidized glutathione 0.1 mL and enzyme source 0.1 mL and water in a final volume of 2 mL, kept for 3 min. afterwards 0.1 mL of NADPH was included. The absorbance was measured at 340 nm was recorded at an interval of 15 secs for 2-3 min. for each series of measurement; controls were done that contained water instead of oxidized glutathione.

4.2.15.5. Assay of glutathione-s-transferase activity (GST, EC.3.5.1.18) (Habig *et al*., 1974)

Glutathione-s-transferase activity was measured by following the increase in absorbance at 340 nm, using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate.

Reagents

- 0.03 M phosphate buffer, pH 6.5.
- 30 mm glutathione.
- 30 mm 1-chloro-2, 4-dinitrobenzene (CDNB) in 95% alcohol.

Procedure

The following solutions were taken in a given proportion: 1.0 mL of phosphate buffer, 0.1 mL CDNB and 0.1 mL tissue homogenate. The volume was adjusted to 2.9 mL with glass distilled water. The reaction mixture was pre-incubated at 37°C for 5 mints. The response on was initiated by the inclusion of 0.1 mL 30 mM glutathione. The absorbance was followed for 5 mints measured at 340 nm, without the enzyme was used as a blank.

Glutathione-s-transferase activity as expressed as mM of CDNB-GSH conjugate formed/minute/mg protein which was calculated using the formula,

$$\frac{\text{ODX3X1000}}{\text{9.6X5Xprotein in mg}}$$

9.6 is the difference in the mM extinction coefficient between CDNB-GSH conjugate and CDNB.

4.2.15.5. Estimation of glutathione (GSH) (Ellman *et al*., 1959)

The yellow colour was developed by treating 5,5'-dithio bis-2-nitro benzoic acid (DTNB) with compounds containing sulphydryl groups.

Reagents

- Phosphate buffer, 0.2 M, pH 8.0.
- TCA, 5% (w/v).
- Ellman's reagent.
- Disodium hydrogen phosphate (Na_2HPO_4). 0.3 M, pH 8.0.
- Standard glutathione solution.
- Precipitating reagent.

Procedure

A known weight of tissue was homogenized in phosphate buffer. 0.5 mL of the sample was taken. With this 3.0 mL of precipitating reagent was added, mixed thoroughly and allowed to stand for 5 minutes and centrifuged. A set of standards (20-100 μg) were chosen and used distilled water for make up the volume 2.0 mL. The 2.0 mL of supernatant along with 2.0 mL of blank containing distilled water was also taken. To all the tubes 4.0 mL 0.3 M disodium hydrogen phosphate and 1 mL of DTNB reagent were included. The colour developed was read at 412 nm. Reduced glutathione levels were expressed as mg/g wet tissue.

4.2.16. Estimation of protein (Lowry *et al*., 1951)

Proteins react with Folin-Ciocalteau reagent to give a complex. The colour so formed was due to the reaction of alkaline copper with protein and the reduction of phospho molybdate by the amino acids like tyrosine, phenyl alanine and tryptophan also present in the protein. The strength of the colour formation depends on the quantity of these aromatic amino acids present.

Reagents

- Alkaline copper reagent.

- Folin's phenol reagent.
- Standard bovine serum albumin (BSA).

Procedure

0.1 mL of plasma and tissue homogenate was make up to 1 mL with saline, after that 1 mL 10% TCA was included. The mixture was centrifuged, supernatant was disposed and the precipitate was disintegrated in 1 mL of 0.1N NaOH. From this aliquots were chosen for the evaluation. 4.5 mL of alkaline copper reagent was included and the contents were permitted to remain at 37ºC for 10 mints. Then 0.5 mL dilute Folin's phenol reagent was included and mixed. A sequence of standards of concentration range 20-100 μg and a blank were processed as for the test. The blue colour formation was measured at 620 nm after 20 mints.

4.2.17. Histopathological study

When the tissues are exposed to any toxicants, alterations in the tissue architecture are more prominent. This can be monitored by histochemical examination (Luna, 1968).

4.2.17.1. Tissue processing

A fraction of the tissues (aorta, heart and liver) were fixed in 10% formalin immediately after autopsy. The mixed tissues were placed in 10% saline (10% formalin in 0.9% NaCl) for 1 hour to remedy shrinkage because of higher concentration of formalin. They were left overnight in running water securing the mouths of the vessels with cotton gauze. The tissues were got dried out in ascending grades of isopropanol (by immersing in 80% isopropanol overnight followed by 100% isopropanol for 1 hour).The got dried out tissues were cleaned in two progressions of xylene, one hour each. Then the tissues were impregnated with histology grade paraffin wax (melting point 58-60ºC). The wax impregnated tissues were inserted in paraffin blocks utilizing the similar grade wax. The paraffin blocks were placed and incise with rotary microtome at 3μ thickness. The tissue sections were floated on a tissue floatation bath at 40ºC and taken on a glass slide spread with equivalent parts of egg albumin and glycerol. The tissue sections were afterward melted in an incubator at 60ºC and allowed to cool for 5 minutes.

4.2.17.2. Tissue staining

The tissue sections were deparaffinised by submerging in xylene for 10 minutes in a staining jar. The deparaffinised sections were washed in 100% isopropanol and stained in Ehrlich's haematoxylin for 8 minutes. After staining in haematoxylin, the tissue sections were washed in normal water and immersed in acid alcohol (8.3% HCl in 70% alcohol) to eliminate surplus stain. The tissue sections were afterward sited in running tap water for 10 mints. Then after, the sections were counter-stained with 1% aqueous solution of eosin for 1minute. The surplus stain was removed in tap water and the section was allowed to dry. Complete dehydration of the stained section was ensured by placing the section in an incubator at 60ºC for 5 mints. While the tissue sections were cooled and the sections were placed in DPX mountant. The cell architecture in the liver was observed under high power objective in a microscope.

4.2.17.3. Statistical analysis

Results were expressed as mean ± SE of 6 rats in each group. One ways analysis of variance (ANOVA) with Scheffe's multiple comparisons test were used to determine the statistical significance. Significance level was fixed at 0.05.


RESULTS

CHAPTER V

RESULTS AND DISCUSSION

5.1. Preparation of extracts and the calculation of percentage yield of *Cordia obliqua.*

The shade dried leaf plant of *Cordia obliqua* was collected from Tenkasi, Tirunelveli District of Tamil Nadu, India. The powdered plant materials were successively extracted with petroleum ether, ethyl acetate and ethanol by hot continuous percolation method in soxhlet apparatus (Harborne, 1984) for 24 hours. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer until dry power was obtained. The extract was stored in screw cap vials until further use. The percentage yield of the leaf plant of *Cordia obliqua* is shown in Table No. 4

Table No. 4: Percentage yield of the leaf plant of *Cordia obliqua* willd

Plant name	Parts used	Method of extraction	Solvent system	Percentage yield (% W/W)
Cordia obliqua	Leaf powder	Continuous hot percolation in Soxhlet Apparatus	Pet. ether	10.48 %
			Ethyl acetate	5.47 %
			Ethanol	4.25 %

5.2 Physicochemical parameter of *Cordia obliqua* willd

Physicochemical parameters of whole plant of *Cordia obliqua* were estimated based on the methods recommended by World Health Organization (WHO) as apparent from Table No. 5, Percentage weight loss on drying and foreign organic matter value was found to be 12.30 ± 0.82 and 8.66 ± 0.46. The swelling index and foaming index value was found to be 7.46 ± 0.31 and less than 100. The ash values of total ash, water soluble ash, acid insoluble ash and sulphated ash value were found to be 6.50 ± 0.48, 8.30 ± 0.57, 9.46 ± 0.36 and 9.10 ± 0.37 respectively. Ash values used to find out quality, authenticity and purity of unsophisticated drug and also these values are important quantitative standards.

Table No. 5: Physicochemical parameters of *Cordia obliqua* Willd.

Experiment	% Values			Average yield (%) ± SEM
	1(%) W/W	2(%) W/W	3(%) W/W	
Total ash	5.9	6.5	7.1	6.50 ± 0.48
Acid insoluble ash	9.0	9.5	9.9	9.46 ± 0.36
Water soluble ash	7.8	8.0	9.1	8.30 ± 0.57
Sulphated ash	8.7	9.0	9.6	9.10 ± 0.37
Loss on drying	11.2	12.5	13.2	12.30 ± 0.82
Foreign organic matter	8.2	8.5	9.3	8.66 ± 0.46
Swelling index	7.0	7.5	7.9	7.46 ± 0.36
Foaming index	Less than 100			

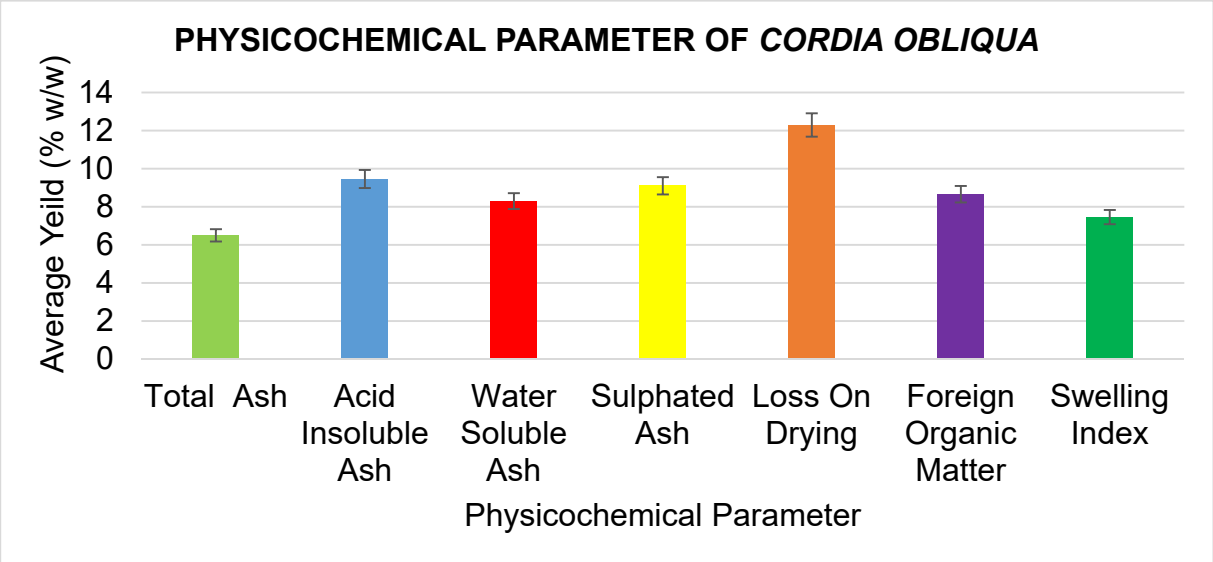


Fig. 18: Physicochemical parameters of *Cordia obliqua*

5.3. Fluorescence Analysis of *Cordia obliqua*

Fluorescence is an important phenomenon displayed by various phytoconstituents present in plant materials. Some show fluorescence in the visible range in daylight. The UV light produces fluorescence in many natural products, which do not visibly in daylight. Some of the substances such as chemical reagents and

solvents (they are not fluorescent) which may be often converted into fluorescent derivatives. The results of fluorescent analysis of whole plant of powder of polyherbal formulation was depicted in Table No. 6 and 7. The herbal formulation showed the characteristic coloration upon treatment with multi various chemical reagents and different solvents.

Table No. 6: Fluorescence analysis of *Cordia obliqua* in different reagents.

S. No	Chemical / Reagents	UV	Short WL	Long WL
1.	1N HCl	Brown	Violet	Pale blue
2.	1N NaOH	Pale yellow	grey	Pale brown
3.	1 N NaOH in alcohol	Pale yellow	Pink	Pink
4.	50% HNO_3	Pale pink	Brown	Brown
5.	Con. H_2SO_4	Yellow	Dirty white	Creamy white
6.	50% KOH	Green	Pale blue	White
7.	50% H_2SO_4	Dirty white	Pale brown	Brown
8.	Con.HNO_3	Pink	Brown	Brown
9.	0.01 M I_2 Soln.	Pale yellow	Pale blue	White

Table No. 7: Fluorescence analysis of *Cordia obliqua* in different solvents.

S. No	Solvents	UV	Short WL	Long WL
1.	Powder	Pale green	Pale blue	Pale blue
2.	CH_3OH	Pale brown	Pale pink	Pink
3.	Aqueous	Light green	Pale green	Pale green

5.4. Extractive values of *Cordia obliqua* willd

The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in particular solvent. Extractive values were recorded in pet.ether, ethyl acetate, chloroform, methanol, acetone, ethanol and aqueous with a view to study the distribution of various constituents of *Cordia obliqua*. Highest range of extractive values obtained in aqueous extract is **4.60% w/w**

Table No. 8: Extractive values of *Cordia obliqua* Willd.

Experiment	% Values			Average yield (%) ± SEM
	1 (%) W/W	2 (%) W/W	3 (%) W/W	
Pet. ether extract	2.6	3.0	3.4	3.00 ± 0.32
Ethyl acetate extract	1.1	1.5	2.1	1.56 ± 0.41
Chloroform extract	3.0	3.5	4.2	3.56 ± 0.49
Methanol extract	2.2	2.5	3.0	2.56 ± 0.32
Acetone extract	1.6	2.0	2.2	1.19 ± 0.24
Ethanol extract	1.8	2.0	2.9	2.23 ± 0.47
Aqueous extract	4.2	4.5	5.1	4.60 ± 0.37

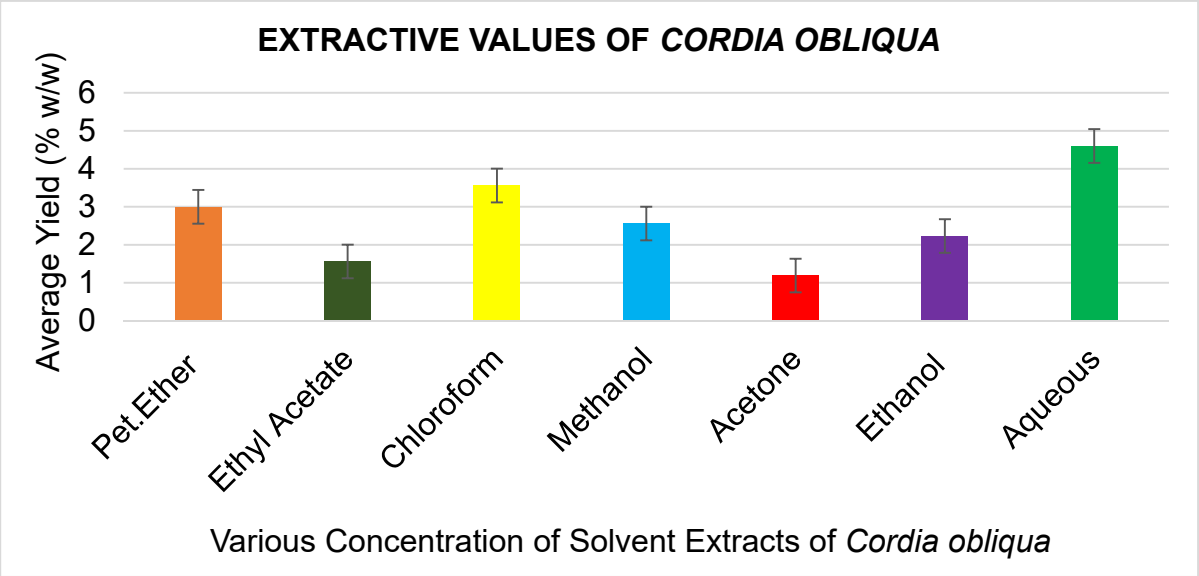


Fig. 19: Extractive values of *Cordia obliqua*

5.5. Phytochemical screening of various extracts of *Cordia obliqua* Willd.

The preliminary phytochemical analysis of various extract of aerial plant of *Cordia obliqua* showed the presence of alkaloids, glycosides, tannins, flavonoids, phytosterols, saponins, fixed oil, sterols, phenolic compounds and terpenoids.

In **petroleum ether** extract it showed the presence of fixed oils.

In **ethyl acetate** extract it showed the presence of alkaloids, flavonoids, phenolic compounds.

In **ethanol** extract it showed the presence of carbohydrates, phytosterols, flavonoids, tannins, phenolic compounds, proteins, aminoacids and saponins.

Table No. 9: Phytochemical screening of various extracts of *Cordia obliqua* Willd.

S. No	Fractions of various extracts	Components											
		Alkaloids	Carbohydrates	Glycosides	Phytosterols	Sterols	Flavonoids	Tannins	Phenolic compounds	Protein	Amino acids	Saponins	Fixed oil	Terpenoiods
1.	Pet. ether extract	-	-	-	-	-	-	-	-	-	-	-	+	-
2.	Ethyl acetate extract	+	-	-	-	-	+	-	+	-	-	-	-	-
3.	Ethanolic extract	-	+	-	+	-	+	+	+	+	+	+	-	-

Note

(+ indicates the presence of constituents)

(- indicates the absence of constituents)

5.6. GC-MS Analysis

From GC-MS analysis 16 active compounds were detected from ethyl acetate extract and another 4 active components were detected from ethanolic extract, totally 20 components are detected. The identification of phytochemical compounds was based on retention time, molecular formula, peak area, molecular weight and medicinal activity.

1. GC-MS analysis in ethyl acetate extract

The presence of GC-MS analysis showed the existence of ethyl acetate extract with different phytochemical compounds are Didodecyl phthalate having peak area (3.06%) with the retention time (11.797), 2-Heptadecenal having peak area (2.38%) with the retention time (13.443), 9,12-Octadecadienoic acid (Z,Z)- having peak area (1.79%) with the retention time (13.490), Cyclobarbital having peak area (10.13%) with the retention time (15.126), Trans-2,3-methylenedioxy-b-methyl-b- nitrostyrene having peak area (1.49%) with the retention time (15.334), 1,1,1,3,5,5,5-Heptamethyltrisiloxane having peak area (2.98%) with the retention time (16.260), 1H-indole, 1-methyl-2-phenyl- having peak area (2.48%) with the retention time (16.327), Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]- having peak area (2.55%) with the retention time (16.582), Benzo [h] quinolone, 2,4-dimethyl- having peak area (0.40%) with the retention time (16.695), 2-methyl-7-phenylindole having peak area (2.11%) with the retention time (16.837), 2-Ethylacridine having peak area (17.53%) with the retention time (17.253), Suprane having peak area (7.81%) with the retention time (17.537), 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)- having peak area (19.03%) with the retention time (17.622), 1-methyl-3-phenylindole having peak area (0.29%) with the retention time (17.830), 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester having peak area (11.59%) with the retention time (19.456), 6-Octadecenoic acid having peak area (14.36%) with the retention time (19.806).

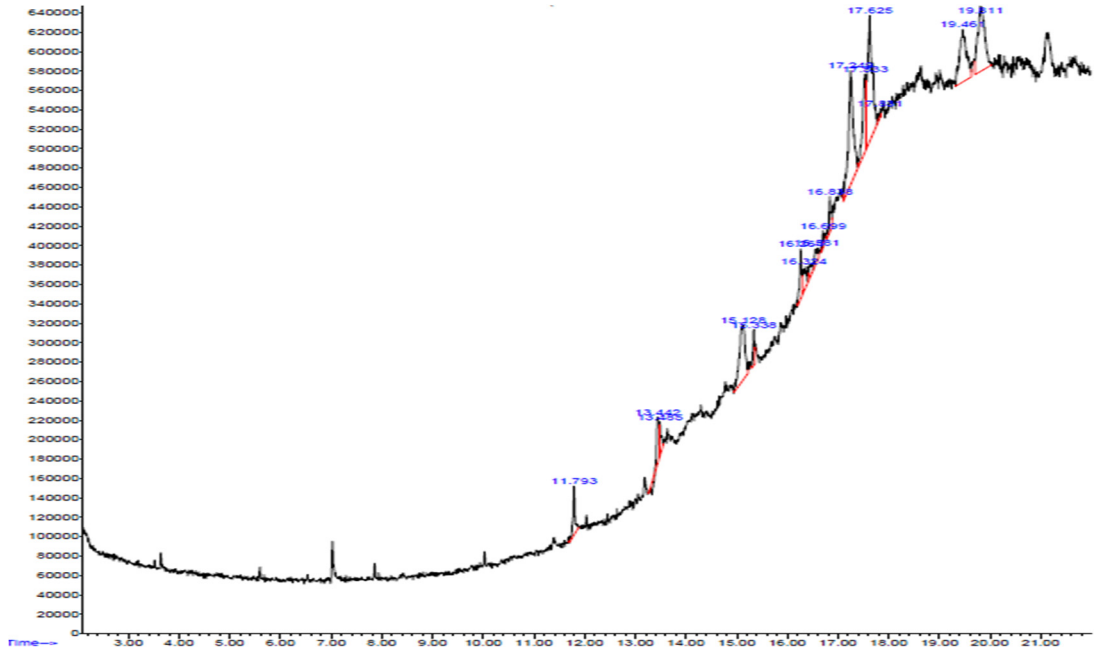


Fig. 20: GC-MS Chromatogram of Ethyl acetate extract of *Cordia obliqua* Willd.

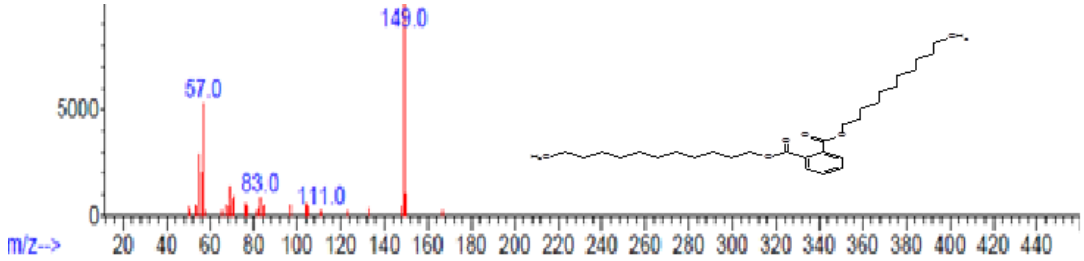


Fig. 21: Didodecyl phthalate

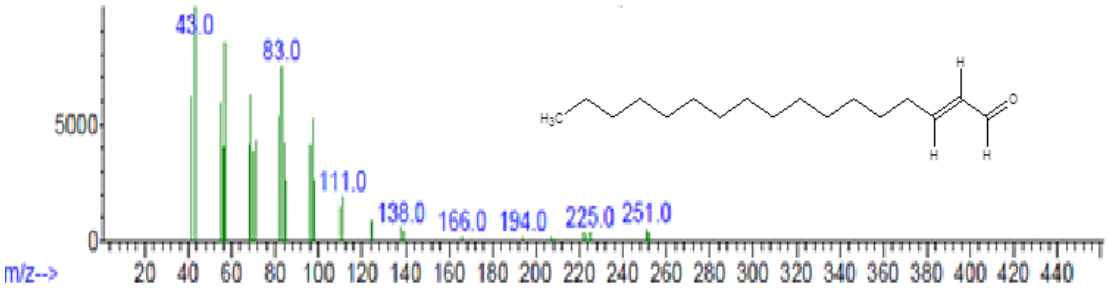


Fig. 22: 2-Heptadecenal

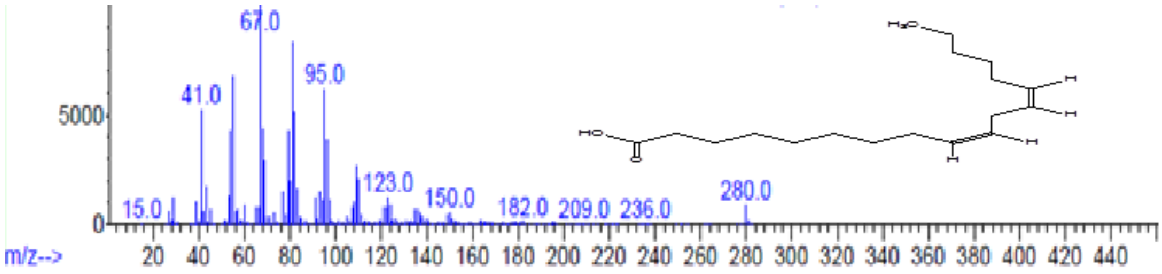


Fig. 23: 9,12-Octadecadienoic acid (Z,Z)-

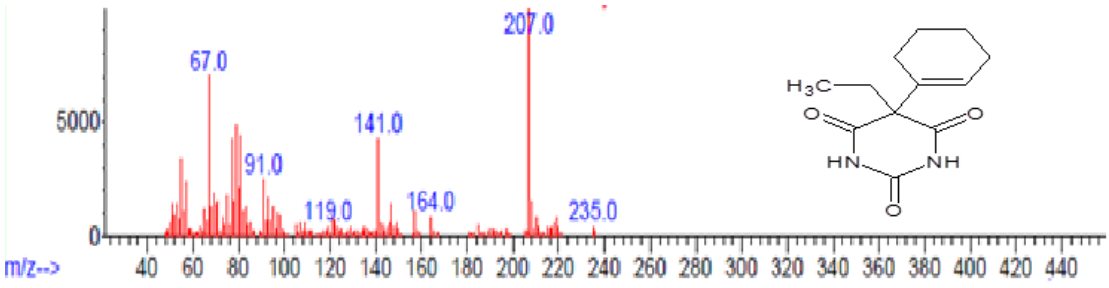


Fig. 24: Cyclobarbital

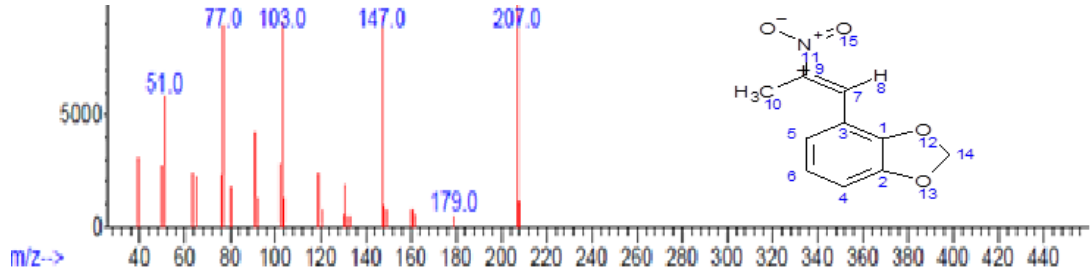


Fig. 25: Trans-2,3-methylenedioxy-b-methyl-b- nitrostyrene

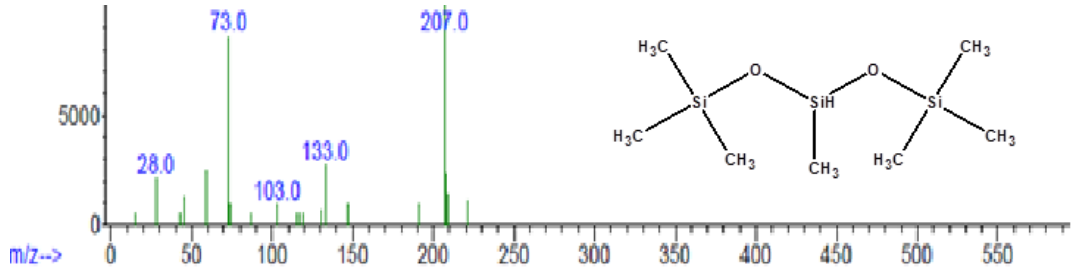


Fig. 26: 1,1,1,3,5,5,5-Heptamethyltrisiloxane

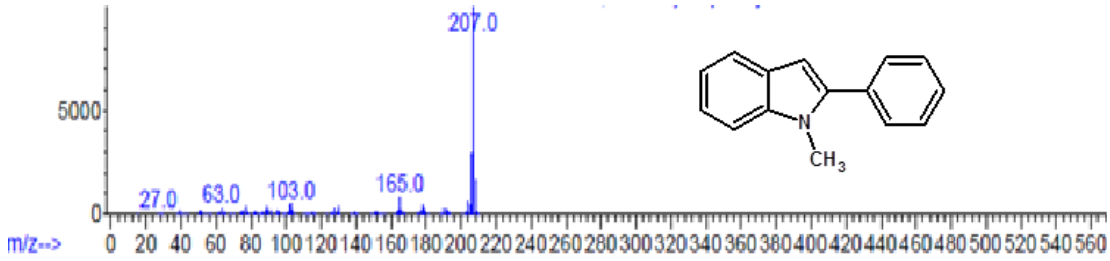


Fig. 27: 1H-indole, 1-methyl-2-phenyl-

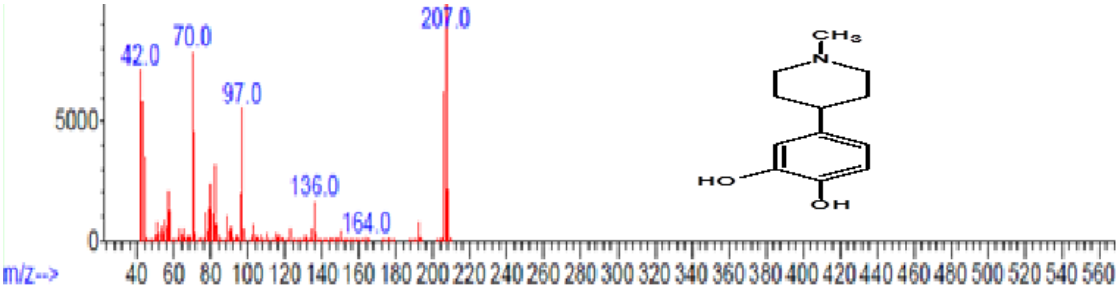


Fig. 28: Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-

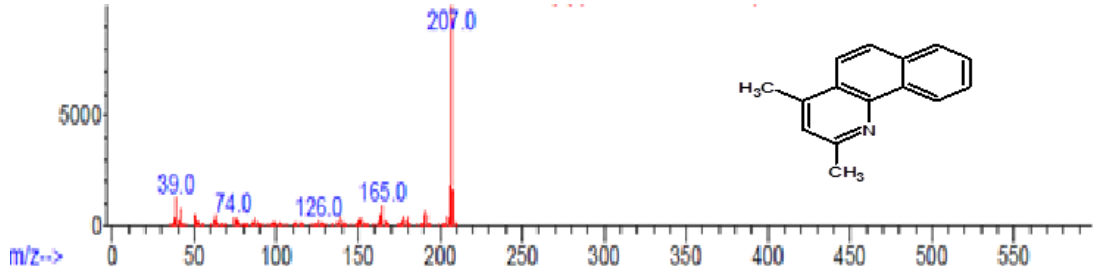


Fig. 29: Benzo [h] quinolone, 2,4-dimethyl-

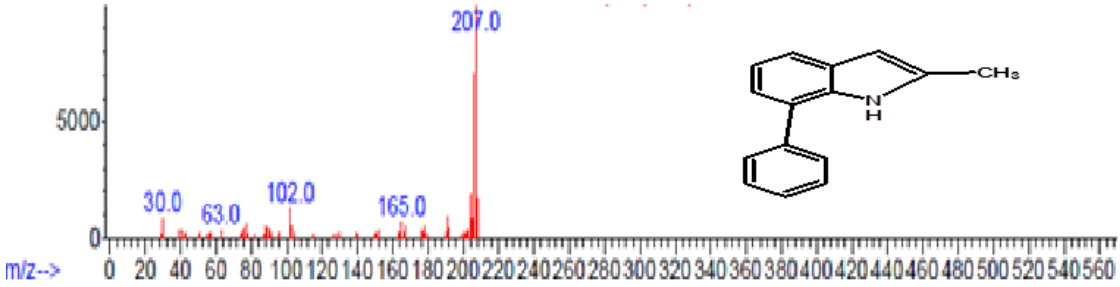


Fig. 30: 2-methyl-7-phenylindole

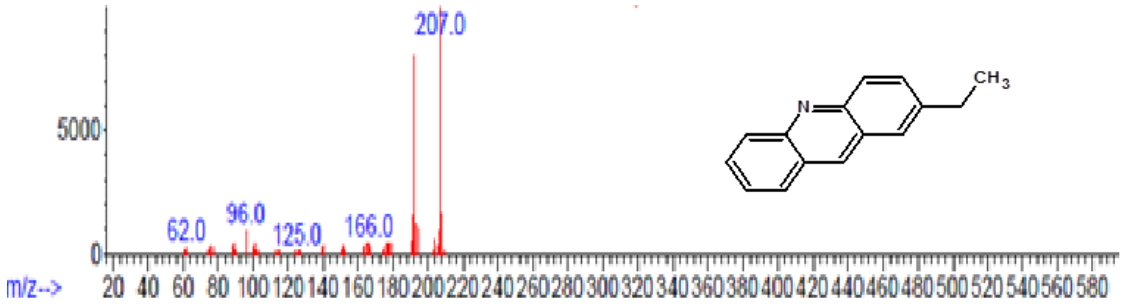


Fig. 31: 2-Ethylacridine

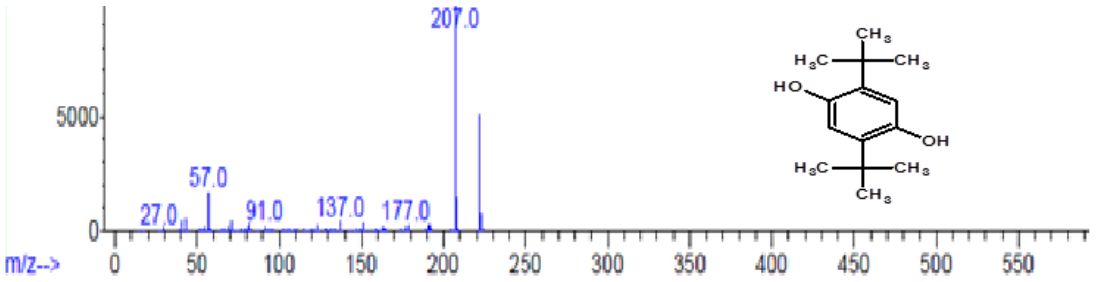


Fig. 32: 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-

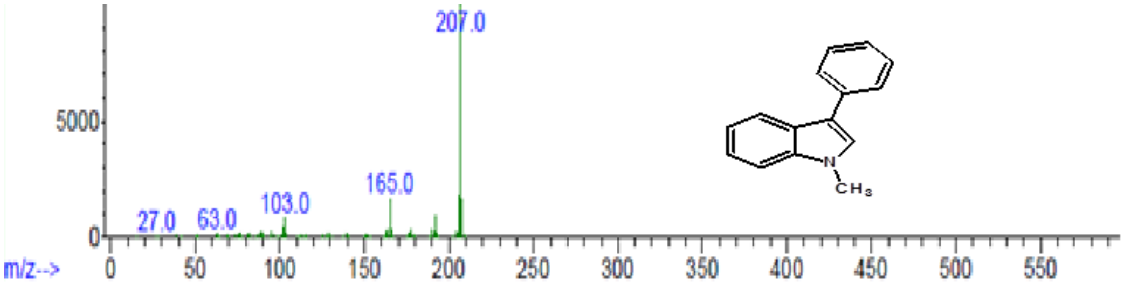


Fig. 33: 1-methyl-3-phenylindole

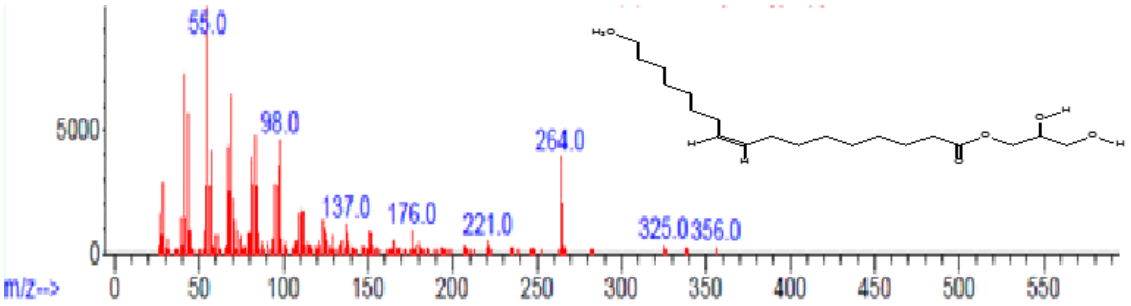


Fig. 34: 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester

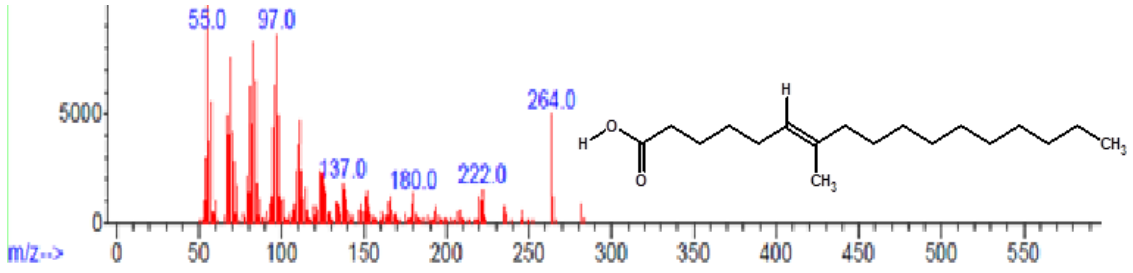


Fig. 35: 6-Octadecenoic acid

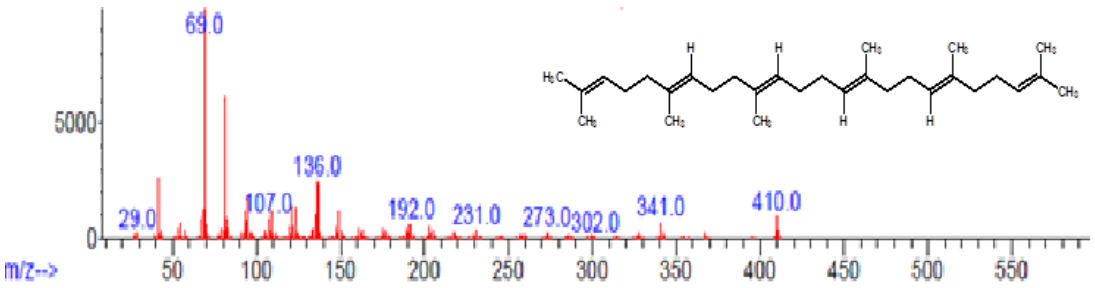


Fig. 36: Suprane

Table No. 10: Chemical composition of Ethyl acetate extract of *Cordia obliqua* Willd.

S. No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	11.797	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	3.06
2.	13.443	2-Heptadecenal	$C_{17}H_{32}O$	252	2.38
3.	13.490	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.79
4.	15.126	Cyclobarbital	$C_{12}H_{16}O_3N_2$	236	10.13
5.	15.334	Trans-2,3-methylenedioxy-b-methyl-b- nitrostyrene	$C_{10}H_9NO_4$	207	1.49
6.	16.260	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_{16}H_{28}OSi$	264	2.98
7.	16.327	1H-indole, 1-methyl-2-phenyl-	$C_{15}H_{13}N$	207	2.48
8.	16.582	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-	$C_{10}H_{28}O_4Si_3$	296	2.55
9.	16.695	Benzo [h] quinolone, 2,4-dimethyl-	$C_{15}H_{13}O_2Cl_2N_2$	285	0.40
10.	16.837	2-methyl-7-phenylindole	$C_{12}H_{17}NO_2$	207	2.11
11.	17.253	2-Ethylacridine	$C_{15}H_{13}N$	207	17.53
12.	17.537	Suprane	$C_{30}H_{50}$	410	7.81
13.	17.622	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	222	19.03

14.	17.830	1-methyl-3-phenylindole	$C_{15}H_{13}N$	207	0.29
15.	19.456	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356	11.59
16.	19.806	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	14.36

Table No. 11: Activity of phytocomponents identified in Ethyl acetate extract of *Cordia obliqua* Willd by GC-MS.

RT	Name of the compound	Nature of compound	Activity
11.797	Didodecyl phthalate	Esters	Non-central analgesic, antipyretic, anti-inflammatory, urinary system disorders.
13.443	2-Heptadecenal	Phenol	Antineoplastic agents, uses of additives.
13.490	9,12-Octadecadienoic acid (Z,Z)-	Monoterpenoid	Hepatoprotective, antiarthritic, hypocholesterolemic, cancer preventive.
15.126	Cyclobarbital	Phenol	Hypnotics and sedatives, nervous system.
15.334	Trans-2,3-methylenedioxy-b-methyl-b- nitrostyrene	Ethene	No activity reported.
16.260	1,1,1,3,5,5,5-Heptamethyltrisiloxane	Siloxane	Antimicrobial,antifungals, anti-infectives.
16.327	1H-indole, 1-methyl-2-phenyl-	Indole	Antiasthmatics, dermatological disorders, antipsoriatics.

16.582	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-	Pyridine	Non-specific cardiovascular stimulants.
16.695	Benzo [h] quinolone, 2,4-dimethyl-	Hydroquinone	Analgesia and anti-inflammatory
16.837	2-methyl-7-phenylindole	Indole	Non-central analgesic, antipyretic, anti-inflammatory, antineoplastic, allergic disorders, bone diseases.
17.253	2-Ethylacridine	Acridine	Antiseptic properties.
17.537	Suprane	Fluorinated Ether	Antineoplastic agents, dermatological disorders, treating wounds, ulcers, burns.
17.622	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	Catechol	Antiasthmatics, dermatological disorders, joint disorders.
17.830	1-methyl-3-phenylindole	Indole	Non-central analgesic, antipyretic, anti-inflammatory, antineoplastic.
19.456	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Long chain fatty acid	Antiasthmatics, bronchodilators, antitussive agents, urinary system disorders.
19.806	6-Octadecenoic acid	Long chain fatty acid	Urinary tract, kidneys, contraceptives, dermatological disorders, treating wounds, ulcers, burns.

2. GC-MS analysis in ethanolic extract

The presence of GC-MS analysis showed the existence of ethanolic extract with different phytochemical compounds are 2-(2-(2-(2-(2-(2-methoxyethoxy) ethoxy) ethoxy) ethoxy) ethoxy) acetic acid having peak area (11.85%) with the retention time (2.579), Phytol having peak area (25.10%) with the retention time (13.188), Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl- having peak area (15.91%) with the retention time (15.122), Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]- having peak area (47.14%) with the retention time (16.459).

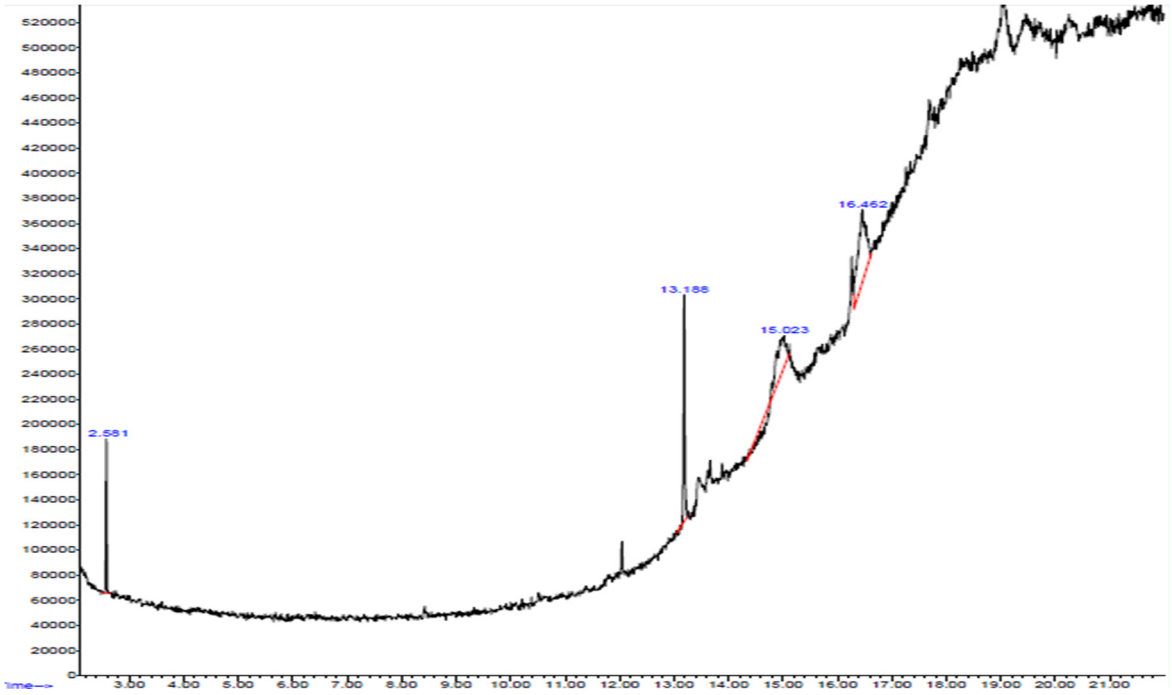


Fig. 37: GC-MS Chromatogram of ethanolic extract of *Cordia obliqua* Willd.

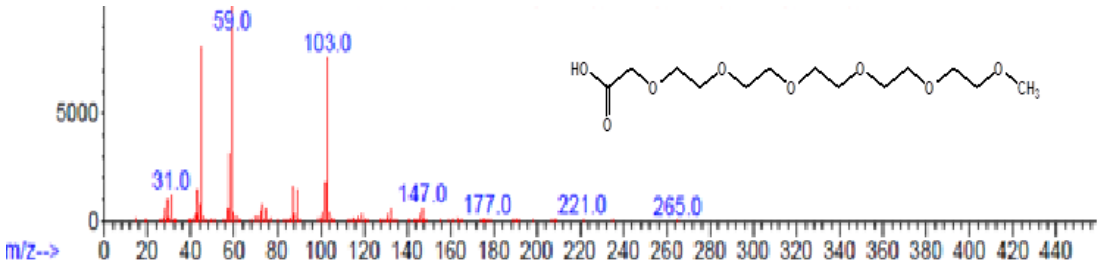


Fig. 38: 2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy) ethoxy)ethoxy)acetic acid

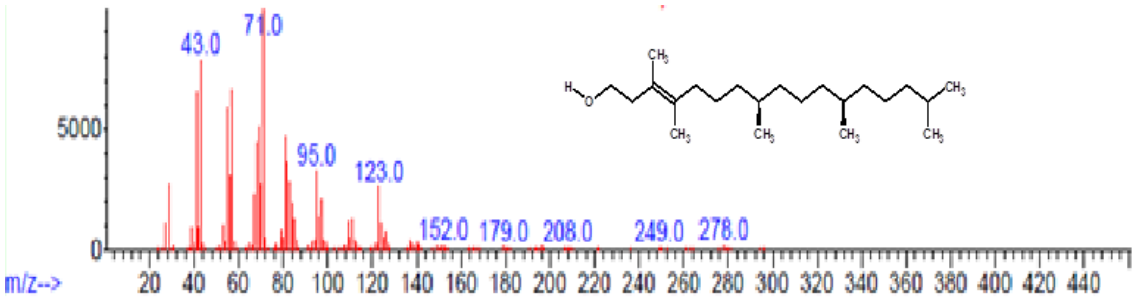


Fig. 39: Phytol

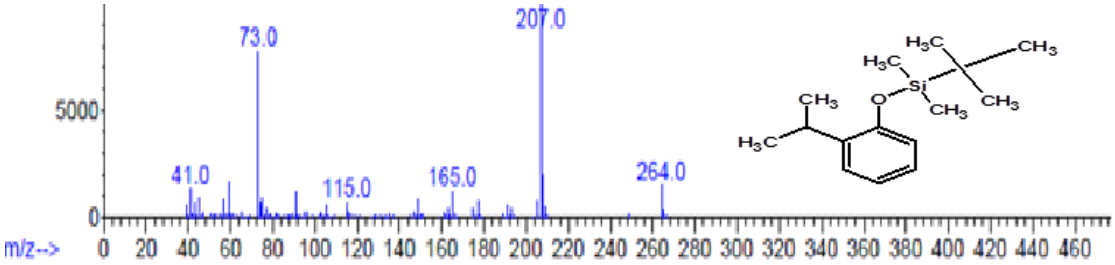


Fig. 40: Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl-

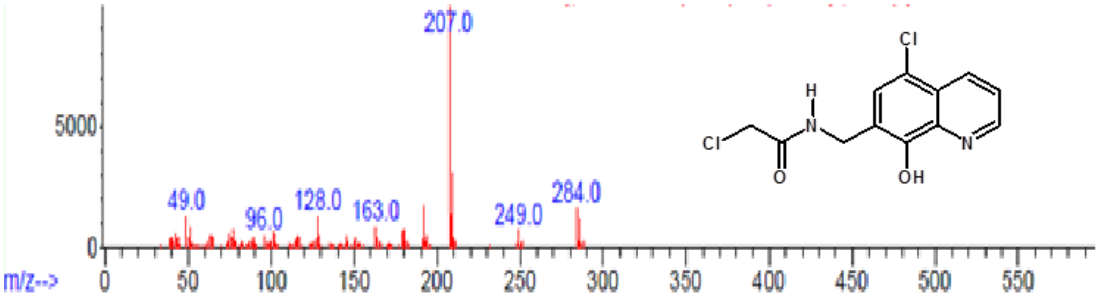


Fig. 41: Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl) methyl]-

Table No. 12: Chemical composition of ethanolic extract of *Cordia obliqua* Willd.

S. No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	2.579	2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy) ethoxy)ethoxy)acetic acid	$C_{13}H_{40}O_2$	310	11.85
2.	13.188	Phytol	$C_{20}H_{40}O$	296	25.10

3.	15.022	Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl-	$C_{16}H_{28}OSi$	264	15.91
4.	16.459	Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-	$C_{12}H_{10}O_2Cl_2N_2$	285	47.14

Table No. 13: Activity of phytocomponents identified in ethanolic extract of *Cordia obliqua* Willd by GC-MS.

RT	Name of the compound	Nature of compound	Activity
2.579	2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy) ethoxy)ethoxy)acetic acid	Ether	Centrally acting analgesics
13.188	Phytol	Diterpene	Antimicrobial, anticancer, cancer preventive, diuretic, anti-inflammatory
15.022	Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl-	Benzene	Antibacterial
16.459	Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-	Ethanamide	Antimicrobial

5.7. *Invitro* Antioxidant

1) DPPH Photometric Assay

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge DPPH photometric assay was calculated as % inhibition and was compared with rutin used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **48.34%** in pet.ether, **54.63%** in ethyl acetate and **65.10%** in ethanol when compared to rutin **69.83%** which is

statistically significant at same concentration. The IC_{50} value was found to be **1120 µg/ml** for pet.ether, **550 µg/**ml for ethyl acetate and **475 µg/ml** for ethanolic extract of *Cordia obliqua* and for rutin it was **480 µg/ml**.

Table No. 14: DPPH Assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Rutin)
1	125	17.55 ± 0.026	25.34 ± 0.035	35.63 ± 0.026	18.85 ± 0.076
2	250	28.68 ± 0.018	36.80 ± 0.028	46.78 ± 0.035	22.08 ± 0.054
3	500	33.73 ± 0.038	49.22 ± 0.030	52.68 ± 0.033	52.21 ± 0.022
4	1000	48.34 ± 0.021	54.63 ± 0.016	65.10 ± 0.028	69.83 ± 0.014
IC_{50}		**1120 µg/ml**	**550 µg/ml**	**475 µg/ml**	**480 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

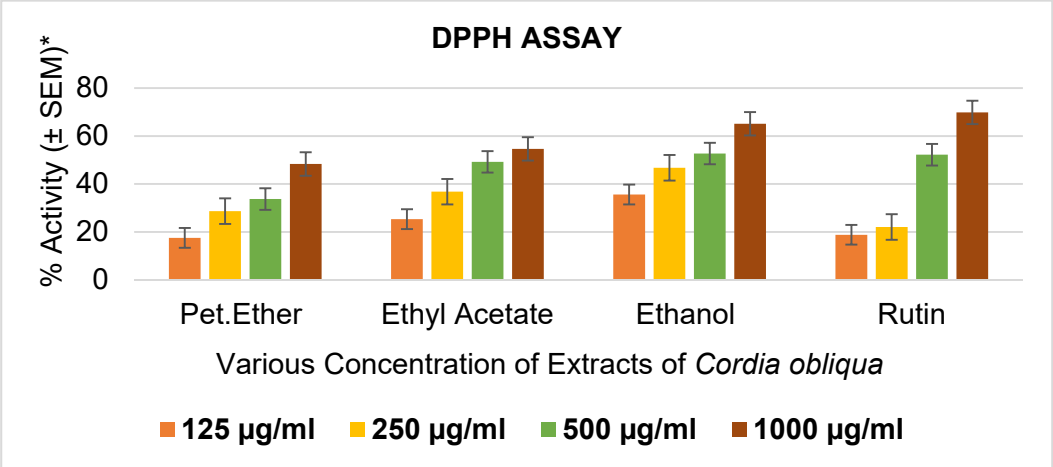


Fig. 42: DPPH assay of *Cordia obliqua*

2. Superoxide anion scavenging activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to superoxide anion scavenging activity was calculated as % inhibition and was compared with quercetin

used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **64.96%** in pet.ether, **68.56%** in ethyl acetate and **72.60%** in ethanol when compared to quercetin **98.01%** which is statistically significant at same concentration. The IC_{50} value was found to be **520 µg/ml** for pet.ether, **450 µg/**ml for ethyl acetate and **75 µg/ml** for ethanolic extract of *Cordia obliqua* and for quercetin it was **60 µg/ml**.

Table No. 15: Superoxide anion scavenging activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Quercetin)
1	125	14.18 ±0 .021	17.64 ± 0.043	57.68 ± 0.035	73.81 ± 0.006
2	250	21.45 ± 0.033	23.08 ± 0.031	60.46 ± 0.046	91.31 ± 0.011
3	500	48.78 ± 0.040	53.20 ± 0.025	65.32 ± 0.028	92.99 ± 0.024
4	1000	64.96 ± 0.022	68.56 ± 0.036	72.60 ± 0.019	98.01 ± 0.012
IC_{50}		**520 µg/ml**	**450 µg/ml**	**75 µg/ml**	**60 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

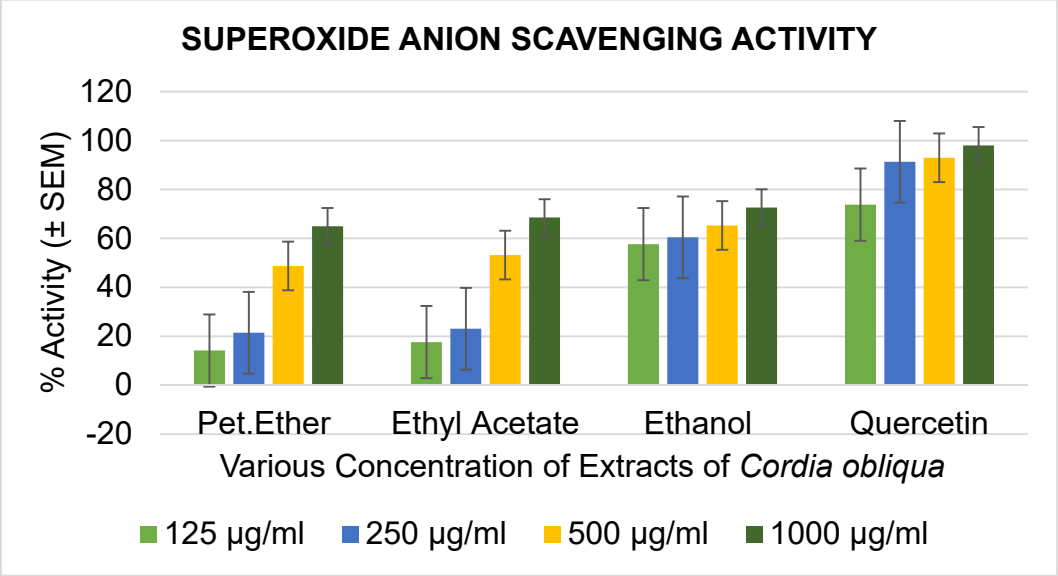


Fig. 43: Superoxide anion scavenging activity of *Cordia obliqua*

3. Iron chelating activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge iron chelating activity was calculated as % inhibition and was compared with EDTA used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **51.46%** in pet.ether, **58.59%** in ethyl acetate and **64.62%** in ethanol when compared to EDTA **97.90%** which is statistically significant at same concentration. The IC_{50} value was found to be **920 µg/ml** for pet.ether, **410 µg/**ml for ethyl acetate and **110 µg/ml** for ethanolic extract of *Cordia obliqua* and for EDTA it was **65 µg/ml**.

Table No. 16: Iron chelating activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (EDTA)
1	125	26.20 ± 0.026	24.34 ± 0.018	51.18 ± 0.030	58.68 ± 0.007
2	250	35.80 ± 0.029	35.28 ± 0.016	55.30 ± 0.028	65.87 ± 0.018
3	500	44.35 ± 0.021	53.45 ± 0.022	60.54 ± 0.033	83.83 ± 0.012
4	1000	51.46 ± 0.025	58.59 ± 0.024	64.62 ± 0.026	97.90 ± 0.019
IC_{50}		**920 µg/ml**	**410 µg/ml**	**110 µg/ml**	**65 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

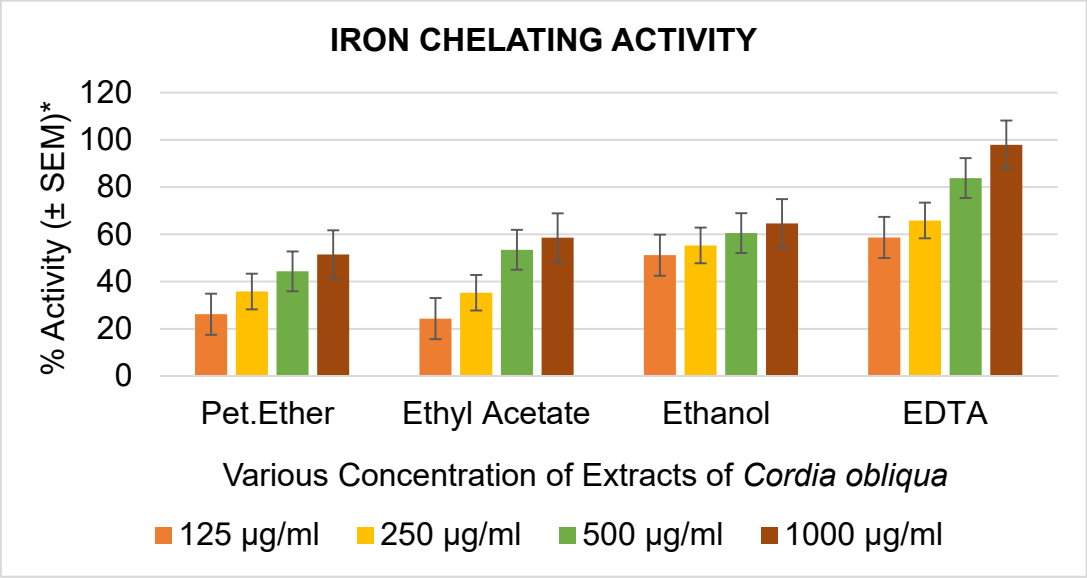


Fig. 44: Iron chelating activity of *Cordia obliqua*

4. Hydroxyl radical scavenging activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to hydroxyl radical scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **52.54%** in pet.ether, **55.82%** in ethyl acetate and **69.51%** in ethanol when compared to ascorbate **75.23%** which is statistically significant at same concentration. The IC_{50} value was found to be **880 µg/ml** for pet.ether, **720 µg/**ml for ethyl acetate and **350 µg/ml** for ethanolic extract of *Cordia obliqua* and for ascorbate it was **410 µg/ml**.

Table No. 17: Hydroxyl radical scavenging activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	23.18 ± 0.021	25.31 ± 0.042	36.82 ± 0.021	26.87 ± 0.076
2	250	39.22 ± 0.033	39.43 ± 0.035	44.94 ± 0.034	30.30 ± 0.054
3	500	45.35 ± 0.041	47.65 ± 0.026	58.76 ± 0.018	60.64 ± 0.022
4	1000	52.54 ± 0.022	55.82 ± 0.038	69.51 ± 0.040	75.23 ± 0.014
IC_{50}		**880 µg/ml**	**720 µg/ml**	**350 µg/ml**	**410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

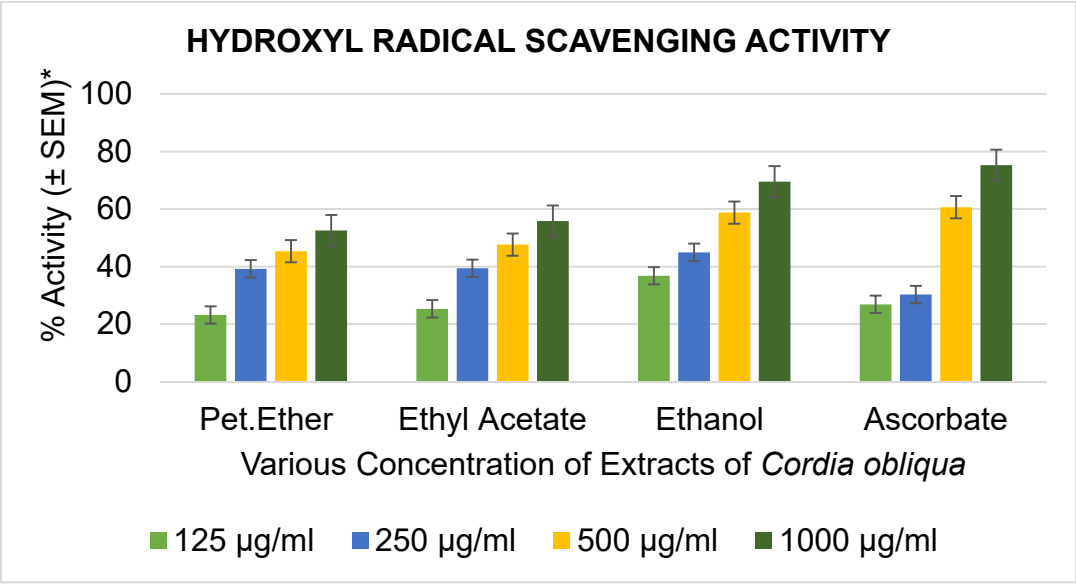


Fig. 45: Hydroxyl radical scavenging activity of *Cordia obliqua*

5. Nitric oxide scavenging activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to nitric oxide scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **49.85%** in pet.ether, **54.06%** in ethyl acetate and **65.26%** in ethanol when compared to ascorbate **75.23%** which is statistically significant at same concentration. The IC_{50} value was found to be **1010 µg/ml** for pet.ether, **820 µg/**ml for ethyl acetate and **450 µg/ml** for ethanolic extract of *Cordia obliqua* and for ascorbate it was **410 µg/ml**

Table No. 18: Nitric oxide scavenging activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	15.21 ± 0 .018	22.32 ± 0.026	33.22 ± 0.020	26.87 ± 0.076
2	250	22.63 ± 0.024	35.40 ± 0.033	48.46 ± 0.026	30.30 ± 0.054
3	500	38.74 ± 0.036	42.85 ± 0.035	52.08 ± 0.034	60.64 ± 0.022
4	1000	49.85 ± 0.042	54.06 ± 0.022	65.26 ± 0.030	75.23 ± 0.014
IC_{50}		**1010 µg/ml**	**820 µg/ml**	**450 µg/ml**	**410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

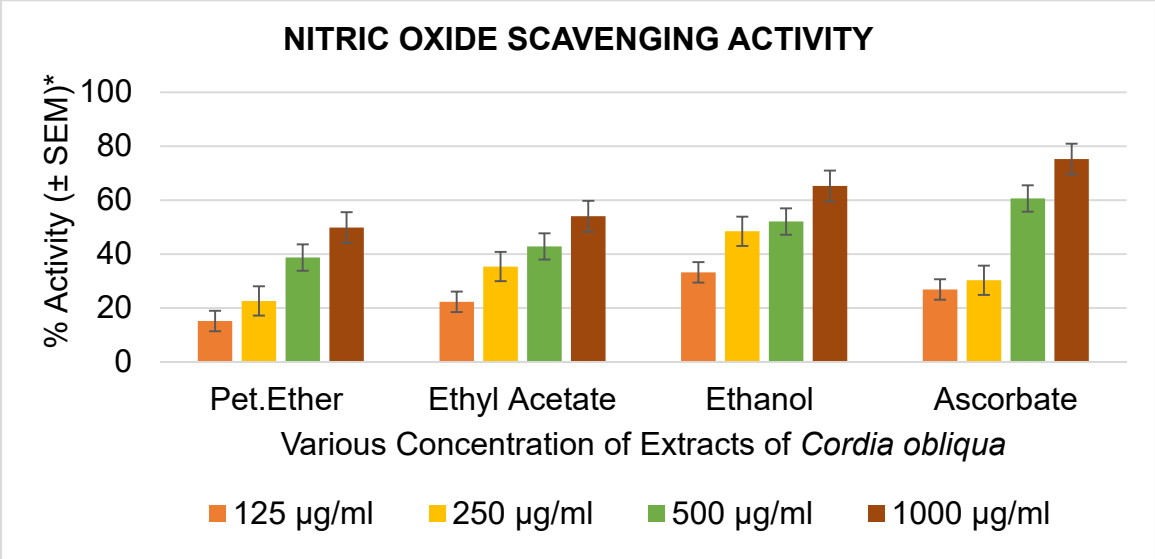


Fig. 46: Nitric oxide scavenging activity of *Cordia obliqua*

6. Total antioxidant activity (Phosphomolybdic acid method

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge superoxide anion scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **64.85%** in pet.ether, **66.35%** in ethyl acetate and **70.95%** in ethanol when compared to ascorbate **65.23%** which is statistically significant at same concentration. The IC_{50} value was found to be **590 µg/ml** for pet.ether, **460 µg/**ml for ethyl acetate and **400 µg/ml** for ethanolic extract of *Cordia obliqua* and for ascorbate it was **410 µg/ml**.

Table No. 19: Total antioxidant activity (Phosphomolybdic acid method)

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	24.93 ± 0.019	29.46 ± 0.027	31.62 ± 0.018	26.87 ± 0.076
2	250	32.82 ± 0.028	42.08 ± 0.035	46.30 ± 0.026	30.30 ± 0.054
3	500	47.74 ± 0.033	51.79 ± 0.028	56.84 ± 0.034	60.64 ± 0.022
4	1000	64.85 ± 0.038	66.35 ± 0.042	70.95 ± 0.040	65.23 ± 0.014
IC_{50}		**590 µg/ml**	**460 µg/ml**	**400 µg/ml**	**410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

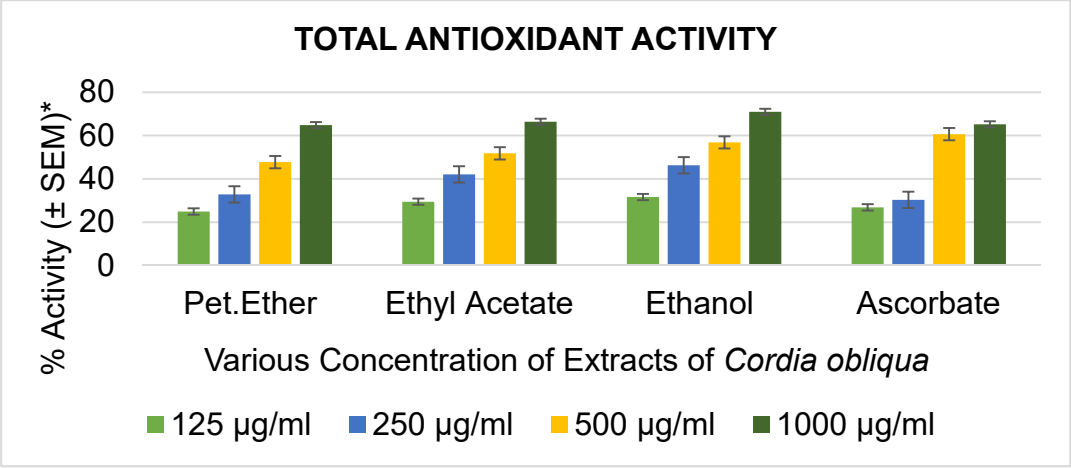


Fig. 47: Total antioxidant activity of *Cordia obliqua*

7. FRAP assay

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge Superoxide anion scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **37.91%** in pet.ether, **53.96%** in ethyl acetate and **76.78%** in ethanol when compared to ascorbate **98.07%** which is statistically significant at same concentration. The IC_{50} value was found to be **1300 µg/ml** for pet.ether, **960 µg/**ml for ethyl acetate and **210 µg/ml** for ethanolic extract of *Cordia obliqua* and for ascorbate it was **50 µg/ml**.

Table No. 20: FRAP Assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Pet. ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	17.56 ± 0.065	12.62 ± 0.021	31.35 ± 0.050	72.04 ± 0.014
2	250	21.88 ± 0.033	32.74 ± 0.016	52.55 ± 0.031	82.05 ± 0.034
3	500	34.04 ± 0.040	48.50 ± 0.028	61.08 ± 0.042	86.04 ± 0.026
4	1000	37.91 ± 0.038	53.96 ± 0.027	76.78 ± 0.026	98.07 ± 0.041
IC_{50}		**1300 µg/ml**	**960 µg/ml**	**210 µg/ml**	**50 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

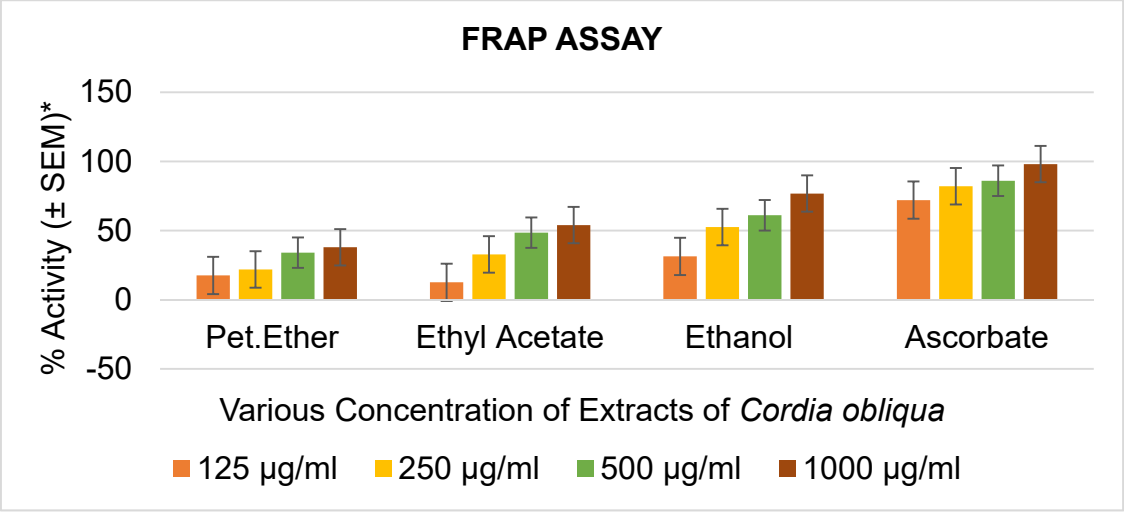


Fig. 48: FRAP assay of *Cordia obliqua*

8. Estimation of total phenol content

TPC showed a sharp pet.ether extract range of **1.43 mg/g**, ethyl acetate extract range of **2.48 mg/g** and ethanolic extract range of **4.91 mg/g** as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

Table No. 21: Total phenol

S.No	Extracts	Total phenolic content (mg/g of Catechol)
1	Pet. ether extract of *Cordia obliqua*	1.43 ± 0.020
2	Ethyl acetate extract of *Cordia obliqua*	2.48 ± 0.042
3	Ethanolic extract of *Cordia obliqua*	4.91 ± 0.044

*All the values are expressed as mean ± SEM for three determinations.

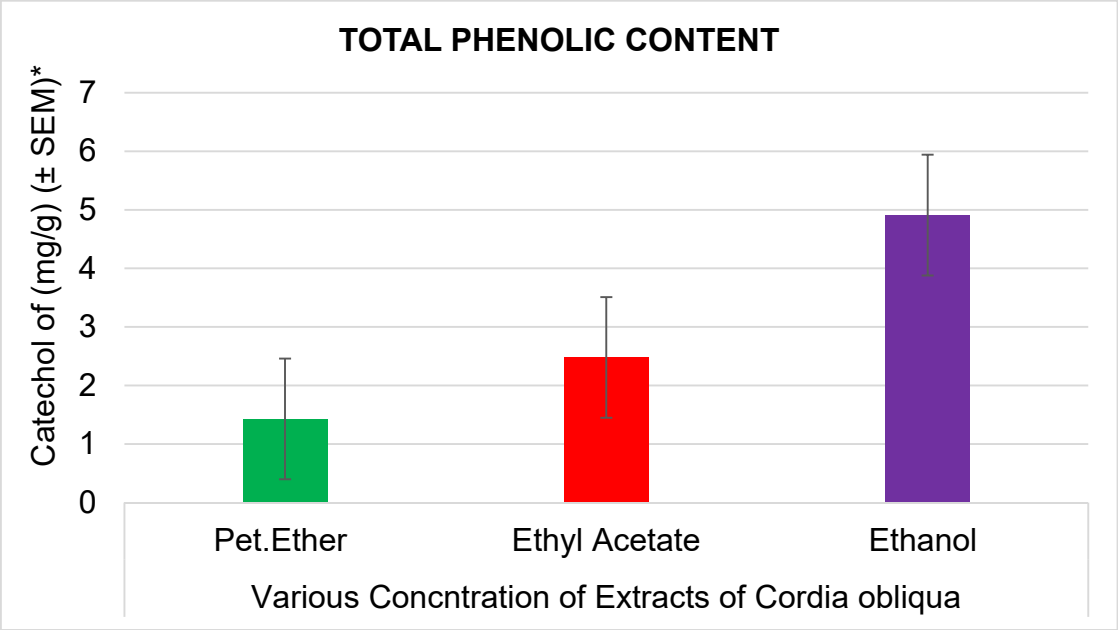


Fig. 49: Total phenolic content of *Cordia obliqua*

9. Estimation of total flavonoids content

The TFC showed a sharp pet.ether extract range of **0.032 mg/g**, ethyl acetate extract range of **1.068 mg/g** and ethanolic extract range of **2.176 mg/g** as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

Table No. 22: Total flavonoids

S. No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Pet. ether extract of *Cordia obliqua*	0.032 ± 0.003
2	Ethyl acetate extract of *Cordia obliqua*	1.068 ± 0.015
3	Ethanolic extract of *Cordia obliqua*	2.176 ± 0.018

*All the values are expressed as mean ± SEM for three determinations

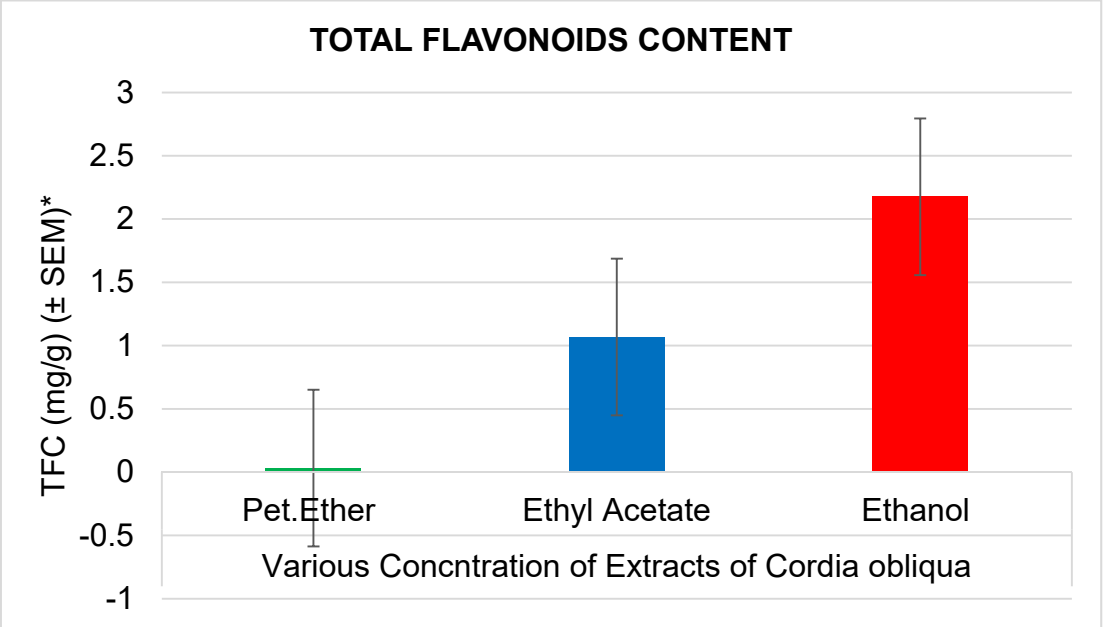


Fig. 50: Total flavonoids content of *Cordia obliqua*

5.8. Molecular Docking

The three-dimensional structure of transport protein (PDB-ID: 3QNT) was transformed from the RCSB protein data bank. The docking study was performed using Molegro Virtual Docker Version 6.0, which focused on molecular docking stimulations. While performing docking, both the ligand and protein are included in the workspace. All the water molecules and other ligands are removed from protein and imported in workspace. Then it undergoes cavity detection in protein to identify cavities.

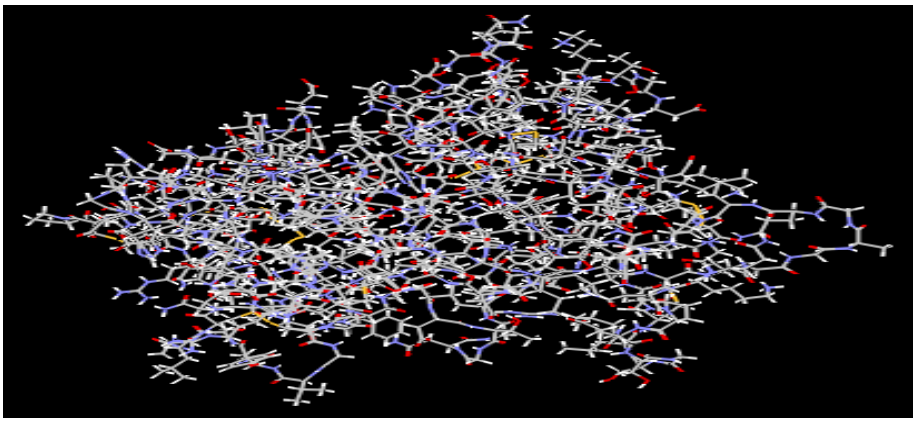


Fig. 51: Protein Structure of 3QNT

While performing docking, the binding radius, grid resolution and maximum parameters are set. In accordance with the targeted enzyme PDB code - 3QNT (NPC_1L_1- Transport protein) was selected for cholesterol lowering drug docking studies of the compounds. Validation of Molegro virtual Docker 6.0 is important because it is a computational approach.

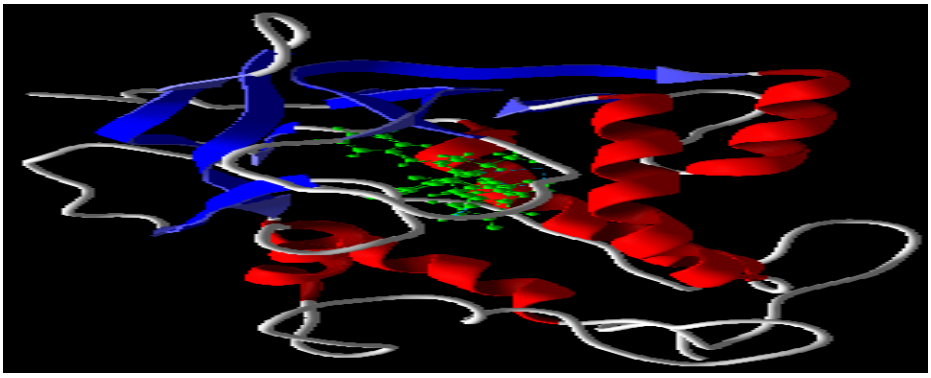


Fig. 52: Protein Structure of 3QNT with cavities

Table No. 23: Docking Scores of Ethyl Acetate Extract of *Cordia obliqua* leaves of Ligands in Cavity 1 of 3QNT

S. No	Phytochemical compound names	Mol. Dock Score	Rerank Score	H Bond
1	Didodecyl phthalate	-158.79	-85.9214	-0.88411
2	2-Heptadecenal	-111.633	-70.8246	-2.5
3	9,12-Octadecadienoic acid (Z,Z)-	-112.666	-34.7376	0
4	Cyclobarbital	-72.977	-58.4834	-2.8445
5	Trans-2,3-methylenedioxy-b-methyl-b- nitrostyrene	-91.6474	-76.2017	-0.723384
6	1,1,1,3,5,5,5-Heptamethyltrisiloxane	-103.071	-89.2597	-3.70706
7	1H-indole, 1-methyl-2-phenyl-	-87.8277	-59.4769	0
8	Hexahydropyridine 1-methyl-4-[4,5-dihydroxyphenyl]-	-94.8281	-80.7934	-11.0039
9	Benzo [h] quinolone 2,4-dimethyl-	-68.8103	-52.2536	-0.911644
10	2-methyl-7-phenylindole	-88.6036	-48.1289	-1.49099
11	2-Ethylacridine	-74.2574	-60.4292	0
12	Suprane	-107.2574	-57.067	0
13	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	-100.818	-76.711	-1.76458
14	1-methyl-3-phenylindole	-90.0469	-54.8693	-1.02016
15	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	-113.099	-92.9621	-3.45615
16	6-Octadecenoic acid	-123.617	-91.1162	-2.21869

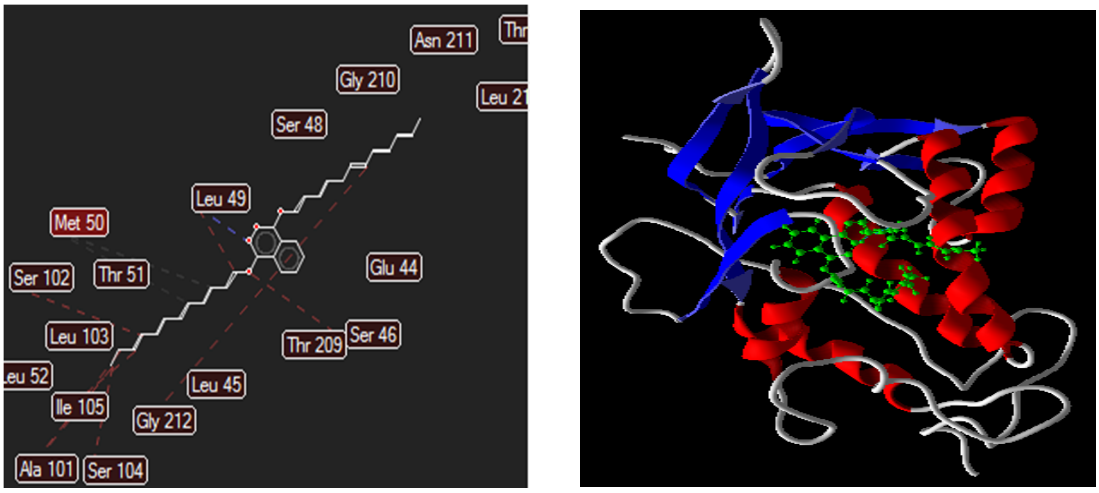


Fig. 53: Stearic interaction and docking pose of didodecyl phthalate.

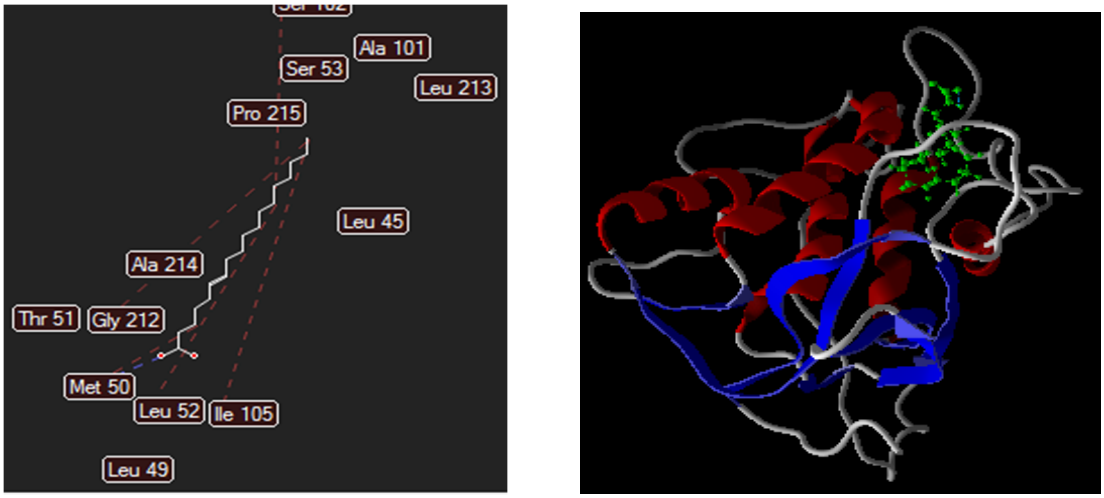


Fig. 54: Stearic interaction and docking pose of 6-octadecenoic acid.

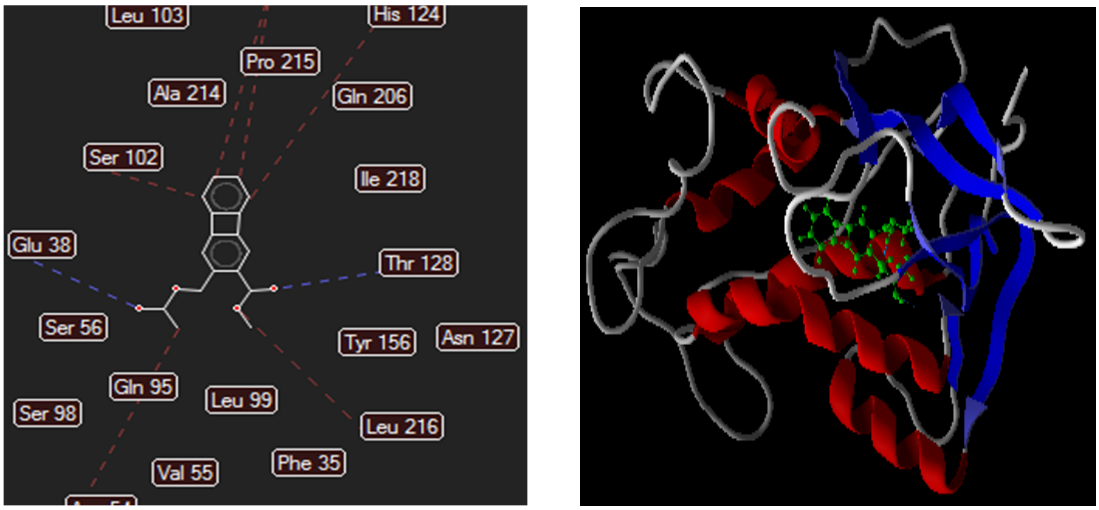


Fig. 55: Stearic interaction and docking pose of 9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester.

Table No. 24: Docking Scores of Ethanolic Extract of *Cordia obliqua* Leaves of Ligands in Cavity 1 of 3QNT

S. No	Phytochemical compound names	Mol. Dock Score	Rerank Score	H Bond
1	2-(2-(2-(2-(2-(2-methoxyethoxy) ethoxy) ethoxy) ethoxy) ethoxy) acetic acid	-71.574	-60.8627	-2.34006
2	Phytol	-121.688	-89.2581	-3.89102
3	Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl-	-103.232	-61.3737	0
4	Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-	-90.2833	-79.9024	-3.78322

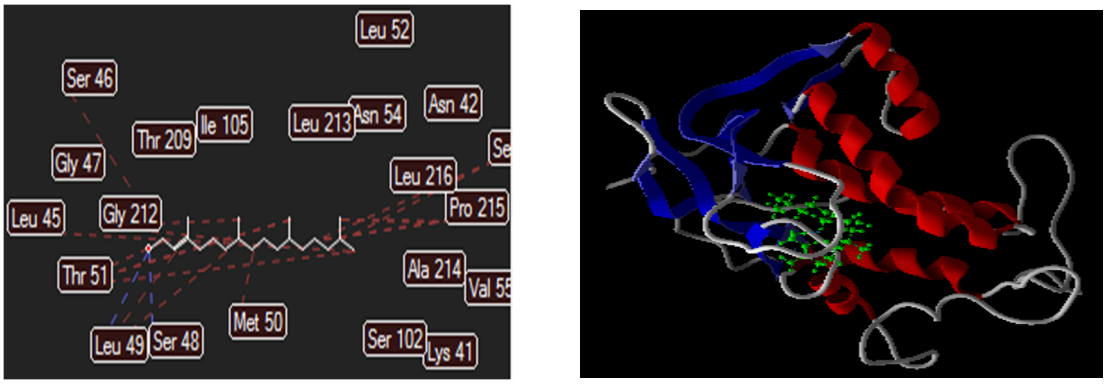


Fig. 56: Stearic interaction and docking pose of phytol.

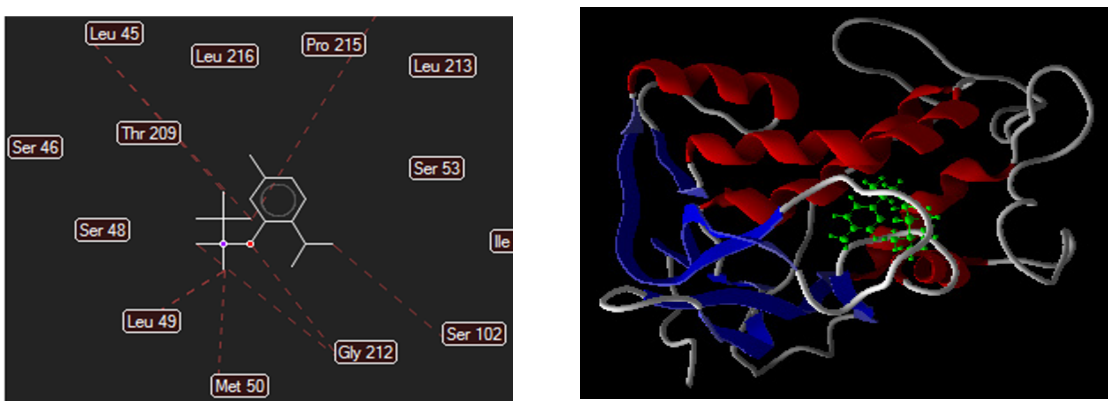


Fig. 57: Stearic interaction and docking pose of benzene, 2-[(tert-butyl dimethyl-silyl) oxy]-1-isopropyl-4-methyl.

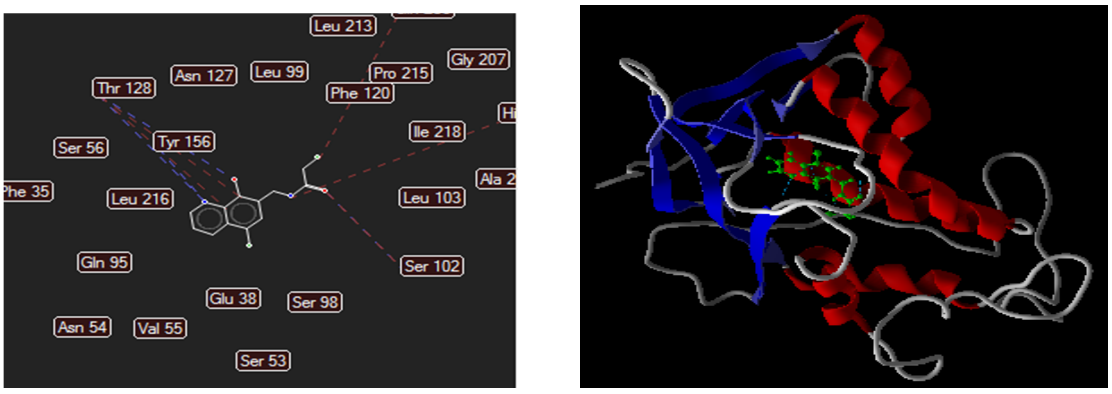


Fig. 58: Stearic interaction and docking pose of acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl) methyl].

Table No. 25: Molecular docking score of standard drug atorvastatin.

Standard drug	Mol. Dock Score	Rerank Score	H Bond
Atorvastatin	-104.402	-555.041	-8.45232

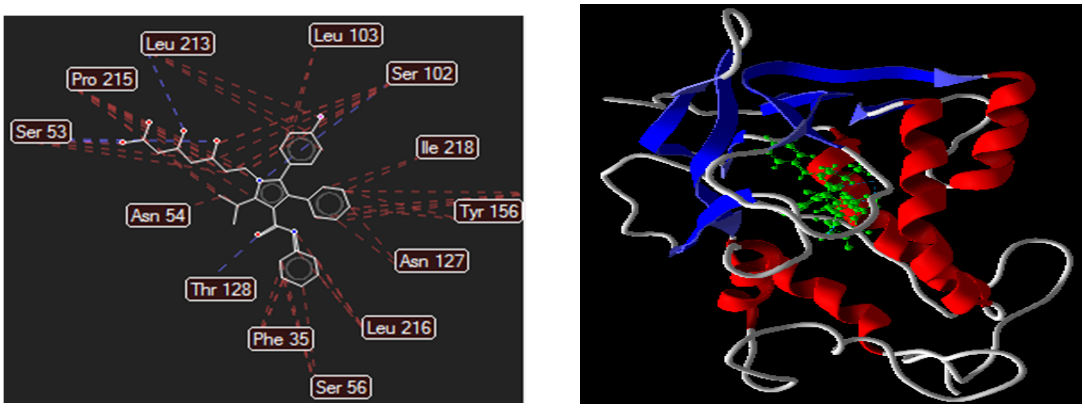


Fig. 59: Stearic interaction and docking pose of standard drug atorvastatin.

The presence of sixteen phytochemical constituents from ethanolic extract and four phytochemical constituents from aqueous extract were identified by GC-MS. These components undergone *in-silico* molecular docking studies using enzyme transport protein. The lead compounds were selected through the docking score. The compounds of ethanolic extract such as didodecyl phthalate (-158.79); 6-octadecenoic acid (-123.617); 9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (-113.099) and the compounds of aqueous extract like phytol (-121.688); benzene, 2-[(tert-butyl dimethyl-silyl) oxy]-1-isopropyl-4-methyl- (-103.232); acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-

7-quinolinyl) methyl] (-90.2833). Each extract have been shown three lead compounds from *insilico* molecular docking using standard atorvastatin (-104.402).

5.9. Effect of various extracts of leaves of *Cordia obliqua* on body weight changes in rats

Table No. 26 shows an average body weight in control and experimental animals in each group. The average body weight gain of rats in all the seven groups was increased after 30 days of experimental period. But high fat diet fed rats (group II) high fat diet a significant increase in body weight compared with other group rats. After high fat administration of various extracts of *Cordia obliqua,* it was found to be decreased in body weight. But the high fat administration of the various extract of *Cordia obliqua* high dose was found to be more significantly decreased ($p<0.001$) the body weight gain when compared to high fat diet rats group (II).

Table No. 26: Effect of various extract of leaves plant of *Cordia obliqua* on average body weight changes in rats

Groups	Initial Weight (g)	Final Weight (g)	Average Body weight gain (g)
Group I	188 ± 0.15	246 ± 0.12	58 ± 0.5
Group II	144 ± 0.8	229 ± 0.6	85 ± 0.7
Group III	166 ± 0.14	237 ± 0.10	71 ± 0.9
Group IV	176 ± 0.14	236 ± 0.15	60 ± 0.5
Group V	177 ± 0.22	231 ± 0.18	54 ± 0.5
Group VI	178 ± 0.31	251 ± 0.26	73 ± 0.8
Group VII	185 ± 0.31	267 ± 0.26	82 ± 0.8

Values are expressed as mean ± SEM (n=6 rats)

P values : $^{*}<0.001$, $^{**}<0.05$

NS : Non significant

a → group I compared with groups II, III, IV, V, VI & VII.

b → group II compared with groups I, III, IV, V, VI & VII.

Group I : Standard chow pellet. (Control)

Group II : High fat diet.

Group III : High fat diet + Standard drug atorvastatin (1.2 mg/kg b.wt)

Group IV : High fat diet + Ethyl acetate extract of *Cordia obliqua* (100mg/kg b.wt)

Group V : High fat diet + Ethyl acetate extract of *Cordia obliqua* (200mg/kg b.wt)

Group VI : High fat diet + Ethanolic extract of *Cordia obliqua* (100mg/kg b.wt)

Group VII : High fat diet + Ethanolic extract of *Cordia obliqua* (200mg/kg b.wt)

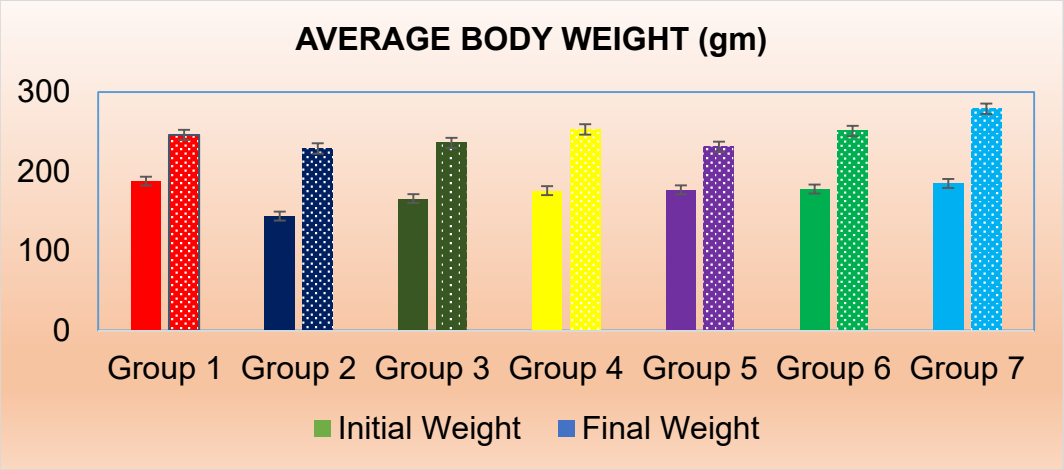


Fig. 60: Effect of various extracts of leaves of *Cordia obliqua* on average body weight changes in rats

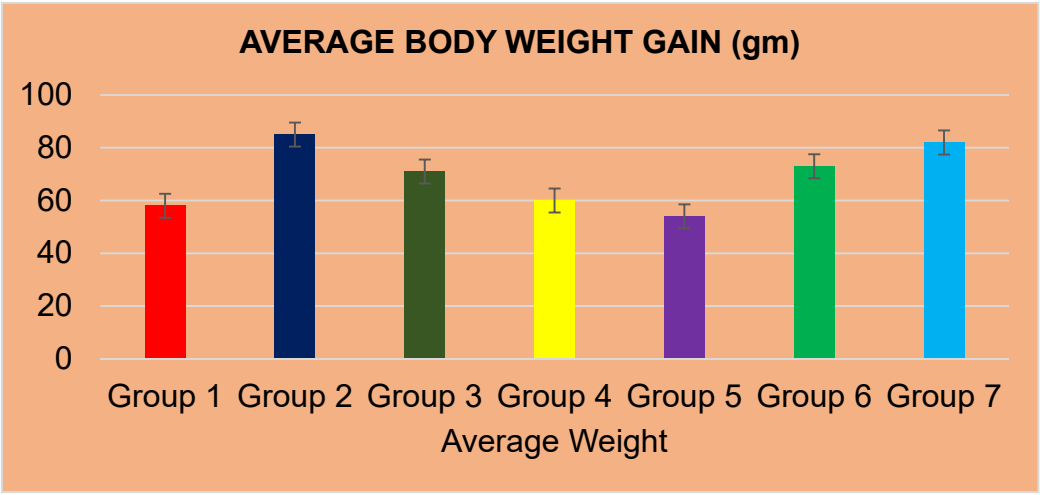


Fig. 61: Effect of various extracts of leaves of *Cordia obliqua* on average weight gain in rats

5.10. Effect of various extracts of leaves of *Cordia obliqua* on plasma lipid in high fat diet rats

Table No. 27 shows the effect of various extracts of *Cordia obliqua* on plasma lipid profile in control and experimental rats in each group. Total cholesterol levels increased in high fat diet fed rats (group II) as compared to control rats (group I). Results show that treatment with high fat diet significantly increased the concentration of plasma and tissue lipids as reported earlier, revealing that significant elevation of plasma and tissue lipid parameters in response to high fat diet and cholesterol feeding (Vijaimohan *et al.*, 2006; Mehta *et al*., 2003; Purohit and Vyas, 2006). High fat administration of various extracts of *Cordia obliqua* at the dose -00mg/kg body weight to rats fed with high fat diet significantly ($p<0.001$) decreased in the concentration of total cholesterol as compared to high fat diet rats (group II). But the high fat administration of various extract of *Cordia obliqua* high dose treated rats with high fat diet showed that the plasma cholesterol was restored to near normal as that of atorvastatin (group VII).

Table No. 27 illustrates the effect of various extracts of *Cordia obliqua* on plasma free and ester cholesterol levels in control and experimental rats in each group. The significant increase in levels of both free and ester cholesterol was also observed in plasma of rats fed with high fat diet. This high cholesterol concentration in circulation may damage the endothelial cells lining the large arteries and aorta and this may be an initial event in the etiology of atherosclerosis (Hennig and Chow, 1988). Increased intake of saturated fatty acids results an increased cholesterol production in liver. Both plasma free and ester cholesterol reduced remarkably on treating the high fat diet rats with various extract of *Cordia obliqua* (group V).

Effects of various extracts of *Cordia obliqua* on plasma free fatty acid levels are depicted in Table No. 27. The concentration of plasma free fatty acids was significantly increased in rats fed high fat diet (group II) as compared to control rats (group I). Increased levels of plasma free fatty acids were observed in high fat diet rats due to the breakdown of membrane phospholipid by the action of oxygen derived free radical induced during hyperlipidemia (Geetha G *et al.,* 2008) or the increased the level of plasma free fatty acids could lead to increase in synthesis of phospholipids and ester cholesterol (Muruganandan S *et al*., 2005). After treatment of various extract of *Cordia obliqua* (dose 200mg/kg body weight) along with high fat diet plasma free fatty acids

levels significantly reduced as compared to high fat diet fed rats (group II). Similar result was not found in other three extracts. Treatment with *Cordia obliqua* extract (group V) decreased the free fatty acids concentration which may be due to the inhibitory action on lipolysis.

Effects of various extracts of *Cordia obliqua* on plasma phospholipids are depicted in Table No. 27. The concentration of plasma phospholipids significantly increased in rats fed high fat diet (group II) as compared to control animals (group I). This may be due to decreased phospolipase activity (Hansen RA *et al*., 2002). High fat administration of various extract of *Cordia obliqua* (200mg/kg body weight) along with high fat diet plasma phospholipids levels significantly reduced as compared to high fat diet fed rats (group II). The various extract of *Cordia obliqua* (200 mg/kg body weight) treated rats with high fat diet showed that the significantly reduced the plasma phospholipids levels when compared to that of other three extracts. The reduced concentration of phospholipids may also be due to the enhanced activity of phospholipases (Kottai Muthu *et al.*, 2005).

The plasma triglyceride levels of various extracts of *Cordia obliqua* are presented in Table No. 27. The concentration of plasma triglyceride elevated in rats fed high fat diet (group II) as compared to control rats (group I). High fat diet rat significant increase in the level of plasma triglyceride was due to decrease in the activity of lipoprotein lipase (Mini S and Rajmohan., 2004). The plasma triglyceride level significantly decreased in rats treated with various extract of *Cordia obliqua* (200mg/kg body weight) and as well as standard drug atorvastatin along with high fat diet when compared with rats fed with high fat diet (group II). Treatment of various extract of *Cordia obliqua* with high fat diet significantly decreased the plasma triglyceride levels when compared to other extracts. The plant extract may have stimulation lipoprotein lipase activities resulting in decrease of plasma triglyceride and might increase the uptake of triglyceride from plasma by skeletal muscle and adipose tissue (El-Hazmi, 2001).

Atherogenic index is used as a marker to assess the susceptility of atherogenesis. It was markedly increased on feeding high fat diet to rats. The various extract of *Cordia obliqua* high dose significantly decreased atherogenic index when compared to other three extracts. But, when the various extract of *Cordia obliqua* high dose was compared with standard group of rats, it was found to have similar result.

Table No. 27: Effect of varoius extracts of leaves plant of *Cordia obliqua* on plasma lipid profile in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Free fatty acid (mg/dl)	Phospho lipid (mg/dl)	Tri glyceride (mg/dl)
Group I	114.26± 1.36^{b*}	26.82 ±1.08^{b*}	88.18 ±0.90^{b*}	44.84± 0.32^{b*}	98.46± 0.44^{b*}	84.34± 1.32^{b*}
Group II	175.76± 1.65^{a*}	43.22± 0.92^{a*}	130.28± 1.58^{a*}	60.80± 0.22^{a*}	148.80± 0.50^{a*}	155.33± 1.26^{a*}
Group III	98.33± 0.86^{a*,b*}	23.98± 0.78^{a*,b*}	75.55± 0.34$^{a*,\ b*}$	39.65± 0.62^{a*,b**}	104.28± 0.33^{a*,b*}	68.46± 0.76^{a*,b*}
Group IV	106.10± 0.88^{a*,b*}	24.56± 0.48^{a*,b*}	82.64± 1.10^{a*,b*}	42.66± 0.32^{a*,b**}	120.46± 0.58^{a*,b*}	102.42± 0.36^{a*,b**}
Group V	100.55± 0.62^{a*,b*}	23.55± 0.55^{a*,b*}	71.92± 0.68^{a*,b*}	40.10± 0.22^{a*,b*}	110.86± 0.46^{a*,b*}	78.98± 0.56^{a*,b*}
Group VI	155.82± 3.51^{a**,b*}	41.56± 0.88$^{a*,\ b*}$	124.68± 1.98^{a*,b**}	53.44± 0.58^{a**,b**}	145.08± 0.60^{a*,b*}	145.33± 1.55^{a*,b*}
Group VII	116.68± 0.56^{a*,b*}	28.34± 0.42^{a*,b*}	87.96± 1.22^{a*,b*}	48.66± 0.26^{a*,b**}	128.06± 0.48^{a*,b*}	117.82± 0.68^{a*,b**}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

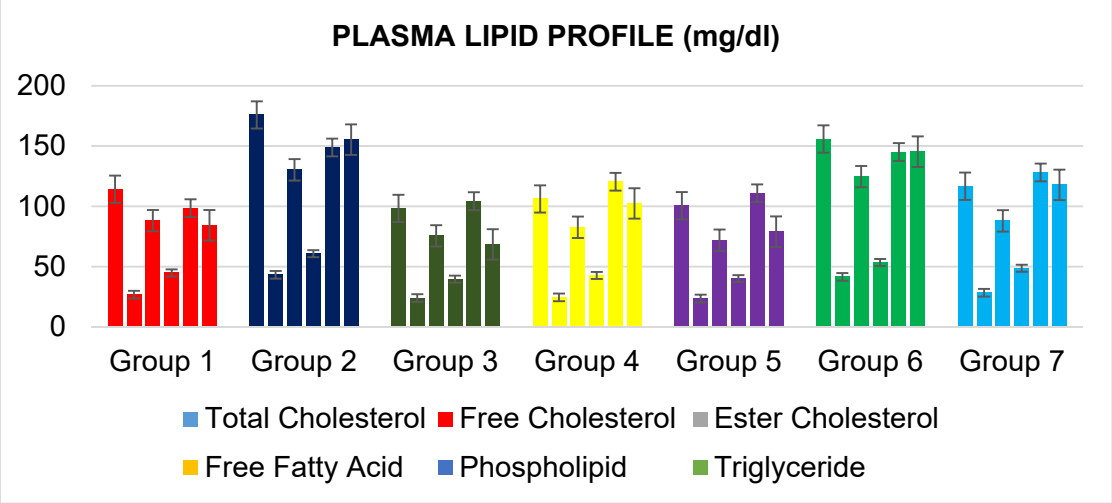


Fig. 60: Effect of various extracts of leaves plant of *Cordia obliqua* on plasma lipid profile in high fat diet rats

Table No. 28: Effect of various extracts of leaves plant of *Cordia obliqua* on plasma lipid profile in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Atherogenic index	Cardiac risk ratio	Atherogenic Coefficient
Group I	2.07± 0.01 ^{b*}	2.08± 0.02 ^{b*}	1.08±0.01 ^{b*}
Group II	4.58± 0.03 ^{a*}	4.50±0.48 ^{a*}	3.28± 0.05 ^{a*}
Group III	1.74± 0.03 $^{a*, b*}$	1.75±0.03 $^{a*, b*}$	0.75±0.03 $^{a*, b*}$
Group IV	1.78± 0.03 $^{a*, b*}$	1.80±0.04$^{a*, b*}$	0.82±0.03 $^{a*, b*}$
Group V	1.75± 0.02 $^{a*, b*}$	1.78±0.02$^{a*, b*}$	0.82±0.02 $^{a*, b*}$
Group VI	2.30± 0.04 $^{a*, b*}$	2.65±0.33 $^{a*, b**}$	1.67±0.02 $^{a*, b*}$
Group VII	2.18± 0.04 $^{a*, b*}$	2.65±0.28 $^{a*, b**}$	1.70±0.02 $^{a*, b*}$

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

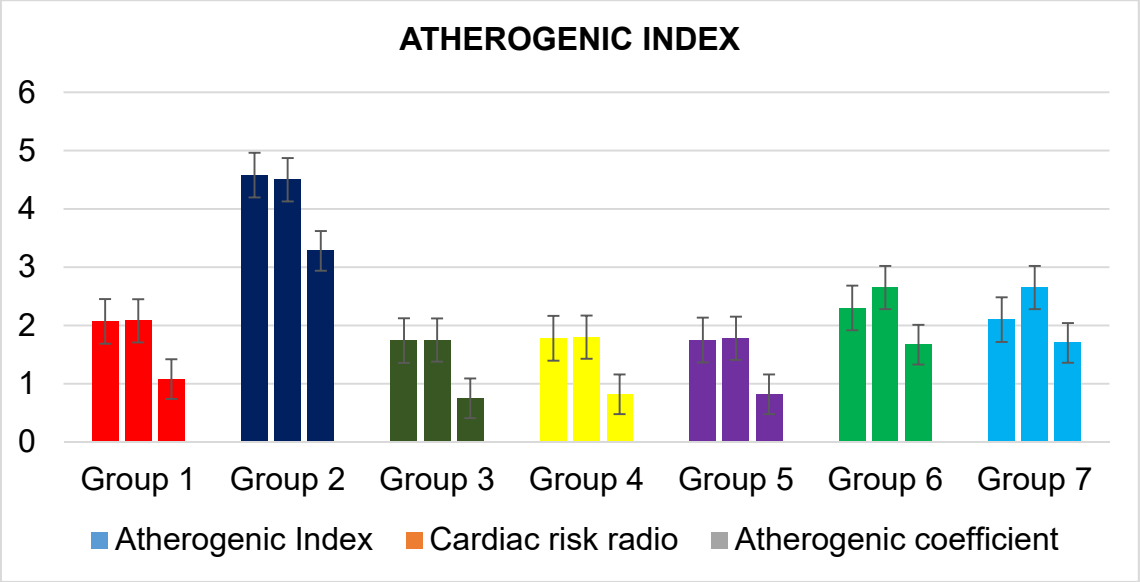


Fig. 61: Effect of various extracts of leaves plant of *Cordia obliqua* on plasma lipid profile in high fat diet rats

5.11. EFFECT OF VARIOUS EXTRACTS OF LEAVES OF *CORDIA OBLIQUA* ON PLASMA LIPOPROTEIN IN HIGH FAT DIET FED RATS

Table No. 29 shows the levels of HDL cholesterol in plasma of control and experimental rats in each group. The HDL cholesterol levels increased in high fat diet rats (Group II) as compared to control rats (group I). But the high fat administration of various extracts of *Cordia obliqua* high dose were found significantly elevated the HDL-cholesterol levels when compared with other extracts. Several studies show that an increase in HDL –cholesterol is associated with a decrease in coronary risk (Jenkins PJ *et al*., 1978).

Effect of various extracts of *Cordia obliqua* on plasma LDL-cholesterol and VLDL-cholesterol levels are presented in Table No. 29. High fat diet fed rats (group II) are elevated levels of LDL and VLDL-cholesterol when compared with the control (group I). Studies show that both LDL and VLDL have a positive role in atherogenesis (Parthasarathy *et al*., 1989). High fat administration of various extract of *Cordia obliqua* markedly reduced the level of LDL-cholesterol and VLDL-cholesterol in plasma when compared with other extracts. Reduced levels of LDL and VLDL-cholesterol in various extract of *Cordia obliqua* on high fat diet fed rats may be possibly due to increase with catabolism of LDL.

The ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol are presented in Table No. 29. High fat diet rats caused significant (P<0.001) increase in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol. These results are consistent with earlier reports (Arca M *et al*., 2007). Administration of various extract of *Cordia obliqua* along with high fat diet was found significantly (P<0.001) reduced the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol when compared to high fat diet group (II). But the various extract of *Cordia obliqua* along with high fat diet (group V) showed similar result to standard group rats (VI).

Table No. 29: Effect of various extracts of leaves plant of *Cordia obliqua* on plasma lipoprotein in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	LDL-c/HDL-c ratio	HDL-c/ TC ratio
Group I	61.38 ± 0.78^{b*}	37.59 ± 1.10^{b*}	17.55 ± 0.38^{b*}	0.68 ± 0.04^{b*}	0.55 ± 0.005^{b*}
Group II	43.58 ± 2.08^{a*}	102.42 ± 1.58^{a*}	31.35 ± 0.44^{a*}	2.88 ± 0.22^{a*}	0.28 ± 0.010^{a*}
Group III	59.46 ± 0.40^{a*,b*}	28.58 ± 0.68^{a*,b*}	14.40 ± 0.32^{a*,b*}	0.50 ± 0.02^{a*,b*}	0.61 ± 0.003 ^{a*,b*}
Group IV	50.86 ± 0.72^{a*,b*}	38.43 ± 1.12^{a**,b*}	22.46 ± 0.15^{a*,b*}	0.78 ± 0.04^{a*,b*}	0.43 ± 0.004^{a**,b*}
Group V	56.98 ± 0.82^{a*,b*}	28.66 ± 1.25^{a*,b*}	16.10 ± 0.24^{a*,b*}	0.49 ± 0.03^{a*,b*}	0.59 ± 0.012 ^{a*,b*}
Group VI	44.88 ± 0.69^{a*,b*}	55.46 ± 1.58^{a*,b**}	29.76 ± 1.44^{a*,b*}	1.30 ± 0.05^{a*,b*}	0.39 ± 0.006^{a*,b**}
Group VII	53.66 ± 0.42^{a*,b*}	40.28 ± 1.22^{a**,b*}	25.12 ± 0.15^{a*,b*}	0.79 ± 0.04^{a*,b*}	0.50 ± 0.004^{a**,b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

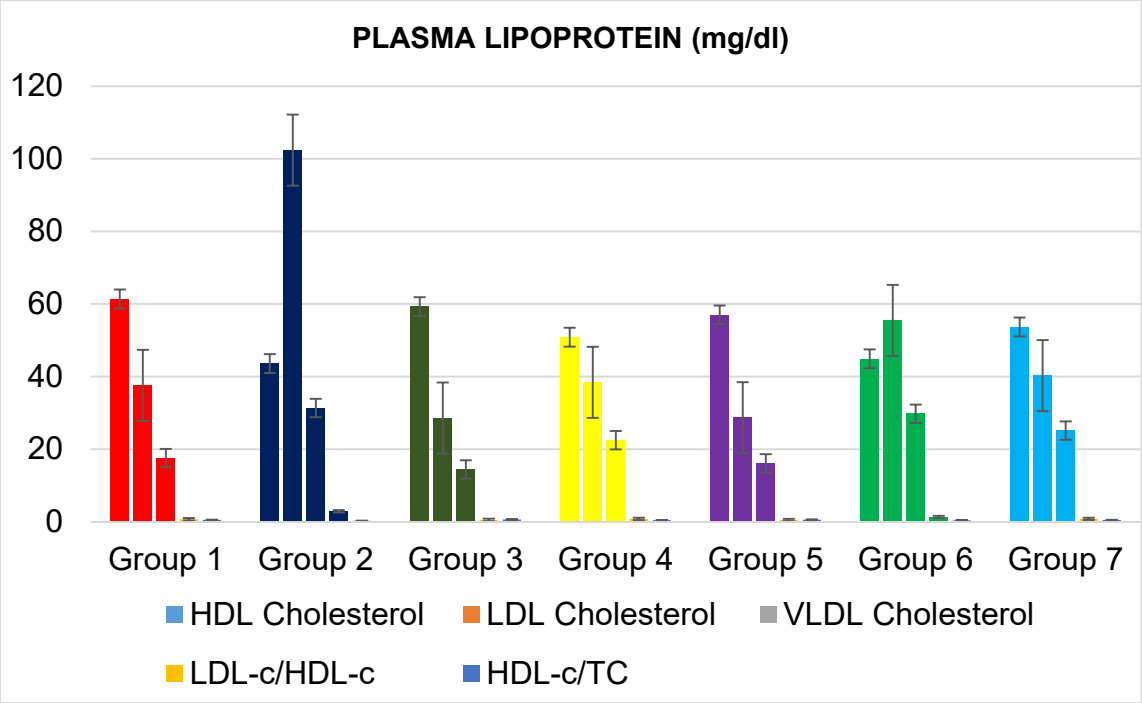


Fig. 62: Effect of various extracts of leaves plant of *Cordia obliqua* on plasma lipoprotein in high fat diet rats

5.12. EFFECT OF VARIOUS EXTRACTS OF LEAVES PLANT OF *CORDIA OBLIQUA* ON TISSUE LIPID CONTENT IN HIGH FAT DIET RATS

Effect of various extracts of leaves plant of *Cordia obliqua* on tissues (Aorta, Heart and Liver) free and ester cholesterol levels are presented in Table No. 30. And 31. A Significant (P<0.001) increase in levels of both free and ester cholesterol were also observed in tissue of rats fed with high fat diet (group II). The tissues free and ester cholesterol levels were reduced remarkably on treating the high fat diet rats with various extract of *Cordia obliqua.* This lipid lowering effect may be due to either the inhibition of hepatic cholesterogenesis or the increase in excretion of fecal sterol (Purohit and Vyas, 2006).

Effect of various extracts of *Cordia obliqua* on tissues phospholipids levels are depicted in Table No. 33. The concentration of tissues phospholipids significantly increased in rats fed high fat diet (group II) as compared to control animals (group I). Administration of various extract of *Cordia obliqua* (200 mg/kg body weight) treated rats with high fat diet showed that the significantly reduced the plasma phospholipids levels than that of other three extract treatment groups. The reduced concentration of phospholipids may also be due to the enhanced activity of phospholipases (Kottai Muthu *et al.*, 2005).

Effect of the various extracts of *Cordia obliqua* on tissues triglyceride are presented in Table No. 32. The concentration of tissues triglyceride elevated in rats fed with high fat diet (group II) as compared to control rats (group I). High fat diet rats had significant increase in the level of plasma triglyceride due to the decrease in the activity of lipoprotein lipase (Mini S and Rajmohan., 2004). The tissues triglyceride levels were significantly reduced in rats treated with various extract of *Cordia obliqua* (400mg/kg b.wt) and as well as standard drug atorvastatin along with high fat diet when compared with rats fed with high fat diet (group II). High fat diet ministration of various extract of *Cordia obliqua* high dose significantly reduced the triglyceride when compared to other three extracts.

Effect of the various extracts of *Cordia obliqua* on tissues free fatty acid were presented in Table No. 34. An elevated level of tissues free fatty acid was observed in rat fed with high fat diet than control rats. It indicates the accumulation of lipid in tissues due to the increased consumption of saturated fats. Previous studies indicated that high fat diet containing saturated fatty acid altered lipid composition fluidity and permeability of membrane. Treatment of various extract of *Cordia obliqua* in high fat diet rats (group IV) significantly decreased the tissues free fatty acid level than that of other three extract treatment group. Many herbal species active principles have been found to stimulate hepatic microsomal cytochrome P_{450} aryl hydroxylase activity, which is involved in the hydroxylation of endogenous steroid such as cholesterol (Sambaiah *et al*., 1989) and may have inhibitory action on the lipogenic enzymes.

Table No. 30: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues free cholesterol level in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Free cholesterol (mg/g tissue)		
	Aorta	Heart	Liver
Group I	0.52 ± 0.04 ^{b*}	0.77 ± 0.03 ^{b*}	0.82 ± 0.04 ^{b*}
Group II	2.50 ± 0.12 ^{a*}	1.09 ± 0.06 ^{a*}	1.43 ± 0.06 ^{a*}
Group III	0.66 ± 0.06 ^{a*b*}	0.66 ± 0.04 ^{a*b*}	0.85 ± 0.05 ^{a*b*}
Group IV	1.06 ± 0.04 $^{a*\ b*}$	0.85 ± 0.02 ^{a*b*}	1.05 ± 0.03 ^{a*b**}
Group V	0.72 ± 0.08 $^{a*\ b*}$	0.69 ± 0.05 ^{a*b*}	0.88 ± 0.06 ^{a*b*}
Group VI	1.50 ± 0.06 ^{a*b**}	1.08 ± 0.04 ^{a*b**}	1.24 ± 0.07 ^{a*b**}
Group VII	1.23 ± 0.10 ^{a*b*}	0.98 ± 0.04 ^{a*b*}	1.10 ± 0.09 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

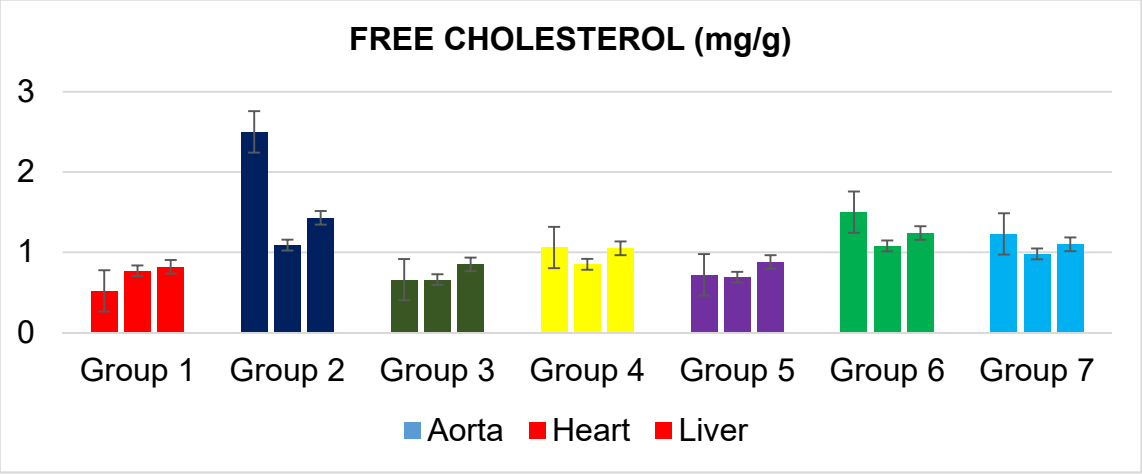


Fig. 63: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues free cholesterol level in high fat diet rats

Table No. 31: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues ester cholesterol level in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Ester cholesterol (mg/g tissue)		
	Aorta	Heart	Liver
Group I	2.08 ± 0.36 ^{b*}	2.70 ± 0.08 ^{b*}	1.98 ± 0.06 ^{b*}
Group II	6.92 ± 0.33 ^{a*b**}	7.08 ± 0.22 ^{a*}	3.42 ± 0.15 ^{a*}
Group III	2.79 ± 0.12 ^{a*b*}	2.90 ± 0.06 ^{a*b*}	1.90 ± 0.10 ^{a*b*}
Group IV	4.58 ± 0.58 $^{a* b*}$	4.70 ± 0.14 ^{a*b**}	2.66 ± 0.05 ^{a*b*}
Group V	2.85 ± 0.10 $^{a* b*}$	2.96 ± 0.04 ^{a*b*}	1.98 ± 0.06 ^{a*b*}
Group VI	6.08 ± 0.56 ^{a*b**}	5.34 ± 0.09 ^{a*b**}	2.98 ± 0.10 ^{a*b**}
Group VII	4.10 ± 0.43 ^{a*b*}	4.12 ± 0.10 ^{a*b*}	2.50 ± 0.09 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

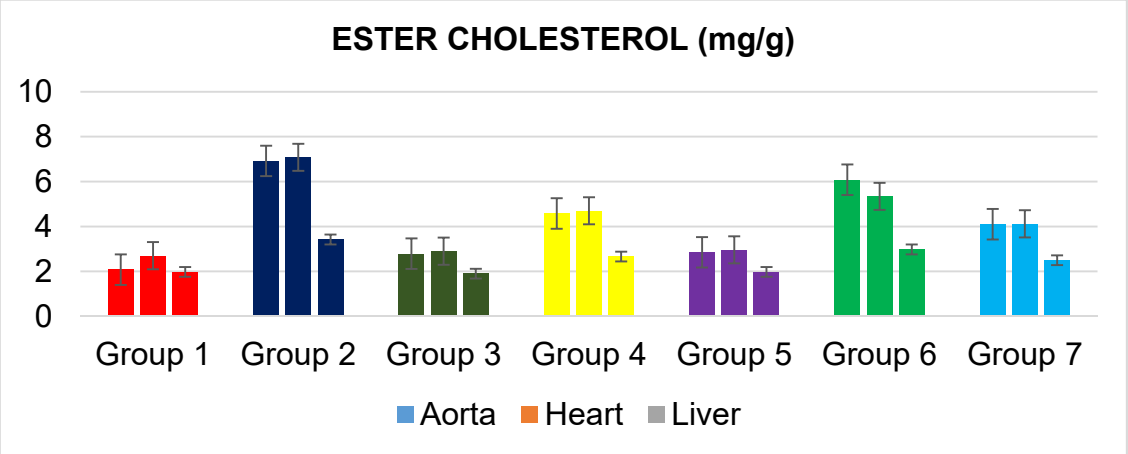


Fig. 64: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues ester cholesterol level in high fat diet rats

Table No. 32: Effect of various extracts of leaves plant of *Cordia obliqua* on tissue triglyceride content in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Triglyceride (mg/g tissue)		
	Aorta	Heart	Liver
Group I	11.28 ± 0.20 ^{b*}	11.45 ± 0.12 ^{b*}	9.14 ± 0.12 b*
Group II	24.26 ± 0.16 ^{a*b**}	49.20 ± 0.15 ^{a*}	29.66 ± 0.18 ^{a*}
Group III	13.94 ± 0.10 ^{a*b**}	22.24 ± 0.14 ^{a**b*}	13.10 ± 0.14 ^{a*b*}
Group IV	15.88 ± 0.90 $^{a* b**}$	32.42 ± 0.12 ^{a*b**}	20.44 ± 0.10 ^{a**b*}
Group V	14.10 ± 0.19 $^{a* b*}$	22.55 ± 0.10 ^{a*b*}	13.76± 0.08 ^{a*b*}
Group VI	17.26 ± 0.08 ^{a*b*}	34.87 ± 0.14 ^{a**b*}	24.55 ± 0.08 ^{a*b*}
Group VII	15.45 ± 0.06 ^{a*b*}	32.61 ± 0.12 ^{a*b*}	20.22 ± 0.06 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 32.

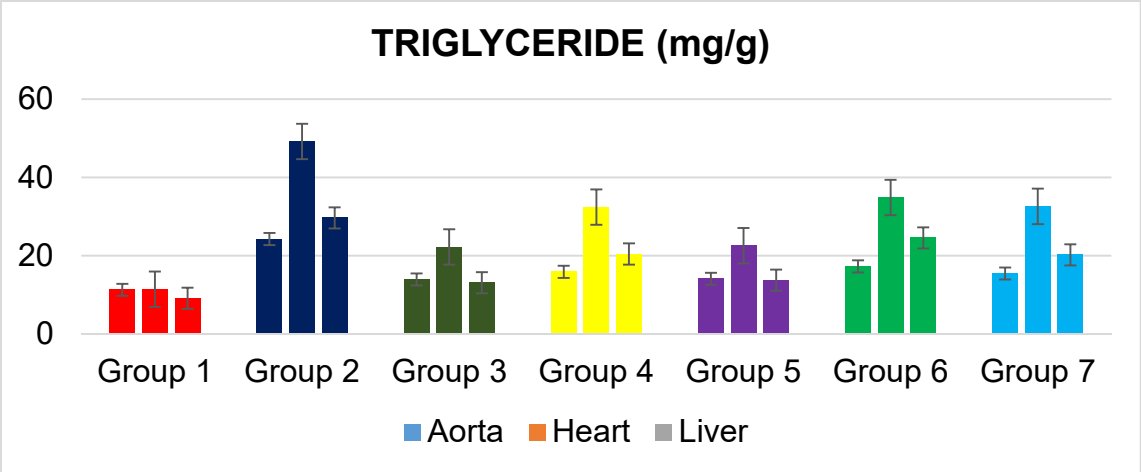


Fig. 65: Effect of various extracts of leaves plant of *Cordia obliqua* on tissue triglyceride content in high fat diet rat

Table No. 33: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues Phospholipid content in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Phospholipids (mg/g tissue)		
	Aorta	Heart	Liver
Group I	8.92 ± 0.15 ^{b*}	24.44 ± 0.20 ^{b*}	18.94 ± 0.10 ^{b*}
Group II	17.98 ± 0.10 ^{a*}	38.00 ± 0.28 ^{a*}	27.10 ± 0.28 ^{a*}
Group III	11.28 ± 0.14 ^{a*b*}	28.34 ± 0.22 ^{a*b*}	19.58 ± 0.35 ^{a*b*}
Group IV	12.76 ± 0.12 $^{a*\ b*}$	31.07 ± 0.20 ^{a*b*}	22.50 ± 0.30 ^{a*b*}
Group V	11.98 ± 0.10 $^{a*\ b*}$	29.10 ± 0.20 ^{a*b*}	20.01 ± 0.18 ^{a*b*}
Group VI	13.26 ± 0.09 ^{a*b**}	33.76 ± 0.18 ^{a*b**}	24.33 ± 0.18 ^{a*b**}
Group VII	12.45 ± 0.10 ^{a*b*}	31.00 ± 0.30 ^{a*b*}	22.38 ± 0.22 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

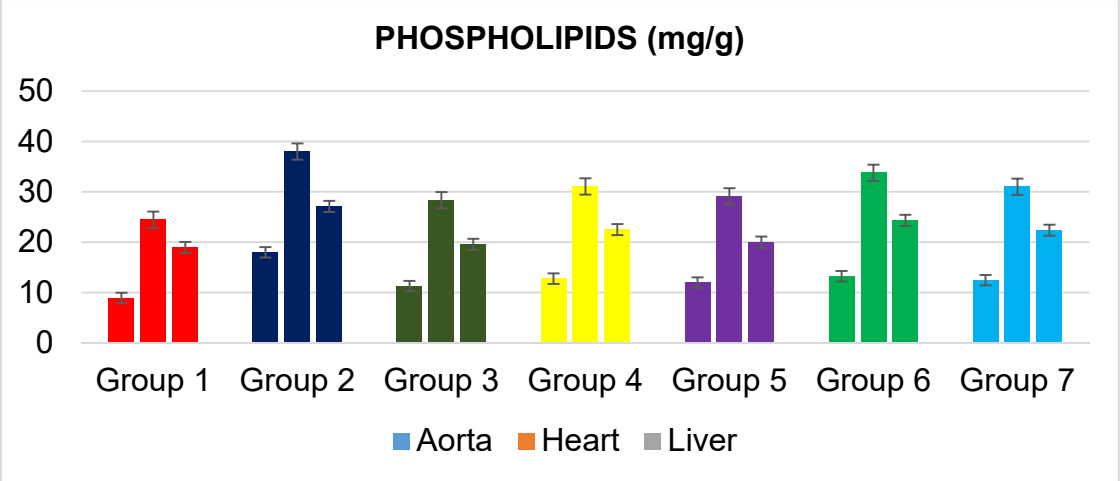


Fig. 66: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues Phospholipid content in high fat diet rats

Table No. 34: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues free fatty acids in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Free fatty acids (mg/g tissue)		
	Aorta	Heart	Liver
Group I	12.28 ± 1.96 ^{b*}	14.48 ± 0.22 ^{b*}	10.56 ± 0.15 b*
Group II	27.20 ± 0.26 ^{a*}	48.55 ± 0.18 ^{a*}	30.46 ± 0.11 ^{a*}
Group III	14.42 ± 0.20 ^{a*b**}	18.12 ± 0.24 ^{a*b*}	11.12 ± 0.12 ^{a*b*}
Group IV	19.48 ± 0.10 $^{a\ ns\ b*}$	30.28 ± 0.26 ^{a*b**}	17.92 ± 0.23 ^{a*b**}
Group V	15.28 ± 0.22 $^{a*\ b*}$	20.10 ± 0.15 ^{a*b*}	12.56 ± 0.15 ^{a*b*}
Group VI	22.98 ± 0.14 $^{a\ ns\ b*}$	37.53 ± 0.12 ^{a*b**}	22.68 ± 0.22 ^{a*b**}
Group VII	20.35 ± 0.18 ^{a*b*}	32.68 ± 0.16 ^{a*b*}	17.78 ± 0.16 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

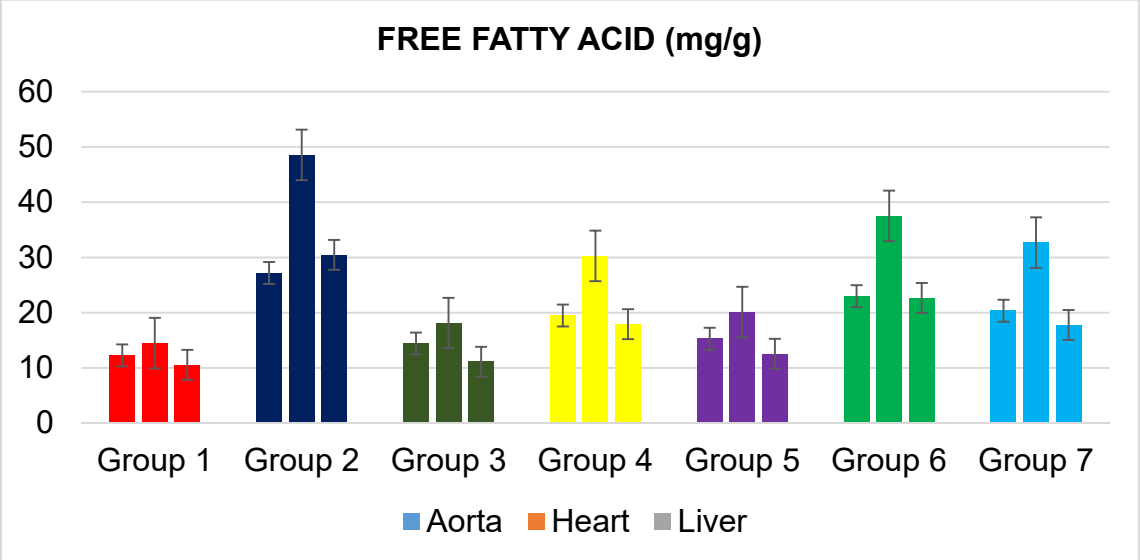


Fig. 67: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues free fatty acids in high fat diet rats

5.13. EFFECT OF VARIOUS EXTRACTS OF LEAVES PLANT OF *CORDIA OBLIQUA* ON TISSUES LIPID PEROXIDATION IN HIGH FAT DIET RATS

Effect of various extracts of leaves plant of *Cordia obliqua* on tissues TBARS and conjugated dienes in high fat diet rats results are shown in Tables No. 35 & 36 respectively.

The elevated levels of TBARS and conjugated denies were observed in aorta, heart and liver of rats fed with high fat diet (group II) rats are a clear manifestation of excessive formation of free radical and activation of lipid peroxidation. The high fat diet is known to induce oxidative stress in the cells by producing reactive oxygen species (ROS) (Khan SA *et al*., 2004). This results in increased lipid peroxidation leadting to elevated concentration of TBARS and conjugated dienes (Boccio *et al*., 1990). The significant decline in the level of TBARS and conjugated dienes in rat high fat diet ministered with various extract of *Cordia obliqua* along with high fat diet (group IV) when compared to other extracts treated groups.

Table No. 35: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues TBARS in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	TBARS (n mol of MDA formed/g tissue)		
	Aorta	Heart	Liver
Group I	19.22 ± 2.10 ^{b**}	45.76 ± 2.982 ^{b*}	26.74 ±2.50 b*
Group II	69.48 ± 4.22 ^{a*}	89.26 ± 4.24 ^{a*}	81.36 ±4.72 ^{a*}
Group III	19.78± 1.62 ^{a*b*}	45.10 ± 2.96 ^{a*b*}	25.52 ± 2.12 ^{a*b*}
Group IV	26.82 ± 2.82 ^{a**} ^{b*}	52.77 ± 4.04^{a**b*}	31.22 ± 4.62 ^{a**b**}
Group V	24.68 ± 2.20 ^{a*} ^{b*}	49.56 ± 2.06 ^{a*b*}	26.82 ± 3.68 ^{a*b*}
Group VI	31.22 ± 3.42 ^{a**} ^{b*}	59.54 ± 3.10 ^{a*b*}	39.58 ± 5.90 ^{a**b*}
Group VII	26.56 ± 2.14 ^{a*b*}	52.98 ± 3.42 ^{a*b*}	32.42 ± 2.10 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

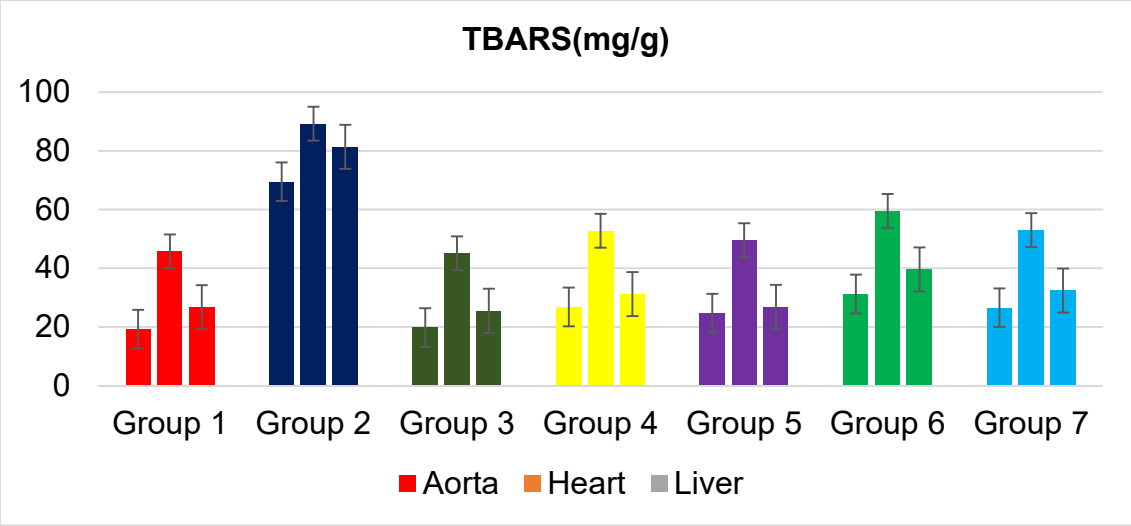


Fig. 68: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues TBARS in high fat diet rats

Table No. 36: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues conjugated diene in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Conjugated diene (µ moles/g tissue)		
	Aorta	Heart	Liver
Group I	177.52± 0.70 ^{b*}	170.22. ± 0.40	184.28 ± 0.50 b*
Group II	756.66 ± 2.02 ^{a*}	272.44 ± 0.25	285.92± 0.82 ^{a*}
Group III	480.26 ± 0.64 ^{a**b*}	178.32± 0.70	196.52± 0.30 $^{a\ ns\ b*}$
Group IV	522.44 ± 2.04 $^{a*\ b*}$	204.80 ± 0.68	220.48± 0.42 ^{a**b*}
Group V	490.08 ± 1.04 $^{a*\ b*}$	180.42 ± 0.64	198.55 ± 0.34 ^{a*b*}
Group VI	620.54 ±2.44 $^{a*\ b*}$	244.36± 0.90	249.39± 0.44 ^{a*b**}
Group VII	504.35 ± 1.14 ^{a**b*}	202.27 ± 0.50	220.12± 0.58^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

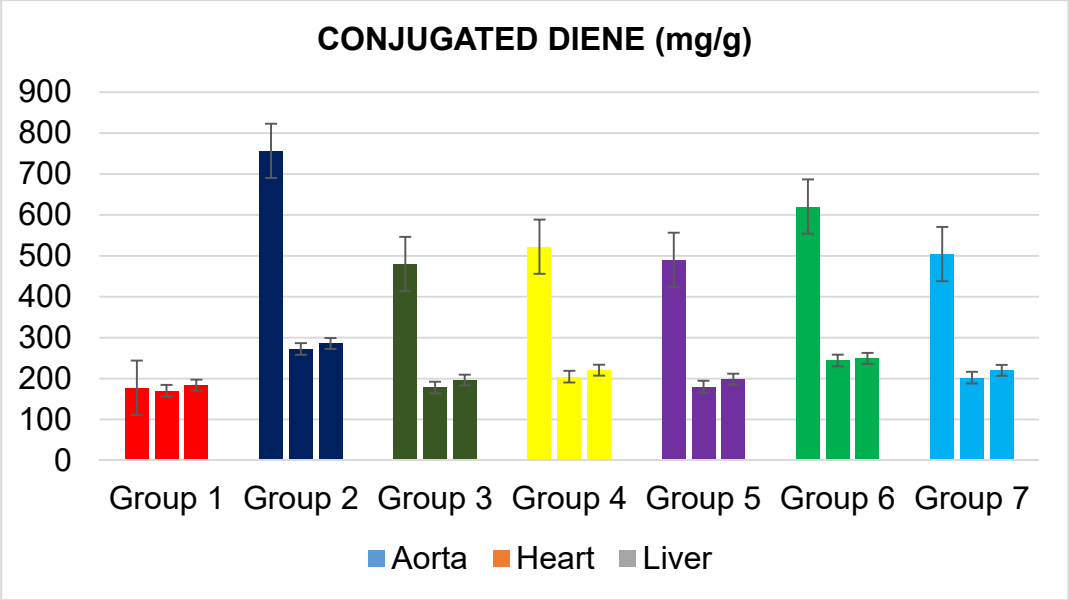


Fig. 69: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues Conjugated diene in high fat diet rats

5.14. EFFECT OF VARIOUS EXTRACTS OF LEAVES PLANT OF *CORDIA OBLIQUA* ON TISSUE ENZYMIC ANTIOXIDANTS IN HIGH FAT DIET RATS

Effect of various extracts of leaves plant of *Cordia obliqua* on tissues like liver, heart and aorta SOD and CAT level results is shown in Table No. 37 & 38 respectively. The activities of SOD and CAT in the tissue like liver, heart and aorta significantly (P<0.001) lowered in rats fed with high fat diet (group II) than control group animals. High fat diet can cause the formation of toxic intermediates that can inhibit the activity of antioxidant enzymes (Conner EM and Grisham MB, 1996) and the accumulation of O_2^- and H_2O_2 which in turn forms hydroxyl radicals (Valko M *et al*., 2004). Catalase decomposes hydrogen peroxide and helps to protect the tissues from highly reactive hydroxyl radicals. Administration of ethanolic extract of *Cordia obliqua* along with high fat diet significantly increased the activities of SOD and CAT in tissues of rats when compared to other extracts treated groups.

Effect of various extracts of *Cordia obliqua* on tissue glutathione peroxidase (GPX), glutathione reductase and glutathione s-transferase results are shown in Table No. 40 & 41. The results indicated that the concentration of glutathione peroxidase (GPX), glutathione reductase and glutathione-s transferase significantly decreased in tissues (aorta, heart and liver) of rats fed with high fat diet as compared to the control rats. High fat diet decreased the ratio of oxidized glutathione/ reduced glutathione in tissue (De La Cruz *et al*., 2000). Administration of various extract (400 mg/kg body weight) of *Cordia obliqua* along with the high fat diet increased the activities of glutathione peroxidase, glutathione reductase and glutathione S-transferase in all the tissues as compared with high fat diet. A standard drug atorvastatin administered rats also showed elevated level of glutathione peroxidase, glutathione reductase and glutathione-s-transferase. It might be due to help to propagation of biological membranes found to be associated with increase in the activities of GPX. Glutathione peroxidase (GPX) mainly detoxifies H_2O_2 and is involved in the reduction of a variety of hydroperoxides such as phospholipid hydroperoxides, fatty acid hydroperoxides.

Table No. 37: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues superoxide dismutase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	SOD (unit min/mg protein)		
	Aorta	Heart	Liver
Group I	2.98 ± 0.14 ^{b*}	1.82 ± 0.15 ^{b*}	3.74 ± 0.25 b*
Group II	1.64 ± 0.20 ^{a*}	0.80± 0.08 ^{a*}	1.72 ± 0.30 ^{a*}
Group III	2.90 ± 0.18 ^{a*b*}	1.80 ± 0.16 ^{a*b*}	3.72 ± 0.18 ^{a*b*}
Group IV	2.45± 0.24 $^{a**\ b**}$	1.39 ± 0.12 ^{a**b*}	3.26 ± 0.26^{a**b*}
Group V	2.72 ± 0.14 $^{a*\ b*}$	1.68 ± 0.14 ^{a*b*}	3.61± 0.32 ^{a*b*}
Group VI	2.30 ± 0.22 $^{a**\ b*}$	1.24 ± 0.09 ^{a**b**}	2.90 ± 0.32 ^{a**b**}
Group VII	2.42 ± 0.16 ^{a**b*}	1.42± 0.12 ^{a*b*}	3.22 ± 0.18 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

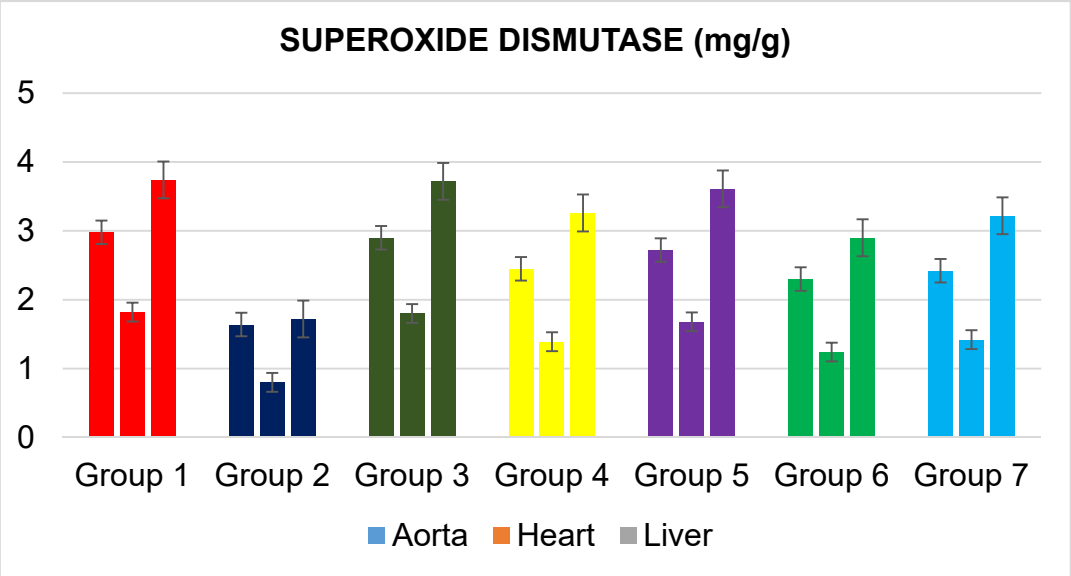


Fig. 70: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues SOD in high fat diet rats

Table No. 38: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues catalase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	CAT (µ moles of H_2O_2, consumed min/mg protein)		
	Aorta	Heart	Liver
Group I	32.35 ± 2.76 ^{b*}	49.22 ± 2.42 ^{b*}	29.76 ± 1.22 b*
Group II	20.96 ± 2.02 ^{a*}	32.40 ± 3.20 ^{a*}	18.10 ±1.46 ^{a*}
Group III	31.08 ± 2.95 ^{a*b*}	48.12 ± 2.90 ^{a**b*}	29.08 ± 1.50 ^{a*b*}
Group IV	25.92± 2.53 $^{a**\ b*}$	42.72± 3.04 ^{a**b*}	22.68 ± 2.30 ^{a**b*}
Group V	27.30± 2.44 $^{a*\ b*}$	46.24 ± 3.92 ^{a*b*}	26.76 ± 2.10 ^{a*b*}
Group VI	24.10± 2.04 $^{a**\ b**}$	39.88± 2.54 ^{a**b*}	21.90 ±1.54 ^{a**b**}
Group VII	25.63 ± 2.33 ^{a*b*}	43.00 ± 2.22^{a*b*}	22.90 ± 1.85 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

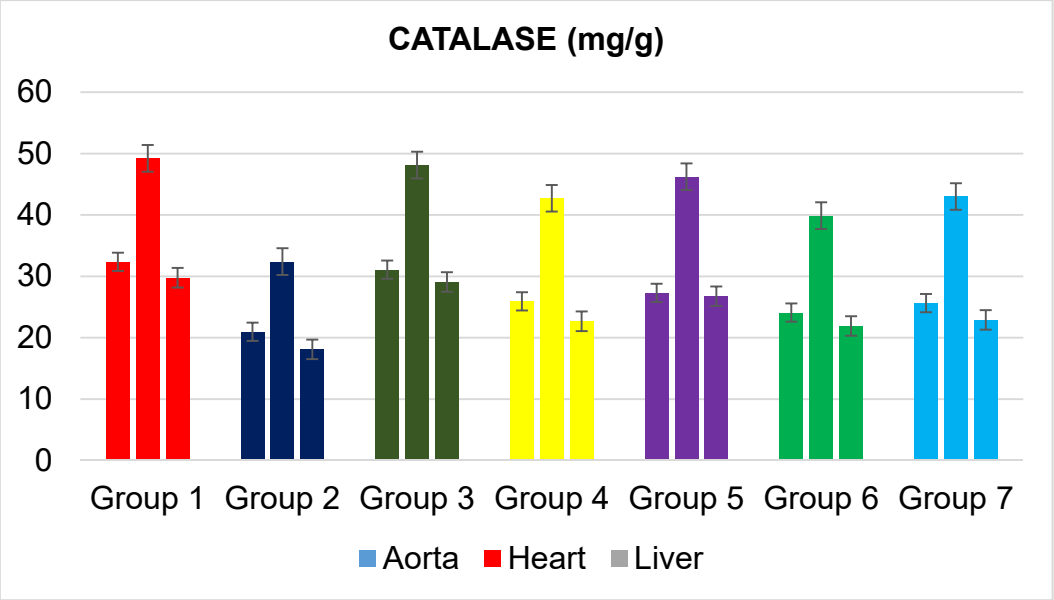


Fig. 71: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues CAT in high fat diet rats

Table No. 39: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues glutathione peroxidase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	GPx (mg of GSH consumed/min/mg protein)		
	Aorta	Heart	Liver
Group I	14.04 ± 1.22 ^{b*}	15.78 ± 1.10 ^{b*}	8.92 ± 0.45 b*
Group II	6.84 ± 0.15 ^{a*}	7.82 ± 0.50 ^{a*}	5.42 ± 0.36 ^{a*}
Group III	15.96 ± 1.26 ^{a*b*}	15.12 ± 0.42 ^{a*b*}	8.79 ± 0.55 ^{a*b*}
Group IV	11.45± 0.32 $^{a**\ b*}$	12.36 ± 0.44 ^{a**b*}	7.62 ± 0.42 ^{a**b*}
Group V	13.74 ± 1.16 $^{a*\ b*}$	14.44 ± 0.53 ^{a*b*}	8.52 ± 0.48 ^{a*b*}
Group VI	9.98 ± 0.20 ^{a**b**}	10.72± 0.47 ^{a**b**}	6.92± 0.55 ^{a**b**}
Group VII	11.84 ± 0.32 ^{a*b*}	12.55 ± 0.44 ^{a*b*}	7.66 ± 0.56 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

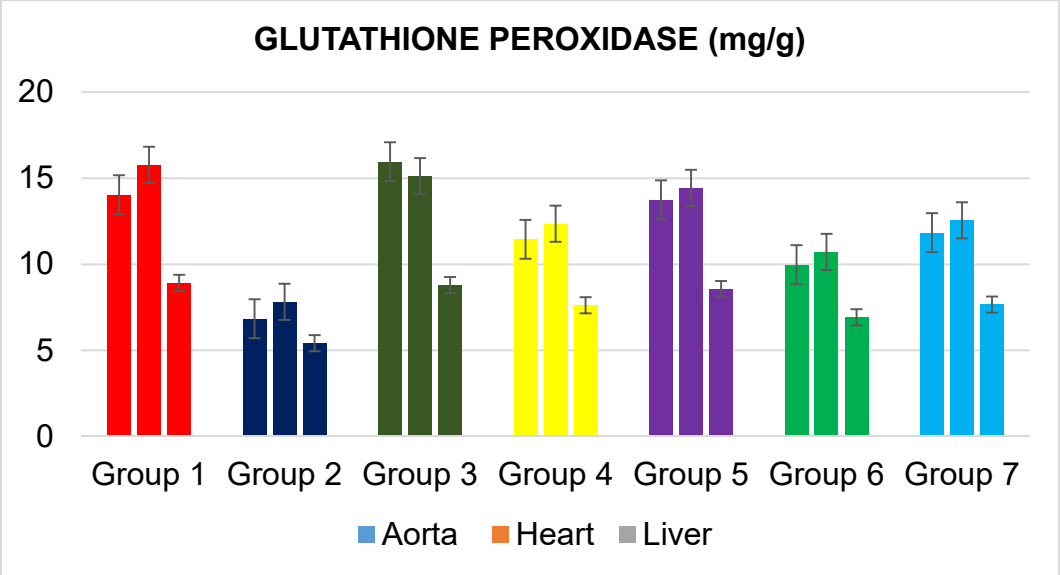


Fig. 72: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues glutathione peroxidase in high fat diet rats

Table No. 40: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues glutathione reductase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	GR (mg of GSH consumed/min/mg protein)		
	Aorta	Heart	Liver
Group I	1.82 ± 0.18 ^{b*}	2.81 ± 0.21 ^{b*}	1.52 ± 0.15 b*
Group II	0.84 ± 0.10 ^{a*}	1.38 ± 0.12 ^{a*}	0.72± 0.08 ^{a*}
Group III	1.80 ± 0.16 ^{a*b*}	2.78 ± 0.16 ^{a*b*}	1.46 ± 0.10 ^{a*b*}
Group IV	1.38 ± 0.24 $^{a**\ b*}$	2.48 ± 0.14 ^{a**b*}	1.29 ± 0.11 ^{a**b*}
Group V	1.62 ± 0.11 $^{a*\ b*}$	2.69± 0.16 ^{a*b*}	1.35 ± 0.10 ^{a*b*}
Group VI	1.29 ± 0.24 $^{a**\ b**}$	2.26 ± 0.10 ^{a**b**}	1.10 ± 0.12 ^{a**b*}
Group VII	1.45 ± 0.20 ^{a*b*}	2.49 ± 0.12 ^{a*b*}	1.28 ± 0.10 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

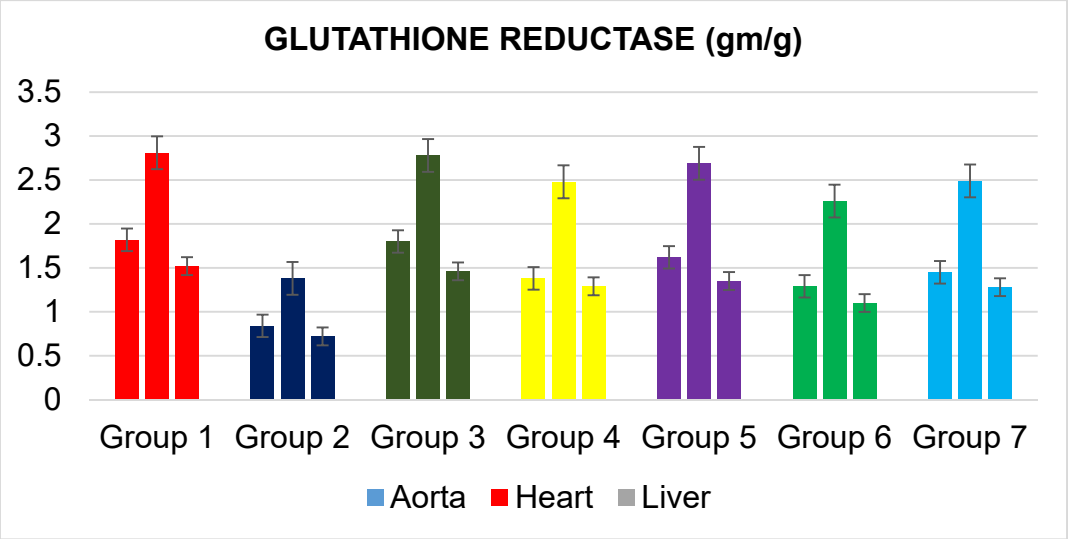


Fig. 73: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues glutathione reductase in high fat diet rats

Table No. 41: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues glutathione-S-transferase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Glutathione-S-transferase(GST) (µ mole of CDNB-GSH-conjugate/min/mg protein)		
	Aorta	Heart	Liver
Group I	16.21 ± 0.22 ^{b*}	18.80 ± 0.48 ^{b*}	26.18 ± 0.25 b*
Group II	7.90 ± 0.20 ^{a*}	8.82 ± 0.22 ^{a*}	9.25 ± 0.30 ^{a*}
Group III	12.40 ± 0.25 ^{a*b*}	16.50 ± 0.50 ^{a*b*}	2.10 ± 0.28 ^{a*b*}
Group IV	9.76 ± 0.20 ^{a**} ^{b*}	13.24 ± 0.28 ^{a**b*}	16.42 ± 0.20 ^{a*b*}
Group V	10.98 ± 0.24 ^{a*} ^{b*}	14.22 ± 0.30 ^{a*b*}	18.76 ± 0.24 ^{a*b*}
Group VI	7.98 ± 0.18 ^{a**} ^{b**}	10.88± 0.35 ^{a*b*}	11.68 ± 0.28 ^{a*b*}
Group VII	9.88 ± 0.15 ^{a*b*}	13.68 ± 0.28 ^{a*b*}	15.90 ± 0.28 ^{a*b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table 5.18.

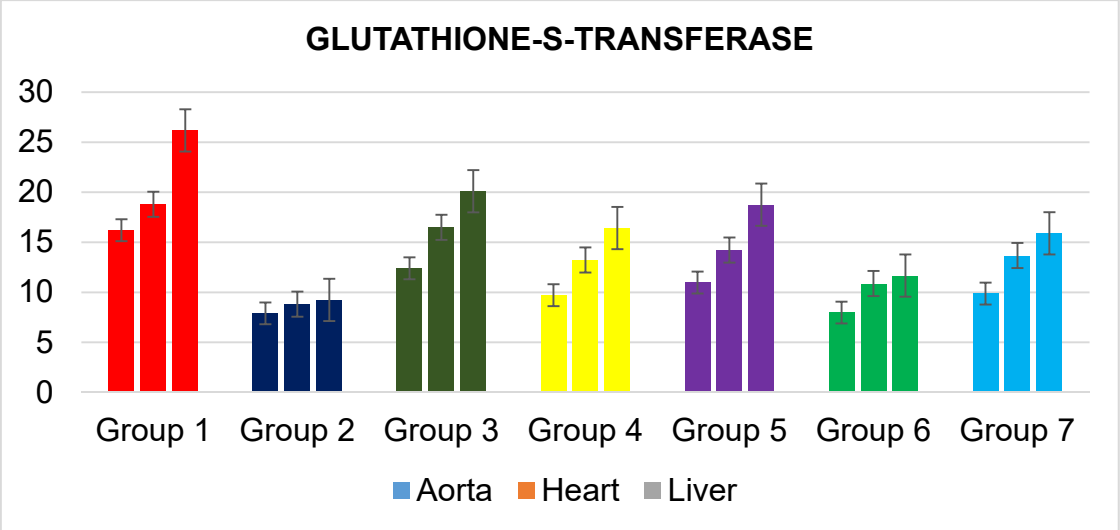


Fig. 74: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues glutathione-s-transferase in high fat diet rats

5.15. EFFECT OF VARIOUS EXTRACTS OF LEAVES PLANT OF *CORDIA OBLIQUA* ON TISSUE NON – ENZYMIC ANTIOXIDANTS

Effects of various extracts of leaves plant of *Cordia obliqua* on aorta, heart and liver of glutathione in rats fed high fat diet results are shown in Table No. 42. Glutathione (GSH), a tripeptide which is present in all the cells is an important antioxidant (Lee DH *et al*., 2006). GSH also functions as free radical scavenger in the repair of radical caused biological damage. The activities of glutathione concentration in tissues significantly decreased in high fat diet rats (group II) as compared to the control rats (group I). The reduced levels may be an attempt by the tissue to counteract the increased formation of lipid peroxides that are handled by antioxidant enzymes such as Glutathione peroxidase which scavenges H_2O_2 utilizing GSH as substrate (Neumann CA *et al*., 2003). Treatment of ethanolic extract (400mg/kg body weight) of *Cordia obliqua* along with high fat diet rats (group IV) increased the levels of glutathione when compared with other three extracts. Increase in glutathione concentration in various extracts high dose treated rats with high fat diet might be due

to the increase in the activity of the enzyme glutathione reductase which catalyses the conversion of oxidized glutathione to reduced glutathione in liver (or) might be due to enhanced synthesis of GSH(Sethupathy *et al*., 2002).

Table No. 42: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues glutathione in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Glutathione (mg/g tissue)		
	Aorta	Heart	Liver
Group I	5.64 ± 0.23 ^{b*}	7.65 ± 0.50 ^{b*}	4.40 ± 0.42 b**
Group II	2.91 ± 0.20 ^{a*}	4.22 ± 0.32 ^{a*}	1.88 ± 0.30 ^{a**}
Group III	5.64 ± 0.30 ^{b*}	7.72 ± 0.36 ^{b*}	4.35 ± 0.28 ^{b*}
Group IV	4.21 ± 0.26 $^{a**\ b*}$	6.48 ± 0.32 ^{a**b*}	3.80 ± 0.32 ^{a**b*}
Group V	5.22 ± 0.28 ^{b*}	7.10 ± 0.33 ^{a*b*}	3.99 ± 0.20 ^{b*}
Group VI	3.72± 0.22 $^{a**\ b**}$	5.94 ± 0.30 ^{a**b**}	3.46 ± 0.31 ^{a**b**}
Group VII	4.44 ± 0.18 ^{b*}	6.93 ± 0.33 ^{b*}	3.76 ± 0.28 ^{b*}

p values : * < 0.001, ** < 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Details of group I-VII are same as in Table No. 26.

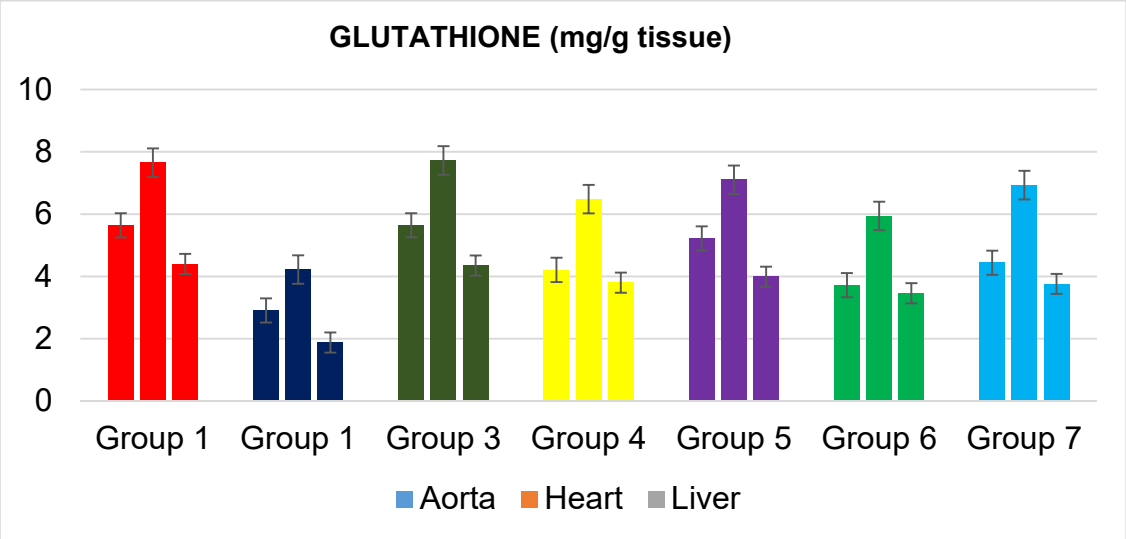


Fig. 75: Effect of various extracts of leaves plant of *Cordia obliqua* on tissues glutathione in high fat diet rats

5.16. HISTOPATHOLOGICAL STUDY

5.16.1. Histological changes of various extract of *Cordia obliqua* on liver

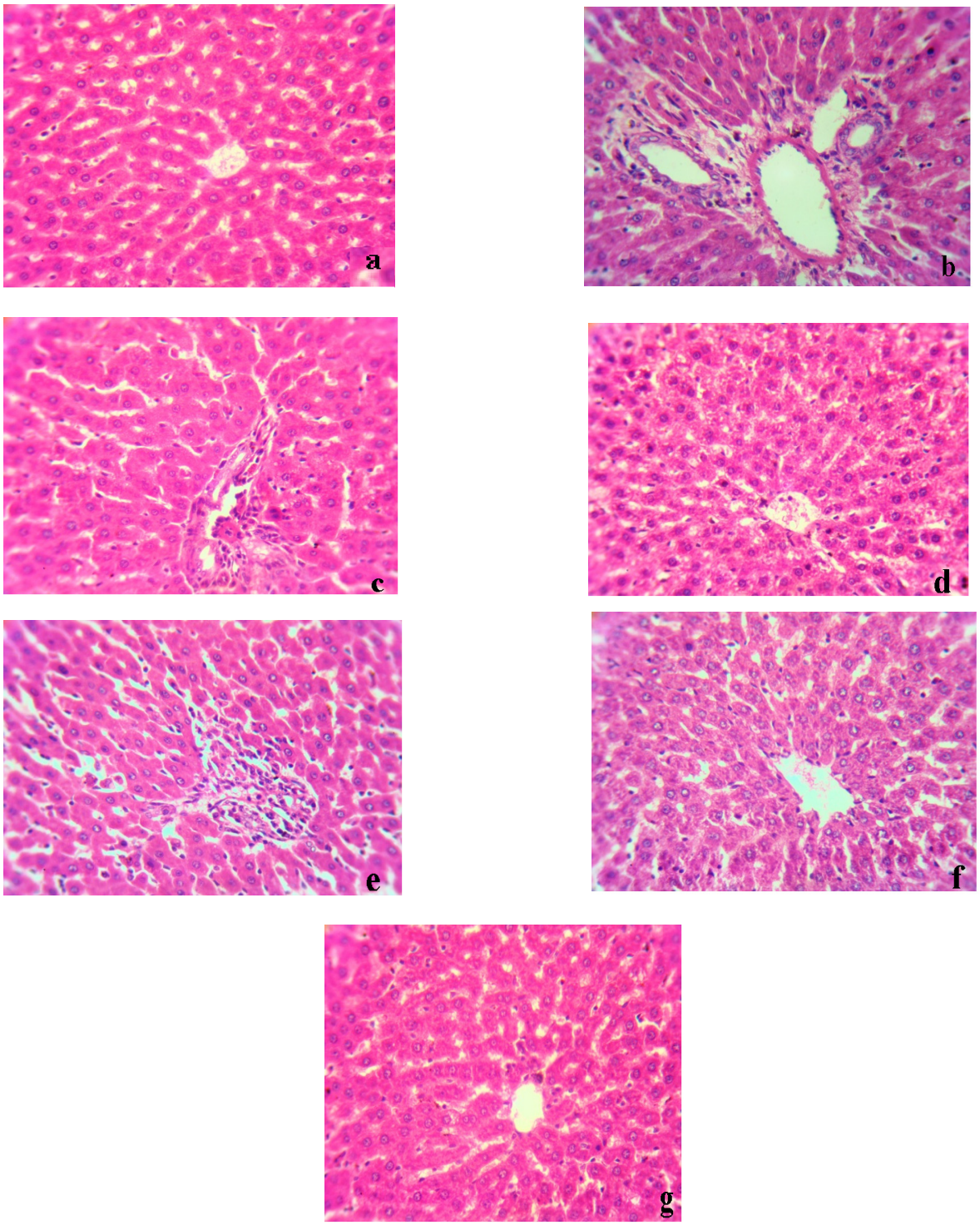


Fig. 76: Histopathological studies for Liver

a) Liver of control rats.

b) Liver of HFD fed rats - (Congestion of the blood vessels and steatosis of micro vesicular type were shown).

c) Liver of HFD + Standard (Atorvastatin 1.2mg/kg b.wt) (Significantly reduced in congestion of blood vessels vesicular and steatosis of micro vesicular type).

d) Liver of HFD + Ethyl acetate extract of *Cordia obliqua* (100 mg/kg b.wt) (Moderately reduced in congestion of blood vessels vesicular and steatosis of micro vesicular type).

e) Liver of HFD + Ethyl acetate extract of *Cordia obliqua* (200 mg/kg b.wt) (Significantly reduced in congestion of blood vessels vesicular and steatosis of micro vesicular type).

f) Liver of HFD + Ethanolic extract of Ethyl acetate extract of *Cordia obliqua* (100 mg/kg b.wt) (Moderately reduced in congestion of blood vessels vesicular and steatosis of micro vesicular type).

g) Liver of HFD + Ethanolic extract of Ethyl acetate extract of *Cordia obliqua* (200 mg/kg b.wt) (Significantly reduced in congestion of blood vessels vesicular and steatosis of micro vesicular type).

5.16.1. Histological changes of various extract of *Cordia obliqua* on aorta

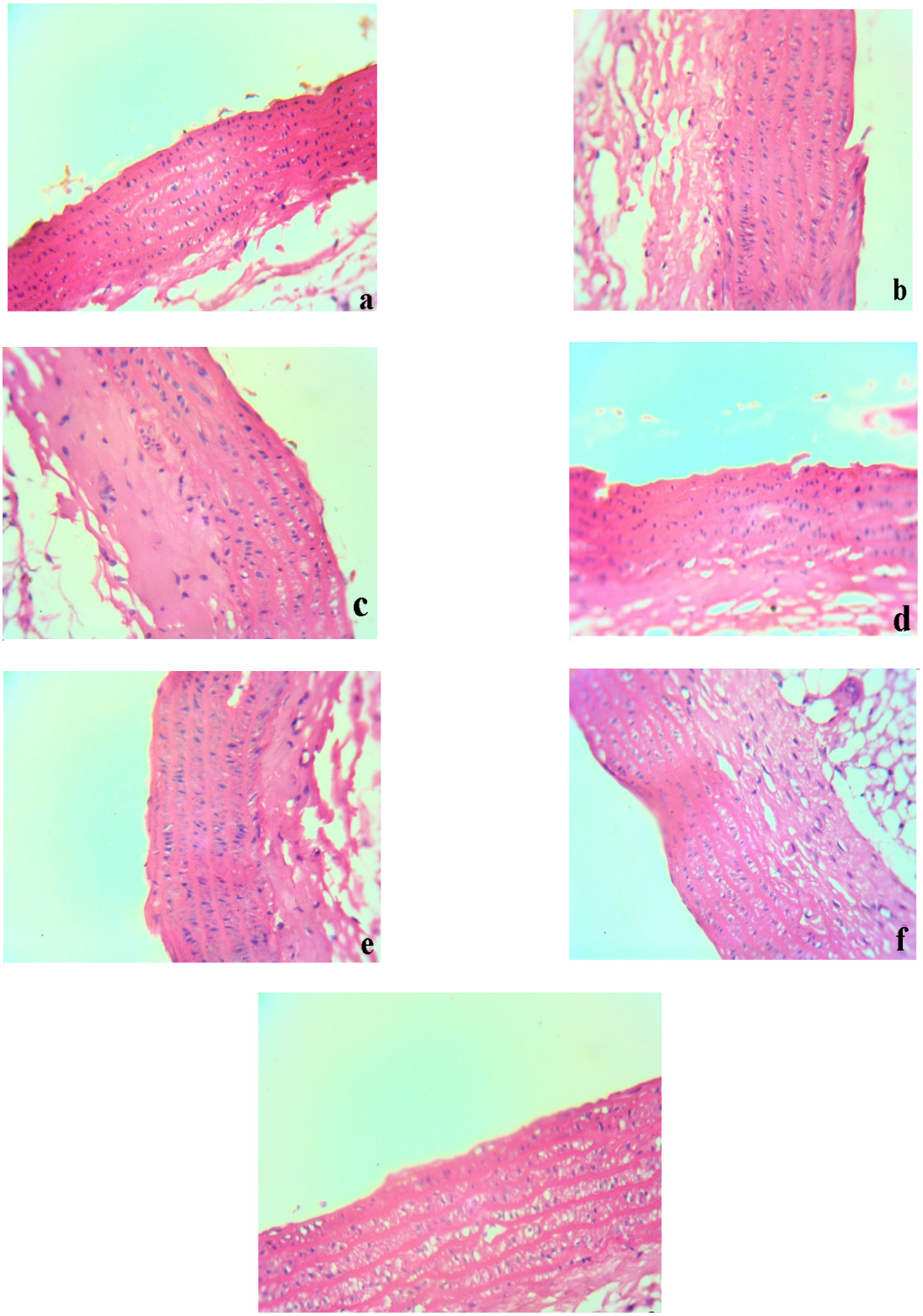


Fig. 77: Histopathological studies for Aorta.

a) Aorta of control rats.

b) Aorta of HFD fed rats - (Deposition of fat).

c) Aorta of HFD + Standard (Atorvastatin 1.2 mg/kg b.wt) (Significantly Disappearance of fat deposition).

d) Aorta of HFD + Ethyl acetate extract of *Cordia obliqua* (100 mg/kg b.wt) (Moderately disappearance of fat deposition).

e) Aorta of HFD + Ethyl acetate extract of *Cordia obliqua* (200 mg/kg b.wt) (Significantly disappearance of fat deposition).

f) Aorta of HFD + Ethanolic extract of *Cordia obliqua* (100 mg/kg b.wt) (Moderately disappearance of fat deposition).

g) Aorta of HFD + Ethanolic extract of *Cordia obliqua* (200 mg/kg b.wt) (Significantly disappearance of fat deposition).

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

The thesis entitled "**PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTI-ATHEROGENIC & ANTIOXIDANT ACTIVITIES OF *CORDIA OBLIQUA* IN WISTAR RATS FED WITH HIGH FAT DIET"** submitted by the author is divided into 6 chapters.

Chapter 1 deals with introduction where in details of the disease and lipidaemic diseases have been highlighted. The details about the herbal remedies for hyperlipidaemic diseases. Numerous variables, for example, modifiable risk factors, non modifiable risk factors, other risk factors (genetically determined), can result to atherosclerosis. Nonetheless, no successful hypolipidemic treatments are accessible up to now. Plant-based medications assume a significant part in the treatment of hyperlipidemic disorders. Without dependable hypolipidemic medications in current pharmaceutical, various restorative plants and their formulations are utilized to cure hyperlipidaemic in conventional medicine.

Chapter 2 deals with review of literature of plants of *Cordia obliqua* and their family and the profiles of plants are also presented.

Chapter 3 deals with scope and objectives of the study and the aim to find out the anti atherosclerosis activity of medicinal plants along with methodology have been detailed out. The work plan included:

- Preliminary phytochemical study.
- *In vitro* antioxidant activity of DPPH assay, Hydroxyl radical scavenging activity, Nitric oxide free radical scavenging capacity, Superoxide anion scavenging activity, Iron-chelating assay, FRAP assay, Total antioxidant activity.
- Estimation of plasma lipid profiles, plasma lipoproteins, tissue lipid levels, tissue lipid peroxidation(TBARS Level), *in vivo* antioxidants like Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPX), Glutathione -S-transferase (GST), Reduced Glutathione (GSH).
- Histopathological examination of aorta, heart and liver.

Chapter 4 deals with materials and methods. The complete list of materials, chemicals, equipments and instruments used for the present studies have been listed. The methodology of research has been described:

Selection of Plant material and extraction

The leaves of *Cordia obliqua* were gathered, pulverized and the powdered plant materials were kept in an airtight polythene bags. The above plant powdered materials were consecutively extracted with ethanol for one day.

Animal Experimentation

16-19 weeks-old adult male Wistar rats, weighing approximately 150 to 200g, were acclimatized for 7 days at temperature (25±2°C) and relative humidity (55±1%) in a 12-hour light/dark cycle at room under hygienic condition. They were given access to water and fed with standard pellet diet *ad libitum.* The experiments were done as per the strategy of CPCSEA, New Delhi, India and accepted by the IAEC, AKCP (**Approved number: AKCP/IAEC/08/19-20**).

Rats were divided randomly into seven groups of six animals each and treated for 30 days as follows.

S. No	Groups	Treatment
1.	Group I	Standard chow diet (Control).
2.	Group II	High fat Diet (HFD).
3.	Group III	HFD + Standard drug atorvastatin (1.2 mg/kg b.w.)
4.	Group IV	HFD + Ethyl acetate extract of *Cordia obliqua* (100mg/kg b.w.)
5.	Group V	HFD + Ethyl acetate of *Cordia obliqua* (200mg/kg b.w.)
6.	Group VI	HFD + Ethanolic extract of *Cordia obliqua* (100mg/kg b.wt)
7.	Group VII	HFD + Ethanolic extract of *Cordia obliqua* (200mg/kg b.wt)

Groups IV and V were fed orally with the ethyl acetate extract of *Cordia obliqua,* group VI and VII were fed orally with the ethanolic extract of *Cordia obliqua* and group III rats were fed with drug atorvastatin as a standard. The both extracts

and standard were suspended in 2% of tween 80 (Waynforth, 1980) independently and sustained to the particular rats by oral intubation. Thirty later all the animals were sacrificed by cervical dislocation after overnight fasting. Blood was collected in previously labeled centrifuging tubes and serum was separated. Aorta, heart and liver was cleared of adhering fat, measured correctly and utilized for the homogenate. Animals were given adequate care as per the AEC's recommendations.

Chapter 5 deals with a detailed account of results with corresponding interpretation and discussion on the outcome of each phase of experimentation:

The percentage of pet.ether, ethyl acetate, ethanolic extracts of leaves of *Cordia obliqua* were 10.48%, 5.47% and 4.25%w/w respectively. The phytochemical screening of pet.ether extract of *Cordia obliqua* contains fixed oil only. Ethyl acetate extract contains alkaloids, flavonoids, and phenolic compounds. The ethanolic extract *Cordia obliqua* contains carbohydrates, phytosterols, flavonoids, phenolic compounds, tannins, protein and amino acids, and saponins.

The pet. ether, ethyl acetate, ethanolic extract of *Cordia obliqua* and standard on *in vitro* antioxidant activities were estimated and IC_{50} values of DPPH method (1120µg/ml, 550µg/ml, 475µg/ml & 480µg/ml), super oxide anion scavenging method(520µg/ml , 450µg/ml, 75µg/ml & 60µg/ml), iron-chelating method (920µg/ml, 410µg/ml, 110µg/ml & 65µg/ml), hydroxyl radical scavenging method (880µg/ml, 720µg/ml, 350µg/ml & 410µg/ml), nitric oxide free radical inhibition method (1010µg/ml, 820µg/ml, 450µg/ml & 410µg/ml), total antioxidant method (590µg/ml, 460µg/ml, 400µg/ml & 410µg/ml) and FRAP assay (1300µg/ml , 960µg/ml, 210µg/ml & 50µg/ml) respectively. The total phenolic compounds and total flavonoids content were estimated. The pet.ether, ethyl acetate and ethanolic extracts of *Cordia obliqua* having higher content of phenolic compounds (1.43 ± 0.020), (2.48 ± 0.042) & (4.91 ± 0.044) and flavonoids (0.032± 0.003), (1.068 ± 0.015) & (2.176 ± 0.018) component among them.

Evaluation of anti-atherogenic activity of ethyl acetate and ethanolic extracts of *Cordia obliqua* in rats fed with HFD.

The average body weight of rats in all the seven groups increased after 30 days of experimental period. Atherogenic diet feeding rats (group II) had a significantly increased body weight compared with control group rats (group I). The body weight significantly reduced in the ethyl acetate extract of *Cordia obliqua* treated groups.

The concentration of free cholesterol, ester cholesterol, triglyceride and phospholipid were significantly increased in plasma and tissues of rats fed HFD (group II) as compared with the control groups of rats (group I). Treatment of ethyl acetate extract of *Cordia obliqua* at the dose of 200mg/kg body weight along with HFD significantly reduced the plasma and tissues free cholesterol & ester cholesterol and triglyceride & phospholipid levels in as compared to rats fed with HFD (group II).

Atherogenic index was elevated on treatment with HFD rats, but it was reduced markedly by ethyl acetate extract of *Cordia obliqua*.

The LDL, VLDL cholesterol concentration increased in rats fed with HFD (group II) compared to control rats (group I). Rats treated with ethyl acetate extract of *Cordia obliqua* at the dose of 200mg/kg body weight significantly reduced the levels of LDL and VLDL cholesterol as compared to HFD rats (group II). The HDL cholesterol concentration significantly reduced in HFD rats (group II) compared to the control group of rats (group I). HDL cholesterol level significantly increased in rats treated with ethyl acetate extract of *Cordia obliqua* at the dose of 200mg/kg body weight (group V) and it was comparable with standard drug atorvastatin (group III).

The TBARS and conjugated diene levels significantly elevated in aorta, heart and liver of rats fed with HFD (group II) as compared to control rats (group I). However, the treatment of ethyl acetate extract of *Cordia obliqua* (200mg/kg body weight) significantly reduced the levels of TBARS and conjugated diene as compared with HFD rats (group II).

The activities of the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), glutathione reductase (GRX) and Glutathione-S-transferase (GST) and non enzymatic antioxidant glutathione levels in tissues significantly decreased in HFD rats (group II) when compared with control rats (group I). Treatment of ethyl acetate extract of *Cordia obliqua* (200mg/kg body weight) with HFD rats significantly elevated both enzymatic and non enzymatic antioxidant level than that of other lower dose (100mg/kg body weight) extracts treatment group.

Histopathological changes (aorta, heart and liver) of ethyl acetate and ethanolic extracts of *Cordia obliqua* in HFD rats.

Histological results of liver showed that degenerative changes, characterized with very small intracytoplasmic fat vacuoles at the periacinar and centrilobular areas were observed in high fat diet fed rats. Administration of high dose of ethyl acetate extract of *Cordia obliqua* along with high fat diet had showed the best liver conditions than that of other extracts treated groups. Despite, histology results of aorta showed clearly thickened wall, and endothelial cells had shed. Vascular internal elastic membrane was seriously damaged, with a small amount of foam cells were observed in high fat diet fed rats. In the treatment of high dose of ethyl acetate extract of *Cordia obliqua* the rupture of vascular internal elastic membrane was significantly less than that of lower dose and ethanoilc extracts treated groups.

SCOPE FOR FUTURE STUDY

The present study scientifically established the antiatherogenic and antioxidant activities of *Cordia obliqua.* The leaves of *Cordia obliqua* can be further investigated on

- A detailed pharmacodynamic and pharmacokinetic studies of the ethyl acetate and ethanolic extracts.
- Feasibility aspects of formulation of the crude drug and also the active constituents.
- Taking the present trends of herbal technology, the process can be developed to make herbal products of *Cordia obliqua* may be carried out for the management of hyperlipidaemic diseases.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CHAPTER VII

REFERENCE

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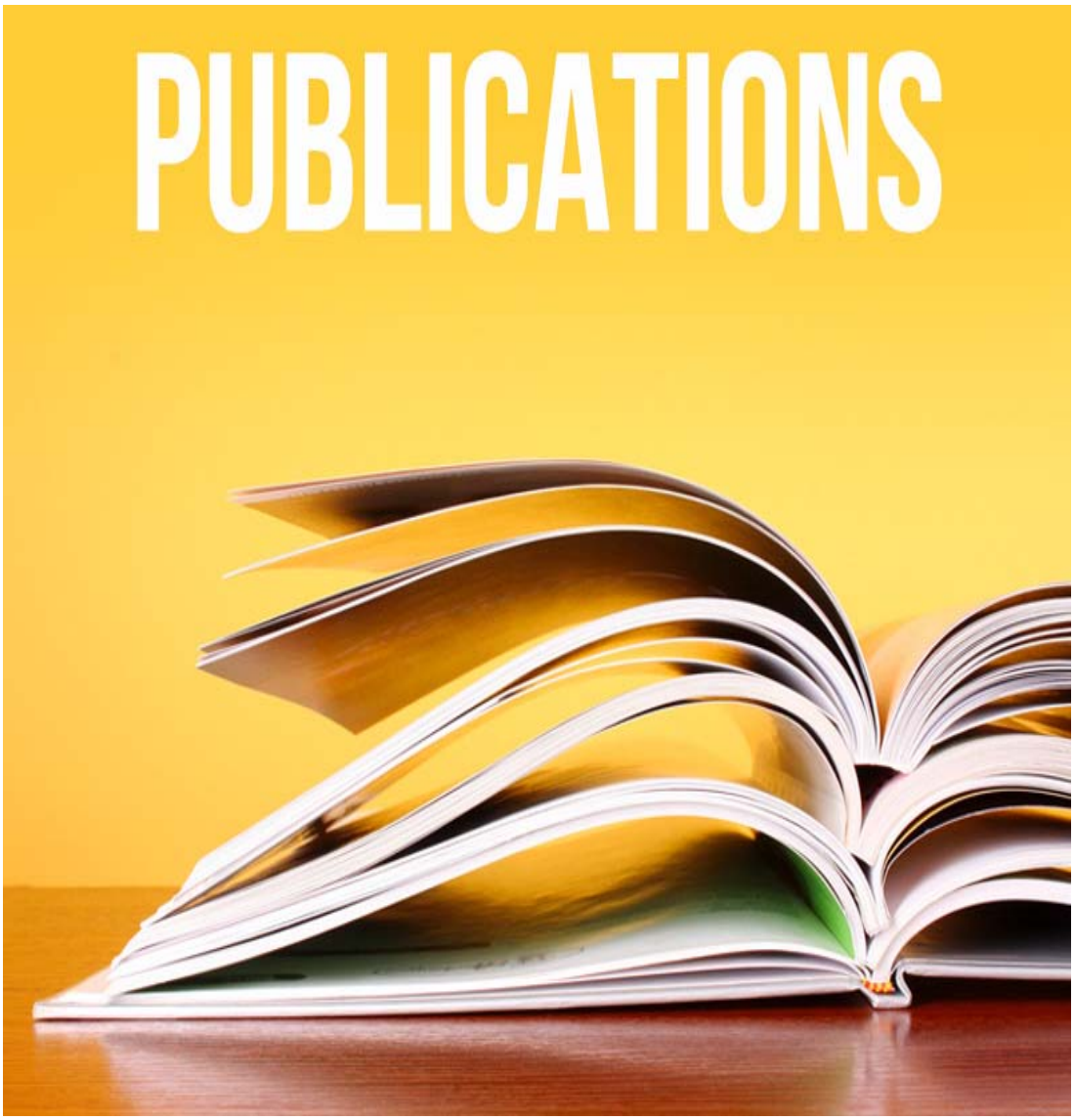
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Review on *Cordia oblique* Willd

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ABSTRACT

The *Boraginaceae* family consists of about 2,700 species, which are distributed in tropical, sub-tropical and warmer regions around the world. About 300 species of genus *Cordia* have been identified worldwide. There are 13 species of this genus found in India. The *Cordia obliqua* Willd., is a medium-sized deciduous tree, found scattered throughout the mid-Himalayas up to elevations of 1,470 meters, this plant having small fruits is commonly found. Its fruit is sweet and possess diuretic, anthelmintic, purgative, expectorant, maturant, useful in dry cough, in the diseases of chest and urethra, in biliousness and chronic fever and pains in the joints.Phytochemical investigation shows the presence of alkaloids, carbohydrates, flavonoids, glycosides, protein & amino acids, saponins, fixed oils, terpenoids, phenolics and steroids. Chemical examination of its seeds, roots, fruits and stem bark resulted in isolation of various constituents like alpha-amyrin, betulin, lupeol, octacosanol, beta-sitosterol, hentricontanol, hesperitin-7-rhamnoside and taxifolin-3,5-dirhamnoside.GC-MS analysis of various extracts of leaf showed several active components. The studies performed on various parts of this plant are anti-inflammatory activity, diuretic activity, hypotensive, respiratory stimulatory activity, antimicrobial activity and anti-diabetic activity.

Keywords: Boraginaceae, Clammy cherry, *Cordia obliqua*,Lassora.

1. INTRODUCTION

Medicinal plants are the nature's gift to human beings to help them pursue a disease-free healthy life. The world's cultures have an extensive knowledge of herbal medicine. Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. Traditionally the fruit has been used for the treatment of coughs, chronic fever, to remove joint pains and spleendiseases. Natural polysaccharides and their derivatives are widely used in pharmaceutical and food industry as biodegradable and biocompatible polymers for a large number of applications such as binding, thickening, emulsifying, gelling agents, etc. (Bhardwaj*et al*., 2000).

Gum *cordia,* an anionic gum obtained from fruits of *Cordia obliqua*Willd (Family: *Boraginaceae*), is one such polysaccharide with potential applications. *C. obliqua*, commonly known as **lassora**, is the medium-size deciduous tree native to Indiansubcontinent. The tribal population traditionally eats the ripefruits of the plant, while the raw fruits are used as vegetable and for making pickles (Parmar*et al*., 1982). The seeds of the plant contain anti-inflammatory constituents (Agnihotri*et al*., 1987).

1.1. Plant description

Cordia obliqua Willd.,plant belongs to genus *Cordia* and family *Boraginaceae.* Commonly it is known as "Clammy Cherry" and hindilasora. It is a medium sized deciduous tree and very vigorous in growth. The ripe fruits are traditionally eat by local tribes and raw fruits are used as pickle. *Cordia obliqua* is otherwise called as "Narivili" in tamil. It is one of the most traditional system of medicine in Ayurvedic and Siddha.

Botanical name: *Cordia obliqua* Willd.

Family : *Boraginaceae*

Kingdom : *Plantae*

Order : *Boraginales*

Subfamily : *Cordioideae*

Genus : *Cordia*

Species : *Cordia obliqua* Willd.

Domain : *Eukaryota*

Phylum : *Spermatophyta*

Subphylum : *Angiospermae*

Class : *Dicotyledonae*

Common name : Clammy Cherry, Mookuchalipazham.

Synonyms : *Cordia wallichii*, *Cordia myxa*,*Cordialatifolia*, *Cordia dichotoma.*

1.2. Vernacular names

Tamil	:	Naruvili, Virisu.
Sanskrit	:	Bhukarvudara.
Kannada	:	Chellamara, Nakkera.
Malayalam	:	Pasakamaram.
Punjab	:	Laswara.
Telugu	:	Bankanakkera, Chinnabotuku.
Bengal	:	Bahubara, Bohari.
Gujarati	:	Gundomoto, Lepistan.
English	:	Sebesten Plum.
Hindi	:	Lasura, Bhirala, Chhota, Laslasa, Gondi, Rasalla.

1.3. Medicinal uses

1. Pharmacological activities confirmed *Cordia obliqua* plant as and antimicrobial, hypotensive, respiratory stimulant, diuretic, and anti-inflammatory drug.
2. Medicinal activities like expectorant, antipyretic, anthelmintic, and maturant.
3. It is used purgative, hepatoprotective, and analgesic action.
4. The fruits are edible and used as pickle.
5. The raw fruits are used as vegetable.
6. The mucilaginous substance of the fruits can be used as a gum for pasting sheets of paper and cardboard.

Fig. - 1: Fruit and leaves of *Cordia obliqua*Willd.

1.4. Folk medicinal uses

1. The gum obtained from mucilage is used for pasting sheets of paper and as matrix forming material in tablet formations.

2. The juice of the bark is given in gripes, along with coconut oil. The bark and unripe fruit are used as a mild tonic.

3. The leaves are useful as an external application to treat ulcers and headache.

1.5. Physicochemical investigation

Physicochemical screening of *Cordia obliqua* revealed that, it possess good physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, loss on drying, swelling index and foaming index. The extractive values such as alcohol soluble extractive and water soluble extractive are also determined **Richa Gupta *et al.*, 2016.**

Table - 1: Physicochemical parameters of leaves of *cordiaobliqua.*

Parameters	Observation
Total ash	11.35 % w/w
Acid insoluble ash	2.76 % w/w
Water soluble ash	2.24 % w/w
Loss on drying	11.67
Swelling index	4.55 cm
Foaming index	Less than 100

Table - 2: Extractive values of leaves of *cordia oblique.*

Parameters	Observation
Alcohol soluble extractive	4.16 % w/w
Water soluble extractive	19.68 % w/w

1.6. Preliminary phytochemical investigation

The petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts were subjected to preliminary phytochemical screening for detection of various plant constituents present. They are individually performed using different qualitative tests for alkaloids, carbohydrates, flavonoids, glycosides, protein & amino acids, saponins, fixed oils, terpenoids, phenolics and steroids **ReenuYadav*et al.*, 2015.**

Table - 3: Phytochemical investigation of leaves of *cordia obliqua*

Type of Phyto Constituents	Petroleum ether extract	Ethyl acetate Extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	-	+	+	+	-
Carbohydrates	-	-	-	+	+
Flavonoids	-	+	-	+	-
Glycosides	-	-	-	+	+
Protein & amino acids	-	-	-	+	+
Saponins	-	-	-	-	+
Fixed oil	+	-	-	+	+
Terpenoids	-	-	+	+	+
Phenolics	-	+	-	+	+
Steroids	-	-	+	+	+

Table - 4: Review of *cordia obliqua*willd

Parts of plant used	Solvents	Activity	Author Name
Leaf	Hexane, Chloroform, Methanol and Water extract	Anti-inflammatory	Richa Gupta *et al.,* 2017
Stem and bark	Ethanolic extract	Anti-inflammatory	Bindu AR *et al.,* 2016
Seed oil	Petroleum ether extract	Anti-inflammatory	Jalalpure SS*et al.,* 2009
Leaf	Hexane, Chloroform, Methanol and Water extract	Anti-oxidant	RichaGupta *et al.,* 2016.
Fruits	Methanolic extract	Anti-microbial	Sung Kwon Park *et al.,* 2014.
Seed and leaf	Methanolic extract	Anti-microbial	ReenuYadav *et al.,* 2015
Fruits	Aqueous extract	Muco adhesive evaluation	Munish Ahuja *et al.,* 2013
Fruits	Aqueous extract	Ophthalmic	Monika Yadav*et al.,* 2010.
Leaf	Methanolic extract	Analgesic and Antipyretic	Richa Gupta *et al.,* 2017.
Unripe fruits	Aqueous extract	Hypotensive and respiratory stimulation	Abou-Shaaban RR *et al.,* 2015
Fruits	Alcoholic extract	Diuretic activity	Aswal BS *et al.,* 2015.
Whole plant	Methanolic extract	Anti-diabetic	Prakash ramakrishnan*et al.,* 2017.

1.7. Pharmacological uses

1.7.1. gas Chromatography - Mass spectroscopy analysis

GC-MS analysis of ethanolic extract of leaves of *Cordia obliqua* showed 11 bioactive compounds, the highest compound was named as 4-pentadecyne, 15-chloro-(13.57%) and lowest was named as 2-isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate (6.46%).**Sivakrishnan S *et al*., 2019.**

GC-MS analysis of hexane extract of leaves of *Cordia obliqua* revealed that it contains large number of phytochemicals such as 3-Hexen-2-one, n-Hexadecanoic acid, Phytol, cis-9-Hexadecenal, Glycidylpalmitate, Tetracontane, Squalene, Hexatriacontane, beta-Amyrin, Stigmasterol, Lupeol, Sitosterol etc. **Tilak Raj *et al.,* 2018.**

GC-MS analysis revealed the existence of various types of constituents in *Cordia obliqua* leaf methanol extract fraction showed different bioactive compounds such as Oleic acid, Oleanolic acid, Sesquiterpene lactone, triterpenoids, galactopyranoside, Hexadecanoic acid, Phthalic acid, Azafrin, Decatrienoic acid, Docasenamide, Morphinan and Carotenoids. Among these Morphinan is an opioid alkaloid and good psychoactive drug. **Richa Gupta *et al*., 2017.**

2. RESULT AND DISCUSSION

The survey of literature revealed that the *Cordia obliqua* having effective pharmacological activities such as anti-oxidant activity, anti-microbial activity, muco adhesive evaluation, anti-inflammatory activity, ophthalmic, analgesic activity, anti-pyretic activity, hypotensive, respiratory stimulation activity, diuretic activity and anti-diabetic. It having important medicinal phytochemical compounds such as amino acid, oleic acid, oleanolic acid, sesquiterpene lactone, triterpenoids, various steroids, galactopyranoside, hexadecanoic acid, phthalic acid, azafrin, decatrienoic acid, docasenamide, morphinan and carotenoids*Cordia obliqua*extract was prepared into nanoparticles. The results from this review are quitepromising for the use of *Cordia obliqua*as a multi-purposemedicinal agent, while *Cordia obliqua*has been usedsuccessfully in Siddha medicine for centuries, moreclinical trials should be conducted to support its therapeuticuse. Moreover, the therapeutic potential of the plant shouldalso be checked when used in combination with otherherbal drugs.

3. CONCLUSION

Ethno botanical and traditional uses of natural compounds, especially of plant origin received much attention in recent years as they are well tested for their efficacy and general believed to be safe for human use. Traditionally, plants are used in the treatment of many infections and systemic disorders. More than hundreds of chemical compounds are derived from plants which have medicinal values due to their health-enhancing and therapeutic properties are referred as herbs.Throughscreening of literature available on *Cordia obliqua* depictedthe fact that it is a popular remedy among the variousethnic groups Siddha and Ayurvedic properties.

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EVALUATION OF *INVITRO* ANTIOXIDANT ACTIVITIES OF VARIOUS EXTRACT FROM LEAF *CORDIA OBLIQUA* WILLD

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ABSTRACT

Objective: The study was designed to examine the *in vitro* antioxidant activities of pet. ether, ethyl acetate and ethanolic extract of plant leaves of *Cordia obliqua* Willd. The shade dried leaves plant powder was extracted with pet. ether, ethyl acetate and ethanol by continuous hot percolation method using Soxhlet apparatus. The antioxidant activity was determined by DPPH assay, Superoxide anion scavenging activity, iron chelating activity, hydroxyl radical scavenging activity, nitric oxide scavenging activity, FRAP assay, total antioxidant activity (phosphomolybdic acid method) at four different doses 125 to 1000 µg/ml with reference natural standard rutin respectively and total phenolic and total flavonoid content was analysed. An IC_{50} value was found that ethanolic extract of *Cordia obliqua* is more effective all antioxidant activity. These *in-vitro* assays indicate that this plant extract are better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

KEYWORDS: Antioxidant, *Cordia obliqua*, *In-vitro* antioxidant, Total flavonoid content, Total phenolic content.

INTRODUCTION

Cordia obliqua Willd. Plant belongs to genus *Cordia* and family *Boraginaceae.* Commonly it is known as “Clammy Cherry” and “Lasora” in hindi. It is a medium sized deciduous tree and very vigorous in growth. The ripe fruits are traditionally eat by local tribes and raw fruits are

used as pickle. *Cordia obliqua* is otherwise called as "Narivili" in tamil. It is one of the most traditional system of medicine in Ayurvedic and Siddha.

The *Boraginaceae* family consists of about 2,700 species, which are distributed in tropical, sub-tropical and warmer regions around the world. About 300 species of genus *Cordia* have been identified worldwide. There are 13 species of this genus found in India. The tribal population traditionally eats the ripe fruits of the plant, while the raw fruits are used as vegetable and for making pickles. The seeds of the plant contain anti-inflammatory constituents.

Traditionally the fruit has been used for the treatment of coughs, chronic fever, to remove joint pains and spleen diseases. Natural polysaccharides and their derivatives are widely used in pharmaceutical and food industry as biodegradable and biocompatible polymers for a large number of applications such as binding, thickening, emulsifying, gelling agents, etc.

MATERIALS AND METHODS

a. Collection and authentication of plant material

The leaves plant of *Cordia obliqua* was collected from Tenkasi, Tirunelveli District of Tamil Nadu, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamilnadu, India. The plant powdered materials were put away in a hermetically sealed holder. The leaves were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

b. Preparation of plant extract

The leaves powder samples of *Cordia obliqua* were extracted with pet.ether, ethyl acetate and ethanol at temperature between 60-70ºC by using soxhlet extractor. The solvent was evaporated by rotavapor to obtained viscous semi solid masses.

In vitro antioxidant studies

1. DPPH radical scavenging effect

The DPPH assay of whole part of the plant was measured using the method described by (Mensor *et al.,* 2001). The ethanolic extract was taken in different concentrations varying between 125 to 1000µgmL and results showed that the antioxidant activity, the percentage of inhibition. The absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging\ activity\ (\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

2. Nitric oxide radical scavenging activity

The ability of the plant extracts to scavenge the nitric oxide radical activity were determined by the method described by Green *et al*., (1982). Nitric oxide radical generated from sodium nitroprusside in aqueous solution at optimum pH conditions interacts with oxygen to produce nitrile ions which can be estimated by the use of Griess Ilosvay reaction at 540 nm.

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

3. Iron chelating activity

The iron chelating assay of aerial plant extracts of *Cordia obliqua* were described by (Benzie and Strain, 1996). The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

4. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging properties of ethanolic extract were determined by (Elizabeth and Rao, 1990). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-Ascorbate-EDTA-H_2O_2 system (Fenton reaction). The scavenging activity on hydroxyl radical was calculated as follows:

Scavenging activity (%) = (1 - absorbance of sample/absorbance of control) × 100

5. Superoxide radical scavenging activity

The assay for superoxide anion radical scavenging activity was supported by riboflavin-light-NBT system (Winterbourne *et al.*, 1975). Ascorbic acid was used as standard. The scavenging ability of the plant extract was determined by the following equation:

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

6. Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activity of the ethanolicextract of *Cordia obliqua* was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto *et al.*,

1999). Ascorbic acid was used as standard. The antioxidant capacity was estimated using following formula:

$$Total antioxidant\ activity\ (\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

7. FRAP assay

The FRAP assay of whole plant extracts of *Cordia obliqua* were described by modified method of (Benzie and Strain, 1996). Readings of the coloured product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results were expressed in µM (Fe (II) /g) dry mass and compared with that of ascorbic acid.

8. Estimation of total phenol content

Total phenolic content of aerial part of the plant was measured using the method described by Mallick and Singh *et al.*, (1980).

9. Estimation of total flavonoids content

Total flavonoid content of aerial part of the plant was measured using the method described by Cameron *et al.*, (1943).

RESULTS AND DISCUSSION

Invitro Antioxidant

1) DPPH Photometric Assay

The ability of pet. ether, ethyl acetate and ethanolic extracts to scavenge DPPH photometric assay was calculated as % inhibition and was compared with Rutin used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be 48.34% in pet. ether, 54.63% in ethyl acetate and 65.10% in ethanol when compared to Rutin 69.83% which is statistically significant at same concentration. The IC_{50} value was found to be 1120 µg/ml for pet. ether, 550 µg/ml for ethyl acetate and 475 µg/ml for ethanolic extract of *Cordia obliqua* and for rutin it was 480 µg/ml.

Table 1: DPPH Assay.

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Pet. ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Rutin)
1	125	17.55 ± 0.026	25.34 ± 0.035	35.63 ± 0.026	18.85 ± 0.076
2	250	28.68 ± 0.018	36.80 ± 0.028	46.78 ± 0.035	22.08 ± 0.054
3	500	33.73 ± 0.038	49.22 ± 0.030	52.68 ± 0.033	52.21 ± 0.022
4	1000	48.34 ± 0.021	54.63 ± 0.016	65.10 ± 0.028	69.83 ± 0.014
		IC_{50}=1120 µg/ml	**IC_{50}=550 µg/ml**	**IC_{50}=475 µg/ml**	**IC_{50}=480 µg/ml**

*All the values are expressed as mean ± SEM for three determinations.

2) Superoxide anion scavenging activity

The ability of pet. ether, ethyl acetate and ethanolic extracts to superoxide anion scavenging activity was calculated as % inhibition and was compared with quercetin used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be 64.96% in pet. ether, 68.56% in ethyl acetate and 72.60% in ethanol when compared to quercetin 98.01% which is statistically significant at same concentration. The IC_{50} value was found to be 520 µg/ml for pet. ether, 450µg/ml for ethyl acetate and 75 µg/ml for ethanolic extract of *Cordia obliqua* and for quercetin it was 60 µg/ml.

Table 2: Superoxide anion scavenging activity.

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Pet. ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Quercetin)
1	125	14.18 ±0 .021	17.64 ± 0.043	57.68 ± 0.035	73.81 ± 0.006
2	250	21.45 ± 0.033	23.08 ± 0.031	60.46 ± 0.046	91.31 ± 0.011
3	500	48.78 ± 0.040	53.20 ± 0.025	65.32 ± 0.028	92.99 ± 0.024
4	1000	64.96 ± 0.022	68.56 ± 0.036	72.60 ± 0.019	98.01 ± 0.012
		IC_{50}=520 µg/ml	**IC_{50}=450 µg/ml**	**IC_{50}=75 µg/ml**	**IC_{50} = 60 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

3) Iron chelating activity

The ability of pet. ether, ethyl acetate and ethanolic extracts to scavenge iron chelating activity was calculated as % inhibition and was compared with edta used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be 51.46% in pet. ether, 58.59% in ethyl acetate and 64.62% in ethanol when compared to EDTA 97.90% which is statistically significant at same concentration. The IC_{50}

value was found to be 920µg/ml for pet.ether, 410µg/ml for ethyl acetate and 110 µg/ml for ethanolic extract of *Cordia obliqua* and for EDTA it was 65 µg/ml.

Table 3: Iron chelating activity.

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Pet. ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (EDTA)
1	125	26.20 ± 0.026	24.34 ± 0.018	51.18 ± 0.030	58.68 ± 0.007
2	250	35.80 ± 0.029	35.28 ± 0.016	55.30 ± 0.028	65.87 ± 0.018
3	500	44.35 ± 0.021	53.45 ± 0.022	60.54 ± 0.033	83.83 ± 0.012
4	1000	51.46 ± 0.025	58.59 ± 0.024	64.62 ± 0.026	97.90 ± 0.019
		IC_{50}=920 µg/ml	**IC_{50}=410 µg/ml**	**IC_{50}=110 µg/ml**	**IC_{50}=65 µg/ml**

*All the values are expressed as mean ± SEM for three determinations.

4) Hydroxyl radical scavenging activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to hydroxyl radical scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be 52.54% in pet.ether, 55.82% in ethyl acetate and 69.51% in ethanol when compared to ascorbate 75.23% which is statistically significant at same concentration. The IC_{50} value was found to be 880µg/ml for pet. ether, 720 µg/ml for ethyl acetate and 350 µg/ml for ethanolic extract of *Cordia obliqua* and for ascorbate it was 410 µg/ml.

Table 4: Hydroxyl radical scavenging activity.

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	23.18 ± 0.021	25.31 ± 0.042	36.82 ± 0.021	26.87 ± 0.076
2	250	39.22 ± 0.033	39.43 ± 0.035	44.94 ± 0.034	30.30 ± 0.054
3	500	45.35 ± 0.041	47.65 ± 0.026	58.76 ± 0.018	60.64 ± 0.022
4	1000	52.54 ± 0.022	55.82 ± 0.038	69.51 ± 0.040	75.23 ± 0.014
		IC_{50}=880 µg/ml	**IC_{50}=720 µg/ml**	**IC_{50}=350 µg/ml**	**IC_{50}=410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

5) Nitric oxide scavenging activity

The ability of pet. ether, ethyl acetate and ethanolic extracts to nitric oxide scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It

was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be 49.85% in pet. ether, 54.06% in ethyl acetate and 65.26% in ethanol when compared to ascorbate 75.23% which is statistically significant at same concentration. The IC_{50} value was found to be 1010 µg/ml for pet. ether, 820 µg/ml for ethyl acetate and 450 µg/ml for ethanolic extract of *Cordia obliqua* and for ascorbate it was 410 µg/ml.

Table 5: Nitric oxide scavenging activity.

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	15.21 ± 0 .018	22.32 ± 0.026	33.22 ± 0.020	26.87 ± 0.076
2	250	22.63 ± 0.024	35.40 ± 0.033	48.46 ± 0.026	30.30 ± 0.054
3	500	38.74 ± 0.036	42.85 ± 0.035	52.08 ± 0.034	60.64 ± 0.022
4	1000	49.85 ± 0.042	54.06 ± 0.022	65.26 ± 0.030	75.23 ± 0.014
		IC_{50}=1010µg/ml	**IC_{50}=820µg/ml**	**IC_{50}=450µg/ml**	**IC_{50}=410µg/ml**

*All the values are expressed as mean ± SEM for three determinations

6) Total antioxidant activity (Phosphomolybdic acid method)

The ability of pet. ether, ethyl acetate and ethanolic extracts to scavenge Superoxide anion scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be 64.85% in pet. ether, 66.35% in ethyl acetate and 70.95% in ethanol when compared to ascorbate 65.23% which is statistically significant at same concentration. The IC_{50} value was found to be 590µg/ml for pet. ether, 460 µg/ml for ethyl acetate and 400 µg/ml for ethanolic extract of *Cordia obliqua* and for ascorbate it was 410 µg/ml.

Table 6: Total antioxidant activity (Phosphomolybdic acid method).

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	24.93 ± 0.019	29.46 ± 0.027	31.62 ± 0.018	26.87 ± 0.076
2	250	32.82 ± 0.028	42.08 ± 0.035	46.30 ± 0.026	30.30 ± 0.054
3	500	47.74 ± 0.033	51.79 ± 0.028	56.84 ± 0.034	60.64 ± 0.022
4	1000	64.85 ± 0.038	66.35 ± 0.042	70.95 ± 0.040	65.23 ± 0.014
		IC_{50} =590 µg/ml	**IC_{50}=460 µg/ml**	**IC_{50}=400 µg/ml**	**IC_{50}=410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

7) FRAP assay

The ability of pet. ether, ethyl acetate and ethanolic extracts to scavenge Superoxide anion scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be 37.91% in pet. ether, 53.96% in ethyl acetate and 76.78% in ethanol when compared to ascorbate 98.07% which is statistically significant at same concentration. The IC_{50} value was found to be 1300 µg/ml for pet. ether, 960µg/ml for ethyl acetate and 210 µg/ml for ethanolic extract of *Cordia obliqua* and for ascorbate it was 50 µg/ml.

Table 7: FRAP Assay.

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Pet.ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	17.56 ± 0.065	12.62 ± 0.021	31.35 ± 0.050	72.04 ± 0.014
2	250	21.88 ± 0.033	32.74 ± 0.016	52.55 ± 0.031	82.05 ± 0.034
3	500	34.04 ± 0.040	48.50 ± 0.028	61.08 ± 0.042	86.04 ± 0.026
4	1000	37.91 ± 0.038	53.96 ± 0.027	76.78 ± 0.026	98.07 ± 0.041
		IC_{50}=1300 µg/ml	**IC_{50}=960 µg/ml**	**IC_{50}=210 µg/ml**	**IC_{50}=50 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

8) Estimation of total phenol content

TPC showed a sharp pet.ether extract range of 1.43 mg/g, ethyl acetate extract range of 2.48 mg/g and ethanolic extract range of 4.91 mg/g as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

Table 8: Total phenol.

S.No	Extracts	Total phenolic content(mg/g of Catechol)
1	Pet. ether extract of *Cordia obliqua*	1.43 ± 0.020
2	Ethyl acetate extract of *Cordia obliqua*	2.48 ± 0.042
3	Ethanolic extract of *Cordia obliqua*	4.91 ± 0.044

*All the values are expressed as mean ± SEM for three determinations.

9) Estimation of total flavonoids content

The TFC showed a sharp pet.ether extract range of 0.032 mg/g, ethyl acetate extract range of 1.068 mg/g and ethanolic extract range of 2.176 mg/g as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

Table 9: Total flavonoids.

S. No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Pet. ether extract of *Cordia obliqua*	0.032 ± 0.003
2	Ethyl acetate extract of *Cordia obliqua*	1.068 ± 0.015
3	Ethanolic extract of *Cordia obliqua*	2.176 ± 0.018

*All the values are expressed as mean ± SEM for three determinations

CONCLUSION

The present study was clearly indicated ethanolic extract of *Cordia obliqua* showed strong antioxidant activity when compared with pet. ether and ethyl acetate extracts. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the ethanolic plant extract.

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