BIOLOGICAL SCREENING METHODS OF ETHANOL AND AQUEOUS EXTRACTS OF LEAVES OF *ALBIZIA LEBBECK (L.)* BENTH. IN ALBINO MICE

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IN

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OCTOBER-2021

EVALUATION CERTIFICATE

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DECLARATION

I do hereby declared that the dissertation **"BIOLOGICAL SCREENING METHODS OF ETHANOL AND AQUEOUS EXTRACTS OF LEAVES OF *ALBIZIA LEBBECK (L.)* BENTH. IN ALBINO MICE"**submitted to **"The Tamil Nadu Dr.M.G.R Medical University", Chennai,** for the partial fulfillment of the degree of **Master of Pharmacy in Pharmacology**, It is a bonafide research work has been carried out by me during the academic year 2021-2022, under the guidance and supervision of **Dr. R. SHANMUGA SUNDARAM , M.Pharm,Ph.D.,** Professor & Head, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title.

The information furnished in this dissertation is genuine to the best of my knowledge.

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1. INTRODUCTION

Introduction:

Since the birth of mankind there has been a relationship between life, disease and plants. India has an ancient heritage of traditional system namely Ayurveda, Siddha, Unani and Homeopathy. Herbal drugs play an important role in health care to treat the various diseases, there are uncountable plants available around globally. The history of herbal medicine is as old as human civilization. The Herbal medicines are practical and holistic set of guidelines to maintain balance and harmony of our system. The knowledge of herbal medicines has been transferred from generation to generation and this is the root of allopathic medicine and its derivatives. Almost 30% of the pharmaceutical preparation and 40% of the world's population still depends directly or indirectly on plant based medicines. The traditional knowledge and experiences are the support for discovery of variety of natural and semi synthetic compounds. [(1)]

According to World Health Organization (WHO) majority of the world's population use traditional medicines for their primary health care needs. Medicinal plants are the most important resource of life saving drugs. Plant secondary metabolites possesses various biological properties.

Currently, the usage of medicinal plants has increased day by day not only for primary healthcare but also to treat chronic diseases like cancer, diabetes, liver disorder, anxiety, rheumatic pains etc,. A large number of potent compounds have been reported from plant sources like ergometrine, narcotine, morphine, strychnine, emetine, brucine, piperine, quinine, colchicines etc. Constantly increases the usage of the medicinal plants around the world because easy availability, economic viability, harmless and more compatibility comparably synthetic drugs.

Most of the Ayurvedic practitioners in India formulate and dispense their own recipes; hence it requires proper documentation and research. In recent years the use of herbal medicine is steadily growing with approximately 40 per cent of population in western world reporting use of herb to treat medical illnesses. Due to the increase in incidence of the adverse drug reactions and economic burden of the modern system of medicine the Public and government both have interest in use of traditional medicines.

1.1 BACKGROUND OF THE PRESENT STUDY

The background of the present study is to focus the role of medicinal plants in management of anxiety, in addition discuss the significance of the Physiochemical, Phytochemical and Biological screening of the ethanol and aqueous leaves extracts of *Albizia lebbeck* (L.) Benth.

1.1.1 Anxiety

The word anxiety is derived from the Latin “anxietas” (to choke, throttle, trouble, and upset) and encompasses behavioral, affective and cognitive responses to the perception of danger. Anxiety is a normal human emotion. In moderation, anxiety stimulates an anticipatory and adaptive response to challenging or stressful events. In excess, anxiety destabilizes the individual and dysfunctional state results. Anxiety is considered excessive or pathological when it arises in the absence of challenge or stress, when it is out of proportion to the challenge or stress in duration or severity, when it results in significant distress, and when it results in psychological, social, occupational, biological, and other impairment.

Anxiety is a feeling of uneasiness and worry, usually generalized and unfocused as an overreaction to a situation that is only subjectively seen as menacing. It is often accompanied by muscular tension, restlessness, fatigue and problems in concentration. Anxiety is closely related to fear, which is a response to a real or perceived immediate threat; anxiety involves the expectation of future threat. People facing anxiety may withdraw from situations which have provoked anxiety in the past.

Fear and anxiety can be differentiated in four domains:

1. Duration of emotional experience

2. Temporal focus

3. Specificity of the threat and

4. Motivated direction.

Fear is short-lived, present-focused, geared towards a specific threat, and facilitating escape from threat; anxiety, on the other hand, is long-acting, future-focused, broadly focused towards a diffuse threat, and promoting excessive caution while approaching a potential threat and interferes with constructive coping.

These disorders alter how a person processes emotions and behave, also causing physical symptoms. Mild anxiety might be vague and unsettling, while severe anxiety may seriously affect day-to-day living.

The American Psychological Association (APA) defines anxiety as “an emotion characterized by feelings of tension, worried thoughts and physical changes like increased blood pressure.”

Anxiety, fear and worry are all completely natural human feelings. If these feelings occur and endure for an extended period, it affects both physical and mental health. This leads to clinical anxiety disorders. [(2)]

1.1.2 Symptoms

While a number of different diagnoses constitute anxiety disorders, the symptoms of generalized anxiety disorder (GAD) will often include the following:

- Increased irritability
- Concentration difficulties
- Restlessness, and a feeling of being “on-edge”
- Uncontrollable feelings of worry
- Sleep difficulties, such as problems in falling or staying asleep

While these symptoms might be normal to experience in daily life.

The physiological symptoms of anxiety may include:

- Respiratory, as shortness of breath or sighing breathing
- Muscular, as fatigue, tremors, or tetany
- . Cardiac, as palpitations, tachycardia, or chest pain

- Digestive, as abdominal pain, nausea, diarrhea, indigestion, dry mouth, or bolus
- Cutaneous, as perspiration, or itchy skin
- Neurological, paresthesias, as headache, fasciculations, vertigo, or presyncope [3]

1.1.3. Types of Anxiety

There are various types of anxiety, there are:

01. Mathematical anxiety, somatic anxiety

02.Existential anxiety can occur when a person faces angst, an existential crisis, or nihilistic feelings.

03. Social anxiety refers to a fear of rejection and negative evaluation by other people

04. Stage fright, or test anxiety. [4]

1.1.4. Causes

The causes of anxiety disorders are impenetrable, Possible causes include:

- Withdrawal from an illicit substance - the effects of which might intensify the impact of other possible causes
- Genetics - as people who have family members with an anxiety disorder are more likely to experience one themselves
- Environmental stressors - such as difficulties at work, relationship problems, or family issues
- Brain chemistry - as psychologists define many anxiety disorders as misalignments of hormones and electrical signals in the brain
- Medical factors - such as the symptoms of a different disease, the effects of a medication, or the stress of an intensive surgery or prolonged recovery

1.2. Status of Anxiety in India

An estimated one in seven Indians suffered from mental disorders of varying severity in 2017 with depression and anxiety being the commonest, according to a study.

The first comprehensive estimates of disease burden due to mental disorders and their trends in every state of India from 1990 published in the Lancet Psychiatry by the India State-Level Disease Burden Initiative show that the contribution of mental disorders to the total disease burden has doubled between 1990 and 2017.

These include depression, anxiety disorders, schizophrenia, bipolar disorders, idiopathic developmental intellectual disability, conduct disorders, and autism.

In 2017, 197 million Indians were suffering from mental disorders, of whom 46 million had depression and 45 million anxiety disorders, according to the study whose findings were released.

Depression and anxiety disorders are the commonest mental disorders and their prevalence is increasing across India and is relatively higher in the southern states and in women.

The prevalence of depression is the highest in older adults, which has significant implications for the aging population of India. Depression is also associated with suicide deaths in India, the study stated.

The prevalence of childhood onset mental disorders such as idiopathic developmental intellectual disability, conduct disorders, and autism was found to be higher in the northern states but is decreasing across India.

The contribution of mental disorders to the total disease burden has doubled in India from 1990 to 2017, indicating the need for implementing effective strategies to control this increasing burden.

The state-specific findings described in this paper highlight the extent of the effort needed in each state to address mental health which could serve as a reference for policy makers to plan approaches for reducing the growing burden of mental disorders in a systematic way.

The trends over about three decades reported in this research paper utilized all available data sources from India, which enables more robust estimates than the estimates based on individual data sources in isolation.

This research paper reports that a large proportion of India's population is impacted by mental health issues and systematically highlights the variations between the states, which can guide efforts for more specific health services planning for mental health in each state.

According to Professor Balram Bhargav, Director General of Indian Council of Medical Research (ICMR), the findings of this research demonstrate important differences between the states — the prevalence of adult mental disorders is higher in the southern states and that of childhood onset mental disorders is higher in the northern states.

"Mental illnesses contribute significantly to the burden of disease in India as reported by this study. There is an urgent need to strengthen mental health services, integrate these with general healthcare, treated with allopathic and herbal drugs for this treatment. [5]

1.3. COVID related anxiety or Corona-anxiety

COVID related anxiety is a major form of anxiety. Especially during the early days of the pandemic, when India was under lockdown, individuals faced major anxiety at the thought of themselves or their families being infected. Popularly known as ***'coronaphobia'*** this fear of the virus it causes a lot of anxiety over the possibility of being hospitalized, or even dying. A study done on this fear among the Indian population using a scale revealed that 54.8% of the population reported low fear, however female married healthcare workers with low educational status reported high levels of fear in this context. Media information via news channels and social media have witnessed a lot of sensationalism and even fake news. This constant access to news updates about rising numbers, buildings being sealed and first-hand accounts of hospitals are a source of anxiety and stress. The advent of social media journalism has led to fake news that does not come from credible sources.

There have thus been many factors that leads to anxiety during the COVID scenario that has been seen in the Indian scenario. The pandemic has caused new anxieties, revoked healed anxieties and exacerbated existent anxieties such as

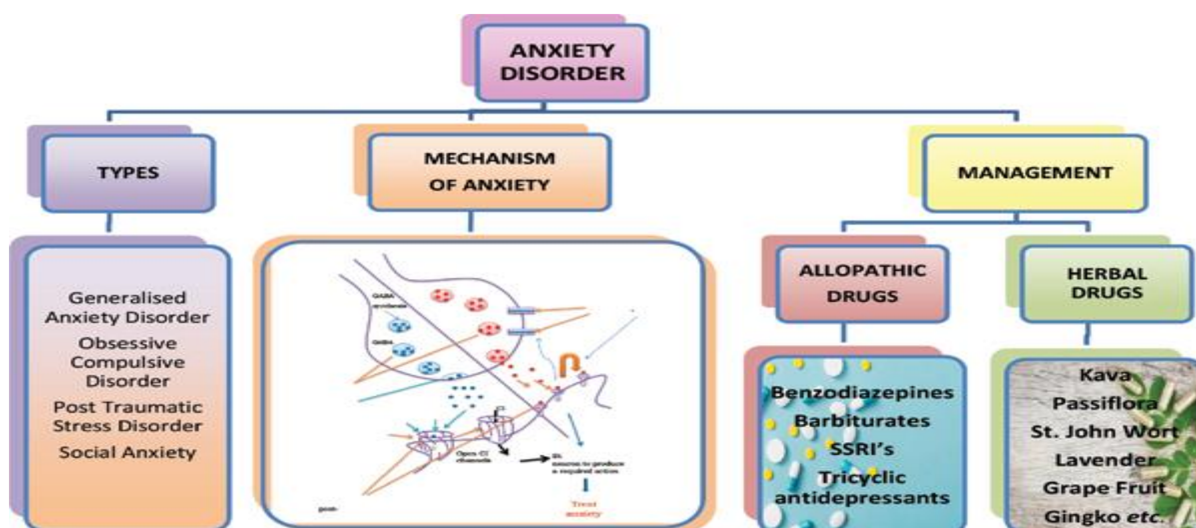
Work from home induced anxiety, Quarantine anxiety, COVID recovery anxiety, Generalized anxiety disorder, panic and pre-existing anxiety, Anxiety in patients with pre-existing psychiatric disorders, Anxiety in frontline health workers and their families, Psychosocial factors leading to anxiety, COVID-19 and pregnancy related anxiety, COVID vaccine related anxiety, Financial and economic factor related anxiety and Second wave COVID anxiety. (6)

1.4. Herbal Remedies for Anxiety

People with anxiety sometime consider herbal remedies as an alternative to prescription drugs. This may be because some medications, for example, beta-blockers or benzodiazepines, can have unwanted side effects. It is important to talk to a doctor before reducing or stopping prescription medication or starting an herbal supplement. Many herbs have been reported for the treatment of anxiety.

Herbal supplements aren't monitored by the FDA the same way medications are despite enhanced quality control regulations in place since 2010, the quality of some supplements may still be an issue.

Several ethnobotanical reports explicitly indicate nine plants used for the treatment of depression, or sadness, a symptom of depression. These reports are from seven different states of the country. Almost all species are prepared as infusions of the aerial parts; however, in two cases roots are used. (7)



1.4.1. Common Herbal Remedies for Anxiety

Ayurveda, the Indian traditional system of medicine uses herbs and their preparations to treat various neuropsychiatric disorders. Numerous herbs have been used for centuries in folk and other traditional medicine to calm the mind and positively enhance mood. Herbal medicine which plays an important role in developing countries, are once again becoming popular throughout developing and developed countries.

Study by revealed that use of herbal medicine is increasing enormously in the Western world. In spite of the large number of animal studies evaluating the potential anxiolytic effects of plant extracts, very few controlled studies have been conducted in a clinical setup.

Chamomile is a flowering herb; there are two types of chamomile that people can use medicinally: Roman chamomile and German chamomile. For instance, both Kava-kava (*Piper methysticum*) and St. John's wort (*Hypericum perforatum*) showed beneficial effectiveness in double blind, randomized placebo controlled trials to treat anxiety and depression.

Lavender is a flowering plant belonging to the mint family. Many people use lavender to help calm the nerves and alleviate anxiety. *Galphimia glauca* is a plant species native to Mexico. People traditionally used it as a tranquilizer to reduce anxiety. Also, extracts of valerian, hops, lemon balm and passion flower preparations have been employed for the prevention and treatment of psychiatric disorders such as anxiety, sleep disorders, convulsions, cognitive impairment and depression. Valerian or *Valeriana officinalis* is a plant native to Europe and Asia. For many centuries, people have used the root to help treat sleep problems, anxiety, and depression. (8)

The commonly used herbal remedies for treating anxiety are:

1.4.1.1. Passion flower

Passiflora incarnata is a folk remedy for anxiety. The anxiolytic effects of passionflower are well documented in rodents. In randomized double-blind study, passion flower extract was effective in 18 generalized anxiety disorder (GAD) out-patients as compared to oxazepam. Also, impairment in the job performance was increased in oxazepam group as compared to Passiflora extract treated group.

1.4.1.2. Kava kava (*Piper methysticum*)

There is substantial evidence that kava has a positive effect on the symptoms of anxiety disorders. Animal studies have demonstrated anti-anxiety activity of kava. Several randomized double-blind clinical studies in GAD patients showed beneficial effect of kava-kava in reducing anxiety.

Kava-kava was used in numerous controlled clinical studies to treat anxiety disorders. In the study by Connor & Davidson, kava extract was compared with placebo in GAD patients. In another 8-week randomized, double-blind multi-center clinical trial, the efficacy of *Piper methysticum* was compared with two anxiolytic drugs opipramol and buspirone in GAD patients. Meta-analysis study by Pittler and Ernst reinforced the anxiolytic effect of kava in generalized anxiety patients and indicated a significant reduction in anxiety parameters evaluated by the Hamilton Anxiety (HAMA) scale.

1.4.1.3. St. John's wort (*Hypericum perforatum*)

St. John's wort is a popular supplement for treating depression but is much less popular for treating anxiety disorders. Studies conducted by Flausino et al. and Singewald et al. have shown that chronic administration of *Hypericum perforatum* induced an antidepressant-like effect in Magnesium-depleted mice in the forced swim test and anxiolytic effect in the elevated T-maze and the light/dark transition test. St. John's wort administration resulted in anti-anxiety effect in animal models of restraint stress and sleep deprivation. *Hypericum perforatum* inhibits the reuptake of serotonin, noradrenaline, dopamine and modulates neuronal excitability via glutamatergic and GABAergic mechanisms.

Hypericum extract treated to 149 out patients diagnosed with somatization disorder, undifferentiated somatoform disorder, or somatoform autonomic dysfunctions, significantly reduced anxiety scores in HAMA scale. Another open-label uncontrolled observation with 500 subjects showed beneficial effect of St. John's wort extract in reducing anxiety disorder symptoms in patients diagnosed with depression comorbid with anxiety. However, stronger evidence is needed before St. John's wort should be considered as a treatment option for patients with diagnosable anxiety disorders.

1.4.1.5. *Valeriana officinalis*

Valerian is one of the most popularly used herbal medicines for insomnia and is also used to treat anxiety. Hydroalcoholic and aqueous extracts of valerian roots have shown affinity for the GABA-A receptor in the brains of rats. In humans, valerian has been successful in the treatment of insomnia and tension. Compared the extract of *Valeriana officinalis* L. (81mg of valepotriates as active ingredients) with placebo and diazepam (6.5 mg) in patients with GAD (DSM-III-R, 12 patients per group). Only the diazepam and valepotriates groups showed a significant reduction in the psychic factor of HAMA scale and the preliminary data obtained in the present study suggest that the valepotriates may have a potential anxiolytic effect on the psychic symptoms of anxiety. The limitations of this study are small sample size and a low dose of diazepam, such studies should be replicated with improved methodological design.

1.4.1.6. *Ginkgo biloba*

Extract of *Ginkgo biloba* (EGb 761) significantly reduced the detrimental effect of learned helplessness in a subsequent conditioned avoidance task. In the elevated plus maze, senescent mice treated with EGb 761 spent more time in open arms than those treated with vehicle control compared a standardized extract of *Ginkgo biloba* L. (EGb 761) in doses of 480mg and 240mg with placebo for four weeks, involving patients with GAD and adjustment disorder with anxious mood (DSM-III-R). The two doses of EGb 761 showed a greater reduction in HAMA scores compared to placebo, as well as a statistically significant reduction in somatic symptoms compared to baseline (which was not observed in the placebo group).

1.4.1.7. *Galphimia glauca*

Galphimia glauca Cav. is a plant used in Mexican traditional medicine as a "nerve tranquilizer". Previous studies have demonstrated anxiolytic effect of methanolic extract from this plant species. Conducted a controlled study comparing the extract *Galphimia glauca* Cav. with lorazepam in patients with GAD with 72 and 80 patients per group, respectively. Both groups of patients showed a significant reduction in scores of HAMA, without any difference between treatments.

1.4.1.8. *Matricaria recutita* (chamomile)

Chamomile is one of the most popular single ingredients of herbal teas, or tisanes. Chamomile tea, brewed from dried flower heads is used traditionally for several medicinal purposes like gastrointestinal tract ailments. Other uses include allergic rhinitis, attention deficit-hyperactivity disorder (ADHD), restlessness, insomnia, dysmenorrhea, mastitis and varicose ulcers. Chamomile contains flavonoids, which exert benzodiazepine-like activity and also has a phosphodiesterase inhibitory action, which leads to increased cAMP levels. A recent study evaluated the efficacy of a standardized extract of *Matricaria recutita* (L), compared with placebo for eight weeks in patients with mild to moderate GAD (DSM-IV). There was a statistically significant reduction in the scores of HAMA in the group treated with extract compared to placebo-treated group.

1.4.1.9. *Astragalus membranaceus*

Astragalus membranaceus (AM) is a useful Korean herb that has been clinically prescribed for stress-related illness. AM significantly restores learning and memory deficits in chronically stressed rats. In the elevated plus maze, AM treatment significantly increases the time spent in the open arms compared to control group. It also enhanced choline acetyltransferase (ChAT) expression in stressed rats. No clinical data is available for its anxiolytic effect. But one clinical study demonstrated the protective effect of astragalous on oxidative stress status in maintenance of hemodialysis patients.

Plant Posses anti anxiety Properties

(1) (2)

(3) (4)

(5) (6)

1. Passion flower 2. Kava kava 3. St. John's wort

4. *Valeriana officinalis* 5. *Ginkgo biloba* 6. *Galphimia glauca*

(7)

(8)

(9)

(10)

(11)

7. *Matricaria recutita* 8. *Astragalus membranaceus* 9. *Centella asiatica*

10. *Bacopa monnieri* 11. *Withania somnifera*

1.5. Marketed Polyherbal Formulations for the Treatment of Anxiety

In Ayurveda, many herbal formulations are generally used in the therapy as the combination of many drugs provides a synergistic therapeutic effect and also includes ingredients which help to minimize the adverse effects. A few example of anti anxiety herbal formulation available in the Indian market are given below.

1.5.1. Mentat (BR-16A)

The main herbs present in the mentat are Brahmi *(Bacopa monnieri),* Mandookparni *(Centella asiatica).* Ashwagandha *(Withania somnifera),* Jatamansi *(Nardostachys jatamansi),* Shankhapuspi *(Evolvulus alsinoides),* Tagar *(Valeriana wallichi).* Vach *(Acorus calamus),* Guduchi *(Tinospora cordifolia),* Malkangni *(Celastrus paniculatus),* Kuth *(Saussurea lappa)*

Some of these plants namely, *B. monnieri, C. asiatica, W. somnifera, N. jatamansi, E. alsinoides, V. wallichi, A. calamus, T. cordifolia and C. paniculatus,* have been classified in Ayurveda as Medharasayanas and claimed to improve memory and intellect. Polyherbal formulations are generally used in Ayurveda, based on the concept that such combinations provide synergistic therapeutic effect.

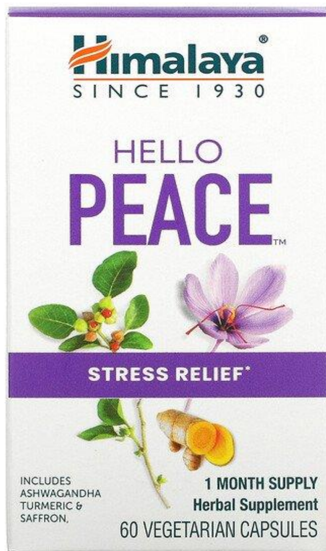
1.5.2. Geriforte

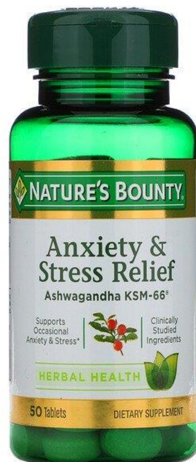
It showed significant anxiolytic effect in clinical studies. Geriforte contains Chyavanprash concentrate and the extracts of *Asparagus adscendens, Withania somnifera, Glycyrrhiza glabra, Centella asiatica, Mucuna pruriens*, Shilajeet, *Asparagus racemosus, Terminalia arjuna*, Makardhwaj and *Piper longum*, besides some others.

1.5.3. Unmadvati

Unmadvati is essentially a **stress and anxiety relief** medication, created using some of the choicest herbs. Formulated with the aim of offering relief from insomnia, this medicine comprises of various brain toners such as Bramhi, Jatamansi, and Shatavari, which help improve the memory while reducing mental stress.Maf By:DR. Vaidyas, Herbo Lab India (P) Ltd, Mumbai.

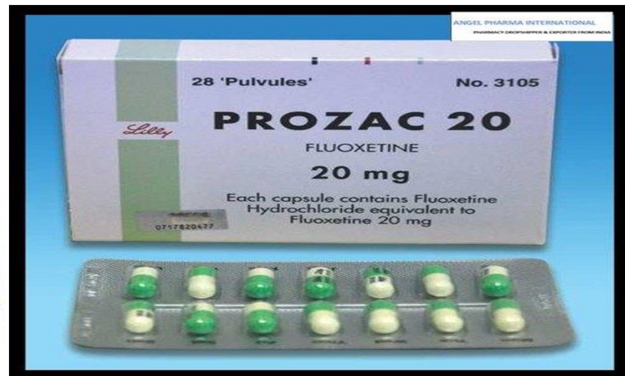
Marketed Polyherbal Products Posses anti antixety





1.6. Allopathic Marketed brands available for anti-anxiety

- Citalopram (Celexa) **Sertraline** (**Zoloft**)
-
- Escitalopram (Lexapro) Fluvoxamine (Luvox)
-
- Fluoxetine (Prozac) Paroxetine (Paxil)
- Vilazodone (Viibryd) etc..



1.6.1. Benzodiazepines

Benzodiazepines are sedatives that can help relax your muscles and calm your mind. They work by increasing the effects of certain neurotransmitters, which are chemicals that relay messages between your brain cells.

Benzodiazepines help treat many kinds of anxiety disorders, including panic disorder, generalized anxiety disorder, and social anxiety disorder. Examples of these drugs include:

- Alprazolam (Xanax), Lorazepam (Ativan)
- Chlordiazepoxide (Librium)
- Clonazepam (Klonopin)
- Diazepam (Valium)
- Benzodiazepines are typically used for short-term treatment of anxiety. This is because they can increase drowsiness and cause problems with balance and memory. They can also be habit-forming. There's an increasing epidemic of benzodiazepine misuse.
- It's important to only use these drugs until your doctor prescribes other treatment. However, if you have panic disorder, your doctor may prescribe benzodiazepines for up to one year.
- Side effects
- In addition to drowsiness and memory problems, taking benzodiazepines can also cause confusion, vision problems, headaches, and feelings of depression. [10]

1.6.2. Buspirone

Buspirone is used to treat both short-term anxiety and chronic (long-lasting) anxiety disorders. It's not fully understood how buspirone works, but it's thought to affect chemicals in the brain that regulate mood.

Buspirone can take up to several weeks to become fully effective. It's available as a generic drug as well as the brand-name drug Buspar.

Side effects

Side effects can include dizziness, headaches, and nausea.Some people also report strange dreams or difficulty sleeping when they take buspirone.

How do anti-anxiety drugs and Benzodiazepines work

Antidepressants reduce anxiety by increasing the concentration of chemicals (neurotransmitters) that the brain uses to communicate. These neurotransmitters include serotonin, norepinephrine, and dopamine.

Buspirone may reduce anxiety by stimulating serotonin and dopamine receptors on nerves, thereby altering the chemical messages that nerves receive.

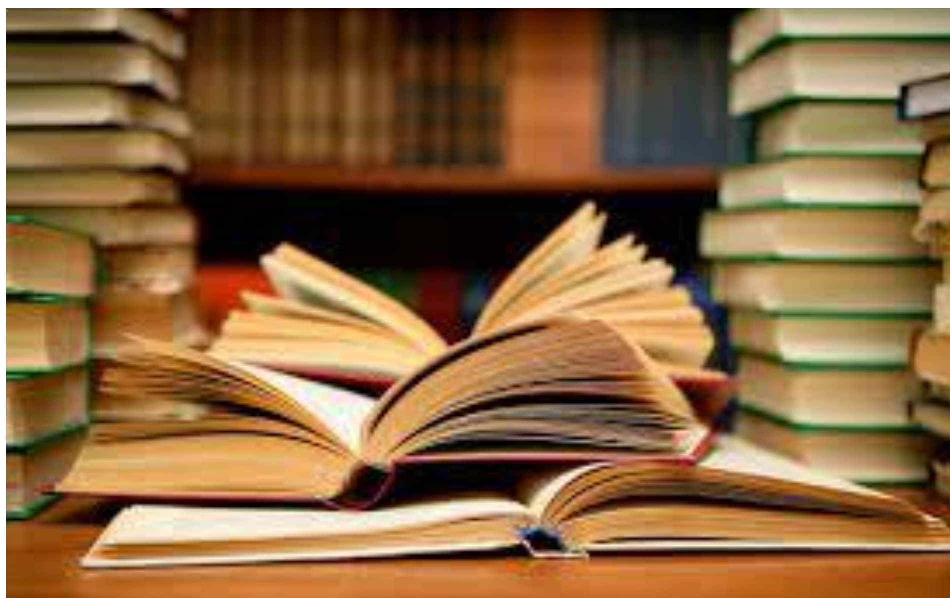
Benzodiazepines reduce symptoms of anxiety by increasing the action of a brain chemical called gamma-aminobutyric acid (GABA). GABA is a chemical that nerve cells use to communicate with each other and it r educes brain activity. It is believed that excessive activity in the brain may lead to anxiety or other psychiatric disorders.

Pregabalin is an anticonvulsant. Like benzodiazepines, Pregabalin also increases the action of GABA, and this may be its main mechanism for reducing anxiety.

Hydroxyzine is an antihistamine that causes sedation. It helps treat insomnia caused by anxiety, and other medical conditions.

Side effects of anti-anxiety drugs:

- Elevated blood pressure, Dry mouth, Blurred vision, Constipation, Orthostatic hypotension
- Drowsiness, Sedation, Confusion, Dependence and withdrawal symptoms
- Increased heart rate, Abnormal heartbeat, Weight gain
- Stomach upset, Nausea, Diarrhea, Sexual dysfunction, Headache, Suicidal thoughts



2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

S.C. Verma *et al* (2013) stated that *Albizia lebbeck* has been of keen interest due to varied phytochemicals and Ayurvedic research due to their excellent medicinal values. Traditionally, it is used as antiasthmatic, anti-inflammatory, anti-fertility, anti-diarrhoeal, antiseptic, anti-dysenteric and anti-tubercular**.** It is also used in the treatment of ringworms and wounds by washing the affected areas, gonorrhea, leucorrhoea, bronchitis, leprosy, paralysis, helminth infection and other genital diseases. The phytoconstituents reported in the plants are melacacidin, D-catechin, β-sitosterol, *albizia*hexoside, betulnic acidand echinocystic acid glycosides, which are responsible for various potent physiological and pharmacological activities. *Albizia lebbeck* appear to have a broad spectrum of activity on several ailments. The various parts of the plant have been explored for antiasthmatic, anti-inflammatory, anti-fertility, anti-diarrhoeal, antiseptic, anti-dysenteric, anti-tubercular and many other activities.

B Sivakumar *et al* (2013) discussed on Methanol extract of *Albizzia lebbeck* were found to be active on the renal system in rodents. Preliminary phytochemical investigation of methanolic extract of albizia lebbeck showed the presence of alkaloid, carbohydrate, protein, tannins, flavonoids, and amino acids. Barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhea. Ethanolic extract of pods possesses antiprotozoal, hypoglycemic and anticancer properties.

V.S. Kasture *et al* (1999) stated The ethanolic extract of *Albizia lebbeck* exhibited anticonvulsant activity. The methanolic fraction of chloroform soluble part of ethanolic extract of *A. lebbeck* exhibited anticonvulsant activity, whereas the other fractions were inactive. The bioassay guided fractionation indicated that the anticonvulsant activity lies in the methanolic fraction of chloroform soluble part of ethanolic extract of the leaves of *A. lebbeck.* The fractions protected animals from maximum electro shock, electrical kindling and pentylenetetrazole-induced convulsions in mice. The fractions also inhibited convulsions induced by lithium–pilocarpine and electrical kindling. However, they failed to protect animals from strychnine induced convulsions. The fractions antagonised the behavioral effects of D-amphetamine and potentiated the pentobarbitone-induced sleep. The fractions raised brain contents of gamma-aminobutyric acid (GABA) and serotonin. These fractions were found to be anxiogenic and general depressant of central nervous system.

Roni Yankelevitch-Yahav *et al* (2015) described the forced swim test (FST), which is one of the most commonly used assays for the study of anxiety-like behavior in rodents. The FST is based on the assumption that when placing an animal in a container filled with water, it will first make efforts to escape but eventually will exhibit immobility that may be considered to reflect a measure of behavioral despair. This test has been extensively usedbecause it involves the exposure of the animals to stress, which was shown to have a role in the tendency for major depression. Additionally, the FST has been shown to share some of the factors that are influenced or altered by depression in humans, including changes in food consumption, sleep abnormalities and drug-withdrawal-induced anhedonia. The main advantages of this procedure are that it is relatively easy to perform and that its results are easily and quickly analyzed. Moreover, its sensitivity to a broad range of antidepressant drugs that makes it a suitable screening test is one of the most important features leading to its high predictive validity. Despite its appeal, this model has a number of disadvantages. First, the issue of chronic augmentation is problematic in this test because in real life patients need to be treated for at least several weeks before they experience any relief from their symptoms. Last, due to the aversiveness of the FST, it is important to take into account possible influences it might have on brain structure/function if brain analyses are to be carried out following this procedure.

John F. Cryan *et al* (2005) described tail suspension test has become one of the most widely used models for assessing antianxiety-like activity in mice. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Various antidepressant medications reverse the immobility and promote the occurrence of escape-related behaviour. They discuss the inherent difficulties in modeling depression in rodents. They describe how the tail suspension differs from the closely related forced swim test. Further, they address some key issues associated with using the TST as a model of antidepressant action. They discuss issues regarding whether it satisfies criteria to be a valid model for assessing depression-related behavioural traits. They elaborate on the tests' ease of use, strain differences observed in the test and gender effects in the test. They focus on the utility of the test for genetic analysis. Furthermore, they discuss the concept of whether immobility maybe a behavioural trait relevant to depression. All of the available pharmacological data using the test in genetically modified mice is collated. Special attention is given to selective breeding programs such as the Rouen 'depressed' mice which have been bred for high and low immobility in the tail suspension test. They provide an extensive pooling of the pharmacological

studies published to date using the test. Finally, they provide novel pharmacological validation of an automated system (Bioseb) for assessing immobility. Taken together, they conclude that the tail suspension test is a useful test for assessing the behavioural effects of antidepressant compounds and other pharmacological and genetic manipulations relevant to depression.

Mohamed Farag *et al* (2013) Reviewed the importance of the plant *Albizia lebbeck* L. growing worldwide revealed many biological interests. However, the species growing in Saudi Arabia has not received due attention. The pre- sent study was undertaken to study antipyretic, analgesic, estrogenic and anti-inflammatory activities of five different fractions from successive extraction of *Albizia lebbeck* flowers: *n* hexane, dichloromethane, ethylacetate, *n*-butanol as well as the 70% total alcohol. The flowers showed reasonable antipyretic, analgesic, estrogenic and anti-inflammatory activities.

N. Prakash Babu *et al* (2007) The purpose of this study is to *Albizia lebbeck* Benth. is used both in Indian traditional system and folk medicine to treat several inflammatory pathologies such as asthma, arthritis and burns. The aim of the study was to evaluate the scientific basis of anti-inflammatory activity of different organic solvent extracts of *Albizia lebbeck*. The petroleum ether and ethanol extracts at 400 mg/kg, showed maximum inhibition of inflammation induced by carrageenan (petroleum ether—48.6%; ethanol—59.57%), dextran (petroleum ether—45.99%; ethanol—52.93%), cotton pellet (petroleum ether—34.46%; ethanol—53.57%) and Freund'sadjuvant (petroleum ether—64.97%; ethanol—68.57%). The petroleum ether extract of *Albizia lebbeck* had shown positive results for steroids, terpenoids and coumarins. The ethanol extract showed the presence of tannins, flavanoids, anthraquinones, and saponins.

Srivastav Neeti *et al* (2016) *Albizia lebbeck* Linn (Fabaceae) is a medicinal herb used in the traditional health care system of Uttara khand (India). The present study reports the anticonvulsant activities in the ethanolic extracts of the leaves of *Albizia lebbeck* Linn on the rats, induced both chemically and electrically. The models chosen for the activity were Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced convulsions in rats. The test dose studied were 200 and 400 mg/kg body weight orally of ethanolic extract of the plant. Acute toxicity studies show that the extract was nontoxic up to the recommended dose 2000mg/kg body weight orally as per OECD guideline no 423. In PTZ induced seizures, onset of clonic convulsions were studied while in MES model, reduction in the mean duration of extensor phase

was noted. The ethanolic extracts showed anticonvulsant activities against both MES and PTZ animal models.

D. Shirode et al (2008) determined the gastro-protective effect of 70% ethanolic extract of leaves of Albizzia lebbeck in pylorus ligation, ethanol and indomethacin induced models in rat. The 70% ethanolic extract of leaves of Albizzia lebbeck was prepared and subjected to acute toxicity study as per CPCSEA guideline no. 420. Two doses i.e. 100 mg/kg and 200 mg/kg were selected for the further study. In pylorus ligation induced ulcer model, the parameters studied were gastric volume, pH, free acidity, total acidity and ulcer index. Ulcer index was also determined in ethanol and indomethacin induced ulcer models. Pre-treatment with the extract (100mg/kg, 200mg/kg) has shown dose dependant decrease in ulcer index in all the experimental models of ulcers (indomethacin, ethanol and pylorus ligation induced ulcers). The prior administration of extract (100 mg/kg and 200 mg/kg doses) also reduced the total acidity (58.50 and 46.17), free acidity (51.48 and 40.05) and increased the pH (2.58 and 5.15). However, the gastric volume was not reduced with 100mg/kg dose and significantly reduced with 200mg/kg dose. The 70% ethanolic extract of leaves of the plant possess antiulcer properties. The antiulcer properties of the extract may be attributed to the polyphenolic compounds that are present in it.

3.PLANT PROFILE

3. PLANT PROFILE

The genus *Albizia* (Fabaceae) comprises approximately 150 species, mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa . *Albizia lebbeck* (AL) is a member of this genus and used in folk medicine to treat inflammatory conditions as asthma, arthritis, burns allergic rhinitis, bronchitis and leprosy and it have been claimed to be useful in treatment of Alzheimer`s and Parkinson`s diseases .

Moreover the extracts of *Albizia lebbeck* exhibited versatile biological effects as antioxidants, Cardiotonic , Hepatoprotective, , Lipid-lowering, Hypoglycemic activities antimicrobial, mental disorders and antihistaminic. Literature survey on *A. lebbeck* revealed the presence of sterols and triterpenes, phenolic compounds, flavonoids, isoflavone , alkaloids, miscellaneous compounds and saponins. [20]

3.1 Synonyms: *Acacia lebbeck Willd, Acacia macrophylla, Acacia speciosa ,*

Tamil - Vaagai, Vellaivenkai, Kattuvakai

3.2 Biological Classification:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Clade: Mimosoid clade

Genus: *Albizia*

Species: *Albizia lebbeck*

3.3 Distribution:

Albizia lebbeck (AL) occurs extensively throughout the Indian subcontinent and in Thailand, Pakistan, Bangladesh, the Andaman Islands, Burma, S China, NE Thailand, Malaysia and Malaysia. *Albizia lebbeck* is probably native to southern and south-eastern Asia and northern Australia, and possibly also parts of eastern Africa.

It is widely distributed all over India, mostly in Maharashtra, Punjab, Madhya Pradesh, Gujarat, Karnataka and Tamilnadu.

3.4 Ethnobotany:

Albizia lebbeck is an astringent, also used by some cultures to treat boils, cough, to treat the eye, flu, gingivitis, lung problems, pectoral problems, is used as a tonic, and is used to treat abdominal tumors. The bark is used medicinally to treat inflammation. This information was obtained via ethno botanical records, which are a reference to how a plant is used by indigenous peoples, not verifiable, scientific or medical evaluation of the effectiveness of these claims.

3.5 Uses:

Albizia lebbeck has many medicinal properties like Antiseptic, antibacterial, anti-allergic, antidermatosis, antidysenteric etc. Used in the treatment of Bronchitis, piles, hemicranias, cough, tropical pulmonary eosinophilia, asthma also Psychoactive.

4. AIM AND OBJECTIVES

4. AIM AND OBJECTIVES

In modern society, people suffer from various psychiatric disorders, especially depression, anxiety and insomnia. As one of the most prevalent forms of mental illness, depressive disorders have a huge influence on individuals and society.

Anxiety is a feeling of fear, dread, and uneasiness. It might cause you to sweat, feel restless and tense, and have a rapid heartbeat. It can be a normal reaction to stress.

According to the World Health Organization **(WHO),** major depressive disorders (MDD) will account for the second major illness in the world by the year **2020**.

The prevalence and comorbidity of psychiatric disorders such as depression, anxiety and insomnia are very common. These well-known forms of psychiatric disorders have been affecting many people from all around the world. In **2017, 197·3 million** (95% UI 178·4–216·4) people had mental disorders **in India,** including **45·7 million** (42·4–49·8) with depressive disorders and **44·9 million** (41·2–48·9) with anxiety disorders.

Herb alone and herbal formulations are commonly prescribed for the therapies of mental illnesses around the world. Since various adverse events of western medication exist, the number of people who use herbs to benefit their health is increasing. Over the past decades, the exploration in the area of herbal psychopharmacology has received much attention.

The plant *Albizia lebbeck* traditionally has been reported for the treatment of depression, cardiac activity, hepatoprotective etc. The aim and objectives of the present study is to screen and report the biological properties of the plant extract. [22]

5.PLAN OF WORK

5. PLAN OF WORK

The plant *Albizia lebbeck* (L.) Benth., was selected based on its traditional information, therapeutic values and literature review.

The Present work scheduled as follows:

Part 1. Pharmacognostical Studies

- Collection and authentication
- Processing and drying of plants
- Organoleptic evaluation

Part 2. Determination of Physiochemical Properties

- Determination of Ash values
- Determination Extractive values
- Determination of Foreign Organic Matter (FOM)
- Determination of Crude fiber content
- Determination of loss on drying
- Determination of Fluorescence analysis
- Determination of Microbial content
- Determination of Heavy metals

Part 3. Phytochemical Studies

- Preparation of extracts
- Qualitative chemical tests
- Estimation of total Phenolic content (TPC)
- Estimation of total Flavonoids
- Thin layer chromatographic study of the extracts

Part 4.Invitro Studies

- In vitro anti oxidant studies
- Antimicrobial Studies
- *Invitro* cyto toxicity Studies

Part 5. Pharmacological Studies

- Acute toxicity studies
- Anti anxiety studies

6. MATERIALS AND METHODS

6. MATERIALS AND METHODS

6.1. PHARMACOGNOSTICAL STUDIES

- Collection and authentication
- Processing and drying
- Organoleptic evaluation

6.1.1. Collection and authentication of the Plant

The plant *Albizia lebbeck* leaves were collected in and around the Tiruchengode district, Tamilnadu. The taxonomical identification of the plant was authenticated by Dr. N.Karmegam, Botanist, Asst Professor, Dept of Botany, Government Arts College, Salem.

6.1.2. Processing and drying methods of the Plant

Medicinal plants are extracted and processed for direct consumption as herbal or traditional medicine or prepared for experimental purposes. The concept of preparation of medicinal plant for experimental purposes involves the proper and timely collection of the plant, authentication by an expert, adequate drying, and grinding.

The freshly collected leaves were shade dried and pulverized. The powder (500 g) was treated with petroleum ether for the removal of chlorophyll and waxy material. Then it was air dried and extracted and macerated with methanol and water respectively. The percentage yield was noted.

6.1.3. Organoleptic Evaluation

Macroscopic identity of botanical materials is based on parameters like shape, size, color, texture, surface characteristics, fracture characteristics, odor, taste and such organoleptic properties that are compared to a standard reference material Organoleptic evaluation provides simplest and quickest way to identify crude drug. Macroscopic evaluation encompass morphologic description of plant parts by using naked eye.

The plant *Albizia lebbeck* morphological characters were studied and reported.

6.2. DETERMINATION OF PHYSICOCHEMICAL CHARACTERS

Physicochemical constants to be used to evaluate the crude drug from their constant values. The dried leaves of *Albizia lebbeck* (AL*)* powder was subjected to the physicochemical studies. The following parameters were determined and reports are tabulated.

- **Determination of Ash values**
- **Determination of Extractive values**
- **Determination of Foreign Organic Matter (FOM)**
- **Determination of crude fiber content**
- **Determination of Loss on Drying (LOD)**
- **Determination of Fluorescence analysis**
- **Determination of Microbial content**
- **Determination of heavy metals**

6.2.1. Determination of Ash values

The ash values are meant for determining the quality and purity of a crude drug especially in the powder form. The residue left after incineration of a drug is designated as ash. The residue originating from inorganic elements present in the plant may be designed as physiological ash. It varies with in definite limits according to types of soil. The purpose of ashing the vegetable drugs are to remove all traces of organic matters which may otherwise interfere in an analytical determination. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. that can be removed during incineration. Sometimes inorganic variables like calcium oxalate, silica, and carbonate content of the crude drug affects the total ash value. Such variables can be determined by different methods which measure total ash, acid insoluble ash and water soluble ash.

6.2.1.1. Determination of total ash

The total ash is designed to measure the total amount of material remaining after inciniration. This includes both the "physiological ash" which is derived from the plant tissue itself, and "non physiological ash", which is the residue of the extraneous matter such as sand and soil adhering to the plant surface 2g of each sample of crude powder was accurately weighed in a silica crucible and weighed. All the four samples were incinerated separately in the furnace gradually increasing the temperature up to 600°c and maintained for 3hours. Cooled in desiccators and incinerated ash was weighed. Percentage of ash value of each sample with reference to the air dried drug was calculated.

% of total ash= Weight of ash Weight of samplex 100

6.2.1.2. Determination of acid insoluble ash

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and incinerating the remaining insoluble matter. The total ash obtained from each sample was boiled separately with 25ml of dilute hydrochloric acid for 5minutes. The insoluble matter was collected on an ash less filter paper and washed with hot water. It was then incinerated again cooled and weighed. The percentage of acid insoluble ash values of each samples were calculated with reference to the air dried drug.

% of acid insoluble ash= Weight of ash Weight of samplex 100

6.2.1.3. Determination of water soluble ash

Water soluble ash is the difference in weight between the total ash and the residue after the treatment of the total ash with water. The total ash of each sample obtained previously was boiled separately with 25ml of distilled water for 5 minutes. The insoluble matter of each sample was collected in an ash less filter paper and washed with hot water. Then it was incinerated in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of this residue is subtracted from the weight of the total ash and the content of water soluble ash in mg / g of air dried material is calculated and the values were tabulated IP (1996).

% of Water soluble ash = water soluble ash Total ash x 100

6.2.2. Determination of extractive values

The extractive value determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material.

6.2.2.1. Determination of petroleum ether extractive values

Accurately weighed (5g) of dried powdered material of leaves and stems of both plants and it was placed separately in a 250ml conical flask. Petroleum ether (100ml) was added into each flask and it was attached with shaker. All the four flasks were allowed to shake for 24hrs with repeated intervals and the solution was filtered separately from each flask. The filtrate (25ml) was taken from each flask and placed in china dish and allowed to evaporation water bath. The obtained residue of each sample was weighed and the percentage w/w of extractive with reference to the air dried drug was calculated using the following formula.

% of Pet ether extractive matter = Weight of residue Weight of sample x 4 x 100

6.2.2.2. Determination of ethyl acetate soluble extractive values

The dried powdered material of leaves and stems of both plants were weighed (5g) and placed in a Stoppard 250ml conical flask. Ethyl acetate (100 ml) was added into each flask and it was attached with shaker. All the four flasks were allowed to shaken for 24hrs with frequent intervals, than solution was filtered separately from each flask. 25ml of filtrate was taken from each flask and placed in china dish and allow to evaporate on water bath. The obtained residue of each sample was calculated and percentage w/w of extractive with reference to the air dried drug was determined by using the formula Subban Murugesan et al. (2012). % of ethyl acetate extractive matter = Weight of residue Weight of samplex 4 x 100

6.2.2.3. Determination of alcohol soluble extractive values

The dried powdered material of leaves and stems of both plants were weighed (5g) and placed in a 250ml conical flask. 100 ml of alcohol was added into each flask 53 and it was attached with shaker. All the four flasks were allowed to shake for 24hrs with frequent intervals, than solution was filtered separately from each flask. 25ml of filtrate was taken from each flask

and placed in china dish. The solvent was evaporated on electric water bath finally the residue of each sample was calculated the percentage w/w of extractive with reference to the air dried drug.

6.2.2.4. Determination of water soluble extractive values

The dried powdered material of leaves and stems of both plants were weighted (5g) and placed in a 250ml conical flask. Distilled water (100 ml) was added into each flask and it was attached with shaker. All the four flasks were allowed to shake for 24hrs with frequent intervals, than solution was filtered separately from each flask. 25ml of filtrate was taken from each flask and placed in china dish and evaporated on water bath. The residue of each sample was calculated and percentage w/w of extractive with reference to the air dried drug was calculated Kamalesh Upreti et al. (2013).

% of Water extractible matter $= weight\ of\ residue\ weight\ of\ sample$ X 4 X 100

6.2.3. Determination of Foreign Organic Matter (FOM)

100 gm of dried leaves of *Albizia lebbeck* was taken on glass plate and spread uniformly. The samples was examined under open light and foreign organic matters separated manually after completion of separation weighed the matter and determined % w/w, present in the sample.

6.2.4. Determination of crude fiber content

2 grams of each samples of defatted powdered materials of dried leaves of AL was taken in a beaker consist of 50 ml of 10% v/v of nitric acid. The solution was warmed, filtered and the residue of each sample was washed with boiling water. The residue was boiled with 50 ml of 2.5% v/v of sodium hydroxide solution and filtered .The residue was washed with boiling water and dried.

6.2.5. Determination of loss on drying

Loss on drying is the loss in weight in percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. Weighed 54 about 2 gm of each powdered drug and placed each sample separately in a flat and thin porcelain dish. Distribute the samples evenly and it was placed in oven at 100-105°C for 30minutes. Cool the

samples at room temperature and weighed. The loss in weight was recorded for all the samples and it is determined as loss on drying.

% of Moisture content = $Final\ weight\ of\ sample\ Initial\ weight\ of\ sample$ x 100

6.2.6. Determination of Fluorescence analysis

Fluorescence analysis was carried out for all the four samples, the powdered samples were treated separately with different chemical reagents and determined the colour reactions of each samples at day light and under ultra violet light. The differences in colour reactions among the samples were observed and recorded.

6.2.7. Determination of minerals and heavy metal content

The human body requires a number of minerals in order to maintain good health which is the good sources of plants. Heavy metal toxicity is a wide topic throughout the world and very challenges in herbal industries. Generally the herbs are collected from different environment which might be contain contaminants includes heavy metals. The heavy metals are generally being induced in to the herbs from soil, water and atmosphere these may lead to metal poisoning. It is necessary to analyses the raw materials and finished preparations, the WHO also recommend the same. In the mineral and heavy metal analysis, initially all the samples were prepared using microwave assisted wet digestion method and the analysis of each element was carried out using Inductively coupled plasma- mass spectrometry (ICP-MS). The crude powdered leaves of *Albizia lebbeck* was used in the present study.

Microwave assisted wet digestion of sample

The powder of *Albizia lebbeck* (0.5 gm) was taken in glass tube consist of 7ml HNO3 and 7ml of HCl and kept at 150˚C in a microwave oven. The mixture was left to cool down and the content of the tubes were transferred to 50 ml of volumetric flask and the volume was made up to 50 ml with deionized water Rania Dghaim et al. (2015). 3.2.8.2. Inductively coupled plasma- mass spectrometry (ICP-MS) The concentration of dietary minerals, dietary trace elements and heavy metals in wet digested samples of leaves and stems were determined by an ICP-Mass spectrometer (made by Thermo Scientific Co, I cap Q model). Collision reaction cells

were adopted for removing spectral interferences in ICP-MS. The operating conditions employed for determination were an ICP RF power of 1500 W, an argon nebulizer gas flow rate of 0.9 1/min and a nebulizer pump speed of 0.1 rps. The data are presented as the mean of triplicate determinations and the results are representative of three independent experiment. Validation method For the quantitative ICP-MS analysis of the calibration was most commonly achieved by external standardization. The signal intensities of all isotopes were 56 measured in a blank as well as in 6 different concentrations of standard solutions which covered the concentration range of interest. Standard solutions were prepared in 5% v/v nitric acid solution by diluting an ICP multi element standard solution. The parameters of method validation including range of linearity, limits of deduction, limits of quantification , specificity, precision under repeatability conditions and within laboratory reproducibility were evaluated. The procedure and calculations were modified according to the European standard for the analysis of heavy metals WHO (2006).

6.3. PHYTOCHEMICAL STUDIES

Phytochemistry is mainly deals with vast variety of secondary metabolites which are biosynthesized by plants. The information on the constituents of the plant clarifies the biological properties of the plants but only ten percentages of the plant species have been investigated. Identification and evaluation of herbal extracts is a fundamental procedure and parts of quality control protocol. The presences of various phytoconstituents in ethanol and aqueous leaf extracts of *Albizia lebbeck* was identified evaluated and reported by qualitatively and quantitatively.

The following studies were carried out in Phytochemical analysis

- Preparation of extracts
- Qualitative chemical test
- Estimation of total phenol
- Estimation of total flavanoids
- TLC

6.3.1. Preparation of extracts

In the present study the leaves extracts were prepared by continuous hot extraction method and cold maceration method by using ethanol and water respectively. The Soxhlet apparatus was used for hot extraction and cold maceration process at room temperature was used to made extracts.

The shade dried leaves of *Albizia lebbeck* (500g) was used for the preparation of ethanol extract. The coarse dried powdered leaves was initially defatted with petroleum ether (60-80) and extracted with 95% ethanol in a Soxhlet extractor for 72hrs. Than the extract was concentrated separately by rotary vacuum evaporator and stored in desicator over sodium sulphite. The obtained extract was weighed and the percentage yield was calculated.

The colour and consistency of the extracts was noted. Analytical reagent grade (Merck, Mumbai) solvents were used for the entire study.

The powdered plant material (500 g) was extracted thrice in distilled water on shaker for 48 hours. The extract was filtered using a Buchner funnel and Whitman's No.1 filter paper. The filtrate of aqueous extract obtained was used for the present study.

6.3.2. Qualitative chemical tests

Test for alkaloids:

Mayer's test

Small quantities of the solvent free extract of each sample was separately treated with 3 drops of dilute hydrochloric acid and filtered. The filtrate treated with Mayer's reagent (mercuric chloride and potassium iodide) and colour reaction was noted.

Dragendroff's test

A small quantity of solvent free extract of each sample was separately treated with few drops of Dragendroff's reagent (Sodium iodide, basic bismuth carbonate, glacial acetic acid and ethyl acetate) and color reaction was observed. Wagner's test

Small quantities of solvent free each extract of each sample was separately treated with dilute hydrochloric acid and filtered. The filtrate treated with Wagner's reagent and developed colour was observed.

Hager’s test

Small quantities of solvent free extract of each sample was separately treated with dilute hydrochloric acid and filtered. The filtrate treated with Hager's reagent and inference was noted.

Test for Flavanoids and Glycosides:

A small quantity of solvent free each extract was separately dissolved in alcohol and it was hydrolyzed with 10% sulphuric acid and cooled. Then it was extracted with diethyl ether and divided in to three portions of each extracts in two separate test tubes. 1ml of diluted sodium carbonate, 1 ml of 0.1M sodium hydroxide and 1ml of diluted ammonia solution were added to the first and second test tubes respectively and findings were noted. Ferric chloride test To a small quantity of each extract with few drops of neutral ferric chloride solution was added and the colour reactions of each extracts were noted. [32]

Shinod’s test

To the each extract was placed with a small piece of magnesium ribbon and few drops of concentrated hydrochloric acid was added and the findings of each sample was noted. Test for steroids and triterpenoids Libermann-Burchard reaction

Small quantities of solvent free each of each sample was separately dissolved in 1 ml of chloroform and 1 ml of acetic anhydride followed by 2ml of concentrated sulphuric acid and colour formed between the solvent layer was noted. Salkowski's test

Chloroform solution to the extracts (l0mg of extract in 1 ml of chloroform) of each sample was treated with concentrated Sulphuric acid and a finding was noted. Test for carbohydrates

Molish test

To Small quantities of solvent free extract of each sample was treated with few drops of 1% α-naphthol in ethanol and concentrated sulphuric acid to the sides of the test tube and results of each extracts was noted.

Benedict's test

Small quantities of solvent free extract of each sample was separately dissolved in minimum amount of distilled water and filtered. To the filtrate equal volume of Benedict's reagent was added in a test tube and heated for few minutes the colour reaction was noted.

Fehling's test

Small quantities of solvent free extract of each sample was separately dissolved in minimum amount of distilled water and filtered. To the filtrates equal volume of Fehling's solution was mixed in a test tube and heated for few minutes and findings were noted.

Test for Tannins

Small quantities of solvent free extract of each sample was separately dissolved in minimum amount of distilled water and filtered. The filtrate is treated with 10% aqueous potassium dichromate solution and results were noted. Small quantities of solvent free extract of each sample was separately dissolved in minimum amount of distilled water and filtered. The filtrate was treated with 1ml of 5% Ferric chloride solution and the developed of colours of each extract was noted.

Test for proteins and amino acids:

Ninhydrin test

A small quantity of each extract solution was boiled with 0.2% solution of ninhydrine blue colour and developed colour from each extract was reported.

Million's test

To the each extract solution, 2ml Million's reagent [mercuric nitrate in nitric acid containing traces of nitrous acid] was added and appearance of colours from each sample was noted.

Biuret test

The each extract was treated with equal volume of 40% sodium hydroxide and 2 drops of 1% copper sulphate solution and reaction was observed.

Test for saponins :

Foam test

To the each extract, 20ml of distilled water was added and agitated on a graduated cylinder for 15 min and findings of each sample results were noted. [36]

6.3.3. Estimation of total phenol content (TPC)

The determination of total phenol content of ethanol extract of leaves of *Albizia lebbeck* was estimated by Folin-Ciocalteu (F-C) assay some modifications. The Folin-Ciocalteu reagent produces blue colour complex when react with polyphenol compounds if present in the sample. The assay relies on the transfer of reducing electrons in the alkaline medium, from phenolic compounds to phospho-molybdic acid complexes, manifested in the formation of blue colour that are estimated by UV-visible spectrophotometer (Thermo Fischer model Evolution 201) Wolfe (2003). The sample was prepared 1mg/ml concentration as stock and prepared volume of 1ml of each sample was taken in 2ml centrifuge tube followed by 0.5ml F-C reagents (1:10diluted with distilled water) was added and allowed to react for 5 min before adding 0.4ml 20% Na2CO3.The above solutions were mixed and allowed to stand 15 min at room temperature than measured absorbance of each samples at 765nm. The blank was prepared in similar manner without sample and standard. Calibration curve was plotted using gallic acid as standard (10, 20, 40, 60, 80,100µg/ml).The results were expressed as milligram of gallic acid equivalents (GAE) per gram of extract. [39]

6.3.4. Estimation of total flavanoids

Total flavanoids content was estimated for the ethanol extract of leaves of *Albezia lebakk* by Aluminium chloride colorimetric assay with some modifications. 1 ml aliquot of appropriately diluted each sample or standard solution of quercetin (10, 20, 40, 60, 80 and 100µg/ml) was mixed with 50µL of $NaNO_2$ in 2ml micro centrifuge tube. After 6 min, 50µL of a 10% aluminium chloride solution was added and allowed to stand for 6 min, and then 50µl 1M potassium acetate solution was added to the mixture. The final volume was made up with distilled water to 2ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm against prepared blank. Blank was prepared in the same above manner omitting sample and standard. All values were expressed as milligrams of quercetin equivalent per 1g of sample.

6.3.5. Thin layer chromatographic study for the extracts (TLC)

Chromatography techniques are one of the most useful techniques for separation and identification of phytoconstituents. The separation of the compound occurred between stationary and moving mobile phase. The compound has separated based on their affinity towards stationary phase.

TLC is one of important analytical method for separation of two or more compounds. The separation is accomplished by the distribution of the mixture of compounds between two phases. One that is fixed phase or solid stationary phase and other is moving liquid phase. The separation behavior of the compound is dependent upon affinity between stationary phase and mobile phase. If the compound has more affinity towards the stationary phase the travelling speed is slow than that of less affinity compounds. The stationary phase was selected on the basis of high eluting power. The silica gel G and alumina are generally used as stationary phase.

Preparation of TLC plate

The carrier plates of thickness 3.8 mm and size 20 cm x 5 cm were taken. The plates were carefully cleaned and completely free from grease. The plates were rinsed with running tap water and dried at room temperature. Silica gel G was made into a homogenous suspension with distilled water (25 gm of silica gel in 50 ml of water). This suspension was poured in TLC

applicator which was adjusted approximately to 0.25 mm thickness. The suspension was spread on the carrier plates in uniform thickness through the spreader. The plates were kept as such for air drying until the transparence of the layer disappears. Then the plates were activated by heating in hot air oven at 110˚C for 45 min. The plates were then stored in a dry atmosphere and used whenever required.

Mobile phase

The migration rate of the compound on the plate is based on the solubility of the compound in mobile phase; selection of solvents is major role in separation of the mixture of compounds. Since the mixture of solvents with different polarity gives better separation than homogeneous solvents, mixture of solvents with high eluting power was selected for the present determination. Solvents were selected for the present study by available literatures and trial and error method. Finally establish that n butanol-Glacial acetic acid and Water (4:1:1) as perfect mobile phase was for the present study, it was selected based on the resolution.

6.3.6. Preparation and application of extracts

The ethanol and aqueous leaves extracts of *Albizia lebbeck* (AL) was dissolved in n butanol separately at concentration of 1mg/ml and filtered. The filtered extract of each sample was spotted on a TLC plate by using capillary tube, samples were placed approximately 1.5 cm above its bottom of the plate. Then the spot was dried at room temperature until it was completely free from solvent.

Preparation of chromatogram

The developing chambers made up of glass contain the mobile phase up to 1cm depth. The chamber was fully saturated with solvent vapour which fastened the development. After drying the spots, the TLC plates were placed into developing chamber vertically. The TLC plates were removed after 3/4th plate was saturated with solvent and the distance travelled by the solvent was noted. TLC separation was performed for the all four extracts using silica gel GF as stationary phase.

Detection of compounds

After the development the plates were dried at room temperature. The spots were detected by irradiating the plate in UV light (mercury light) and placing in Iodine chamber. The colour intensity of the spots was observed under iodine chamber and UV light. Wagner et al. (1996).

6.5. Invitro Studies

- *Invitro a*ntioxidant Study - DPPH and ABTS Methods
- Anti microbial studies
- Cytotoxicity studies – MTT Assay on NH 3 T 3 Mouse embryonic cell line

6.5.1. Invitro antioxidant study

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease etc.

Various studies have been done to identify antioxidants from plant sources and efforts have been taken to incorporate it in conventional therapy. In our present study, the ethanolic and aqueous leaves extracts of *Albizia lebbeck* have been evaluated against standard. The *invitro* antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis,3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging and reducing power assay. [49]

6.5.1.1. DPPH$^{\cdot}$ (2, 2′-diphenyl-1-picrylhydrazyl radical) assay

The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore d The molecule 1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an

absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.eterminate free radical scavenging capacity. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. According to Manzocco et al., 1998 the sample extract (0.2 mL) is diluted with methanol and 2 mL of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below:where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

$$\% \text{ inhibition of DPPD radical} = (Abr - Arr/Abr) \times 100$$

6.5.1.2. ABTS radical cation decolorization assay

This method, uses a diode-array spectrophotometer to measure the loss of color when an antioxidant is added to the blue–green chromophore ABTS· + (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)). The antioxidant reduces ABTS·+ to ABTS and decolorize it. ABTS·+ is a stable radical not found in the human body. Antioxidant activity can be measured as described by Seeram et al. (2006). ABTS radical cations are prepared by adding solid manganese dioxide (80 mg) to a 5 mM aqueous stock solution of ABTS (20 mL using a 75 mM Na/K buffer of pH 7). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analog of vitamin E, can be used as an antioxidant standard. A standard calibration curve is constructed for Trolox at 0, 50, 100, 150, 200, 250, 300, and 350 µM concentrations. Samples are diluted appropriately according to antioxidant activity in Na/K buffer pH, 7. Diluted samples are mixed with 200 µL of ABTS·+ radical cation solution in 96-well plates, and absorbance is read (at 750 nm) after 5 min in a microplate reader. TEAC values can be calculated from the Trolox standard curve and expressed as Trolox equivalents (in mM).

6.5.2. Anti microbial studies

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Several bioassays such as disk-diffusion, well diffusion and broth or agar dilution are well known and

commonly used, but others such as flow cytofluorometric and bioluminescent methods are not widely used because they require specified equipment and further evaluation for reproducibility and standardization, even if they can provide rapid results of the antimicrobial agent's effects and a better understanding of their impact on the viability and cell damage inflicted to the tested microorganism.

The antibacterial efficacy of ethanol and aqueous leaf extracts of Albizia lebbeck was tested by agar well diffusion method.

In the present study four gram positive, four gram negative antibacterial and two anti fungal strains were used. The list of strains used in the antimicrobial studes are Bacillus lintus (NCIM 2018), Salmonella paratyphi (NCIM 2501), Candida albicans (MTCC3100), Bacillus subtilis (NCIM 2063), Escherichia coli (NCIM 2065), Aspergillus Niger (MTCC 1344), Staphylococus albus (NCIM 2178), Pseudomonas auroginosa, (NCIM 2200), Staphylococus aureus (NCIM 2079) and Klebsiellapnemonia (NCIM 2707).

6.5.2.1. Antibacterial study

In this study the initial work has been carried out by disc diffusion method. In the disc diffusion method the drug potency is based on measurement of the diameter of zones of inhibition surrounding cylindrical discs. The extracts was placed at on the surface of the solid nutrient media previously inoculated with a culture of a suitable microbe. Inhibition produced by the each test compound is compared with known concentration of reference standard. Ciprofloxacin at the concentration of 10µg/disc were used for bacterial study. Ethanolic and aqueous extracts of leaves of *Albizia lebbeck* was screened at 300µg/disc concentration. The each extract was separately dissolved in 1ml of DMSO and the sterile discs were impregnated with respective extract and placed on agar medium against various strains Buwa and Van Staden (2006). The Beef extract 10gm, Peptone, 10gm Sodium Chloride and 5gm Distilled water were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers or tins etc. Sterilize forceps by dipping in alcohol and burning off the alcohol. Procedure Accurately weighed quantities of the above ingredients were suspended in 1000ml of distilled water. They were boiled to dissolve completely. The pH was adjusted to 7.3± 0.2 at 25°C. It was then sterilized by autoclaving at 15lbs pressure at 121°for 15mins. Preparation and standardization of test inoculam for bacteria The culture obtained from the

laboratory stocks of the department of pharmaceutical biotechnology, one loopful each strains were transferred into 100ml of nutrient broth using a sterilized Pasteur loop. The inoculated broths were incubated at 37°C for 24 hrs. After incubation, inoculums are standardized to 108 CFU/ml for colony forming unit method.

Preparation of test drug solution The Ethanolic and aqueous extracts of AL was dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 300µg/disc. The sterile disc were impregnated with 10µl of the extracts and tested against selected strains. Muller Hinton agar media was prepared and transferred into sterile Petri-plates. 200µl of the standardized bacterial inoculums was spread on agar medium using sterile cotton swab. The extracts impregnated discs were placed on the inoculated agar media. Ciprofloxacin (10µg/disc) disc was used as a positive reference standard to determine the sensitivity of each microbial species tested. All the Petri-plates were incubated at 37°C for 24 hrs. After incubation, diameter of zone of inhibition was measured. 3.4.2.2. Anti fungal activity Preparation of inoculum The inoculums for the experiment were prepared in fresh sabouraud's broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth if excessive or by further incubation to get required turbidity Srinivasahan and Durairaj (2014). Preparation of sterile swabs and forceps Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers or tins etc. Sterilize forceps by dipping in alcohol and burning off the alcohol. Experimental Procedure The standardized inoculums is inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60ºC after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed.

Each Petri dish is divided into 3 parts, ethanol, aqueous extracts (300µg) and standard Clotrimazole 10µg, are placed in each quadrant with the help of sterile forceps. Then Petri dishes were placed in the refrigerator at 4º C or at room temperature for 1 hour for diffusion and the plates were incubate at 28º C for 48hours. The zone of inhibition produced by different samples

and standard was measured by using a scale and record the average of two diameters of each zone of inhibition. The measured zone of inhibitions was tabulated.

6.5.3. Cytotoxicity studies – MTT Assay on NH 3 T 3 Mouse embryonic cell line

Materials and methods

Dulbecco's Modified Eagles Medium containing, 10% fetal bovine serum, ethylene diaminetetraacetic acid (EDTA), 5% FBS, dimethylsulfoxide and phosphate buffered saline were purchased from Sigma.NIH 3T3 cell lines was procured from NCCS, Pune.

Procedure

The cytotoxicity activity was screened by MTT assay using NIH 3T3 cell lines. The cells were grown in Dulbecco's Modified Eagles Medium containing 10% fetal bovine serum and maintained at 370C, 5%, Co2 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x105 cells/ml. One hundred micro litres per well of cell suspension were seeded into 96 well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 370C, 5% CO2, 95% air and 100% relative humidity. After 24 h, the cells were treated with serial concentrations of the ethanol and aqueous extracts of *Albizia lebbeck*. They were initially dissolved in dimethylsulfoxide and an aliquot of the extract was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations (6.25, 12.5, 25, 50 and 100µg/mL). Aliquots of 100 µl of these different dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final concentrations.

Following sample addition, the plates were incubated for an additional 48 h at 370C, 5% CO2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 370C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were

solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows.

% Cell viability = [A] Test / [A]control x 100

6.6. PHARMACOLOGICAL SCREENING

- **Determination of acute oral toxicity study**
- **Evaluation of anti anxiety study**

6.6.1. Determination of acute oral toxicity study

Toxicology traditionally known as the Science of Poison began with early mans who recognized poisonous plants and their extracts for hunting. The usage of plants and its derived products are increased day by day so it must be ensured the measurement of toxicity is needed to confirm the safety and efficacy of the plants. Toxicity may be acute or chronic and may vary from one organ to another as well as with age, genetics, gender, diet, physiological condition, or the health status of the organism. As opposed to experimental animals, which are highly inbred, genetic variation is a most important factor in human toxicity since the human population is highly out bred and shows extensive genetic variation. Even the simplest measure of toxicity, the LD50 (the dose required to kill 50% of a population under stated conditions) is highly dependent on the extent to which the above variables are controlled. LD50 is the amount of a chemical given all at once, which causes the death of 50% of a group of test animals. The LD50 is one way to measure the short term poisoning potential of a material Sabbani et al. (2015). 3.6.1.1. Procedure Acute oral toxicity was performed as per the Organization for Economic Co-operation and Development (OECD) guideline 423 methods. The healthy mice between 25-30g of both sex were used for the study and maintain the animals at room temperature 22 -25 C by provided standard laboratory animal food pellets with water. The animals were made in to four groups 3mice in each group. The ethyl acetate extract of HHL, HHS, HML and HMS were administrated orally at a single dose at 2000mg/kg. After administration of each extracts food was withheld 2h to all the groups. All the animals were observed individually after at least once during the first 30 minutes, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter for a total of 14 days. During the study the following activities

such as tremors, convulsion, salivation, diarrhea, skin and fur, eyes, and behavior patterns were closely monitored and recorded as per OECD guideline (2001).

6.6.2. Evaluation of Anti anxiety Studies

The present study was planned to evaluate the anti-anxiety activity of ethanolic extract of Albizia lebbeck in Swis albino mice were divided into four groups of six animals each, comprising of both male and female in each group. Group I received water swerved as normal control (WC), group II received vehicle and served as vehicle control (VC), group III received ethanolic extract of Albizia lebbeck and group IV received standard drug diazepam (2mg/kg) for anxiolytic study in elevated plus maze. This formulation can be used in prevention and treatment of anxiety.

7. RESULTS AND DISCUSSION

7. RESULTS AND DISCUSSION

7.1. PHARMACOGNOSTICAL STUDIES

Organoleptic characters:

The *Albizia lebbeck* is deciduous trees grow upto 25 m high, bark 20-25 mm thick, surface yellowish-brown, rough, deeply fissured, exfoliating in irregular semi brittle scales blaze pinkish-yellow.

Leaves: The leaves are Bipinnate, alternate, stipulate; stipules small, free, lateral, cauducous; rachis 7-9 cm long, slender, grooved above, with a gland at its base, glabrous, pulvinate, pinnae 2-4 pairs, 5.5-12 cm long, slender, glabrous, opposite, even pinnate; leaflets 8-20, opposite, estipellate, even pinnate, petiolule to 1 mm long, a gland in between each leaflets; lamina 2.7-5 x 1-2.5 cm, obliquely oblong, base of one half cuneate, other round, apex obtuse, margin entire, glabrous above and slightly pubescent beneath, coriaceous; midrib towards distal margin, lateral nerves 6-8 pairs, pinnate, slender, prominent, intercostae reticulate.

Flowers: The flowers are Bisexual, greenish-white, in subglobose heads, 3.5-3.7 cm long, solitary or 2-4 together in axillary corymbose racemes; peduncle 6-8 cm; bracts linear, cauducous; pedicels to 3 mm; calyx tube funnel-shaped pubescent, 3-4.5×1.5-3 mm, teeth triangular; corolla infundibuliform 7-10 mm long, greenish, lobes 5, lanceolate, pubescent without; stamens many, filament tube shorter than corolla tube, filaments long exserted, green or pink; anthers very small; ovary subsessile, glabrous, style 2.5 cm, filiform; stigma minute. Fruit a pod 20-30 x 4-5 cm, flat, oblong, compressed, straw coloured, base and apex obtuse, turgid above the seeded region;

Seeds: 8-12, 6-10 x 5-8 mm, ovate, dull dark brown, flattened.

***Albizia lebbeck* (L.) Benth**

7.2. DETERMINATION OF PHYSICOCHEMICAL CHARACTERS

Physicochemical studies were performed to determine the identity and purity of the plant materials. Physico-chemical properties are the intrinsic physical and chemical characteristics of a substance. These include appearance, boiling point, density, volatility, water solubility and flammability etc.

7.2.1. Determination of ash values

Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave ash usually consisting of carbonates, phosphates, and silicates of sodium, potassium, calcium, magnesium.

Determination of total ash,acid insoluble and water soluble ash values were carried out for the leaves of ***Albizia lebbeck*** was foud to be 7.6,2.4 and 2.10 respectively.

Table 1.Determination of Ash values %w/w

S.No	Types of ash	Content %w/w
1	Total ash	7.6
2	Acid insoluble ash	2.4
3	Water soluble ash	2.10

7.2.2. Determination of extractive values

Extractive value determines the amount of active constituents extracted with solvent from a given amount of medicinal plant. It gives an idea about the nature of the chemical constituents present.

It gives an idea about the nature of the chemical constituents present in the crude drug. Useful for the estimation of constituents extracted with the solvent used for extraction. Extraction of phytoconstituents from plant materials has been of tremendous interest and

potential. Extractive value of the crude drug also indicates the quality as well as purity of the drug. For most extractions, relatively simple techniques, such as percolation and maceration are effective and economical.

The extractive values were determined with different solvents and the percentage yield of soluble matters were observed in decreasing order of water, ethanol, ethyl acetate and petroleum ether respectively. The extractive values of *Albizia lebbeck* aqueous leaves extract were found to be higher than that of ethaloic extract; the results are tabulated in the Table

Table 2. Determination of extractive values %w/w

S.No	Name of the solvent	Extractive value %w/w
1	Ether	16.5
2	Ethyl acetate	15.6
3	Ethanol	19.6
4	Water	21.8

7. 2.3. Determination of Foreign organic matters, crude fiber content and loss on drying

The foreign matter should be detected by inspection with the unaided eye or by the use of a lens. Separate and weigh it and calculate the percentage present. Herbs drugs should be free, as much as possible, of mold, insects and other animal contaminants. The amount of foreign matter should not be higher than the standard regulated for each product according to the pharmacopoeia monograph. Over the last decade, significant developments have been made in our understanding of crude fiber and its role in the promotion of health and disease risk reduction. A wealth of scientific evidence demonstrates that adequate dietary fiber intake has a number of health benefits, including maintenance of healthy laxation and the reduced risk of cardiovascular disease and cancer. The crude fiber content is one of the physical parameters used to determine the quality of the crude drug and is also useful to distinguish and identify the crude drug. The estimation of crude fiber comprises the measurement of length of fiber, lignin, cellulose and cork cell.

The determination of moisture content is one of the important physical parameters to minimize the error in the estimation of actual weight of the drug material. Further, moisture content is essential to minimize the microbial content or microbial growth and this can maintain better stability of crude drugs during storage.

The determination of foreign organic matters, crude fibre content and loss on drying were carried out for the leaves of *Albizia lebbeck* and results were tabulated in the Table no.

Table 3.Determination of foreign organic matters, crude fiber content and loss on drying

S.No	Parameters	%w/w
1	FOM	4.4
2	Crude fibre	6.2
3	Loss on drying	8.4

7.2.4. Determination of Fluorescence analysis

Fluorescence is the phenomenon exhibited both in visible and UV- light by various chemical constituents present in the plant material. Some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.

The powdered drug was examined under UV and ordinary light with different reagent and results are reported in the ginen table.

Table 5.Determination of Fluorescence analysis

Solvent used	Colour reaction observed
Conc. H_2SO_4	Blacksh
Aqueous $FeCl_3$	Reddish
Iodine solution	Blueish
Picric acid solution	Yellowish
Aqueous $HgCl_2$ solution Brownish	Aqueous KOH No change
Dilute HCl	No change
Aqueous $AgNO_3$ solution	No change
Dilute NH_3 solution	No change
Aqueous KOH	No change

7. 2.5. Determination Microbial content

The determination of total microbial limit tests is the most important physical evaluation. WHO guideline also recommends to determine the microbial limits in raw materials and finished products. The determination of total microbial limit tests was carried out as per the WHO guideline. The molten agar medium Soybean-Casein Digest agar (SCD) for aerobic organisms and Sabouraud Dextrose Agar (SDA) for fungi were used against the raw materials of both the plants. All the plates were incubated at 35-37°C &20-25°C for about 24hrs for bacteria and 48 hrs for fungi respectively. No colonies were found in the sample of *Albizia lebbeck* after respective incubation time. The present study was concludes the absence of bacteria, fungi and moulds in the selected plant of *Albizia lebbeck*.

7. 2.6. Analysis of metals and minerals

The contamination of the environment requires the content of heavy metals in medicinal plants to be limited and controlled. Heavy metal contamination in herbal medicines is a global threat to human beings especially at levels above known threshold concentrations.

The heavy metals are not easily metabolized by body and are found to accumulate in the soft tissues. They produce toxic effects due to their interference in many known normal

biochemical and metabolic processes. Several health problems were linked to excessive uptake of dietary heavy metals, including decreased immunity, cardiac dysfunction, fetal malformation, impaired psychosocial and neurological behavior. Pb and Cd are not essential elements that are required neither in the human body nor in plants, and which cause various bimolecular adverse functional effects at low level doses.

In the present study, initially the powdered materials of *Albizia lebbeck* (AL) was prepared using microwave assisted wet digestion method and the present elements were analyses by using Atomic Absorption Spectrometer (FAAS- erkin Elmer 400).The determined metal content present in leaves of *Albizia lebbeck* was within the limits. The results were tabulated in the given Table no.

Table 6. Heavy metal analysis of AL leaves

S.No	Name of the Trace elements	Amount in ppm
1	Fe	20.06
2	Cd	1.26
3	Pd	6.20
4	Ni	12.24
5	Mn	8.02
6	Zn	32.44

7. 3. PHYTOCHEMICAL STUDIES

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. Different qualitative phytochemical analyses are known that allow, by using standard analytical techniques, the determination of chemical groups, or compounds in the extracts from different plants.

The plant extracts are then analyzed for the presence of secondary metabolites like alkaloids, terpenes, and flavonoids. Standard tests are available in the literature for each class of compounds to be analyzed.

The phytochemical evaluation of the present study was measured qualitatively and quantitatively from the aqueous and methanol leaves extract of *Albizia lebbeck.*

7. 3.1. Preparation of extracts

Dried, coarse powdered leaves of *Albizia lebbeck* (*AL*) was used for the preparation of extracts by successive solvent extraction process in Soxhlet apparatus. Petroleum ether (60-80%) and ethanol (95%) were used for hot extraction and aqueous extract was prepared by cold maceration process. The plant material (500g) was extracted separately with respective solvents (1.5Lt) and each extraction was carried out for 72hrs. Both the leaf extracts were found dark green to brown in colour with light odour. The percentage yield of the aqueous extract was high (21%), than that of ethanol (19%).

7. 3.2. Qualitative chemical tests

The phytochemical studies play an important role in the identification of active constituents in the extracts. The leaves of ethanol and aqueous extracts of Albizia lebbeck content the phytoconstituents were identified and discussed in the below table.

Table 7 Phytoconstituents in the ethanol and aqueous extract of *Albizia lebbeck*.

S.No	Phytochemical Constituents	Ethanol Extract	Aqueous Extract
1	Carbohydrates	+	++
2	Alkaloids	+ +	-
3	Glycosides	+	+
4	Triterpenoids	+	+
5	Flavonoids	+++	+
6	Phenols	++	-
7.	Tannins	+	+
7	Steroids and sterols	+	-
8	Saponins	+++	+

+++More intense in colour, ++ Moderate, + less and – Absence

The carbohydrate present in all the extracts were confirmed from the formation of brown purple ring in Molish test and formation of brick red precipitate in Benedict's test. Fehlings test gives brick red precipitate which further conform the carbohydrate present in the ethanol and aqueous extracts.

The present alkaloids in ethanol extract was identified by Mayer's (Yellowish ppt), Dragondroff's (orange brown ppt), Wagner's (reddish brown ppt) and Hager's tests (yellow ppt) and the absences of alkaloids were noticed in aqueous extract.

The flavanoids present in both the extracts were confirmed by the appearance of blackish red colour in ferric chloride test and magenta colour by Shinod's test also observed more indense colour in ethanol extract than that of aqueous extract. The Legals and Baljet tests were confirmed the presence of glycosides in both the extracts.

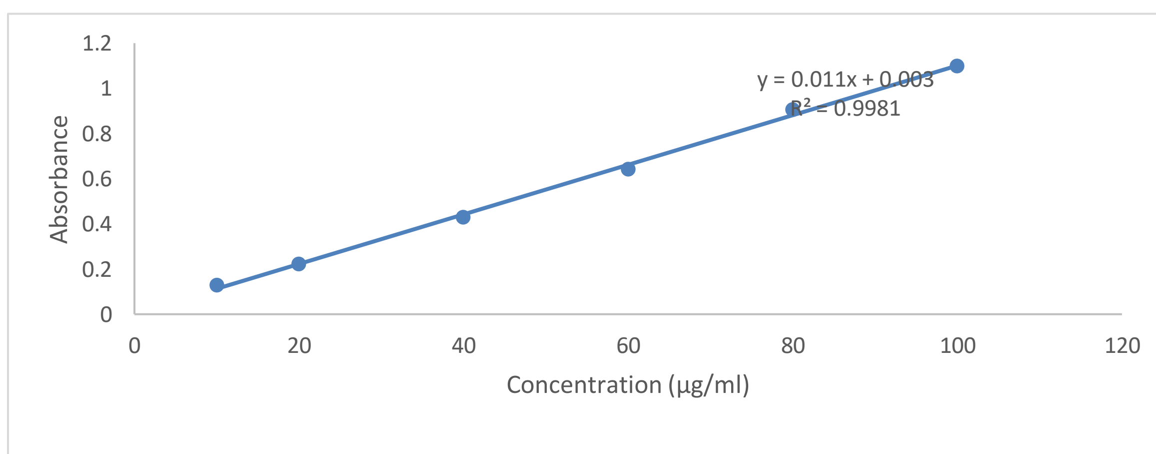
Phenolic and tannins were also confirmed in all the extracts from ferric chloride and lead acetate tests and absence of Phenol in aqueous extract was noticed. The absence of steroids in the aqueous extract was confirmed by Libermanns and Burchard and Salkowskis tests.

The saponins present in more in ethanol extract than that of aqueous extract s were confirmed by foam test.

7. 3.3. Estimation of Total Phenols in Ethanol extract of AL

Table 8 Estimation of total Phenol content of Test

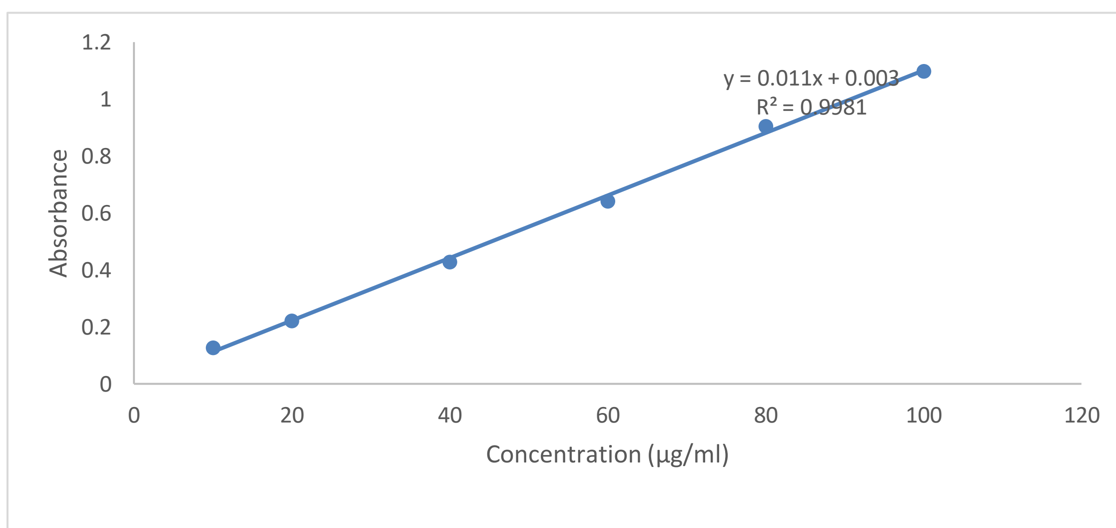
	Concentration (µg/ml)	**Absorbance at 725 nm**
Standard (Gallic Acid)	10	0.097
	20	0.127
	40	0.195
	60	0.348
	80	0.412
	100	0.502
Test	100	0.278



7. 3.4. Estimation of Total flavanoids in Ethanol extract of AL

Table 9 Estimation of total flavonoid content of Test

Sample	Concentration (µg/ml)	Absorbance at 415nm
Standard (Quercetin)	10	0.127
	20	0.221
	40	0.428
	60	0.642
	80	0.905
	100	1.098
Test	100	0.489



7. 3.5. TLC studies

The thin layer chromatography (TLC) was used as a preliminary study in identification and separation of the components in the extracts. The Silica gel G and GF were used as stationary phase and the chromatogram was developed with different solvent systems and as a final point Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:27) has selected an ideal mobile phase for identify the present galic acid and Quercetin in the ethanol extract of AL. The gallic acid and present Quercetin were identified with standard reference and Rf value. The report was discussed in the given table.

Table 10 Identification of Gallic acid and Quercetin in ethanol extract of AL

S.No	Name of the Sample	Rf Value
1	Gallic acid	0.68
2	Quercetin	0.8
3	EA extract of AL	0.66 0.78

7. 4.1. IN VITRO STUDIES

***In vitro* antioxidant studies**

Various studies have been done to identify antioxidants from plant sources and efforts have been taken to incorporate it in conventional therapy. In our present study, ethanolic and aqueous extracts of Albezea libbic leaves extracts have been evaluated for *in vitro* antioxidant activity using DPPH radical scavenging and ABTS reducing power assay.

Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by ROS.Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects.

Table 11 DPPH and ABTS radical scavenging activity of EH and aqueous extracts of leaves of AL

S. No	Sample	IC 50 (µg/ml) DPPH	IC 50 (µg/ml) ABTS
1	Ascorbic acid	27.1±1.5	115±7.8
2	EH extract	67.7±2.6**	197±8.6*
3	AQ extract	44.3±3.2***	176±8.2*

(Values represent means ± standard deviation, n= 6)

7.4.4.1. Estimation of DPPH (2,2 diphenyl-1 picrylhydrazyl) radical scavenging assay

The DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radical was initiated by the lipid auto-oxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable dia-magnetic molecule. The reduction capability of DPPH was determined by decrease in its absorbance at 517 nm, which is induced by anti-oxidants. The scavenging effect of EH and AQ extracts of *Albizia lebbeck* was compared with standard ascorbic acid. On the DPPH radical the EH extract has significant scavenging effects than that of aqueous extract when compared with respect to standard.

Free radicals are known to be a major factor in biological damages and DPPH radical is extensively used to evaluate the free radical scavenging activity of natural compounds. DPPH which is a radical itself gives a purple colour those changes into a stable yellow colour compound by reacting with an antioxidant. The extent of the reaction depends on the hydrogen donating ability of the antioxidants. The DPPH scavenging ability of both the extracts were reported given table. The concentration of antioxidants needed to decrease the initial DPPH concentration by 50% (IC_{50}) it is a key parameter widely used to measure the antioxidant activity.

7.4.4.2. Estimation of ABTS radical scavenging assay

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activity is used for the determination of complex antioxidant mixtures in the plant extracts. ABTS is a protonated radical that has characteristic absorbance maxima at 734nm, **Emad *et al.* (2013)** which decreases with the scavenging of the proton radicals. ABTS scavenging activity of both the extracts is presented in table. The concentration of antioxidant needed to neutralise the ABTS radicals by 50% (IC_{50}). As the IC_{50} value of the extracts decreases, the free radical scavenging activity increases. The investigated EH extract of AL exhibited ability to scavenge the stable DPPH free radical reaching 50% of reduction with an IC_{50} value of 67 μg/ml, whereas the standard ascorbic acid showed IC_{50} value of 115 μg/ml. Further, it was found that the EH extract showed better scavenging activity compared to the aqueous extract of *Albizia lebbeck*.

The decolorization of ABTS+ radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. Thus monitoring the antioxidant activity by ABTS+ radical scavenging assay gives good prediction of their ORAC and DPPH radical scavenging capacity. EH extract of AL showed potential activity in ABTS decolorization further 62 % inhibition was noted for EH extrac. Similar to DPPH, it was found that the EH extract showed significant scavanging activity than queous extract. Decolorization of ABTS+ in the present study reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical cation.

The findings of the study suggest these plants have a potential scavenging activity and excellent sources of antioxidant properties due to presence of flavanoids in the extracts.

7.4.4.3. Antimicrobial studies

Plant products and its derivatives serve as a protype to develop less toxic and more effective medicines in controlling the growth of various pathogens including bacteria, fungi and virus. Numerous studies have been conducted and reported with the various plant extracts and screened the antimicrobial activity as well as for the discovery of new antimicrobial compounds. Therefore, medicinal plants are findings their way into pharmaceuticals.

In present investigation was screened the antibacterial and antifungal studies were carried out by disc diffusion method against four pathogenic Gram +ve, Gram –ve bacteria and two fungi respectively as discussed in below table. The ethanol and aqueous extracts of leaves of AL were used at a concentration of 250µg/disc. Ciprofloxacin (10µg/disc) and Clotrimazole (10µg/disc) were used as standard for bacteria and fungi respectively. Disc diffusion method was carried out by using Muller-Hinton agar as bacterial and Sabournds Dextrose agar as fungal media. The antibacterial and antifungal potential of extracts were measured by zone of inhibition of the microbial growth. This study represents the preliminary report on the antimicrobial activity of the crude extracts of ethanol and aqueous extract of AL.

The anti bacterial activity of leaves of AL extracts shows potential activity against the following strains in order of *Staphylococus albus, Staphylococus aureus, Bacillus subtilis, Bacillus lintus, Salmonella paratypi, Pseudomonas auroginosa, Klebsiella pneumoniaand, Escherichia coli* respectively and moderate antifungal activity was found against *Candida albicus* and *Aspergillus niger.*

The findings of the antimicrobial study prove that the EH extract of AL crude extract exhibited considerably higher antimicrobial activity against various strains. The maximum zone of inhibition was produced by EH extract of AL crude extract against *staphylococcus albus.* The EH extract showed more effective than that of aqueous extract. The antifungal activity were noticed less in both the extract when compared to anti bacterial activity.

Table 12 Antimicrobial study zone of inhibition report

Name of the extract	Diameter of zone of inhibition in mm									
	G+ve strains				G-ve strains				Fungi strains	
	B.lints	***B.Subtilis***	***S.aureus***	***S.albus***	***Salmonella paratyphi***	***P.auroginosa***	***K.pneumonae***	***E.coli***	***Candida albicans***	***Aspergillus niger***
STD (Ciprofloxacin) Clotrimazole	11	20	26	32	27	18	20	14	5	6
EH extract of AL	10	14	22	28	22	17	18	10	12	6
AQ extract of AL	10	10	14	18	12	10	10	12	10	12
									8	8
									12	12
									8	8
									11	8

EH – Ethanol extract and **AQ** – Aqueous extract

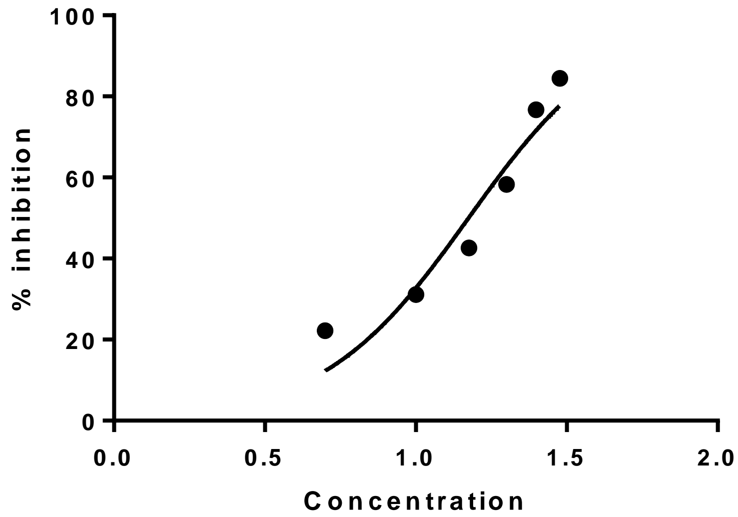
7. 4.4.4. In vitro cytotoxicity studies

Cell cytotoxicity assays are commonly used *in vitro* bioassay methods to predict the toxicity of substances in various tissues and the ability of a chemical to elicit a corrosive response is easily predicted using appropriate end point of the study because its reveal the degree of damage caused by test substances. The MTT assay is most widely used to determine the cytotoxic potential of medicinal agents and other toxic materials. The reduction of yellow to purple coloured formation takes place only when mitocodrial redactase enzymes are active and therefore the conversion can be directly related to number of viable cells **Sanchoz *et al*.**

The ethanol extract of leaves of *Albizia lebbeck* was screened for cytotoxicity study by MTT assay method. The study revealed that none of the extracts possess cytotoxic effects on NIH 3T3 cell lines during the 48 hrs exposure. It was found that more than 95% cell viability was observed at different concentrations used (6.25, 12.5, 25, 50 and 100µg/ml).

Table 13 Percentage inhibition and IC50 values of DPPH

Sample	Concentration (µg/ml)	% Inhibition	IC_{50}(µg/ml)
Standard (Quercetin)	5	22.20	14.95
	10	31.14	
	15	42.63	
	20	58.26	
	25	76.68	
	30	84.45	
Test EH extract of AL	10	24.43	33.69
	20	37.50	
	40	47.51	
	60	57.75	
	80	78.12	
	100	86.12	



DPPH radical scavenging activity of quercetin

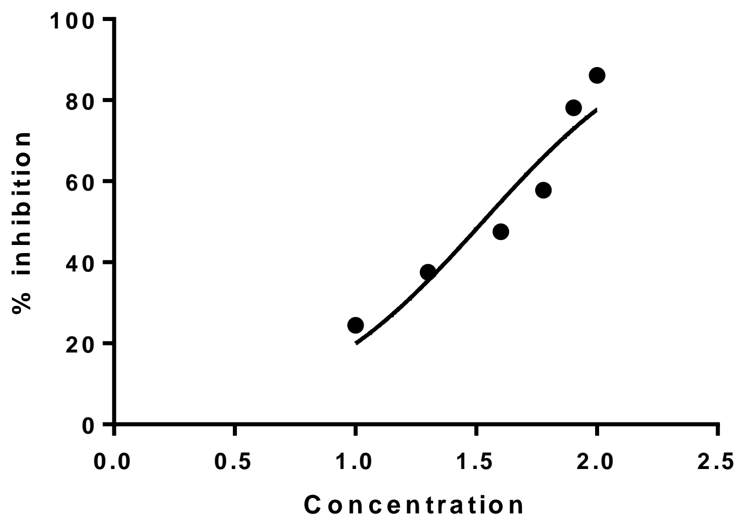


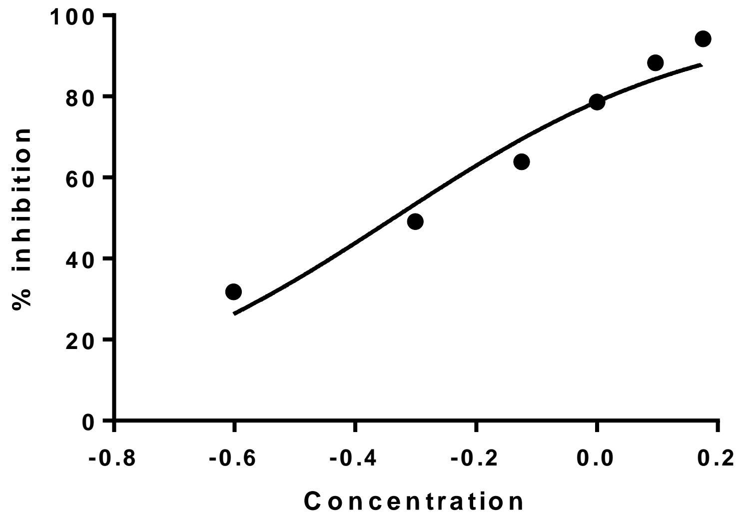
Figure 9: DPPH radical scavenging activity of Test

7.4.4.1.2 Estimation of ABTS radical scavenging assay

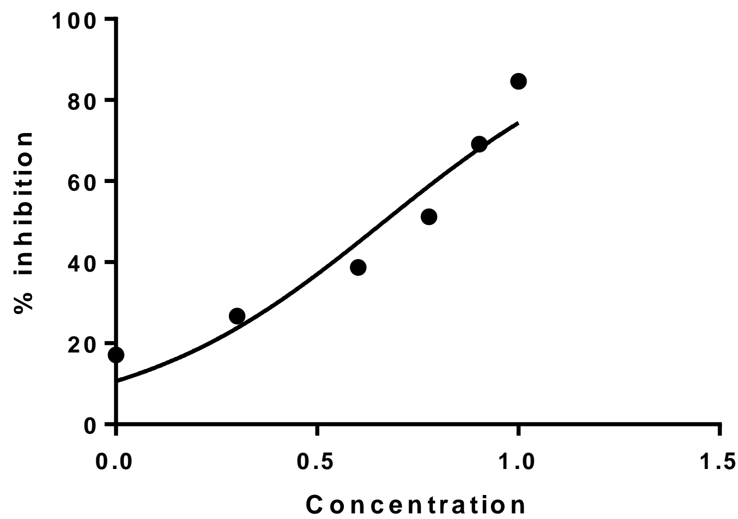
ABTS RADICAL SCAVENGING ACTIVITY

Table 14 Percentage inhibition of ABTS

Sample	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50}(µg/ml)**
Standard (Quercetin)	0.25	31.76	0.46
	0.5	49.14	
	0.75	63.89	
	1.0	78.64	
	1.25	88.33	
	1.5	94.21	
Test EH extract of AL	1	17.13	4.64
	2	26.72	
	4	38.7	
	6	51.22	
	8	69.14	
	10	84.66	



ABTS radical scavenging activity of quercetin



ABTS radical scavenging activity of Test

7. 4.5. PHARMACOLOGICAL SCREENING

Evaluation of acute toxicity studies

Table General appearance and behavioral study of Ehanol extract of leaves AL

S.No	Response	Before	After
1	Alertness	Normal	Normal
2	Grooming	Absent	Absent
3	Anxiety	Absent	Absent
4	Roaming	Normal	Normal
5	Sniffing	Normal	Normal
6	Tremors	Absent	Absent
7	Convulsion	Absent	Absent
8	Depression	Normal	Absent
9	Gripping strength	Normal	Normal
10	Scratching	Present	Present
11	Defecation	Absent	Present
12	Writhing	Absent	Absent
13	Pupils	Normal	Normal
14	Urination	Normal	Present
15	Salivation	Normal	Normal
16	Skin cloure	Normal	Normal
17	Lacrimation	Normal	Normal

Antianxity Study:

Open Field Test

The open field apparatus is constructed of white polywood that measures 72 × 72 cm with 36 cm walls. Red lines were drawn on the floor with a marker and were clearly visible through the clear Plexiglas floor. Mice were injected (i.p) with the test once per day for 7 days. The test EH extract was performed 30 min after the last administration of the test or saline (10 mL/kg). The standard drug diazepam (1 mg/kg, i.p.) was given once 30 min before the test. The mice were placed in the open field box for 6 min, and their behaviors were recorded. The behaviors scored included time spent at the center square, number of the lines crossed in the floor of the maze, rearing frequency (number of times the animal stood on its hind legs), and grooming (duration of time the animal spent licking or scratching itself while stationary).

Open-field test

Group	**Number of line traversed**	**Rearing Time (s)**	**Time Spent at the centre (s)**
Control	38 ± 3.69	30.54 ± 3.69	8.70 ± 0.23
Diazepam (1mg/kg)	6 ± 0.23*	10.21 ± 0.87*	123.02 ± 2.25*
Test I	18 ± 1.98*	17.01 ± 1.32*	65.23 ± 4.23*
Test II	14 ± 1.68*	13.21 ± 1.05*	81.32 ± 4.85*

Values represent mean ± SD, n=6.Data were analyzed by one-way analysis of variation (ANOVA) followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns- non significant. Standard and test were compared with control group.

Elevated Plus-Maze Test

Behavior in the elevated plus maze (EPM) is used to assess exploration, anxiety, and motor behavior. The EPM consists of four arms, 49 cm long and 10 cm wide, arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 50 cm above the floor. Two arms were enclosed by walls 30 cm high and the other two arms were exposed. Rats were injected i.p. with the test or saline (10 mL/kg, i.p) once per day for 7 days. The positive control diazepam (1 mg/kg, i.p) was given once 30 min before the test. Thirty minutes after the i.p. injection of the last dose of extract or saline, each animal was placed at the center of the maze facing one of the enclosed arms. During a 5 min test period, the number of open and enclosed arms entries, as well as the time spent in open and enclosed arms, was recorded as previously described. Entry into an arm was defined as the point when the animal places all four paws into the arm. After the test, the maze was carefully cleaned with 10% ethanol solution and allowed to dry before the next animal.

Elevated Plus-Maze Test

Group	Time Spent in Open Arm	Time Spent in Closed Arm
Control	51 ± 1.26	249 ± 5.89
Diazepam (1mg/kg)	232 ± 4.68	68 ± 1.28
Test I	198 ± 3.69	102 ± 2.58
Test II	210 ± 4.58	90 ± 1.02

Values represent mean ± SD, n=6.Data were analyzed by one-way analysis of variation (ANOVA) followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns- non significant. Standard and test were compared with control group.

8 CONCLUSION

8. CONCLUSION

In fact, in humans, it is a common emotion intimately associated with proper fear and probably functions as a mechanism to adapt the environment psychologically. Worldwide, one in five people meet with clinical criteria of anxiety disorder at least once in their lives. The study of anxiety has developed into a key area of psychopharmacological research during this decade. It has been observed that people who suffer from anxiety often accompanied with sleep disorder. The most common sleep disorder is a subjective complaint that it has an inability to initiate or maintain sleep, or the sleep is non-restorative with poor quality and quantity. It is estimated that about 9 to15% of the people in the world suffer from insomnia which causes severe after effects in the day time.

Anxiety disorders affect over 18 percent of people in the United States every year. Over the years, treatment options for anxiety disorders have widened to include both prescription medications and natural alternatives.

Research trusted source suggests that several herbal supplements may be helpful for conditions such as anxiety, depression, and more. Scientific studies indicate that certain herbs may help to alleviate the symptoms of anxiety. As with the evidence the present study provides valuable information of Phrmacognostical, Pphysicochemical, Phytochemical, Cytotoxic and Pharmacological values of the selected plant extract *Albizia lebbeck.*

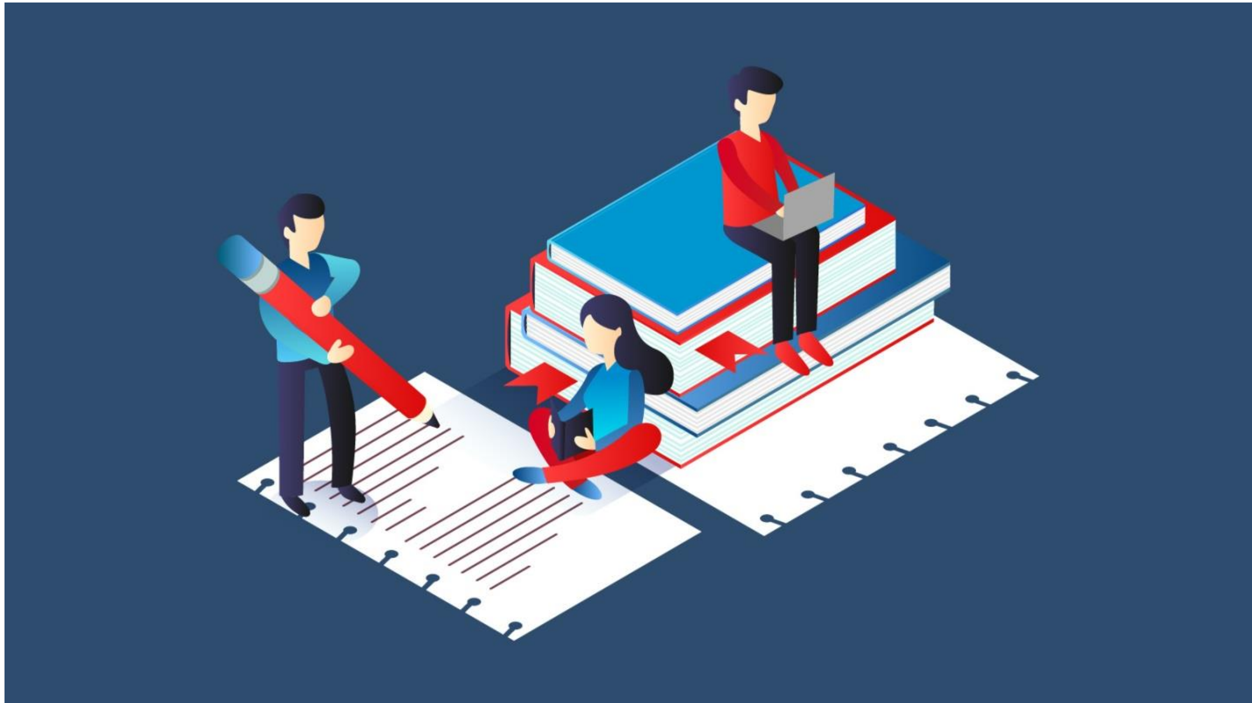
The Pharmacognostical studies provide the morphological characters that could be useful to identify and evaluate these plants from adulteration.

The Phytochemical studies confirmed the presence of alkaloids, glycosides, phenols, triterphenoids, saponins and flavanoids in the ethanolic extract of AL and the Present flavonoid Quercetin has confirmed from the TLC study.

The *in vitro* studies includes antioxidant, antimicrobial and cytotoxicity was carried out for the EH and aqueous extracts of AL. In vitro antioxidant activity of EH extract of AL was clearly evident from the DPPH and ABTS radical scavenging activity. The antimicrobial studies were carried out against selected bacterial and fungal strains by disc diffusion method. The EH extract posses moderate activity than that of aqueous extract,

The EH extract of leaves of AL was non toxic, and it was confirmed by Cytotoxicity study on 3T3 cell lines.Acute toxicity studies for EH extract of AL was carried out upto 2000mg/kg, and they did not cause any mortality in experimental animals. The pharmacological studies confirmed that EH extract leaves of AL possesses significant anti anxiety activity confirmed with the screened parameters.

The mechanism of action of anti anxiety activity of these plant extract is yet to be explored in future studies.



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10 ANNEXURE

10. ANNEXURE