ANTIMICROBIAL AND PHARMACOLOGICAL PROPERTIES OF SOME MEDICINAL PLANTS

By

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A THESIS

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Dedication

I dedicate this thesis to my loving parents

Who painted my bootsteps to the path of learning

To my sisters, brothers and my lovely son Abdullah

The real meaning of the life, for thoughtfulness,

Support and encouragement.

Manal Ibrahim.
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Abstract

Comparative antimicrobial effects of extracts from seeds of *Linum usitatissimum* and *Lepidium sativum*, leaves and stems of *Monechma ciliatum* against standard organisms (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*) were investigated. *Monechma ciliatum* was chosen for further pharmacological and toxicological effects because of its potent antimicrobial activity.

Methanolic extract of *Lepidium sativum* caused significant antimicrobial activity while chloroformic and water extracts have low activity. *Linum usitatissimum* had very low activity. *Monechma ciliatum* stems methanolic extract was shown significantly active more than chloroformic extract. Water extract caused inhibition zone against *Psedomonas vulgaris* only. Methanolic extract of *Monechma ciliatum* leaves was the most active extract against standard organisms.

The pharmacological activity of Methanolic extract of *Monechma ciliatum* leaves was investigated on the isolated tissues of Rabbit jejunum, Guinea pig illeum and rat fundus and uterus. There were contraction on the rabbit jejunum and guinea pig ileum. The contraction caused by the guinea pig illeum was not blocked by Atropine and Chlorophenramine, but completely blocked by serotonergic blocker, Cyproheptadine. Also rat fundus and uterus caused significant contraction that blocked by Cyproheptadine.

Toxicity study of methanolic extract of *Monechma ciliatum* leaves showed that the extract was less toxic.
لوجر، وحمة، وسقان، مراج، بذرة، طبية، نبات، ثلاثة، على، دراسة، هذه، أجرى، أفضل، اختر، ثم، ومن، الميكروب، ضد، نشاطها، لمعرفة، وذللك، الأسود، الحليب، وأوراق، الدوائي، نشاط، الإختبار، فعال، مسثلص، (الفارمولوجيا، وسمته).

على، النبات، اختبار، تتم، الذهبية، المصدرة، البيئية، القياسية، الميكروب، ضد، له، والكلوروفوري، المياه، مسثلص، النباتين، بمساكن، مقارنة، الأثار، ضعيفة، الاختبار، التي، الكنان، بذرة، نبات، مسثلص، الأثرين.

الذي، البذرة، الببتيدية، خاصة، نشاط، أكثر، كانت، الأسود، الحليب، نبات، أوراق، أما، الميكروب، ضد، متوسطة، نشاطاً، الأسود، الحليب، بذرة، نبات، الأعطر، وقد، النتائج، أفضلاً، قياسية، واختبار، تتم، البائس، جزر، ورم، للعدة، الورمير، وجمع، جن، وامع، الرنب، مى، بتحفز، مسثلص، قام، انتفاخ، هذا، الفحل، بينما، الأثر، هذا، الفحل، ومن، والكلوروفورمات، الأرتورين، يتكون، للرغم، إنه، البذرة، الببتيدية،photos/best沦为 facilitate the discussion, the document has been translated into Arabic and consists of a text discussing various aspects of plant biology and microbiology. The text is filled with scientific terms and concepts, indicating a focus on research and analysis in the field of plant biology and microbiology.
INTRODUCTION

Plants have been used for thousands of years for many different purposes ranging from medicinal to religious (in rituals) and from protection against spirits to culinary delights, perfumery and cosmetics (Elghazali et al., 2004). Plants have been considered an important source of indispensable drugs.

The relationship between man and plants has always been a very close one throughout the development of human culture, and no doubt, the herbalist is probably one of the first professional in the evolution of human cultures. The uses of the plants as drugs lead researchers to study more about these plants to determine their mode of action and active principles i.e. pharmaceutical value.

Traditional medicine in Sudan is practiced in rural as well as urban areas of the Sudan for many years ago for lack of proper medical care, difficulty in availability of appropriate means of transportation to provide rural areas with medicines and their high price. Sudanese people used many traditional and folklore as the uses of medicinal plants for curing the diseases and relief pain.

The plants selected for this investigation are used in folk medicine for their effects are known for its antimicrobial activities but this has not been confirmed by experiments.

The objective of this study is intended to:-

1- Establish a well documented baseline information about the antimicrobial activities of three medicinal plants named Monechma ciliatum, Lepidium sativum and Linum usitatissimum.
2- To select the most potent antimicrobial plant extract and to test their pharmacological activity in isolated organs.

3- To investigate the toxicity of this selected plant.
CHAPTER ONE
LITERATURE REVIEW

The use of plants for treatment of various diseases, as specific against magic and for religious ceremonies is universal, and has been practiced for many years; even up to now where many people are treated by modern drugs, in East Africans, both literate and illiterate people still use local plants as drugs in many conditions (Kokwaro, 1976).

Chinese medicinal plants, which have been used for centuries, are still being widely used today within the framework of health care services. A recent nation wide survey has shown that there are 7295 species of plants used medicinally in China (Ciba Found, 1994). However, he reported that in an ethno pharmacological investigation of 100 species 15 species with a high proportion of therapeutic agents was found. He also reported that medicinal plants are an important source for new drug development in China. Up to now about 200 new drugs have been developed directly or indirectly from medicinal plants. Nearly one half of the new drugs are from a single medicinal plant, or its active principle and synthetic derivatives, or active fraction or even the total extract.

Through antimicrobial agents of synthetic origin and those made by fermentation processes, commonly used in therapeutics, no use of antibiotic of higher plant origin has been reported. However, plants may represent a potential source of antibiotic (Aizenman, 1978).
Furthermore, in the various health care systems of the world, there are no accurate data available to assess the value and extent of use of plants or their derived active principles. However, the World Health Organization has estimated that 80% or more than four billion inhabitants of the world rely primarily on traditional medicine for primary health care need. It can be presumed that major part of traditional medicine involved the use of plant extracts and their derived active principles, though their use is not always verified by scientific means (Aizenman, 1978).

For many years, Africans people have developed a store of empirical information concerning the therapeutic values of Africans plants (Kokwaro, 1976). He also stated that, some of the plants used for direct or specific treatment of particular disease are worth further investigation in order to determine their chemical composition and their mechanism of action.

In the Sudan medicinal folklore passed from one generation to another but was never documented (Elghzali et al., 1997).

1.1. Plants as source of drugs:–

*Acaia nilotica* is traditionally used to treat sore throat, colds, bronchitis, pneumonia, ophthalmia, diarrhoea, dysentery, leprosy venereal diseases and haemorrhages. Aqueous extract of fruits showed activity against bacteria and fungi (Abdel Nabi et al., 1992). Elgazali et al. (2003) reported that, maceration of the roots of *Acaia polyacantha* is used for diarrhoea and vomiting.

found that *Khaya senegalensis* is used to treat malaria and for wound healing.

Some medicinal plants can destroy helminthic parasites by different mechanisms. *Ananas comosus*, Pineapple (*Bromeliaceae*) contains the proteolytic enzyme bromelain, and can destroy the parasite through lysis (Oliver-Bever, 1986). He also reported that other plants can act specifically on cestodes, especially those containing cucurbitine as from *Cucurbita pepo*, Pumkin and shammam (*Curcurbitaceae*) and saponins from *Opilia cettidifolia*.

The powder of the whole plant of *Barleria ruellioides* mixed with Gossypium seeds are used externally against scabies (Elgazali *et al.*, 2003). They also reported that decoction of the barks of *Anogessus leiocarpus* are used against cough while maceration of whole plant of *Clitoria ternatea* is used against constipation and *Dicoma tomentosa Cass* leaves used to relief tooth pains.

Vernolepin and vernodalalin were isolated from leaves of *Vernonia amygdalina*, both compounds exhibited activity against a panel of bacteria (Almagboul *et al.*, 1997).

The decoction of the barks of *Gynocarpus jacquinii* is used against cancer and renal troubles whereas the powdered fruits mixed with sesame oil for rheumatism (Elgazali *et al.*, 2003). The same authors found that, maceration of whole plant of *Polycarpea corymbosa* is used as antifungal remedy. *Wissadula rostrata* the powdered seeds mixed with (Alkohl) are used to treat conjunctivitis and eye infection.
The effects of Guava leaves extract on bleeding time had been studied. Water extract of Guava leaves extract was used as topical haemostasis (Jaiarj et al, 2000).

Decoction made from stem and leaves of *Anisomeles indica* (*Lamiaceae*) possesses analgesic activity (Dharmasiri, 2003).

*Echinacea angustifolia* rhizome was used by North American Plains Indians, for various herbal remedies. Echinacea was one of the basic antimicrobial herbs of Eclectic medicine in the mid 1800s through the early 1900s and its use was documented for snakebite and anthrax. In the 1930s Echinacea became popular in both Europe and America as an herbal medicine prepared from the above-ground parts and the root. Echinacea has been popularly attributed with the ability to boost the body’s immune system and ward off infections, particularly the common cold (Wikipedia, 2007).

1.2. Antimicrobial plants:-

Samy and Ignacimuthu (2000) screened thirty Indian medicinal plants used by tribal healers to treat infections, for antibacterial properties. They were tested at 10mg/ml concentration using disc diffusion method against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris*, *Pseudomonas aerogenes* and *Staphylococcus aureus*. Twenty plant species showed activity against one or more species of bacteria, ten plant species were found inactive against all tested bacteria. These results were compared with results obtained using standard antibiotics, Chloramphinicol and Streptomycin (30microg/ disc) which served as a reference for inhibition zone diameter.
Two new antimicrobial peptides related to the gamma-thionine family have been isolated by acid extraction from the broad bean *Vicia faba*. The extract was separated by ion exchange chromatography, and a fraction showing antibacterial activity was further purified by reverse-phase HPLC. Material from a single HPLC peak was sequenced and revealed the presence of two peptides differing by one amino acid. The peptides were named fabatins. Fabatins were active against both Gram-negative and Gram-positive bacteria, but were inactive against the yeasts *Saccharomyces cerevisiae* and *Candida albicans* (Zhang and Lewis, 1997).

Mehrabian *et al.* (2000) studied the antimicrobial effects of the aqueous, methanolic and chloroformic extracts of *Rubia tinctorum*, *Carthamus tinctorius* and *Juglans regia* on some airborne microorganisms. It has shown that extracts of these plants have antimicrobial effects on, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus mycoides*, and also on some fungi *Aspergillus niger*, *Penicillium expansum* and *Geotrichum candidum*. The antimicrobial effects which was detected are "microbicidal". The aqueous and the chloroformic extracts had the most and the least microbicidal effects, respectively.


Mohamed (1979) reported a potent antimicrobial effect of *Hibiscus sabdariffa* (Karkadeh). He found that various extracts of the plant have a wide range of antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as yeast. He was also reported that water extract of Karkadeh had inhibitory effects against
the standard organism namely, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. Waffaa (1989) found that all concentrations of the water extract of karkadeh possess antibacterial activity. The diameter of the inhibitory zones increased with the increase in concentration.

A number of essential oils from Mongolian aromatic plants are claimed to have antimicrobial activities. The essential oil of *Dracocephalum foetidum*, a popular essential oil used in Mongolian traditional medicine, was examined for its antimicrobial activity.

The essential oil of *Dracocephalum foetidum* exhibited strong antimicrobial activity against most of these pathogenic bacteria and yeast strains by both the agar diffusion method and the minimum inhibitory concentration (MIC) assay (MIC range was 26-2592 microg/ml). Interestingly, *Dracocephalum foetidum* even showed antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains (Lee, *et al*, 2007).

Molina-Salinas *et al*. (2007) evaluate the potential antimicrobial activity of 14 plants used in northeast Mexico for the treatment of respiratory diseases, against drug-sensitive and drug-resistant strains of *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae* and *Mycobacterium tuberculosis*. Organic and aqueous extracts were tested against these bacterial strains. The aqueous extracts showed no antimicrobial activity, whereas most of the organic extracts presented antimicrobial activity against at least one of the drug-resistant microorganisms tested. Methanol-based extracts from the roots and leaves of *Leucophyllum frutescens* and ethyl ether extract from the roots of *Chrysanthinia mexicana* showed the greatest
antimicrobial activity against the drug-resistant strain of *Mycobacterium tuberculosis*.

Extracts of the stem bark, wood and whole roots of *T. brownii* exhibited antibacterial activity against standard strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi*, and *Bacillus anthracis* and the fungi, *Candida albicans* and *Cryptococcus neoformans* (Zakaria, 2007).

Vlietinck and Vanden Bergh (1991) have noted that the method for assaying antiviral substances used in various laboratories is not standardized, and therefore the results are often not comparable to one another. These authors also point out that researchers must distinguish between merely toxic effects of agents on host cells and true antiviral properties of the plant extracts.

**1.3. Toxicity of the medicinal plants:-**

In tropical countries, the number of naturally occurring medicinal plant is very great. The active principles and chemical properties of many of these plants have not yet been isolated and very little is known about some of them.

In Sudan, poisoning is not quite as rare as indicated by the records (Idris et al., 1979). They also said, criminal poisoning, arrow and fish poisons, and accidental poisoning. The latter type of poisoning may be attributed to Cynogenesis, famine foods, native medicine and dermatitis venenata. A wide array of herbs were identified to cause each of the above mentioned poisons. *Capparis tomentosa* this medicinal plant is eaten by camels in West Africa and Tanzania. However, a mixture of fruits and leaves was toxic for
dromedaries at daily dose of 5g per kg body weight. Nervous signs appeared on day, and the animals died 24 hours later.

Idris *et al.* (1973) were fed fresh green leaves of *Ipomoea carnea* at the rate of 3lb/animal/day to four Nubian goats. All the animals showed general weakness, termors, paresis of the hind limbs and lateral recumbency and died within 75 days after appearance of symptoms. The most outstanding lesions were focal hepatic necrosis and congestion of the brain.

Numerous investigations have been carried out on the toxicity of plants in farm animals. Plants in the genera *Asclepiadaceae*, *Apocynaceae* and *Liliaceae* were found to contain steroid cardiac glycosides which have the ability to contract the heart muscle (Watt and Breyer-Brandwijk, 1962).

Extracts of the roots and stem bark of *Terminalia brownii* exhibited relatively mild cytotoxic activity against brine shrimp larvae. The stem wood extracts exhibited the highest toxicity against the shrimps (Zakaria, 2007).

*Asclepias mexicana* and *A. Eriocarpa*, (*Ascalepiadaceae*), contain a toxic resin, galitoxin (Couch, 1929; Baxter, 1944). Depression, weakness, staggering; convulsions, laboured breathing and recumbency are the important signs of poisoning by *Asclepias* in animals (Kingsbury, 1964).

1. 4. Pharmacological studies:-
1.4.1. Cholinergic agonists and antagonists:

Drugs which stimulate muscarinic receptors cause contraction of the isolated intestine and their effects can be blocked by appropriate
antagonists such as atropine. Contractions can also be induced by stimulation of parasympathetic ganglia with nicotinic agonists, which causes the postganglionic neuron to fire and these results in the release of acetylcholine at the neuroeffector cell junction. These effects can antagonize by muscarinic antagonists as well as ganglion blockers such as hexamethonium (Lan kitchen, 1984).

1.4.2. Cholinesterase inhibitor:

Cholinesterase inhibitors increase levels of acetylcholine, a chemical messenger involved in memory, judgment and other thought processes. Acetylcholine released by certain brain cells to carry messages to other cells. After a message reaches the receiving cell, various other chemicals, including an enzyme called acetylcholine esterase, break acetylcholine down so it can be recycled (John et al, 2006).

They also reported that, Alzheimer’s disease damages or destroy cells that produce and use acetylcholine, reducing amounts available to carry messages. A cholinesterase inhibitor slows the breakdown of acetylcholine by blocking the activity of acetylcholine esterase. By maintaining acetylcholine levels, the drug may help compensate for the loss of functioning brain cells.

The same authors said cholinesterase inhibitors may also have other mechanisms that contribute to their effects. Galantamine appears to stimulate the release of acetylcholine and to strengthen the way certain receptors on message receiving nerve cells respond to it.
1.4.3. Atropine:

Derived primarily from *Atropa belladonna*, atropine is a typical alkaloid; it is insoluble in water but soluble in alcohol, and reacts with acids to form salts, e.g. atropine sulphate and hydrochloride, which are more frequently, used than the parent alkaloid.

In addition to pure atropine salts used in the form of injection, the alkaloid is commonly used as the relatively crude belladonna-plant preparations. The more popular preparations are the powdered leaf, the tincture and a soft extract.

Following absorption the effects of atropine can be divided into two groups;

1. The action on the central nervous system; in normal therapeutic doses no cerebral effect is apparent. Intravenously in small over doses atropine has a stimulant and sometimes convulsing action on the cerebral cortex. Larger doses will excessively stimulate the medulla and still larger doses will affect the spinal cord. Excessive over dosage produces cortical and medullar depression, coma and death.

2. The action on the peripheral nervous system.

This is one of paralysis of muscarinic part of the parasympathetic division of the autonomic nervous system. The muscarinic effects of acetylcholine are seen when the postganglionic parasympathetic receptors are activated, e. g. by the alkaloids muscarinic or pilocarpine. The paralysis is not complete when single therapeutic doses are used, but even partial blocking of the
parasympathetic impulses allows greater action of the sympathetic system (Brander and pugh, 1977).

1.4.4. Histamine:-

The same authors reported that, histamine is widely distributed in plants and is found in animal foodstuffs (silage) and digesta. Histamine occurs in an inactive form in all tissues, but especially in the lungs, skin, intestinal and muscular tissues. It is formed from amino acid histidine by decarboxylation mediated by an enzyme histidine decarboxylase: bacteria decomposition of proteins also produces histamine and fairly high concentrations exist in septic foci where tissue destruction is in progress. When administrated by intra dermal injection, it elicits what is called the triple –response. The small vessels at the site of injection dilate, and then the neighboring vessels dilate via an axon reflex of a sensory nerve. Finally, as plasma escapes through the walls of the most widely dilated vessels, the central area of the reaction becomes edematous and appears raised and blanched.

Histamine can stimulate nerve-endings is well remembered from the itch and pain of a nettle sting and it has been suggested that histamine is the peripheral mediator for sensations of itch and pain. It has also been suggested that wherever tissue is damaged there is a production of histamine which causes either local or general shock.

1.4.5. Chlorpheniramime:

Chlorpheniramine is part of a series of antihistamines including pheniramine and its halogenated derivatives and others including fluorpheniramine, chlorpheniramine, dexchlorpheniramine, brompheniramine, dextromchlorpheniramine, deschlorpheniramine and
dipheniramine. Chlorphenamine has antidepressant properties, inhibiting reuptake of the neurotransmitter serotonin (Bruce, 2005).

1.4.6. 5-Hydroxytryptamine (5-HT):

This base known as serotonin was identified as vasoconstrictor released from platelets lyse in the coagulation process. It is now established that considerable quantities of 5HT occur in the argentaffin cells of the gastro-intestinal tract. It also occurs in the mast cells of rats, mice and bovines in which species it is released during anaphylaxis. It is present in other tissues and is believed to be a transmitter substance in CNS.

5-HT is produced in the body by the hydroxylation of tryptophan to 5-hydroxytryptophan which is then decarboxylated to 5-HT. The blood 5-HT is stored in platelets and these, just like granules of argentaffin and mast cells can accumulate 5-HT against a concentration gradient. Elimination of 5HT by a monoamine oxidase catalysed oxidation to 5-hydroxyindolacetic acid or by acetylation to yield N-acetyl-5-hydroxytryptamine and excretion in the urine.

The actions of 5-HT are constriction of blood vessels, especially in the lungs, and contraction of the smooth muscle of intestine, uterus and bronchial tree. Nerves and ganglion cells may be stimulated and adrenaline is released from the adrenal medulla (Brander and Pugh, 1977).

Tryptamine and its hydroxyl derivative are powerful radioprotective amines in vivo, but they also give rise to physiological changes (Van den brenk and Kathleen, 1958). Bacq and Alexander (1955) state that tryptamine is not a vasoconstrictor, but is nevertheless as good a radiation protector of mice as 5-
hydroxytryptamine. However, tryptamine causes intense smooth muscle contraction, similar to its hydroxyl derivative.

1.4.7. Cyproheptadine (Periactin):

Cyproheptadine is an antihistamine that similar to other antihistamines with which we are more familiar. Histamine, a biochemical mediator of inflammation, works by binding to histamine receptors. The histamine receptors we usually want to inactivate in combating allergic reactions are called H1 receptors and this is the type inactivated by cyproheptadine (Wendy et al, 2005).

They also reported that cyproheptadine also has some other properties of interest. It also antagonizes serotonin, a neurotransmitter, in the brain. This leads to an increase in appetite and often is the reason this medication is used, rather than for its antihistamine effects. Cyproheptadine has been used in the treatment of Cushing's disease, where there is excess cortisone production but not with reliable success.

1.5. Plants used in this study:

1.5.1. Monechma ciliatum

*Monechma ciliatum* belongs to the family Acanthaceae, locally known as "Black Mahlab". It is an annual hispid-scabrous almost glabrous herb 1-2 ft. high; woody below-leave linear or narrowly linear-lanceolate, flowers cream-white with purple and orange streaks, 2-lipped, in short spikes bracts pectinate with long stiff white bristles. Seeds with a tuft of rigid thick hairs at the hilum and a similar tuft at the other end (Fig.1and 2) (Andrews, 1956).

According to Show (1973) *Monechma ciliatum* is approximately native of tropical Africa and India. It is widely
distributed from Senegal to Cameroon and east word to central and southern states of the Sudan and south through E. Africa to Malawi and Zambia (Wichens, 1976). It is also grown in Nigeria (Uguru et al., 1998).

In Botswana, they believed that *Monechma ciliatium* played an important role as a medicine; it is used for general body pain, liver, bowel trouble and sterility in women (Inga Hedberg and Stengard, 1989).

In the Sudan *Monechma ciliatum* is widely distributed in Kassala, Southern Blue Nile, Kordofan, Upper Nile, Bahr Elghazal and Bahr El Jebel States (Broun and Massey, 1929).

Black Mahlab is a famous medicinal plant in western Sudan, especially in Gebel Mara Area. It’s seeds are used as an effective laxative and contain an essential oil which emits a sweet and pleasant odour. It is also used in traditional Sudanese fragrances, lotions and other cosmetics used for wedding preparations and child birth.

Phytochemical and pharmacological characters on *Monechma ciliatum* was studied by Uguru and Evans (2000). The leaves of *M. ciliatum* were found to contain alkaloids, glycosides, proteins, tannins and saponins. The hot methanol extract of the leaves was found to have potent oxytocic effect, which may constitute an amino acidic derivative.

Uguru et al. (1998) investigated the utrotonic properties of methanol extract of *Monechma ciliatum*, the oxytocic activity of hot methanolic extract of leaves of *M. ciliatum* was compared with other uterine stimulants like Ergometrine, Oxytocin, 5-Hydroxy tryptamine,
Acetyl Choline and Prostaglandins (E2 and F2alpha) in the presence of some antagonists in an attempt to explain the mechanism of action of the extract. Atropine partially blocked the effect of hot methanolic extract. Indomethacin inhibited the effects of hot methanolic extract as well as all drugs, except the Prostaglandins and Acetyl Choline. D-600 blocked the effect of all the drugs including hot methanolic extract.

Methyserigide antagonised only the effect of 5-Hydroxytryptamine and partially blocked Ergometrine. Prolonged treatment altered the uterine musculature and the activity profile of the drugs.

These results suggest that the hot methanolic extract may be acting by more than one mechanism to contract the uterus and this explains the mechanism of the anti implantation activity of the plant.

Ayoub and David (1984) stated that *Monechma ciliatum* had anticancer constituents.

1.5.2. *Lepidium sativum*

*Lepidium sativum* belongs to the family *Brassicaceae*. The plant is cultivated in Khartoum and Kordofan states. The common names of the plant are cress, pepper cress, Garden cress, pepper grass and pepper wort, the Arabic name is ELRashad.

The plant is glabrous erect annual, the leaves and seeds contain volatile oils known as cress-oil (Watt and Breyer 1962). The leaves are entirelyto pinnatisect, the flowers small, white and the seeds are siliqua obovate to nearly rotundate, emarginated (Figs. 3-4-5). The growing plant is eaten and the seeds yield oil which are used in treating dysentery and diarrhoea (Broun and Massey, 1929).
The chemical constituents of the plant include phenolic compounds (Ozeker and Esiyok, 1999), imidazole alkaloids (Maier, et al., 1998), hydroxylated glutamic acids (Bell et al., 1981) sinigrin, K-salt (Thies, 1988); flavonoid compounds (Paszkowski and Kremer, 1988), sterols (Bettach et al., 1997) and Benzyl glucosinolate degradation protect (Gil and Macleod, 1980). *Lepidium sativum* contained glucotropaeolin as reported by Songsak and Lockwood (2002).

Phytochemical screening of *Lepidium sativum* studied by Nuha (2006) showed that the plant was positive for Triterpenses, Alkaloids, Flavonoids, Tannins and Cumarins but negative for Saponins, Cynogenicglycoside and Anthraquinoneglycoside.

The plant is traditionally used in Indian lactating women as well as in cases of diarrhoea and dysentery. The seeds of *Lepidium sativum* are used for treatment of fracture healing in Saudi traditional medicine (Ahsan et al., 1989). The mucilage in the outer seed is used as substitute for tragacanth and Gum Arabic (Mathews et al., 1993).

*Lepidium sativum* was toxic to Nubian goats (Amani, 1995). The signs in goats of *Lepidium sativum* poisoning were bloat, loss in condition and recombency.

1.5.3. *Linum usitatissimum*:-

The plant *Linum usitatissimum* belongs to the family *Linaceae*. The plant distributed in the Mediterranean Sea area and was commonly cultivated in North Africa. The Arabic name of the plant is Kettan, Berber, Tifert and Delkmouch. And English name is Commonfax (Kokwaro, 1976). (Fig 6 and 7).
Traditionally the plant was used to treat fever. Seeds used as a laxative, soothing and pain-relieving. 'Infusion of seeds used for inflammation of digestive and urinary tracts, antidiarrhoea, often mixed with Althaea flowers. Seeds used in preparation of cataplasms for their emollient prosperities against boils and inflammations (Kokwaro, 1976). The plant is considered one of the richest dietary source of soluble fiber mucilage and that 50g high lingolenic acid flax seed per day for 4 weeks is palatable, safe and nutritionally beneficial in human by raising n-3 fatty acids in plasma and erythrocytes and decreasing post-prandial glucose responses (Cunnane et al., 1993). Linum usitatissimum contains linamarin which contains a peptide, linatine and has an antipyridoxine action in chickens (Humphreys, 1988).

Bakhiet and Adam (1995) studied the response of Bovans chicks to low dietary levels of Linum usitatissimum seeds. They found that the effect of 10-20 % Linum usitatissimum seeds in their normal diet for 6 weeks increased the growth rate in spite of the development of centrilobular hepatocyte fatty vacuolation and an inflammatory cell response in the intestinal lamina propria and renal cortex. These effects were correlated with alteration in some hepatorenal function and heamo-atolgy parameters.

Furthermore Linum usitatissimum was toxic to Nubian goats (Amani, 1995). The main clinical signs of Linum usitatissimum poisoning in Nubian goats were locomotor disturbances, diarrhoea, and loss in condition, dullness and recumbence.
Fig (1): *Monechma ciliatum* leaves
Fig (2): *Monechma ciliatum* leaves, stems and fruit
Fig (3): *Lepidium sativum* (shoot system)

Fig (4): *Lepidium sativum* leaves
Fig (5): *Lepidium sativum* seeds
Fig (6): *Linum usitatissimum* L. plant

Fig (7): *Linum usitatissimum* L. seeds
CHAPTER TWO
MATERIALS AND METHODS

2.1. Materials and Experimental Designs

2.1.1. Plants

Three plants were used in this experiment; *Monechma ciliatum* (leaves and stems) and *Lepidium sativum* were collected from Medicinal and Aromatic Plants Research Institute Farm. *Linum usitatissimum* was supplied by herbalist.

The plants were authenticated by the scientist of the Medicinal and Aromatic Plants Research Institute.

2.1.2. Animals

*Rabbits*, local strain *Oryctolagus cuniculus*, weighing 1-3 Kg were brought from market- Khartoum North *Guinea pigs*, weighing about ½-1½kg and *Wister albino rats* weighing about 80-120gms were obtained from the Laboratory animal's house, Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum.

Jejunum was dissected from rabbits while ileum from Guinea pig. Uterus and fundic part of stomach were isolated from rat. The animals were scarified.

2.1.3. Microbes

The negative organisms used are *Escherichia coli* (NCTC 8196), *Proteus vulgaris* (ATCC 6380) and *Pseudomonas aeruginosa* (ATCC 27853), while the gram positive organisms were *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923).
Two fungi *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596) are used. All organisms are obtained from National Collection of Culture Type Colindale (NCTC) England and American Type Culture Collection (ATCC) Rockville Maryland, USA.

**2.1.4. Toxicity of *Monechma ciliatum* leaves methanolic extract in albino rats:-**

Twelve Wister albino rats of different sexes weighing from 80-110 gm, obtained from Medicinal and Aromatic Plants Research Institute were used. They were kept in cages within the premises of MARPI. The rats were allowed one week adaptation periods.

**2.1.4.1. Experimental design: -**

At the end of the adaptation period, the rats were weighed and divided into two groups 6 rats in each group. The first group was kept as a control and the other group received a dose of 100mg /kg body weight (B wt) daily of methanolic extract of *Monechma ciliatum* leaves for 21 days.

**2.1.4.2. Parameters:-**

Blood sample were collected after slaughter for serum analysis. Sera were analyzed for total Cholesterol, Bilirubin, Protein and Albumin. Samples from the liver, heart, kidneys, lung, brain, intestine and spleen were collected immediately after slaughter and fixed in 10% formalin for histopathology.

**2.2. Methods**

**2.2.1. Preparation of the plants extraction**

150gm of the dried leaves and 50gm of stem of *Monechma ciliatum* 160gm from dried seeds of *Lepidium sativum* and 50gm from dried seeds of *Linum usitatissimum* were coarsely powdered and
extracted according to Harborne, (1984). They were left for 20 hours soaked in Chloroform in Soxhlet apparatus. The Chloroform extract was filtered and evaporated under reduced pressure using Rota-Vap. The extracted plant material was then air-dried, repacked in the Soxhlet and exhaustively extracted with Methanol. The methanolic extract was filtered and again evaporated under reduced pressure using Rota-Vap.

The yield percentages were calculated after the traces of the solvent were air dried as follows:

\[
\frac{\text{Weight of extract}}{\text{Weight of plant}} \times 100
\]

**2.2.2. In vitro testing for antimicrobial activity:**

**2.2.2.1. Testing for antibacterial activity:**

The cup –plate –agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications, to assess the antibacterial activity of the extracts.

Three ml of each of the five standardized bacterial stock suspensions \((10^8-10^9\ \text{C.F.U. }/\text{ml})\) were thoroughly mixed with 300ml of sterile melted nutrient agar maintained at 45ºC.

20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set for 5-10 minutes. The plates were divided into two halves, two cups in each half (10mm in diameter) using a sterile cork borer (No. 4). The agar disks were removed. Alternate cups were filled with 0.1ml samples of each of the extracts using Transfer adjustable volume automatic microtitre pipette, and allowed to diffuse at room temperature for 2 hour.

The plates were then incubated in the up right position at 37ºC for 18 hours.
Two replicates were carried out for each extract against each of the tested organisms.

Simultaneously positive controls involving the addition of the respective solvents instead of the extracts were carried out separately. After incubation the diameters of the resultant growth inhibition zones were measured and the mean values were tabulated.

2.2.2.2. Testing for antifungal activity:-

The antifungal activity was measured by a method similar for testing antibacterial activity. For the antifungal activity sabouraud dextrose agar was used.

The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for *Aspergillus niger*.

2.2.3. Pharmacological Experiments:-

The animals were starved for 24 hours before killing. Animals were killed by dislocating the neck, and then exsanguinated and the abdomen was opened.

2.2.3.1. Rabbit jejunum:-

The preparation of the isolated jejunum is based on the method of Finkleman (1930). The jejuna area of the intestine in the rabbit was located out and then cut into isolated segments about 4cm in length.

The parts of jejunum were transferred to a Petri dish containing Tyrode solution, the mesentery and fat surrounding the muscle were trimmed away and thread was passed through one wall of the jejunum at both top and bottom. The bottom thread was attached to the tissue holder and the mounted tissue was transferred to the organ bath and attached to an isotonic transducer. The tissue was left for 45 minutes for
adaptation to the new environment and washed several times by outflow.

2.2.3.2. Guinea pig ileum:-

The preparation of the isolated ileum is based on the method of Magnus (1904). The ileum was removed from the caecal end and it was cut into isolated segments about 4 cm in length. The ileum was transferred to a Petri dish containing Tyrode solution and the mesentery and fat surrounding the muscle were trimmed away. The threads were passed through one wall of the ileum at both top and bottom. The bottom thread was attached to the tissue holder. The mounted tissue was transferred to the organ bath and attached to the transducer.

2.2.3.3. Rat fundus strip:-

The preparation of fundus strip is based on the method of Vane (1957). The fundal part of the stomach was isolated. The rest of the stomach was cut away from the fundal region. The fundus was transferred to a Petri dish containing Ringer solution. The fundal end was opened out longitudinally by making two cuts on either side of the dome shaped preparation. The tissue was laid flat in a Petri dish. A strip was prepared from the tissue by making alternate transverse cuts on opposite sides of the muscle, the strip about 4 cm long. Threads were tied around each end of the strip and one end was attached to a tissue holder. The mounted preparation was transferred to an organ bath and the other thread was attached to the transducer.
2.2.3.4. Rat uterus:-

The preparation of isolated rat uterus is based on the method of De Jalon et al. (1945). A female rat was injected with 0.1mg/kg stilboesterole intramuscularly 24 hours before the removal of uterus.

The two uterine horns were exposed by pulling a side the intestine. Each horn was free from surrounding fat and mesenteric attachments. Each horn was cut out separately, and transferred to a Petri dish containing De Jalons Ringer. The two horns were divided by making a transverse cut, into four preparations 3-4cms. The tissue will work quite adequately as a tube, a longitudinal cut was made and the tissue was set up as a sheet of muscle. Threads were passed through one wall of the uterus at both top and bottom. The bottom thread was attached to the tissue holder, and was transferred to an isolated organ bath and the top thread was attached to the transducer.

2.2.5. Blood samples:-

Blood samples were collected from rat during slaughter either into dry clean bottles containing EDTA (Ethylene – diamine tetra acetic acid) as anticoagulant for hematological studies or into clean test tubes without anticoagulant for sera. The tubes were left for 45 minutes and then sera were separated by centrifugation at the centrifuge (HETTICH EBA 35 centrifuge). The sera were stored at -4ºc till analysis.

2.2.5.1. White blood cells Count (WBC):-

Leukocytes were counted according to Schalm (1965) using Neubauer haemocytometer for white blood cell counts. Turk's solution (1% glacial acetic acid, tinged with gentian violet) was used as diluents.
2.2.5.2. Differential leucocytes count:-

Blood films on clean slides were stained with leishman's stain. The Battlement method was used for different leukocyte count. 100 cells were counted in each blood smear and average percentages were recorded.

2.2.6. Biochemical Analysis:-

The biochemical analysis of bilirubin, cholesterol, protein and albumin were estimated to determination of serum constituents.

2.2.6.1. Total Bilirubin:-

The bilirubin in the serum was measured as stated by Jendrassik and Grof (1938).

Test principle:-

Bilirubin is coupled with diazotised sulfanilic acid in presence of caffeine to give azodye. The optical density was measured by JENWAY 6305 UV/VIS spectrophotometer at wave length 540 nm. Total bilirubin calculated as follow:-

Total bilirubin (mg/dl) = Absorbance of sample – Absorbance of sample blank.

2.2.6.2. Total cholesterol:-

Serum total cholesterol was measured by enzymatic colorimetric test according to Richmond (1973). Its concentration was estimated using commercial kit.

Test principle:-

The principle of assay depends on released of cholesterol from its esters after enzymatic hydrolysis and oxidation to cholestenone plus H₂O₂ – The indicator, quinonemine, is formed as a result of reaction between hydrogen peroxide (H₂O₂) and 4- amino antipyrine in the presence of peroxidase (POD).
Cholesterol ester + H₂O → **Cholesterol** → Cholesterol + Fatty acids
Esterase
Cholesterol + H₂O → **Cholesterol** → Cholestenone + H₂O₂
Oxidase
H₂O₂ + 4AP + Phenol Peroxidase Quinoneimine + H₂O

Cholesterol concentration was estimated as follows:-

\[
\text{Cholesterol (mg/dl)} = \frac{\text{A sample}}{\text{A standard}} \times \text{X concentration of standard}
\]

Where the concentration of the standard is 200 mg/dl.

**2.2.6.3. Total Protein:-**

Biuet reagent was used to determine the total serum protein concentration as described by Wiechselbaum (1946).

**Test principle:-**

The method is based on the reaction of protein with copper sulphate in the presence of sodium hydroxide and potassium sodium tartrate (Rochelle salt) which keeps cupric hydroxide in solution for development of blue colour.

A spectrophotometer (Corning Halstead, U.K) was used at a wavelength of 540 nm and values were calculated as follows:-

\[
\text{Total Protein (gm/dl)} = \frac{\text{A sample}}{\text{A standard}} \times \text{X concentration of standard}
\]

Where the concentration of the standard is 8 gm/dl
2.2.6.4. Albumin:-

Serum albumin concentration was measured by the use of Bromo- Cresol Green method as described by Doumas et al. (1971).

Test principle:-

The principle of this method is that the measurement of serum albumin is based on its quantitative binding to the indicator, bromo-cresol green (BCG). The sera and reagents were mixed and incubated at 20-25ºc. The absorbance was measured against the reagent at 578 nm using a spectrophotometer. Albumin concentration was calculated as follows:-

\[
\text{Serum albumin (g/dl)} = \frac{A \text{ sample}}{A \text{ standard}} \times \text{concentration of standard}
\]

Where the concentration of the standard is 5gm/dl

2.2.7. Histopathological methods

Slices of tissues from lungs, small intestine, heart, liver, kidneys, spleen and brain of rats after slaughter were fixed in 10% formalin, embedded in paraffin wax, sectioned at 5µm and stained with haematoxylin and eosin (Drury and Wallington, 1980).

2.2.8. Statistical analysis:-

The student t- test was used for significance of data analysis according to procedure described by Mendenhall (1971).
CHAPTER THREE

RESULTS

3.1. The antimicrobial activities of the plants:-

3.1.1. The antimicrobial activity of *Monechma ciliatum* (stems) extracts against standard organisms are presented in Table (1):-

The methanolic extract of *Monechma ciliatum* stems was showed high antimicrobial activity against *Proteus vulgaris* (24) and *Staphylococcus aureus* (20), moderate activity against *Escherchia coli* (18) and low inhibition zone against *Bacillus subtilis* (12), *Pseudomonas aeruginosa* (12) and *Candida albicans* (12).

The Chloroform extract of *Monechma ciliatum* stems showed moderate activity against *Staphylococcus aureus* (17), *Proteus vulgaris*, and *Bacillus subtilis* (15). It had low activity against *Escherchia coli* (13), *Pseudomonas aeruginosa* (13) and *Candida albicans* (13).

The water extract of *Monechma ciliatum* stems, had high antimicrobial activity against *Pseudomonas aeruginosa* (23), while the activity was absent against all other standard organisms tested.

*Aspergillus niger* was resistant to the all plant extracts.

3.1.2. The antimicrobial activity of *Monechma ciliatum* leaves extracts against standard organisms are presented in Table (2) and Fig (8-13):-

The antimicrobial activity of methanolic extract of *Monechma ciliatum* leaves was moderate against *Proteus vulgaris* (19) and *Escherchia coli* (18), low against *Bacillus subtilis* (17),
Pseudomonas aeruginosa (17) and Staphylococcus aureus (17). The activity was absent against Candida albicans and Aspergillus niger.

The chloroform extract of Monechma ciliatum leaves caused high inhibition zone against Staphylococcus aureus (23) and low against Bacillus subtilis (15) and Candida albicans (14). However, the extract showed low activity against Escherchia coli (13), Proteus vulgaris (13) and Pseudomonas aeruginosa (13).

Water extract of Monechma ciliatum leaves caused moderate activity against Pseudomonas aeruginosa (17) and low inhibition zone activity against Bacillus subtilis (13) and Staphylococcus aureus (13). The activity was absent against Escherchia coli, Proteus Vulgaris, Candida albicans and Aspergillus niger (Table 4).

3.1.3. The antimicrobial activity of Linum usitatissimum seeds extracts against standard organisms is presented in Table (3):-

The methanolic extract of Linum usitatissimum caused an inhibition growth zone diameter. It was moderate in Pseudomonas aeruginosa (18) and Bacillus subtilis (16), low against Proteus vulgaris (15), Staphylococcus aureus (14) and Escherchia coli (13). The activity was absent against Candida albicans and Aspergillus niger.

The antimicrobial activity of Chloroform extract of Linum usitatissimum was low against Pseudomonas vulgaris (13) and inactive against Bacillus subtilis, Staphylococcus aureus, Escherchia coli, Proteus vulgaris, Aspergillus niger and Candida albicans.

Water extracts showed low activity against Candida albicans (14) while there was no activity against other organisms.
3.1.4. The antimicrobial activity of *Lepidium sativum* seeds extracts against standard organisms is presented in Table (3):

The methanolic extract of *Lepidium sativum* had high inhibition against *Candida albicans* (21) and *Proteus vulgaris* (20). The antimicrobial activity of the extract was low against *Bacillus subtilis* (13), *Staphylococcus aureus* (13), *Escherchia coli* (13) and *Pseudomonas aeruginosa* (13). The extract was inactive against *Aspergillus niger*.

The chloroform extract of *Lepidium sativum* had very low activity against *Staphylococcus aureus* (12) and *Pseudomonas aeruginosa* (12) and the activity was absent against *Bacillus subtilis*, *Escherchia Coli*, *Proteus Vulgaris*, *Candida albicans* and *Aspergillus niger*.

The antimicrobial activity of water extract of *Lepidium sativum* was low against *Bacillus subtilis* (15) and *Escherchia coli* (15). The extract had no effect against *Staphylococcus aureus*, *Proteus Vulgaris*, *Candida albicans* and *Aspergillus niger*. 
Table (1):-The antimicrobial activity of *Monechma ciliatum* stems against standard organisms:-

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Yield</th>
<th>Conc.* (mg/ml)</th>
<th>MIZD* (Mm)</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. s</td>
<td>S. a</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.88</td>
<td>100</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Chloroform</td>
<td>6.05</td>
<td>100</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Water</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* MIZD: Mean of inhibition growth zone diameter. (Mm): Millimeter

* Conc.: Concentration of extract in solvent.

*Bacillus subtilis* (*B. s*); *Staphylococcus aureus* (*S. a*); *Escherichia coli* (*E. coli*); *Proteus vulgaris* (*Pro. v*); *Pseudomonas aeruginosa* (*Pseudo. a*); *Aspergillus niger* (*Asp. n*); *Candida albicans* (*C. a*).

Low = $< 15$mm, Moderate = 19-20 mm, High =20-25 mm, V. High = $> 25$mm.
Table (2):- The antimicrobial activity of *Monechma ciliatum* Leaves against the standard organisms:

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Yield %</th>
<th>Conc.* mg/ml</th>
<th>MIZD* (Mm)</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. s</td>
<td>S. a</td>
</tr>
<tr>
<td>Methanol</td>
<td>12.43</td>
<td>100</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Chloroform</td>
<td>11.13</td>
<td>100</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Water</td>
<td>0.09</td>
<td>-</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

* MIZD: Mean of inhibition growth zone diameter. (Mm): Millimeter

* Conc.: Concentration of extract in solvent.

*Bacillus subtilis* (B. *s*); *Staphylococcus aureus* (S. *a*); *Escherichia coli* (E. *coli*); *Proteus vulgaris* (Pro. *v*); *Pseudomonas aeruginosa* (Pseudo. *a*); *Aspergillus niger* (Asp. *n*); *Candida albicans* (C. *a*).  
Low = < 15mm, Moderate = 19-20 mm, High =20-25 mm, V. High = > 25mm.
**Table (3):- The antimicrobial activity of *Linum usitatissimum* against the standard organisms:**

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Yield %</th>
<th>Conc.* mg/ml</th>
<th><strong>Bacteria</strong></th>
<th><strong>Fungi</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MIZD* (Mm)</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>4.6</td>
<td>100</td>
<td>16 14 13 15 18</td>
<td>- -</td>
</tr>
<tr>
<td>chloroform</td>
<td>28.5</td>
<td>100</td>
<td>- - - - 13</td>
<td>- -</td>
</tr>
<tr>
<td>Water</td>
<td>0.041</td>
<td>-</td>
<td>- - - - -</td>
<td>14</td>
</tr>
</tbody>
</table>

* MIZD: Mean of inhibition growth zone diameter. (Mm): Millimeter

* Conc.: Concentration of extract in solvent.

*Bacillus subtilis (B. s); Staphylococcus aureus (S. a); Escherichia coli (E. coli); Proteus vulgaris (Pro. v); Pseudomonas aeruginosa (Pseudo. a); Aspergillus niger (Asp. n); Candida albicans (C. a).*

Low= <15mm, Moderate= 19-20mm, High= 20-25mm, V. High= >25.25.
Table (4):- The antimicrobial activity of *Lepidium sativum* against the standard organisms:

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Yield %</th>
<th>Conc.* mg/ml</th>
<th>MIZD* (Mm)</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. s</td>
<td>S. a</td>
</tr>
<tr>
<td>Methanol</td>
<td>9.82</td>
<td>100</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>chloroform</td>
<td>9.0</td>
<td>100</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Water</td>
<td>0.03</td>
<td>-</td>
<td>15</td>
<td>-</td>
</tr>
</tbody>
</table>

* MIZD: Mean of inhibition growth zone diameter. (Mm): Millimeter.

* Conc.: Concentration of extract in solvent.

*Bacillus subtilis* (*B. s*); *Staphylococcus aureus* (*S. a*); *Escherichia coli* (*E. coli*); *Proteus vulgaris* (*Pro. v*); *Pseudomonas aeruginosa* (*Pseudo. a*); *Aspergillus niger* (*Asp. n*); *Candida albicans* (*C. a*).

Low = < 15mm, Moderate = 19-20 mm, High = 20-25 mm, V. High = > 25m
Fig (8): Antimicrobial activity of methanol and chloroform extract of *Monechma ciliatum* leaves on *Escherichia coli*.

- M = methanol extract
- Ch = chloroform extract
Fig (9): Antimicrobial activity of methanol and chloroform extract of *Monechma ciliatum* leaves on *Staphylococcus aureus*.

- M = methanol extract
- Ch = chloroform extract
Fig (10): Antimicrobial activity of methanol and chloroform extract of *Monechma ciliatum* leaves on *Candida albicans*.

- M = methanol extract
- Ch = chloroform extract
Fig (11): Antimicrobial activity of methanol and chloroform extract of *Monechma ciliatum* leaves on *Pseudomonas aeruginosa*.

- **M** = methanol extract
- **Ch** = chloroform extract
Fig (12): Antimicrobial activity of methanol and chloroform extract of *Monechma ciliatum* leaves on *Bacillus subtilis*.

- M = methanol extract
- Ch = chloroform extract
Fig (13): Antimicrobial activity of methanol and chloroform extract of *Monechma ciliatum* leaves on *Aspergillus niger*.

- M = methanol extract
- Ch = chloroform extract
3.2. The pharmacological activity of Methanolic extract of *Monechma ciliatum* leaves on different isolated tissues:

3.2.1. Rabbit jejunum:

The methanolic extract of *Monechma ciliatum* leaves caused significant contraction in rabbit jejunum at different doses (1.6, 3.2, 6.4 mg/ml) (Fig. 14). Similar contraction in rabbit jejunum caused by doses (0.4, 1, 2, 0.8 mg/ml) (Fig. 15).

3.2.2. Guinea pig ileum:-

On the isolated Guinea pig ileum, the plant extract showed contraction at submaximum dose 3.2 mg/ml. The contraction was refractory to both the muscarinic blocker Atropine (Fig. 16) and the antihistamine antagonist Chlorophenramine (Fig. 17), but effectively blocked by the serotonergic blocker, Cyproheptadine at dose of 4µg/ml (Fig. 18).

3.2.3. Rat fundus:-

The rat fundus strip is highly sensitive to 5-Hydroxytriptamine (5HT). 5HT (1µg/ml) stimulated the rat fundus strip while extract of *Monechma ciliatum* leaves stimulated it at (3.2 mg/ml) (Fig. 19). This contracting activity of both extract and serotonin was effectively blocked by Cyproheptadine (2µg/ml).

3.2.4. Rat uterus:-

The methanolic extract of *Monechma ciliatum* leaves at a concentration of 3.2 mg/ml caused contraction on isolated rat uterus. This effect was completely blocked by Cyproheptadine (2µg/ml) (Fig. 20).

In a similar manner the contraction produced by standard (5HT) was blocked completely by Cyproheptadine (2µg/ml).
3.3. Effect of methanolic extract of *Monechma ciliatum* leaves in rats:

3.3.1. Clinical findings:

There were no clinical signs observed in experimental rats treated with 100mg/kg through out the experiment.

3.3.2. Haematological changes:

The means WBC and differential count are shown in Table (5). The WBC and differential count showed no significant changes.

3.3.3. Postmortem Findings:

There were no postmortem changes noticed in the treated group.

3.3.4. Histopathological findings:

The rats treated with methanolic extract of *Monechma ciliatum* leaves at (100mg/kg) showed congestion of livers, Kidneys, brains and spleens. In some rats the liver showed dilatation of sinusoids, some treated rats showed increase in goblet cells in the intestine. Fig (27- 28-29).

3.3.5. Serum constituents change:

The mean levels of bilirubin, cholesterol, total protein and albumin were shown in Table (6). There was significant increase (p < 0.05) in the values of total protein. In the other hand there were no significant changes in cholesterol, albumin and bilirubin concentration.
Table (5): White blood cell and differential count of rats treated with methanolic extract of *Monechma ciliatium* leaves

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
<th>Eosinophils (%)</th>
<th>Basophils (%)</th>
<th>Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>2.5×10³ ± 170.78</td>
<td>42.83 ± 1.7</td>
<td>18.50 ± 1.7</td>
<td>18.17 ±1.7</td>
<td>1.17± 1.7</td>
<td>17.67 ±1.7</td>
</tr>
<tr>
<td>Group (B)</td>
<td>2.3 ×10³ ± 600.9</td>
<td>42.30 ± 2.51</td>
<td>21.70 ± 2.88</td>
<td>17.00 ± 2.6</td>
<td>1.00 ± 1.00</td>
<td>18.00 ± 2.65</td>
</tr>
</tbody>
</table>

Group A: Treated with methanolic extract of *Monechma ciliatum* leaves 100mg/kg B wt.

Group B: control.
Fig (21): White blood cell count in rats given methanol extract of *Monechma ciliatum*.

![WBCs graph](image)

Fig (22) Differential count in rats given methanol extract of *Monechma ciliatum*.

![Differential count graph](image)

L = Lymphocytes  
N = Neutrophils  
E = Eosinophils  
B = Basophils  
M = Monocytes
Table (6): Serum constituents values in rats treated with methanolic extract of *Monechma ciliatum* leaves.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Protein gm/dl (Means ± S. E.)</th>
<th>Albumin gm/dl (Means ± S. E.)</th>
<th>Cholesterol mg/dl (Means ± S. E.)</th>
<th>Bilirubin mg/dl (Means ± S. E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>11.9±2.65*</td>
<td>2.35 ± 1.26</td>
<td>48.5 ± 17.7</td>
<td>0.05 ± 0.034</td>
</tr>
<tr>
<td>Group B</td>
<td>7.35± 0.65</td>
<td>2.9 ± 0.92</td>
<td>66.00 ± 10.00</td>
<td>0.18 ± 0.173</td>
</tr>
</tbody>
</table>

* *P* < 0.05

Group A: Treated with methanolic extract of *Monechma ciliatum* leaves 100mg/kg B wt.

Group B: control.
Fig (23) Changes in serum Protein in rats given methanolic extract of *Monecma ciliatum* leaves.

![Bar chart showing changes in serum Protein](image)

Fig (24): Changes in serum Albumin in rats given methanolic extract of *Monecma ciliatum* leaves.

![Bar chart showing changes in serum Albumin](image)
Fig (25): Changes in serum Cholesterol in rats given methanolic extract of *Monecma ciliatum* leaves.

![Cholesterol Graph](image)

Fig (26): Changes in serum Bilirubin in rats given methanolic extract of *Monecma ciliatum* leaves.

![Bilirubin Graph](image)
Fig (27): Liver of a rat given methanolic extract of *Monechma ciliatum* leaves. Note congestion and dilatation of sinusoids. H&E ×100

Fig (28): Intestine of a rat given methanolic extract of *Monechma ciliatum* leaves. Note the increase in goblet cells. H&E ×100
Fig (29): Kidney of a rat given methanolic extract of *Monechma ciliatum* leaves. Note congestion. H&E ×40
CHAPTER FOUR
DISCUSSION

In this study the methanol, chloroform and water extracts of *Lepidium sativum*, *Linum usitatissimum* seeds and *Monechma ciliatum* leaves and stems were tested to verify their antimicrobial activities against standard organisms. The present investigation has shown that *Monechma ciliatum* leaves extracts were the most effective against standard organisms than the other tested plants extracts. All five standard bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) were sensitive to the methanol extract of *Monechma ciliatum* leaves and showed moderate inhibitory zone sizes, while chloroform extract of *Monechma ciliatum* leaves shown low activity against standard bacteria except *staphylococcus aureus* which has higher inhibitory zone size. Water extract was shown low inhibitory zone on *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, when *Escherichia coli*, *Proteus vulgaris*, *Aspergillus niger* and *Candida albicans* were resistant to this extract. The *Aspergillus niger* was inactive to all *Monechma ciliatum* leaves extracts. From these findings, it is clear that the methanol extract of *Monechma ciliatum* leaves, as an antimicrobial agent, is more effective than the chloroform and water extracts. Mohamed (1979) reported similar result by using *Hibiscus sabdariffa* (Karkadeh) which showed a potent antimicrobial effect. He found that an aqueous, ethanol, 1% methanol hydrochloric acid, ethereal, chloroform and petroleum ether extracts have a wide range of antimicrobial activity against both Gram- positive and Gram- negative bacteria as well as yeast.
Methanol extract of *Lepidium sativum* showed high activity against Gram-negative *Proteus vulgaris* and the yeast *Candida albicans*, but low against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The chloroform extract of *Lepidium sativum* was inactive against all standard organisms tested. *Aspergillus niger* was resistant to this plant extracts. Asma (2003) recorded similar effects in her study of *Lepidium sativum* seeds for its antimicrobial activities. She found that the methanol extract of *Lepidium sativum* was the most active extract. The clinical isolates exhibited low susceptibility compared with the standard organisms.

In the present investigation the *Linum usitatissimum* seeds extracts showed very low antimicrobial activity against standard organisms compared with the other plants extracts tested in this study. Methanol extract of *Linum usitatissimum* caused inhibitory zone sizes in Gram positive and Gram-negative bacteria. The fungi *Aspergillus niger* and *Candida albicans* did not show any effect to methanolic and chloroform extracts. Water extract of *Linum usitatissimum* had low inhibitory against *Candida albicans*, the activity was absent against *Aspergillus niger* and almost five standard bacteria.

In the other hand the topical wound healing properties of Canisep cream containing oils from *Linum usitatissimum* was studied by Amresh *et al* (2005). This study was conducted on 32 surgical wounds of 8 goats. Wounds were subjected to topical application of cream. Granulation tissue appeared but scab never appeared. This result suggested that Canisep cream had antimicrobial property which makes *Linum usitatissimum* a suitable for wound healing. They may indicate that *Linum usitatissimum* has a synergistic effect as antimicrobial properties.
In this study the methanolic extract of *Monechma ciliatum* stems was found to be more effective as an antimicrobial agent than chloroformic and water extracts. This may be due to the fact that the active constituent which had antimicrobial affect dissolved in methanol rather than chloroform. The water extract had no inhibitory zone which means that the active ingredients were insoluble in water. The fungi responded less to *Monechma ciliatum* stems extracts which indicated that the plant had very little antifungal component. *Monechma ciliatum* leaves extracts are more effective than *Monechma ciliatum* stems this explain that *Monechma ciliatum* plant had different component between the parts of the plant.

In this study the investigations revealed that the methanolic extract of *Monechma ciliatum* leaves contained pharmacologically active components. Small doses of the extract produced contraction on Rabbit jejunum which was increased immediately with increase of the dose. ElTayeb (1996) had found that the aqueous extract of *Hyphaene thebaica* (Doum) also stimulated the rabbit jejunum. It also caused significant contraction at a dose of 3.2mg/ml on isolated Guinea pig ileum. The contraction was neither due to cholinomimetic activity because, it was not blocked by the muscarinic blocker Atropine and nor due to histamine receptors that the antihistamine blocker Chlorophenramine did not block the effect which blocked the standard Histamine. However, the effect was blocked by Cyproheptadine a drug which antagonized the action of both (serotonin) 5-Hydroxytryptamine (Gyermek, 1961) and Histamine (H1) receptors agonists (D’Arcy, 1963).This suggested that the contraction could be probably due to 5- Hydroxytryptamine like substance. Afra’a (2002) reported similar result by water extract of *Amobrosia maritime*
which induced contraction on isolated guinea pig at dose of (20mg/ml) this stimulant action was blocked completely by 2µg/ml cyprohyptadine.

Serotonin causes increased gastrointestinal motility and contraction of isolated strips of intestine, this being partly due to direct effect on smooth muscle cells and partly due to indirect effect on enteric neuron (Rang and Dale, 1987).

The fundus strip was also sensitive to serotonin (Vane, 1957). 5-Hydroxytryptamine (1µg/ml) produced contraction on the rat fundus while methanol extract of *Monechma ciliatum* leaves (3.2 mg/ml) caused similar contraction to that produced by 5-Hydroxytryptamine. Furthermore, the contraction of both the extract and 5-Hydroxytryptamine were completely blocked by Cyproheptadine. Einas (2000) reported that the decoction of *Striga hermonthica* stimulated the isolated rat fundus strip at (2mg/ml); this stimulant effect was blocked partially by (2µg/ml) cyprohyptadine.

Moreover, the extract of *Monechma ciliatum* leaves showed significant contraction on isolated rat uterus at dose of 3.2 mg/ml, similar contraction was caused by the standard 5-Hydroxytryptamine. This contracting activity on the rat uterus was blocked with Cyproheptadine at dose of 2µg/ml. Uguru et al. (1998) in their investigated of the utrotonic properties of methanol extract of *Monechma ciliatum* leaves, the contracted activity of hot methanolic extract of leaves of *Monechma ciliatum* was compared with other uterine stimulants like Ergometrine, Oxytocin, 5-Hydroxy tryptamine, Acetyl Choline and Prostaglandins (E2 and F 2α) in the presence of some antagonists in an attempt to explain the mechanism of action of the extract.

Atropine blocked partially the effect of hot methanolic extract. Indomethacin inhibited the effects of hot methanolic extract. They
suggested that the hot methanolic extract may be acting by more than one mechanism to contract the uterus. The present finding which showed that contracting activities of rat uterus blocked by Cyproheptadine and this similar to which Uguru et al (1998) suggested.

Ronald (1994) stated that serotonin is widely distributed in animals and plants, occurring in vertebrates, fruits and venoms. A number of congeners of serotonin are also found in nature and have been shown to possess variety of peripheral and central nervous activities. The epidermis of growing potions of Panicum virgatum roots was found to contain cytoplasmic inclusions. Evidence was presented that these epidermal inclusions were largely an amino compound similar to 5-Hydroxytryptamine (Lewis, 1974).

A herbal drug (Chot-san) improved performance in mice with procephalic ischaemia, disrupted passive avoidance and this effect was antagonized by NAN-190, a serotonin IA receptor antagonist (Yuzurihara et al., 1999).

Serotonin is found in mushrooms and plants, including fruits and vegetables (Jerome and Ellen, 1985). They reported that the highest values of 25 - 400 mg/kg have been found in nuts of the walnut (Juglans) and hickory (Carya) genus. Serotonin concentrations of 3 - 30 mg/kg have been found in plantain, pineapple, banana, kiwifruit, plums, and tomatoes. Moderate levels from 0.1 - 3 mg/kg have been found in a wide range of tested vegetables. Serotonin is one compound of the poison contained in the stinging hairs of the stinging nettle (Urtica dioica). It should be noted serotonin does not cross the blood-brain barrier unlike its precursors 5-HTP or tryptophan. Several plants contain serotonin together with a family of related tryptamines that are methylated at the amino (NH₂) and hydroxy (OH) groups, are N-oxides, or miss the OH group.
Examples are plants from the *Anadenanthera* genus that are used in the hallucinogenic yopo snuff.

Toxicological experiment was done on methanol extract of *Monechma ciliatum* leaves on albino rats. The daily oral administration of the extract at 100mg/kg was not lethal and less toxic. The investigation showed that the extract had low toxicity on the rat, there was no any signs indicated to toxicity. This result makes the Plant wide safety for its uses.
CONCLUSION AND RECOMMENDATION

1- On the light of the present study it is concluded that the three plants had ability to inhibit the effects of standard organisms or act as an antimicrobial agents in variable inhibition ranges.

2- The stimulatory effect of methanolic extract of *Monechma ciliatum* leaves on isolated rabbit jejunum and contraction on the guinea pig ileum may be due to the presence of 5-Hydroxytryptamine like components. Contraction on the isolated rat uterus suggested that the plant may contain oxytosic proprieties.

3- The toxicity results of *Monechma ciliatum* methanol extract showed that it is not toxic and safer to use.

According to above mention results we recommend the following:-

1. Further antimicrobial studies on clinical isolated and standard antibiotics to know specifically the mode of action of the plants studies.

2. Biological guided fractionation of the extracts to isolate the different active components is recommended.

3. Pharmacological and toxicological investigation on *Lebidium sativum* seeds, *Monechma ciliatum* stems and *Linum usitatissimum* seeds to know the activities of their extracts and mode of action.
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