Pharmacological Studies on three Sudanese Medicinal Plants Possessing Antimicrobial Properties

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Dedication

To my parents,

The one who teach a lot now get some part.

To my brothers,

The one who work for others will get some part.

To my children’s,

When asking for knowledge try to get all and not part.
Acknowledgements

I was and remain even at the conclusion of this work indebted for my supervisor Professor Idris Babekir Eltayeb of the department of pharmacology, faculty of pharmacy, University of Khartoum for his guidance and motivating criticism. I am extremely grateful for his wisdom, encourage and patience.

My deep appreciation is due to Dr. Abd ElWahab Hassan Mohamed, who initiated this work and skilled me with some of the techniques he had. I am in even more in awe of his valuable help.

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خلاصة الأطرحة

Anogeissus leiocarpus

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Abstract

In order to verify or refute the folklorically claimed benefits of some Sudanese plants possessing antimicrobial activity and to detect any side effects that may be associated with their use, the methanol extract of three plants were studied on selected isolated tissue preparations.

The methanol extract of *Loranthus acaciae* (Zucc.) leaves exhibited a dose dependent non-adrenergic inhibition of the isolated rabbit jejunum preparation, since it was not blocked neither by propranolol (Non selective β–blocker) nor tolazoline (Non-selective α blocker). The extract was devoid of any activity on guinea pig ileum preparation and couldn’t antagonize the Histamine (50ng/ml) induced contraction of the tissue. Prior administration of the extract (2mg/ml) markedly potentiated the Acetylcholine (1µg/ml) contracture on Toad rectus abdominis muscle preparation, while the extract alone was without activity on the preparation.

In a dose of 4mg/ml the extract effectively reversed d-tubocurarine (20µg/ml) blockade of Acetylcholine (1µg/ml) contracture on toad rectus abdominis muscle preparation in a manner comparable with that produced by the anticholinesterase Neostigmine (40µg/ml). On both isolated rabbit heart and guinea pig atrium preparations the extract showed a dose dependent inhibition of the force of contraction but without effect on the rate of contraction, its action on the atrium was not affected by prior administration of Atropine sulphate (50µg/ml). Prior administration of the extract (2mg/ml) to the cardiac muscle obviously potentiated acetylcholine (2µg/ml) inhibition of the force of contraction.

The methanol extract of *Nymphaea lotus* (L.) showed a dose dependent (1-4mg/ml) stimulation of the isolated rabbit jejunum preparation, this stimulation was refractory to atropine sulphate (2µg/ml) antagonistic property, but was blocked by Cyproheptadine (40µg/ml) (a non selective 5HT blocker). The extract (2mg/ml) exhibited a contraction of the guinea pig ileum preparation comparable with that of Histamine (10ng/ml) but refractory to the antagonistic action of the antihistamine Chlorpheneramine maleate(40ng/ml), on the other hand the extract(2mg/ml) induced contraction as well as 5Hydroxytreptammine were completely blocked by Cyproheptadine (20ng/ml). On rat fundus strip preparation both...
5-Hydroxytryptamine (25 ng/ml) and Nymphaea lotus methanol extract (2 mg/ml) showed a prominent contraction that was completely blocked by Cyproheptadine. The extract (2 mg/ml) neither blocked nor potentiated the Acetylcholine (1 µg/ml) induced contracture of the toad rectus abdominis muscle preparation. The extract per se showed no activity.

The methanol extract of *Anogeissus leiocarpus (DC.)* showed a dose dependent inhibition of the isolated rabbit jejunum preparation. This relaxant activity was not blocked by Tolazoline (40 µg/ml) while propranolol (4 µg/ml) completely blocked the inhibition. The extract (2 mg/ml) per se was without effect on both isolated guinea pig ileum preparation and isolated toad rectus abdominis muscle preparation. The extract neither antagonized nor potentiated the carbachol (1 µg/ml) contraction on the guinea pig ileum preparation and Acetylcholine (1 µg/ml) contracture on toad rectus abdominis muscle preparation.

On guinea pig atrium preparation the extract (2 mg/ml) decreased markedly the frequency of contraction and was devoid of any activity on the force of contraction. The extract (4 mg/ml) neither had any activity on isolated guinea pig tracheal preparation nor affected histamine (400 ng/ml) induced contraction of the preparation. On rat uterus preparation the extract (2 mg/ml) showed relaxant activity that blocked the Acetylcholine (1 µg/ml) contraction of the preparation, prior administration of propranolol (80 µg/ml) was without effect.
CHAPTER ONE

INTRODUCTION

1.1 Sudan location and vegetations diversity:

Sudan is the largest country in Africa, lying within the junction of civilizations of the Faraoh’s, Greeck and Islam. A position that led to a vast exchange of information, experience and practices of the ethnic groups living in these vicinities and that pass by during trade journeys and pilgrimages. In addition the unique climatic zones which extends between latitudes 4° N and 22° N and the longitudes 22° E and 38° E, the north is a barren desert, the south is equatorial and the east entertains a Mediterranean –like type of climate, this difference provides the natives with a large array of flora. These plants whether cultivated or wildly growing are used effectively particularly in rural area for treatment of various human ailments.

1.2 medicinal plants as potential source of antimicrobial agent:

The search for a noble antimicrobial urged the scientists to dig into the folklorically used plants and to exploit their potential, an effort which was ended by many targeted options; seventy six extracts of 31 Sudanese medicinal plants belonging to 21 families were investigated for their antibacterial activity against four bacteria by Farouk et al. (1983). Out of the 76 extracts tested, 64 exhibited inhibitory effect against at least one of
the tested microorganisms. Of these, seven plants showed significant activity against the four tested organisms, namely *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. These plants were *Achyranthus aspera* L. (Amaranthaceae), *Aristolochia bracteata* Lam., (Aristolochiaceae), *Ethulia conozoides* L., (Asteraceae), *Pulicaria crispa* (Forsk) Oliv., (Asteraceae), *Memordica multiflora* Hook. F., (Cucurbitaceae). *Bergia suffruticosa* (Del.) Fenzl. (Elatinaceae) and *Withania obtusifolia* Pauq. (Solanaceae).

Al Magboul and co workers (1992) screened a total of 573 plant extracts belonging to 111 Sudanese plants, distributed among 46 families for their antibacterial activity. Among the six plants belonging to the family Combretaceae, *Anogeissus leiocarpus* (Dc.) Guill. & Perr. was the most active plant, and its highest activity was observed with the leaf methanolic extract against the four tested organisms. All combretum species methanolic extracts possessed high activity against the tested organisms except *C. hartmannianum* Scheinf which exhibited high activity only against *P. aeruginosa* and low activity against the other three organisms. The stem and root-bark chloroformic extracts of *Anogeissus leiocarpus* showed activity against the four organisms tested, and the leaf extract exerted activity against one Gram-positive bacteria and one gram-negative organism. The leaf chloroformic extract of *Plicosepalus acaciae* (Zucc.) Weins & Pol. had a high activity against *E. coli*, low activity against the two Gram-positive organisms and no activity against *P. aerogenosa*. (Its equivalent methanolic extract was highly active against the four tested organisms), while its aqueous extract had equal high activities against the organisms examined. The chloroform extract of the whole plant of *Nymphaea lotus* (L.) was highly active against *S. aureus* and *E. coli*, and had a low activity towards the other two organisms. Its
methanolic as well as aqueous extracts were highly active against the four tested organisms. The chloroform extracts of the root-bark and stem-bark of *Anogeissus leiocarpus* (DC.) Guill. & Pers. exerted a fungicidal activity against *C. albicans* and a fungistatic effect against *A. niger*. However, the equivalent methanol and aqueous extracts of its different parts tested were inactive against the two test fungi. The chloroform extracts of the leaf as well as the whole plant of *Plicosepalus acaciae* (Zucc.) Weins & Pol., exhibited fungicidal activities against both *A. niger* and *C. albicans*. The chloroform extracts of the stem and root of the same plant exerted a fungicidal action against *C. albicans* and a fungistatic effect on *A. niger*. All methanolic and aqueous extracts of the four different parts of this plant did not show any activity against the two fungi tested. The whole plant chloroform extract of *Nymphaea lotus* L., exerted a fungicidal activity against both *A. niger* and *C. albicans*. However, its methanol and aqueous extracts did not show any activity against the two fungi examined. Tharib SM et al., (1986), screened four compounds isolated from the stems of the desert shrub *S.argel*, they found that only one compound showed reasonable antibacterial properties against both gram-positive and gram-negative bacteria.

Ross SA and co workers (1980) reported that *Solenostemma Argel* has a marked antifungal activity. Geraniin, hydrolysable tannin from *Nymphaea tetragona* Georgi (Nymphaeaceae) Was isolated from a fraction of the 70% aqueous extract of fresh leaves at 0.3% w/w. It was assayed for activity against 2 species of bacteria pathogenic to fish and inhibited the growth of both *Aromonas salmonicide* and *Pseudomonas fluorescens* (Kurihara et al, 1993). The flavonol aglycones quercetin and myricetin were isolated from the ether extract of aerial parts of flowering *N. hybrida* (collected from Egypt). Gallic and ellagic acids were isolated from the aerial parts of N. hybrida (collected from Egypt). They were
identified from spectral data. The compounds showed significant activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* (Saeed and Hamdy, 1996). Crude extract of *Anogeissus schemperi* used traditionally in Nigeria to treat infectious and septic diseases was screened in vitro for antibacterial activity, using the hole-plate diffusion method. The extract exhibited good activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Kudi et al, 1999). The antibacterial properties of a decoction and methanol extract of *Anogeissus leiocarpus* were used in traditional medicine of Mali, to treat fever and respiratory tract diseases, were tested for in-vitro activity against clinically isolated bacterial strains; *Haemophilus influenzae* (6 strains); *Staphylococcus aureus* (5 strains); *Streptococcus pneumoniae* (3 strains); *S. pyogenes* (8 strains) and *Moraxella catarrhalis* (5 strains) responsible for respiratory infections. The extract exhibited significant activity against all strains of bacteria tested. (Sanogo et al, 1998).

1.3 drugs- herbs interaction:

The adverse effects and drug interactions associated with herbal remedies are largely neglected. *Ginko biloba* extract, advertised as improving cognitive functioning, has been reported to cause spontaneous bleeding, and it may interact with anticoagulants and antiplatelet agents. St.John’s wort, promoted as a treatment for depression, may have monoamine oxidase – inhibiting effects or may cause increased levels of serotonin, dopamine and norepinephrine. Although St.John’s wort probably does not interact with foods that contain tyramine, it should not be used with prescription antidepressant. Ephedrine-containing herbal products have been associated with adverse cardiovascular events,
seizures and even death. Ginseng, widely used for its purported physical and mental effects, is generally well tolerated, but it has been implicated as a cause of decreased response to warfarin. (Cupp MJ, 1999).

Concurrent use of herbs may mimic, magnify, or oppose the effect of drugs. Plausible cases of herb-drug interactions include: bleeding when Warfarine is combined with garlic(*Allium sativa*), dong quai(*Angelica sinensis*) or danshen (*Salvia miltiorrhiza*). Decreased bioavailability of Digoxin, Theophylline, Cyclosporine and Phenprocoumon when these drugs are combined with St John’s wort; induction of mania in depressed patients who mix antidepressants and *Panax ginsing*; exacerbation of extrapyramidal effects with neuroleptic drugs and betel nuts (*Areca catechu*); increased risk of hypertension when tricyclic antidepressants are combined with Yohimbine (*Pausinystalia yohimbe*); potentiation of oral and topical corticosteroids by Liquorice (*Glycyrrhiza glabra*); decreased blood concentrations of Prednisolone when taken with the Chinese herbal product Xaio chai hu tang (sho-salko-to); and decreased concentration of Phenytoin when combined with the Ayurvedic syrup shankhaphushi. Anthranoid –containing plants (including senna[*Cassia senna*] and cascara [*Rhamnus purshiana*] and soluble fibres (including guar gum and psyllium) can decrease the absorption of drugs). (Fugh-Berman A., 2000).

### 1.4 proposed alternatives:

Many plants were studied in Medicinal and aromatic plants research institute (MAPRI) and found to have a potent antimicrobial activity of which three plants, namely *Loranthus acacia*, *nymphaea lotus* and
Anogeissus leiocarpus, are the most active and much available, thus selected for further pharmacological investigations, the topic dealt with in this text. According to Al Magboul and co-workers (1992), the methanolic extracts showed the highest level of antibacterial activity, with about 74% of the total number of methanolic extracts examined being active against the four tested organisms. The lowest level of activity against the tested bacteria was shown by the aqueous extracts, of which only about 20% were active against the four organisms tested. The chloroformic extracts exhibited an intermediate level of activity, with about 46% of the total number of chloroformic extracts screened being active against the four tested organisms.

1.5 Family Loranthaceae taxonomy:

Mainly shrubs parasitic on trees or very rarely erect terrestrial trees or shrubs. Leaves without stipules, usually opposite or whorled, entire, simple, sometimes reduced to scales.

Flowers often brightly coloured, actinomorphic, hermaphrodite or unisexual. Calyx small or rudimentary, adenate to the ovary. Petals valvate, free or united high up into a tube which is often split down on side. Stamens as many as the petals and inserted on them or at their base; anthers 2-locular or rarely 1-locular, some times divided into numerous smaller loculi. Disk present or absent. Rudimentary ovary often present in the male flower, staminodes in the female. Ovary inferior usually without a distinct placenta and ovule.

Fruit a berry or drupe; seed solitary, without distinct testa. (Andrews, 1952).
1.5.1 *Loranthus acacia (Zucc.) Weins & Pol.*

*Syn. Plicosepalus acacia:*

*Family:* Loranthaceae

The vernacular name:  Inab El-nabag,

Botanical Description: it is a succulent semi-parasitic glabrous shrub with solid, cylindrical, striate, brownish stems.

Leaves alternate, opposite or sub-opposite, petiolate, laminas oblonger elliptic-oblong, straight or slightly curved, ceriaceous, 2-4x 0.6-1.4 cm, apex rounded or obtuse, base cuneate, margin entire or slightly undulate; petioles c 3 mm long.

Inflorescences solitary or clustered umbels, axillary; pedicels c. 5 mm long. Flowers reddish, c. 3.5 cm long. Fruit drupe, ovoid, 0.5-1 cm across, reddish-brown, rugose. Fig (1.1)

Habitat: It is a semi parasitic on *Ziziphus spp.* And *Acacia spp.* This plant found in fangouga, el-mazroub, also widespread throughout Sudan.

1.5.2 **Folkloric use:**

The fresh whole plant is used as lactogogue and to enhance wounds healing (El-Gazali et al, 1997). The root is said to be used in Bahr El Gazal province for making a lotion for itch.
1.5.3 **Economic importance:**

Good camel fodder. (Braun & Massey, 1929).

1.5.4 **Chemical constituents:**

The genus expertise many works around the world investigating its medicinal use and constituents.  
*Loranthus yadoriki*, meoh extract showed higher electron donating ability than alpha-tocopherol. Using fractionation on column chromatography and evaluating the reduction of the free radical alpha, alpha-diphenyl-beta-picryl hydrozyl.

It was thought that Phenolic compounds including gallic acid account for antioxidative activity (Choi-WonSil and Ahn-WonYung, 2000).

Lin-terhui et al.,(1999) isolated 8 flavonoids from fresh leaves of *Loranthus kaoi*, an endemic parasitic shrub found only at median altitudes in the central parts of Taiwan.  
These compounds were a mixture of (+)- and (-)-catechin,(-)-epicatechin,2’,4’,6’-trihydroxydihydrochalcone,4’-o-beta-D-glucoside, kaempherol 3-o-alpha-D-rhamnoside,kamferol3,7-di-o-beta-D-glucoside-quercetin 3-o-alpha-D-rhamnoside and quercetin 3-o-beta-D-glucoside. The structure of these compounds were established on the basis of physical properties and spectroscopic evidence (Lin-JerHuei et al,,1999) . Structural features of water soluble polysaccharides from Korean oak mistletoe (*Loranthus yadoriki* Sieb) was studied by Lee-SuHee and Ahn-WongYung,,(1996), the sugars of the water soluble polysaccharide (WSP) from *Loranthus yadoriki*, were mainly arabinose and galactose in
the stem, and very high contents of galactose in the leaves which were
different in composition from those in the stem.

Ahn-WonYung, (1996) noted that Loranthus yadoriki had three types of
triterpenoid, oleanane, lupane, and ursane, irrespective of hosts, sampling
positions and species.
The bark of Loranthus globosus contains 17.4% tannin (pyrogallol ad
catechol) (Haque, 1979).

1.5.5 Biological activity:

Amongst the 122 species screened by yang et al, (1987) for
antihepatotoxic activity in primary cultured hepatocytes, Loranthus
parasiticus showed more than 50% inhibition against cytotoxicity
induced by carbon tetrachloride and D-galactosamine, respectively.

Kusumoto et al, (1992) tested methanol and water extracts of 30
Indonesian plants for inhibitory activity on avian myeloblastosis virus
reverse transcriptase (RT). The most potent inhibition was shown by
extracts of Loranthus parasiticus (whole plant).
The extract showed no cytotoxicity at concentrations where over 90% of
RT activity was inhibited.

Metabolic and renal changes following the ingestion of Loranthus
begwensis in rats was investigated by Obatomi et al (1997). Treatment
with extract significantly and dose-dependently reduced serum glucose
and cholesterol levels, and reduced urine flow rate and serum creatinine.
Increased excretion of urinary enzymes and protein was dose-related and
dependent on extract source.
Obatomi-Dk et al, (1996) administered an aqueous extract (1.32g/kg, p.o.) of leaves of *Loranthus bengwensis* to normotensive (NWR) and spontaneous hypertensive (SHR) rats for 8 days. A significant reduction in the mean arterial pressure in both NWR and SHR was observed. A significant reduction in the serum total cholesterol concentration was also observed on days 6, 7 and 8.

1.5.6 **Anticholinesterase activity in plant kingdom:**

*Evodia rutaecarpa* Bentham showed a strong inhibitory effect on acetylcholinesterase in vitro and an anti-amnesic effect in vivo, it inhibited anticholinesterase in a dose-dependant and non-competitive manner. A single administration of DHED (dehydroevodiamine hydrochloride; the finally identified active component) to rats (6.25mg/kg) significantly reversed the scopolamine–induced memory impairment in a passive avoidance test. The anti amnesic effect of DHED was more potent than that of Tacrine, which is the only drug of DHED, was thought to be due to the combined effects of acetylcholinesterase inhibition and the known cerebral blood flow enhancement.

These results indicate that DHED has novel anti-cholinesterase and anti-amnesic activities and might have therapeutic potential in various disorders including Alzheimer’s disease. (Park et al, 1996).

Galanthamine (or galantamine, REminyl) a tertiary alkaloid is a naturally occurring acetylcholinesterase inhibitor (AChEI) has been approved in several countries for the symptomatic treatment of senile dementia of the Alzheimer’s type. It was early isolated from bulbs of the common
snowdrop and several amaryllidaceae plants, (-)-galanthamine (GAL), and has long been used in anesthetics to reverse neuromuscular paralysis induced by Tubocurarine-like muscle relaxants and more recently, has been used to attenuate drug-and lesion-induced cognitive deficits in animal models of learning and memory. GAL directly inhibits acetylcholinestrase activity, while demonstrating much weaker activity on butyrylcholinestrase (BuChE). GAL also stimulates pre- and postsynaptic nicotinic receptors, although the clinical significance of this finding is yet unclear. Numerous variants and analogues of GAL have also been developed, with varying potency in inhibiting AChE activity. The efficacy of GAL administered to Alzheimer’s disease (AD) patients have been well demonstrated by large-scale clinical trials. Typical of AChEIs , the most common adverse events associated with GAL are nausea and vomiting . In conclusion, evidence to date suggests galanthamine to be similar to other AchEIs in improving cognitive function in AD patients. (Sramek JJ et al, 2000).

As current memory-enhancing/anti-dementia drugs are based on enhancing cholinergic activity by inhibiting cholinesterase, Perry and his co-workers, 2000 studied the effects of Salvia lavandulaefolia Vahl. (It is reputed in European herbal encyclopedias to enhance memory) essential oil and some of its constituent terpines on human erythrocyte acetyl cholinesterase in vitro. The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be non-competitive reversible inhibitors. These findings suggest that if the inhibitory activity of the essential oil is primarily due to the main inhibitory terpenoid constituents identified, there is a major synergistic effect among the constituents. Since no single constituent tested was particularly potent, it remain to be determined whether these in-vitro cholinesterase inhibitory activities are relevant to
in vivo effects of the ingestion of S.lavandulaefolia essential oil on brain acetylcholinesterase activity.

Sung et al, 2002 Found that a total methanolic extract of the underground parts of Caragana chamlague (Leguminosae) had significant inhibition towards AchE, which is specific, reversible and non competitive.

It was demonstrated that the zeatin (one of the derivatives of purine adenine, confirmed by electron impact mass spectrometry) from Fiatoua villosa appeared to be the most potent AchE inhibitor in AD. (Heo HJ et al, 2002)

A fluorometric assay for acetylcholinesterase inhibitory activity was developed in a flow system using the fluorogenic substrate 7-acetoxy-1-methyl quinolinium iodide which is hydrolyzed to highly fluorescent 7-hydroxy-1-methyl quinolinium iodide. The detection limit of galanthamine is 0.5microM, which is about 20 times more sensitive than in the colorimetric flow assay. In the presence of 30%methanol or of 5%acetonitrile, about 70% of the enzyme activity could still be detected. Various plant extracts have been screened using the described system including bulbs of Galanthus nivalis, Eucharis amazonica (E.x grandiflora), Crinum powelli and Nerine bowdenii (all member of amaryllidaceae), which showed strong AchE inhibitory activity. (Rhee et al, 2003).

Bacoba monniera and Ginkgo biloba are well-Known cognitive enhancers in Indian and Chinese traditional medicine systems. Standardized extracts of B.monniera and G.biloba were used to evaluate the antidementic and anticholinesterase activities in adult male Swiss mice. These extracts possess a significant anticholinesterase and antidementic
properties, which may be useful in the treatment of dementia. (Das A et al., 2002).

During the last decade, there has been an explosive growth of research concerning the extract of *Ginko biloba* termed Egb 761. In experimental, animal and clinical studies Ginko biloba has shown a similar pharmacological potency and clinical efficacy like synthetic defined drugs in the therapy of reduced cerebral performance. *Ginkko biloba* special extract Egb 761 is a standardized and highly purified extract of Ginko leaves. Containing as active constituents ginko-flavone glycosides and the terpene-lactones (ginkgolides, bilobalide). The multifactorial principle of action of *Ginko biloba* is characterized by rheological and blood-flow-promoting properties, protective effects against ischaemia and hypoxia, effects on nerve cell energy metabolism, antiedematous and myelin-protective effects, radical-scavenger activity, effects on various cerebral transmitters and receptor systems. These actions explain the rationale for clinical trials in vascular dementia and primary degenerative dementia of the Alzheimer type, and in mixed forms of both. The cerebral bioavailability of *Ginko biloba* extract has been demonstrated by electoencephalography. In clinical trials of different working-groups, effects of *Ginko biloba* on the cognitive performance, global function, and activities of the daily living have been identified. Metaanalysis in the indication—dementia disorders—comparing *Ginko biloba* versus acetylcholinestrase inhibitors have shown a similar clinical efficacy of both therapy regimens with an additional drug safety benefit for Ginko. Due to the clinical efficacy the WHO accepted *Ginko biloba* as anti dementive drug. In future antidementive therapy drugs with different mode of action should be given in combination. Furthermore, it has been stated that clinical trials with fixed combinations of acetylcholinestrase
inhibitors with *Ginko biloba* extracts in moderate or severe dementia would be necessary. (Loed D, 2002)

It was shown that an alkaloid of *Artemisia asiatica* (Korean traditional tea plant), which was metabolized to small molecule in digestive tract and then could pass through the blood-brain barrier, appeared to be an acetylcholinesterase inhibitor with a blocker of neurotoxicity induced by A beta in human brain causing Alzheimer’s disease. (Heo HJ et al, 2000).

Kim SR and his co-researchers, (1999) suggested that protropine (an alkaloid obtained from the methanolic extract of *Corydalis ternata* [papaveraceae]) have both anti-acetylcholinesterase and antiamnesic properties that may ultimately hold significant therapeutic value in alleviating certain memory impairments observed in dementia.

A complex glycoside containing phenol and uronic acid part obtained from *Eugenia caryophyllus* inhibited brain acetylcholinesterase activity. This inhibition was not due to the acidic nature of the extract and suggests that *Eugenia caryophyllus* contains some water-soluble substance(s) with anti-cholinesterase activity. (Akinrimisi and Akinwande, 1976).

Oral administration of the essential oil of *Salvia lavandulaefolia* at two dosage levels to rats for five days inhibited cholinesterase in vitro also has an in vivo effect and this may help to explain its traditional use for ailing memory. (Perry N.S. et al, 2002).
1.6 Anticholinesterases:

They prolong the existence of the acetylcholine after it is released from cholinergic nerve endings. These agents inhibit acetylcholinesterases, which is concentrated in synaptic regions and is responsible for the rapid hydrolysis of acetylcholine. Anticholinesterase agents have therapeutic utility in the treatment of glaucoma and have other ophthalmic indications. The facilitation of gastrointestinal and bladder motility and influencing activity at the neuromuscular junction of skeletal muscle, are desired in myasthenia gravis. The use of anticholinesterase agents in Alzheimer’s disease may be an emerging application of this class of drugs. (Goodman and Gilman’s 1996)

Peripherally acting anticholinesterase drugs fall into three main groups according to the nature of their interaction with the active site, which determines their duration of action. Most of them inhibit AchE and BchE about equally.

1.6.1 Short –acting anticholinesterase:

The only important drug among the short –acting anticholinesterase is Edrophonium, a quaternary ammonium compound that binds to the anionic site of the enzyme only. The ionic bond formed is readily reversible and the action of the drug is very brief. It is used mainly for diagnostic purposes, since improvement of muscle strength by an anticholinesterase is characteristic of myasthenia gravis, but does not occur when muscle weakness is due to other causes.
1.6.2 Medium –duration anticholinesterases:

The medium –duration anticholinesterases include Neostigmine and Pyridostigmine, which are quaternary ammonium compounds of clinical importance, and Physostigmine (Eserine), a tertiary amine which occurs naturally in the dried ripe seed of *Physostegma venenosum* Balfour (Calabar bean), a perennial plant found in tropical West Africa. The Calabar bean was once used by native tribes of West Africa as an ‘ ordeal poison ‘ in trials for witchcraft, a pure alkaloid was isolated by Jobst and Hesse in 1864 and named Physostigmine, the first therapeutic use of the drug was in 1877 by Laqueur, in the treatment of glaucoma, one of its clinical uses today, (Karczmar, 1970).

These drugs all possess strongly basic groups, which bind to the ionic site, but are carbamyl, as opposed to acetyl esters. Transfer of the carbamyl group to the serine-OH of the esteratic site occurs, as with acetylcholine, but the carbamylated enzyme is very much slower to hydrolyze, taking minutes rather than microseconds. The anticholinesterase drug is therefore hydrolyzed, but at a negligible rate compared with acetylcholine, and the slow recovery of the carbamylated enzyme means that the action of these drugs is quite long lasting.

1.6.3 Irreversible anticholinesterases:

Irreversible anticholinesterases are pentavalent phosphorus compounds containing a labile group such as fluoride (in dyflos) or an organic group (in parathion and eclothiophate). This group is released leaving the residue of the molecule attached covalently through the phosphorus atom to the serine-OH group of the enzyme. Most of the organophosphate compounds, of which there are many, developed as war gases and
pesticides as well as for clinical use, interact only with the esteratic site of the enzyme and have no cationic group. Ecothiophate is an exception in having a quaternary nitrogen group designed to bind also to the anionic site. The inactive phosphorylated enzyme is usually very stable. With drugs such as dyflos no appreciable hydrolysis occur and recovery of enzymatic activity depends on the synthesis of new enzyme molecules, a process that may takes weeks. With other drugs such as Ecothiophate slow hydrolysis occurs over the course of a few days, so that their action is not strictly irreversible. Dyflos and parathion are volatile non-polar substances of very high lipid solubility, and are rapidly absorbed through mucous membranes and even through unbroken skin and insect cuticles; the use of these agents as war gases or insecticides relies on this property. The lack of a specificity-conferring quaternary group means that most of these drugs block other serine hydrolases (e.g. Trypsin, Thrombine) though their pharmacological effects result mainly from cholinesterase inhibition. (H.P. Rang, M.M. Dale and J.M. Ritter, 2000).

### 1.6.4 Clinical uses of anticholinesterases:

They are currently used in:

- **Anaesthesia**, to reverse the action of non-depolarizing neuromuscular-blocking drugs; neostigmine intravenously. Neostigmine lacks central effects and causes fewer parasympathetic effects than other anticholinesterases; nevertheless, atropine is given routinely as a precaution.

- **The treatment of myasthenia gravis**; neostigmine and pyridostigmine orally:
  - Pyridostigmine acts for slightly longer (3-6 h) than neostigmine (2-4 h).
Muscarinic side effects tend to wear off with continued use.

Excess use can produce a cholinergic crisis consisting of various muscarinic effects (salivation, gastrointestinal cramps, lacrimation, poor vision, etc) together with muscle weakness, resulting presumably from depolarization block. Injection of edrophonium may be used as a test to distinguish between this drug-induced weakness and the weakness of myasthenia itself; if the weakness transiently improves it is due to myasthenia and more anticholinesterase is indicated; if it gets worse the anticholinesterase dose should be reduced.

Treatment of glaucoma; ecothiophate as eye drops. Systemic side effects may occur, and plasma cholinesterase activity may be reduced, which can cause prolongation of the action of suxamethonium, if this is given concurrently. (H.P. Rang, M.M. Dale and J.M. Ritter, 2000).

1.7 **Family Nymphaeaceae taxonomy:**

Aquatic herbs (water lilies) with peltate or cordate leaves arising from submerged prostrate rhizome. Flowers solitary, large and showy. Often sweet-scented. Sepals 4-6, free, imbricate, sometimes gradually passing into stamens. Stamens numerous. Carpels 8 or more, free and immersed in the receptacle or more or less united into an ovary of as many loculi, fleshy, surrounding the ovaries or ovary and with the petals and stamens inserted on its side. Fruit multilocular, indehiscent, fleshy. (Andrews, 1950).

1.7.1 **Nymphaea lotus L.**

The vernacular name is: Al Suteib,
It is an aquatic pubescent herb with submerged prostrate rhizomes. Leaves radial, peltate, petiolate; laminas rounded, 11-30 cm across, with many strong radiating nerves, apex rounded, base chordate, margin sharply toothed; petiols up to 2m long. Inflorescences solitary, pedicles up to 20cm across. Fruits multi-locular, indehiscent, and fleshy.

Habitat: It is an aquatic plant with floating leaf–laminas, found in Um Hegleiga, also widespread throughout central and southern Sudan. (El Gazali et al, 1997).

1.7.2 Folkloric uses:

the roots are used for dysentery and as a fumigant for rheumatism. (El Gazali et al, 1997). The powdered root is used for piles, dysentery and dyspepsia; the seeds are used in skin diseases, leprosy, etc. (Braun & Massey, 1929).

1.7.3 Chemical constituents:

The alkaloid nymphaeine is found mainly localized in the rhizomes of N. lotus; nymphaline & nupharine in the flowers (Delphant and Balansard, 1943).

Seven flavonols including the novel 3-(2’’-acetyl-rhamnosides) of myricetin and quercetin, the rare kaempferol 3-(2’’-acetyl-rhamnoside), in addition to the 3-rhamnoside of kaempferol and quercetin, were isolated from blue flowers of N. caerulea collected from Uganda by (Fossen et al, 1999).
Two novel acylated anthocyanins were isolated from blue flowers of the African water lily, *N. caerulea* by Fossen and Andersen, 1999, they were identified to be delphinidin 3’-o-(2”-o-galloyl-beta-galactopyranoside) and delphinidin 3’-0-(2”-0-galloyl-6”-0-acetyl-beta-galactopyranoside). Contrary to other anthocyanidin 3’-glycoside reported, both have the monosaccharide galactose instead of glucose on the B-ring and no glycosyl moiety on the anthocyanidin 3-position. The chemotaxonomic significance of anthocyanidin galloyl galactosides in Nymphaeaceae is discussed.

Alpha- and beta- (or gama)-Tocopherols, esterified with saturated monocarboxylic acids ranging from C12 to C18, were identified for the first time in the leaves and stems of *Nymphaea alba*, using high temperature GC-FID and GC-MS mass fragmentography (Klink et al, 1994).

Uncooked / sun-dried (UCSD) and boiled / freeze-dried (BFD) flours of the Malawian water tuber *Nymphaea petersiana* (NYika) were analyzed for selected nutrients and antinutrients. On a wet weight basis, the flours contained 8.1 and 8.0 % crude protein; 0.8 and 1.0 % crude fat; 12.0 and 13.0% dietary fiber and 2.2 and 1.9% ash, respectively. The flours also contained 928 and 1300 mcg/g of calcium; 2600 and 2200 mcg/g of phosphorus; 88 and 20mcg/g of iron; and 22 and 20 mcg/g of zinc, respectively. USCD and BFD flours were limiting in lysine and had amino acid scores of 91 and 84. The predominant fatty acids in the tubers were oleic (47%), linoleic (32%), palmitic (21%), linolenic (7%). Tannin content was 1.5 and 1.0%; phytate content was 5.4 and 3.9mcg/g; trypsin activity was reduced from 400 to 55 TIU/g of tuber and chymotrypsin
activity was reduced from 240 to 50 CIU/g by moist heat. Hydrogen cyanide was below detection limits (Chawanje et al, 2001).

Lavid-N et al, (2001) found that in *Nymphaea aurora*, heavy metals are accumulated primarily in association with glands found in plant organs that have direct contact with water or mud. Deposition and storage of heavy metals by these glands may represent a stage in the sequestration and detoxification of the metals. Our results raise the possibility of utilizing water lilies for the removal of heavy metals from polluted environments.

Jambor and Skrzypczak, (1991) isolated from flower of *Nymphaea alba* the following flavonoids; the a glycone quercetin, kaempferol, isokaempferide and apigenin and the glycosides quercetin 4’-beta-xyloside, 3-methylquercetin 3’-beta-xyloside, quercetin 3-galactoside and quercetin 3-glucoside.

Crystalline ellagic and gallic acids and their methyl and ethyl esters, as well as small amounts of p-hydroxybenzoic, p-coumaric, vannillic and ferulic acids, were isolated from *Nymphaea alba* flowers. The presence of bound forms of the acids was demonstrated by (Jambor and Skrzypczak, 1991).

Three novel flavonols, myricetin-3’-O-(6”-p-coumaroyl)glucoside and two epimeric macrocyclic derivatives, as well as the known myricetin-3-O-rhamnoside and pentagalloyl glucose, have been isolated from the wild water lily *Nymphaea lotus* L. and identified using 2D NMR. This is the first report of such a macrocycle from any source. (Awatif A. Elegami et al., 2003)
1.7.4 **Biological activity:**

A mixture of herbs indigenous to Pakistan containing *Nymphaea lotus* was studied for its hypocholesterolaemic effect in rabbits. The mixture in doses of 1 or 2 g/kg B.W resulted in significant decreases in serum cholesterol levels of hypercholesterolaemic rabbits after 1 week. However, the drug mixture did not decrease the serum cholesterol level of control rabbits after 2 weeks of administration. (Kamran and Ahmed, 1992).

The flavonol aglycones quercetin and myricetin and The flavonol glycosides quercetin 3-glucoside were isolated from the ether and ethyl acetate extract of the aerial parts of flowering *N. hybrida* collected from Egypt. Quercetin and myricetin exhibited antimicrobial (against bacteria and fungi), anti-inflammatory, analgesic and devoid of ulcerogenic property. (Saeed and Hussieny, 1996).

1.7.5 **Serotonin occurrence in plant kingdom:**

The benzene: ethyl acetate fraction (BE) of the leaves of *Sesabania grandiflora* used in Ayurveda for the treatment of epileptic fits, raised the brain contents of gamma-aminobutyric acid and serotonin. Thus the triterpine fraction exhibits a wide spectrum of anticonvulsant profile and anxiolytic activity. (Kasture VS et al, 2002).

*Hypericum perforatum* extract oral acute administration reduced the immobility time of wild type, but not of knockout mice. Hypericum did not modify tryptophan content in all the animal groups. Hypericum
increased serotonin and 5-HIAA diencephalic content in both wild and knockout mice. However, the increase observed in the wild type was greater than in knockout mice. These data indicate that IL-6 could be necessary to the antidepressant action of hypericum, and that this cytokine (probably) mediates the effects of Hypericum through activation of the serotonin system. Calapi G et al, (2001)

The effects of acute administration of hypericum extracts on levels of 5-hydroxytreptamine (5-HT), tryptophan, 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline and dopamine in the cortex, diencephalon and brainstem was evaluated by Calapai G et al, (1999) and confirmed that acute administration of hypericum extracts modifies the levels of neurotransmitters involved in the pathophysiology of mood disorders. When the extract contains a higher concentration of flavonoids the effects are more widespread and involve brain regions such as diencephalon and brainstem that are implicated in depression.

The convulsive response to pentylenetetrazol has been investigated by Cabral-Filho JE et al, (1987) in rats receiving different dietary tryptophan inputs. They found that corn diet facilitated seizures; the low-protein/high-carbohydrate/tryptophan diet prevented seizures. These results suggest that the brain serotonin levels determined by dietary tryptophan, or tryptophan by itself could play a role in the convulsive response.

The effect of saponin containing n-butanolic fraction (BF) extracted from dried leaves of *Albizzia lebbeck* on learning and memory was studied in albino mice using passive shock avoidance paradigm and the elevated plus maze. Significant improvement was observed in the retention ability
of the normal and amnesic mice as compared to their respective controls. The brain concentrations of GABA and dopamine were decreased, whereas the 5-HT level was increased. The data indicate the involvement of monoamine neurotransmitters in the nootropic action of BF of *A. lebbeck*. (Chintawar SD et al, 2002).

Tissue bath techniques were used to examine the effect of cotton bract extract (CBE) on isolated canine airways. The CBE elicited strong contractions that were abolished by the 5-hydroxytryptamine (5-HT) antagonist, methysergide, but were only slightly affected by atropine. Russell JA et al, (1982) concluded that cotton bracts contain a potent 5-HT receptor agonist that appears to be distinct from 5-HT.

Ohia SE et al, (2002) demonstrated the efficacy of *Garcinia cambogia* – derived natural (-)-hydroxycitric acid (HCA) in weight management by curbing appetite and inhibiting body fat biosynthesis. They also showed that in the rat brain cortex a novel HCA extract (HCA-SX, Super CitriMax) increases the release/availability of radio labeled 5-hydroxytryptamine or serotonin, furthermore, HCA-SX can inhibit 5-HT uptake (and also increase 5-HT availability) in isolated rat brain cortical slices in a manner similar to that of SRRIs. And thus may prove beneficial in controlling appetite, as well as treatment of depression, insomnia, migraine headaches and other serotonin-deficient conditions.

Mittra S et al, (2000), showed that Feverfew (*Tanacetum parthenium* having antimigraine activity) powder is more potent than any of its extract or parthenolide alone in its antiserotonergic activity. Degraded Feverfew extracts show a marked decrease in their antiserotonergic activity. With thermally degraded Feverfew powder containing less contents of parthenolide no built-up antiserotonergic responses were
observed after one month. This ascertains that Feverfew should be dispensed in a properly stabilized form wherein its parthenolide content is not degraded to less than 90% of the original content.

1.8 5-hydroxytryptamine:

It has a diverse physiological role as a neurotransmitter in the central nervous system, as a regulator of smooth muscle function in the cardiovascular and gastrointestinal systems, and as a regulator of platelet function. Molecular cloning has revealed an unexpected diversity of receptor subtypes (15 to date), which fall into four structural and functional families,(Sjoerdsma and Palfreyman, 1990). Three subtype families (5-HT1, 5-HT2, and 5-HT4) are coupled via G proteins to a variety of enzymatic and electrical effector systems; the 5-HT3 receptor, in marked contrast, serves as a 5-HT-gated ion channel.

1.8.1 5-Hydroxytryptamine Distribution, biosynthesis and degradation:

The structures, which are rich in 5-HT are; gastrointestinal tract (chromaffin cells and enteric neurons), platelets and central nervous system. Its metabolism closely parallels that of noradrenaline. 5-HT is formed from dietary tryptophan, which is converted to 5-hydroxytryptophan by tryptophan hydroxylase, then to 5-HT by a non-specific decarboxylase. Transportation into 5-HT-containing cells is by a specific transport system. The degradation of 5-HT occurs mainly by MAO, forming 5-HIAA, which is excreted in urine. (H.P. Rang, M.M. Dale and J.M. Ritter, 2000).
1.8.2 **Actions and functions of 5-HT:**

The important actions are:

- Increased gastrointestinal motility (direct excitation of smooth muscle and indirect action via enteric neurons). Motility of gastric and intestinal smooth muscle may be either enhanced or inhibited via at least six subtypes of 5-HT receptors. The stimulatory effect occurs at nerve endings on longitudinal and circular enteric muscle, at postsynaptic cells of the enteric ganglia, and by direct effects of 5-HT on the muscle cells (5-HT\textsubscript{2A} in intestine and 5-HT\textsubscript{2B} in stomach fundus), (Dhasmana et al., 1993).
- Contraction of other smooth muscles (bronchi, uterus).
- Mixture of vascular constriction (direct and via sympathetic innervations) and dilatation (endothelium dependent).
- Platelet aggregation.
- Stimulation of peripheral nociceptive nerve endings.
- Excitation / inhibition of CNS neurons.

1.8.3 **Postulated physiological and pathophysiological roles include:**

- In periphery: peristalsis, vomiting, platelet aggregation and haemostasis, inflammatory mediator, sensitization of nociceptors and microvascular control.
- In CNS: many postulated functions, including control of appetite, sleep, mood, hallucinations, stereotyped behavior, pain perception and vomiting.

1.8.4 5-HT receptors:

- Seven types (5-HT 1-7) with further subtypes (A-D) of 5-HT1 and 5-HT2. All are g-protein coupled receptors, except 5-HT3, which is a ligand gated cation channel.
- 5-HT1 receptors occur mainly in CNS (all subtypes) and some blood vessels (5-HT1D subtype). Effects are neural inhibition and vasoconstriction. Act by inhibiting adenylate cyclase. Specific agonists include: sumatriptan (used in migraine therapy) and buspirone (used in anxiety). Ergotamine is a partial agonist. Specific antagonists include spiperone and methiothepin.
- 5-HT2 receptors occur in CNS and many peripheral sites (especially blood vessels, platelets, autonomic neurons). Neuronal and smooth muscle effects are excitatory. Act through phospholipase C/inositol phosphate pathway. Specific ligands include LSD (agonist in CNS, antagonist in periphery). Specific antagonists: ketanserin, methysergide and cyproheptadine.
- 5-HT3 receptors occur in peripheral nervous system, especially nociceptive afferent neurons and enteric neurons, and in CNS. Effects are excitatory, mediated via direct receptor –coupled ion channels. Specific agonists: 2-methyl-5-HT. Specific antagonist: ondansetron, tropisetron. Antagonists are used mainly as anti-emetic drugs, but may also be anxiolytic.
• 5-HT4 receptors occur mainly in the enteric nervous system (also in CNS). Effects are excitatory causing increased gastrointestinal motility. Act by stimulating adenylate cyclase. Specific agonists include metoclopramide (used to stimulate gastric emptying).
• Little is known so far about the function and pharmacology of 5-HT5-7 receptors.

1.8.5 **Important drugs that act on 5-HT receptors in the periphery include:**

• 5-HT1D receptor agonists (e.g. sumatriptan) used for treating migraine. Selective 5-HT1A agonists, such as 8-OH-DPAT, are potent hypotensive agents, acting by a central mechanism, but are not used clinically.
• 5-HT3 receptor antagonists (e.g. ondansetron, granisetron, tropisetron) used as anti-emetic drugs particularly for controlling the severe nausea and vomiting that occurs with many forms of cancer chemotherapy- a major advance since this side effect is one of the main factors limiting the effective use of chemotherapy.
• 5-HT2 receptor antagonists (e.g. dihydroergotamine, methysergide, cyproheptadine, ketanserine, ketotifen, pizotifen). These classical 5-HT antagonists act mainly on the 5-HT2 receptor. They are however, non-selective, and act also on other targets, such as alpha adrenoceptors and histamine receptors. Dihydroergotamine and methysergide belong to the ergot family and are used mainly for migraine prophylaxis. Ketotifen is used sometimes to treat asthma but the role of 5-HT receptors in the condition is unclear. Other 5-HT2 antagonists are used to control the symptoms of carcinoid tumours.
5-HT4 receptor antagonists (e.g. metoclopramide, cisapride), which stimulate coordinated peristaltic activity (known as a prokinetic action), are used for treating gastrointestinal disorders. (H.P. Rang, M.M. Dale and J.M. Ritter, 2000).

1.9 **Family Combretaceae taxonomy:**

Trees or shrubs, sometimes climbing. Leaves opposite or more rarely alternate or verticillate, without stipules, simple. Flowers spicate or racemose or in heads, mostly small, hermaphrodite or rarely unisexual. Calyx 4-8-fid or –lobed; lobes valvate. Petals 4-5 or more or absent, free, small. stamens 4-10, rarely more ; filaments inflexed in bud ; anthers versatile , opening lengthwise by slits . Ovary inferior or rarely semi-inferior, 1-locular; ovules 2-6, apical. Fruit often winged, rarely dehesent. (Andrews , 1950).

1.9.1 *Anogeissus leiocarpus (DC.) Guill & Perr:*

syn. *Conocarpus leiocarpus* (DC.) Guill & Perr. :

*Anogeissus schimperi* Hochst

Family : Combretaceae

Vern. Name: Al Sahab

Medium sized to large trees up to 20m high.
Bark grayish white becoming dark gray, scaly. Branches often drooping and slender. Leaves alternate, rarely opposite or sub-opposite, elliptic to ovate lanceolate, 2-8 *1.3-5 Cm, densely silky becoming pubiscent beneath. Inflorescence small, greenish-yellow globuse. Heads; petals absent. Fruit in globose cone. Like heads, broadly winged, coriaceous, dark grey, about 3mm across, beaked by the persistent receptacle.
Flowers june-oct; Fruits july-feb. fig (1,4 and 1.5)
Habitat: A common constituent of the gallery forests on high rain fall savanna on well drained or alluvial soils along streams, rivers and valleys in s.kassala, kordofan, s.darfor, blue Nile, upper nile, bahr algazal and equatoria.(Hamza,1990).

1.9.2 Economic value:

Timber, white, strong, elastic, useful for building carts, implements, etc. the roots are rich in tannin and the wood ashes are used in W.Africa as a mordant for fixing dyes. Yields an insoluble gum. (Broun &Massey, 1929).

Folk uses:
The decoctions of the barks are used against cough. (EL Gazali et al, 2003).

1.9.3 Chemical constituents:

The leaves, root and bark contain tannins (Daziel, 1937). The gum exuding from the trunk contains 20% uronic acids which produce via hydrolysis xylose , arabinose, and fructose (Aspinal and Christensen, 1961). The gum contains two distinct polysaccharides Leiocarpan A and Leiocarpan B (Watt and Breyer-Brandwijk, 1962); benzoic acid and Ellagic acid in the leaves and ellagittannin constituents in the sapwood (Nduji et al., 1982;Nduji and Okwute, 1983). More polyphenols have been identified in the bark; 3, 3’, 4’- tri-0-methylellagic acid and a tri-0-methylellagic acid (Nduji and Okwute, 1988).

Anogeissus leiocarpus, a chewing species, used for oral hygiene, from tropical West Africa was analyzed for 11 inorganic elements using
inductively coupled plasma and ion chromatography. Inorganic contents in the whole wood, xylem and bark meals were analyzed. The elements accounted for 26-to74% of the ash content. Calcium and potassium concentrations were highest while phosphorus, aluminum, sodium, magnesium and flourine were found in appreciable amounts. Notable differences in elemental concentrations occurred between the bark and xylem. (Akande and Yamamoto, 1998). Preliminary phytochemical screening of the 7 most active species showed that 5 were rich in tannins and/or flavonoids. The amino acid compositions of the commercial gum ghatti (Anogeissus schemperi) were tabulated. The principal amino acids common to this species were aspartic acid, alanine, and glycine; it was low in hydroxyproline. (Anderson et al, 1987).

1.9.4 Biological activity:

Elegami et al, (2002) screened the Anogeissus schemperi for antibacterial activity against standard organisms (Staphylococcus aureus NCTC 6447, Bacillus subtilis NCTC 8236, Escherichia coli NCTC 8196 and Pseudomonas aeruginosa NCTC 57501), as well as clinical isolates (S. aureus, E. coli, Proteus vulgaris and Pseudomonas aeruginosa). The extract in different solvent systems showed high activity against both standard organisms and clinical isolates. Phytochemical screening revealed that the plant were very rich in tannins to which antibacterial activity may be attributed.

More than 100 extracts of 15 plant species in 6 families were investigated by Almagboul et al (1988). The majority (85%) showed inhibitory effects against one or more of the 4 microorganisms tested (Staphylococcus
Preliminary phytochemical screening of the 7 most active species showed that 5 were rich in tannins and/or flavonoids. The most promising species were *Anogeissus leiocarpus*, *V. venosa* and *Vernonia adoensis*.

The activity of aqueous extract (0.25-50 mg/ml) from *Anogeissus leiocarpa* [A. leiocarpus] showed (LC50= 9.5-84.6 mg/ml) on free-living nematode *Caenorhabditis elegans* as the test species. (Ibrahim, 1992).

During an ethnopharmacological survey of antiparasitic medicinal plants used in Ivory Coast, 17 plants were identified and collected. Polar, non-polar and alkaloidic extracts of various parts of these species were evaluated in vitro in an antiparasitic drug screening. Antimalarial, leishmanicidal, trypanocidal, antihelminthiasis and antiscabies activities were determined. Among the selected plants, *Anogeissus leiocarpus* and *Terminalia glaucescens* were strongly active against *Plasmodium falciparum*. (Okpekon T. et al 2004).

Eight extracts from four Ivorian medicinal plants, traditionally used to treat malaria, were tested for their antiplasmodial activity in vitro by assessing their ability to inhibit the uptake of [3H] hypoxanthine into the *Plasmodium falciparum K1* chloroquine-resistant strain. The most active extract was the methylene chloride extract of *Anogeissus leiocarpus*. (Vonthron-Senecheau C., 2003)

### 1.10 Classification of adrenoceptors:

(Ahlquist, 1948) first proposed the existence of more than one adrenergic receptor; he based his hypothesis on a study of the abilities of adrenaline,
noradrenaline, and other related agonists to regulate various physiological processes, he proposed the designations $\alpha$ and $\beta$ for receptor on smooth muscle where catecholamines produce excitatory and inhibitory responses, respectively.

- Main pharmacological classification into $\alpha$ and $\beta$ subtypes, based originally on order of potency among agonists, later on selective antagonists.

- There are two main $\alpha$-adrenoceptor subtypes ($\alpha_1$, $\alpha_2$) and three $\beta$-adrenoceptor subtypes ($\beta_1$, $\beta_2$, $\beta_3$). Cloning studies show that all belong to the superfamily of G-protein-coupled receptors, this wider family of receptors includes the muscarinic cholinergic receptors and even the visual “photon receptor” rhodopsin. (Dohlman et al., 1991).

- Second messengers: $\alpha_1$-receptors activate phospholipase C, thus producing IP3 and DAG as second messenger, IP3 stimulates the release of Ca 2+ from intracellular stores via a specific receptor mediated process, while DAG is a potent activator of protein kinase C, Berridge (1993); $\alpha_2$-receptors inhibit adenylate cyclase and thus decrease cAMP formation and although $\alpha_2$ adrenergic receptors may activate several different signaling pathways, the exact contribution of each to many physiological processes is not clear, (limbird, 1988); all types of $\beta$-receptor stimulate adenylate cyclase via interaction with Gs. (Taussing and Gilman, 1995). in addition Gs can enhance directly the activation of voltage-sensitive Ca 2+ channels in the plasma membrane of skeletal and cardiac muscle. (brown and birnbaumer, 1988).
The main effects of receptor activation are:

- $\alpha_1$-receptor vasoconstriction, relaxation of gastrointestinal smooth muscle, salivary secretion and hepatic glycogenolysis.
- $\alpha_2$-receptors- inhibition of transmitter release (including NA and Ach release from autonomic nerves), platelet aggregation, contraction of vascular smooth muscle, inhibition of insulin release.
- $\beta_1$-receptors-increased cardiac rate and force.
- $\beta_2$-receptors- bronchodilatation, vasodilatation, relaxation of visceral smooth muscle, hepatic glycogenolysis and muscle tremor.
- $\beta_3$-receptors, lipolysis.


1.11 Clinical uses of adrenergic receptor agonists:

1.11.1 Cardiovascular system:

- Cardiac arrest: adrenaline intravenously, or sometimes via an endotracheal tube.
- Cardiogenic shock: dobutamine ($\beta_1$-agonist) by intravenous infusion for its positive inotropic effect, an infusion of dobutamine in combination with echocardiography is useful in the noninvasive assessment of patients with coronary artery disease, stressing the heart with dobutamine may reveal cardiac abnormalities in carefully selected patients (Madu et al., 1994).
- Low-dose dopamine to increase renal perfusion (via dopamine
receptors in renal vasculature) and maintaining glomerular filtration.

- Heart block: sympathetic heart block is treated by electrical pacing: β-agonists (isoprenaline, dobutamine) can be used temporarily while this is being arranged.

1.11.2 Anaphylactic reactions:

- Acute anaphylactic (or type I hypersensitivity) reactions—sudden and sometimes life-threatening immunological reactions usually caused by bee stings or by hypersensitivity reactions to drugs (especially penicillin).
- The main effects are gross swelling of the skin and mucous membranes, which can obstruct breathing, and cardiovascular collapse due to vasodilatation.
- Adrenaline is the first-line treatment, usually injected intramuscularly: intravenous infusion requires close monitoring, usually in an intensive care unit.

1.11.3 Respiratory system:

- Asthma: selective β2-receptor agonists (salbutamol, terbutaline, salmetrol) by inhalation; salbutamol by intravenous infusion in severe attacks, evidence suggests that β-adrenergic agonists also may suppress the release of leukotrienes and histamine from mast cells in lung tissue (Hughes et al, 1983), enhance mucociliary function, decrease microvascular permeability, and possibly inhibit phospholipase A2 (Seale, 1988). Adrenaline was first used as a bronchodilator at the beginning of this
century, and ephedrine was introduced into western medicine in 1924, although it had been used in China for thousands of years. (Chen and Schmidt, 1930).

- Nasal decongestion: drops containing oxymetazoline or ephedrine for short-term use.

1.11.4 Miscellaneous indications:

- Prolongation of local anaesthetic action: vasoconstrictor agents such as adrenaline can be injected with the local anaesthetic solution; it must not be injected into digits because of the risk of gangrene.

- Inhibition of premature labour (salbutamol).

Miscellaneous indications for $\alpha_2$-agonists (e.g. clonidine) include hypertension, menopausal flushing, lowering of intraocular pressure and migraine prophylaxis. (H.P. Rang, M.M. Dale and J.M. Ritter, 2000).
CHAPTER TWO

MATERIALS AND METHODS

2.2 Materials:

2.2.1 Plant material:

A. *Loranthus accacia* leaves.
B. *Nymphae lotus* leaves.
C. *Anogeissus leiocarpus* bark.

They were obtained from their habitat by a team from MAPRI, classified by Gamal EL-Gazali, and a specimen was deposited at the herbarium of the Medicinal and Aromatic research institute. The required parts were first cleaned carefully, dried in the shade and coarsely powdered.

2.1.2 Chemicals:

2.1.2.1 PHYSIOLOGICAL SALTS:

1- Sodium chloride (BDH, UK)
2- Sodium hydrogen carbonate (BDH, UK)
3- D-Glucose (BDH, UK)
4- Potassium dihydrogen orthophosphate(RIEDEL-DE HAEN AG)
5- Sodium dihydrogen phosphate(BDH, UK)
6- Potassium chloride (RIEDEL-DE HAEN AG)
7- Magnesium sulphate (BDH, UK)
8- Magnesium chloride (BDH, UK)
9- Calcium chloride (RIEDEL-DE HAEN AG)

Physiological media of the following composition had been used:

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<th>Ringer Locke</th>
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2.1.2.1 **Solvents:**

1- Methanol (AVONDALE LABORATORIES, ENGLAND)
2- Pet. Ether (AVONDALE LABORATORIES, ENGLAND)

2.1.2.3 **Standard drugs:**

1- Cyproheptadine HCL (SIGMA, USA)
2- Clorpheneramine maleate (SIGMA, USA)
3- Carbamylcholine Chloride (SIGMA, USA)
4- Atropine sulphate (SIGMA, USA)
5- Acetylcholine bromide (SIGMA, USA)
6- Histamine acid sulphate (SIGMA, USA)
7- Propranolol Hydrochloride (SIGMA, USA)
8- Tolazoline Hydrochloride (SIGMA, USA)
9- Tubocurarine chloride BP (wellcome foundation, London)
10- Oestradiol monobenzoate B.P. (Oestroform,BDH)
11- Serotonin creatinine sulphate (SIGMA, USA)
12- Neostigmine Bromide (SIGMA, USA)

2.1.3 **Apparatus:**

1- Harvard organ bath (Eden bridge, Kent. UK)
2- Harvard universal Oscillograph (Eden bridge, Kent. UK)
3- Water bath (Fisher Scientific, Pittsburgh, PA, USA)
4- Rotavapor Apparatus (Buchi Laboratoriums- Technik AG, Switzerland)
5- Soxhlet apparatus (Electro-mag, Turkey)
6- Analytical balance (A and D company ltd, Japan)
7- Air pump (BioScience, Shreemess, Kent, UK)
8- Langendorff apparatus (BioScience, Shreemess, Kent, UK)
9- Oven (B and T, Searle Company, England)
10- Desiccator (Glaswerk, Wertheim, Germany)

2.1.4 **Laboratory Animals:**

A- Wister albino rats:
Laboratory animal house, Department of pharmacology and toxicology, Medicinal and aromatic research institute (MAPRI), National Center for research, Ministry of Science and Technology.

B- Rabbits: local strain.
C- Guinea pig:
Laboratory animal house, Department of pharmacology and toxicology, Medicinal and aromatic research institute (MAPRI), National Center for research, Ministry of Science and Technology.
D- Frogs: local strains.

2.2 Methods:

2.2.1 Method of extraction:

The method carried out for extraction was the continuous extraction method using Soxhlet apparatus. Coarsely powdered part of each plant was defatted using Soxhlet apparatus with petroluim ether (60°-80° C). The marc was air dried and extracted with 97% methanol. The methanolic extract was filtered while hot and the solvent was evaporated under reduced pressure using rotavapour apparatus, the residue was evaporated to dryness and kept in closed container in a desiccator.

2.2.2 Methods of preparation of isolated organs:

2.2.2.1 The Guinea-pig ileum preparation:

The preparation is based on the method of Magnus (1904). A guinea pig was killed by dislocating the neck and the animal was exsanguinated. The abdomen was opened and the caecum was exposed. The ileum was removed from the caecal end and cut into isolated segments (4cm in length). The ileum was transferred to a petri dish containing Tyrodes
solution and the fat and mesentry was trimmed away. A thread was passed through one wall of the ileum at both top and bottom and the bottom thread was attached to the tissue holder. The preparation was transferred and mounted into the organ bath and attached to the isotonic transducer connected to oscillographic recorder.

2.2.2.2 *Toad rectus abdominis muscle preparation:*

A frog was decapitated after stunning and pith of the animal using a pithing needle. The frog was placed, ventral side up, on a cork board and a cut was made in mid ventral line of the trunk. The skin was separated along this midline, and the recti muscle (which lie underneath) was exposed and moistened with frog ringer solution. Two longitudinal cuts were made on either sides of the xiphoid cartilage and the line of the recti muscles was followed to their attachment to the pubis. A transverse cut through the xiphoid cartilage was made and the tissue was freed from attachment to the pubis. The recti muscles were then transferred to a petri dish containing frog ringer solution at room temperature and separated from xiphoid cartilage. By making a longitudinal cut along the linea alba the two muscles were separated and a thread was passed through one muscle at both top and bottom and the bottom thread was attached to the tissue holder. The mounted preparation was transferred to the organ bath and the top thread was attached to an isotonic transducer. An additional stretching weight was added to the resting tension to ensure that the muscle returns to its baseline after drug induced contracture. Ian Kitchen (1984).
2.2.2.3 *The rat fundus strip preparation:*

Male Wister albino rats (180-200 g) were used. The animal was killed by a blow on the head, bled, the abdomen opened and the fundus part of the stomach removed and placed in a petri-dish containing kreb's solution at room temperature. Gastric contents were washed away with kreb's. The tissue was cut transversally to make a single piece five cm in length. The tissue was suspended in a 25ml organ bath containing oxygenated kreb's solution at 37° C. the free end of the tissue was attached to an isotonic transducer. The preparation was allowed to settle for at least 1-hour period interrupted with washing at 10-minute intervals. The isotonic contractions were recoded using Harvard Ossilograph. Ian Kitchen (1984).

2.2.2.4 *Rabbit jejunum preparation:*

Rabbits of local strains weighing 1.5-2 kg were used. The animal was sacrificed and the abdomen exposed. The first 2-3 cm of the jejunum were taken out and placed in a petri-dish containing tyrode solution at room temperature. The preparation was freed from fats and connective tissues and transferred to an organ bath (25ml) containing an aerated tyrode solution maintained at 37° C. The tissue was allowed to settle for at least 45 minutes. The responses to the extract, the drugs and antagonists were recorded with isotonic transducer connected to Osillographic recorder with attenuation of 0.5, speed 0.25mm/sec under 0.5g tension. Ian Kitchen (1984).
2.2.2.5 *The isolated perfused rabbit heart preparation:*

Rabbits of local strains (1.5-2 kg) were used in this study. The animals were injected intravenously via the ear vein with 1000 IU heparin 5 minutes prior to the experiment. The animal was sacrificed, the chest quickly opened and the heart with a reasonable length of the aorta (1cm) attached, removed and transferred to a beaker containing Ringer-Locke solution (Ice cooled).

The preparation was squeezed several times to remove blood and connected through the aorta to the perfusion apparatus (free from air bubbles) when the aorta was tied strongly onto the glass cannula. The perfusion fluid (Ringer-Locke) was oxygenated, maintained at 37° C, and allowed to circulate through the heart at the rate of 10-12ml/minute. A hook was attached to the apex of the heart and an isotonic transducer connected to an oscillographic recorder registered the isotonic activity. Ian Kitchen(1984).

2.2.2.6 *Guinea pig atria preparation:*

A guinea pig was killed by dislocating its neck and exsanguinated. The thorax was opened immediately and the heart was exposed and removed as rapidly as possible and transferred to petri dish containing ice-cold aerated ringer solution. The fat was removed and the ventricles were dissected off. Threads were tied to the tip of each atrium and the preparation was mounted to a tissue holder, then transferred to an organ bath and the upper thread was attached to the transducer (Ian Kitchen, 1984).
2.2.2.7 The rat uterus preparation:

Female Wister albino rats (190-110g.) were injected with oestradiol (2.5mg/kg) 24 hours prior to the experiment.
The animal was killed by a blow on the head, bled through the jugular veins and the abdomen opened. The intestines were removed aside to expose the uterus. Fats and adventitia were removed and the two uterine horns were dissected and transferred to a petri-dish containing De jalon solution at room temperature. Each horn was opened longitudinally and suspended in an organ bath (25ml) containing aerated De jalon solution maintained at 32° C. the free end of the horn was attached to an isotonic transducer. Connected to ossillographic recorder. The isotonic contractions were recorded under 0.5g tension and a recording speed of 0.25 mm/sec. De Jalon et al.(1945).

2.2.2.8 Guinea pig tracheal Chain preparation:

The preparation is based on the method of Castello and De Beer(1947). A guinea pig was killed by dislocation the neck. The neck and the upper thorax was oppened and the muscles surrounding the trachea were cleared.6 cm of trachea was dissected, cut and placed in cold oxygenated Krebs Ringer in a petri dish. At least six rings of the muscle were cut by making transverse cuts, each ring contained two bands of cartilage.
The rings were tied together with fine thread so that the smooth muscle in the longitudinal plane and each alternate ring has muscle on the opposite sides, in which the ties between rings is close to the smooth muscle as possible. A cut through the cartilage of each ring was made.
between the ties and one end of the chain was attached to a tissue holder. The mounted tissue was transferred to an organ bath and the upper thread was attached to the transducer. The preparation was left for 20 minutes between doses.
CHAPTER THREE

Results

3.2 *Loranthus acacia:*

3.2.1 **Effect of methanolic extract of *Loranthus acacia* on isolated rabbit jejunum preparation:**

Utilizing a dose range starting from 1mg to 4mg/ml of the methanolic extract of *Loranthus acacia*, produced a dose dependent inhibition of the rabbit jejunum muscle tone. The extract showed a rapid onset of action (30 seconds) of the contact with the tissue and attained its maximum relaxing property within one minute. Fig (2.1). Upon each successive wash the tissue retain its normal pattern of rhythmicity.

3.2.2 **Effect of Adrenoceptor blocking drugs on *Loranthus acacia* extract induced relaxation on isolated rabbit jejunum preparation:**

Prior administration of the non selective β Blocker Propranolol (a non selective β Blocker) in a dose up to 8µg/ml did not block the *Loranthus acacia* methanolic extract (2mg/ml) induced relaxation of the muscle, also previous administration of the non selective α blocker Tolazoline (20µg/ml) did not affect the relaxant action of the extract on the mentioned smooth muscle preparation. Fig (2.2)
3.1.3 **Effect of *Loranthus acacia* methanolic extract on isolated guinea pig ileum preparation:**

*Loranthus acacia* methanolic extract 2mg/ml was without effect on guinea pig ileum. Physiological antagonism of Histamine (H=50ng/ml) induced contraction was not shown by up to (2mg/ml) of the extract. Fig (2.3)

3.2.4 **Effect of methanolic extract of *Loranthus acacia* on isolated toad rectus abdominis muscle preparation:**

Up to 4mg/ml of *Loranthus acacia* methanolic extract exhibited no activity on toad rectus abdominis muscle preparation. To identify the sub maximal dose Acetylcholine was added in ascending dose schedule from which 2mg/ml was shown as the dose above which any Acetylcholine dose will give the maximum contracture. Fig (2.4)

3.2.5 **Effect of *Loranthus acacia* methanolic extract on Acetylcholine induced contracture on isolated toad rectus abdominis muscle preparation:**

Prior Addition of *Loranthus acacia* methanolic extract (L.a =1mg/ml) potentiated the contracture resulted from Acetylcholine (Ach= 2µg/ml). The influence of Loranthus acacia on Acetylcholine induced contracture was even more pronounce when using (L=2mg/ml) instead of the previous dose, fig (2.5). Also the extract reduced the time by which Acetylcholine effect reaches the maximum by about 50%.
3.2.6 *Loranthus acacia* methanolic extract reversal of tubocurarine antagonistic effect on Acetylcholine induced contracture on isolated Toad rectus abdominis muscle preparation:

Preliminary addition of d-tubocurarine (T=20µg/ml) blocked the acetylcholine-induced contracture on isolated toad rectus abdominis muscle preparation. Prior administration of *Loranthus acacia* methanolic extract (L=4mg/ml) followed by d-tubocurarine (T=20µg/ml) then Acetylcholine (Ach=1µg/ml), the produced contracture was as same as that of Acetylcholine (Ach=1µg/ml) alone, fig (2.6). These findings were similar to those of Neostigmine in a dose of 40µg/ml, fig (2.7).

3.2.7 Effect of methanolic extract of *Loranthus acacia* on isolated perfused rabbit heart preparation:

The methanolic extract of *Loranthus acacia* was injected in bolus doses ranging from 2.5mg/ml to 10mg/ml to the isolated perfused rabbit heart preparation. A quick response within 10 seconds was shown that involved a negative dromotropic effect that vanishes upon the depletion of the extract. The most characteristic observation was that the heart rate was not affected absolutely. Fig (2.8).

3.2.8 Effect of *Loranthus acacia* methanolic extract on guinea pig atrium:

The methanolic extract of *loranthus acacia* (L=1-4mg/ml) produced a dose dependant inhibition of the force of contraction of the guinea pig atrium preparation without notable effect on the rate of
contraction. This inhibition reach it’s maximum within 20 seconds, fig (2.10). Addition of Acetylcholine in descending manner revealed that 2 µg/ml is the dose above which any Acetylcholine dose will give the maximum decrease in rate and force of contraction, fig (2.9).

3.2.9 Potentiation of Loranthus acacia dromotropic effect on isolated guinea pig atrium preparation using Acetylcholine:

Prior addition of Loranthus acacia methanolic extract (L.a=2mg/ml) for 60 seconds provoked the inhibition induced by Acetylcholine (Ach= 2 µg/ml) of the isolated guinea pig atrium preparation which was obvious compared to that inhibition produced by Acetylcholine (Ach= 2 µg/ml) alone. Fig (2.11).

3.2.10 Influence of Atropine sulphate on Loranthus acacia induced negative dromotropic effect on guinea pig atrium preparation:

Prior administration of Atropine sulphate (At= 50µg/ml) was without effect on the dromotropic activity of the methanolic extract of Loranthus acacia (L.a =2mg/ml) on isolated guinea pig atrium preparation. Fig (2.12).
3.2 **Nymphaea Lotus:**

3.2.1 **Influence of methanolic extract of *Nymphaea lotus* on isolated rabbit jejunum preparation:**

Addition of *Nymphaea lotus* methanolic extract (N=1-4mg/ml) on isolated rabbit jejunum preparation produced a dose dependant contraction of the muscle associated with an upward shift in the baseline. The maximum effect attained in about 1 minute and returned to normal upon washing. Fig (3.1)

3.2.2 **Use of atropine sulphate and Cyproheptadine on *Nymphaea lotus* induced contraction of the isolated rabbit jejunum preparation:**

Prior administration of atropine sulphate (At=1µg/ml) didn’t block the *Nymphaea lotus* methanolic extract (N=2µg/ml) induced contraction, while a dose of Cyproheptadine (Cp=40g/ml) completely blocked the contraction. Fig (3.2)

3.2.3 **Effect of *Nymphaea lotus* methanolic extract on isolated guinea pig ileum preparation:**

*Nymphaea lotus* methanolic extract (N=2mg/ml) produced contraction of the guinea pig ileum preparation comparable to that produced by Histamine (H=10ng/ml), prior administration of Chlorpheneramine maleate (Cp=10-40ng/ml) completely blocked the Histamine (H=10g/ml)
induced contraction, but without any activity on *Nymphaea lotus* methanolic extract (N=2mg/ml) induced contraction. Fig (3.3)

3.2.4 **Effect of 5-hydroxytreptamine and methanolic extract of *Nymphaea lotus* on isolated guinea pig ileum preparation:**

Administration of *Nymphaea lotus* methanolic extract (N=2mg/ml) showed a contraction that is similar to 5-hydroxytreptamine (1µg/ml) induced contraction on isolated guinea pig ileum preparation. Cyproheptadine (Cp=10,20ng/ml) block the contraction of both the extract and 5-HT respectively. Fig (3.4)

3.2.5 **Activity of *Nymphaea lotus* extract on isolated rat fundus strip preparation:**

Both 5-Hydroxytreptamine (5HT=25ng/ml) and *Nymphaea lotus* methanolic extract (2mg/ml) markedly stimulated the isolated rat fundus strip preparation. While 5-Hydroxytreptamine effect need 1 minute to reach it’s maximum, that of the methanolic extract required 2 minutes to show the same level of effect. Fig (3.5)

3.2.6 **Blockade of 5-Hydroxytreptamine and *Nymphaea lotus* induced contraction of isolated rat fundus strip preparation using Cyproheptadine:**

5-hydroxytreptamine and *Nymphaea lotus* methanolic extract showed a contraction on isolated rat fundus strip preparation that reached it maximum in 40 seconds, with the difference that the 5-
Hydroxytreptamine induced contraction is faster in onset of action (10 seconds) than that of the extract (30 seconds).

Cyproheptadine in a dose of 20µg/ml completely blocked the 5-Hydroxytreptamine induced contraction of the preparation, while 40µg/ml was needed to effectively block the extract (2mg/ml) induced contraction. Fig (3.6)

**3.2.7 Effect of Nymphaea lotus methanolic extract on isolated toad rectus abdominis muscle preparation:**

_Nymphaea lotus_ methanolic extract in a dose up to 2mg/ml was without activity on isolated toad rectus abdominis muscle preparation. Prior administration of the extract neither potentiated nor blocked the Acetylcholine (1µg/ml) induced contracture on isolated toad rectus abdominis muscle preparation. Fig (3.7)
3.3 *Anogeissus leiocarpus*: 

3.3.1 Effect of *Anogeissus leiocarpus* methanolic extract on isolated rabbit jejunum preparation:

Incremental addition of Anogeissus *leiocarpus* methanolic extract resulted in a dose dependent relaxation of the isolated rabbit jejunum preparation. The relaxation characterized by a rapid onset (10 seconds) that reaches its maximum within 30 seconds. The tissue reverts to its normal spontaneous activity upon washing. Fig (4.1)

3.3.2 Tolazoline effect on *Anogeissus leiocarpus* methanolic extract relaxation of the isolated rabbit jejunum preparation:

Different doses of Tolazoline (10, 20 and 40 µg/ml) was without activity on the *Anogeissu leiocarpus* methanolic extract induced relaxation on rabbit jejunum preparation. Fig (4.2)

3.3.3 Blockade of *Anogeissus leiocarpus* relaxant activity on isolated rabbit jejunum preparation using propranolol:

Prior administration of propranolol (1, 2 and 4 µg/ml) showed a dose dependant blockade of *Anogeissus leiocarpus* methanolic extract relaxant property. Fig (4.3)

3.3.4 Effect of *Anogeissus leiocarpus* methanolic extract on isolated guinea pig ileum preparation:
Anogeissus leiocarpus methanolic extract in a dose up to 4mg/ml showed no activity on the isolated guinea pig ileum preparation. Prior administration of the extract (2mg/ml) couldn’t reverse the Charbachol (1µg/ml) induced contraction of the preparation. Fig (4.4)

3.3.5 **Effect of Anogeissus leiocarpus methanolic extract on isolated toad rectus abdominis muscle preparation;**

Anogeissus leiocarpus methanolic extract (2mg/ml) was devoid of activity on isolated toad rectus abdominis muscle preparation, prior administration of the extract (2mg/ml) neither potentiated nor antagonized the Acetylcholine (1µg/ml) induced contracture of the preparation. Fig (4.5)

3.3.6 **Activity of Anogeissus leiocarpus methanolic extract on guinea pig atrium preparation:**

The frequency of contraction of the isolated guinea pig atrium preparation was markedly inhibited following the administration of the methanolic extract of Anogeissus leiocarpus in a dose of 1 and 2mg/ml. worth mentioning the force of contraction of the preparation was not altered. Fig (4.6)

3.3.7 **Effect of Anogeissus leiocarpus methanolic extract on isolated guinea pig tracheal chain preparation:**

Methanolic extract of Anogeissus leiocarpus (1,2and 4mg/ml) didn’t relax or contract the isolated guinea pig tracheal chain preparation. Prior
administration of the extract didn’t reverse the Histamine (400ng/ml) induced contraction. Fig (4.7)

3.3.8 **Activity of Anogeissus leiocarpus methanolic extract on isolated rat uterus preparation:**

Slight relaxant activity on spontaneously contracting rat uterus preparation was shown by *Anogeissus leiocarpus* methanolic extract (2mg/ml). This activity attained its maximum within 1 minute and lasted for 2 minutes. Fig (4.8)

3.3.9 **Anogeissus leiocarpus Blockade of Acetylcholine contraction on isolated rat uterus preparation:**

Acetylcholine (1µg/ml) contracted the isolated rat uterus preparation for 2 minutes; prior administration of *Anogeissus leiocarpus* methanolic extract decreased the Acetylcholine contraction to 30 seconds. Fig (4.9)

3.3.10 **Blockade of Anogeissus leiocarpus methanolic extract effect on isolated rat uterus preparation:**

Prior administration of propranolol (80ng/ml) was without effect on *Anogeissus leiocarpus* methanolic extract (2mg/ml) reversal of Acetylcholine (1 µg/ml) contraction of the isolated rat uterus preparation. Fig (4.10)
CHAPTER FOUR

Discussion

In our search for a novel Antimicrobial drug with minimum side effects, many activities were encountered of which we have to characterize the most useful ones and utilize them in alleviating human suffering.

Based on previous works by Al-Magboul and co workers (1992) the methanolic extract of three plants namely *Loranthus acacia*, *Nymphaea lotus* and *Anogeissus leiocarpus* was chosen as it retain the most active antibacterial fraction.

These plant extracts were studied on selected isolated tissue preparations bearing two goals in mind; to provide information on the presence of certain pharmacological activities, and to suggest which other tests should be performed. (Robert A. Turner, 1965)

The methanolic extract of *Loranthus acacia* (Zucc) leaves exhibited a dose dependent inhibition of the isolated rabbit jejunum preparation. Both Propranolol hydrochloride and Tolazoline did not block this activity, which may indicate a non-adrenergic relaxant property of the extract. These results also exclude the possibility of release of adrenergic transmitters as a result of the nicotinic receptors activation by the methanolic extract in the enteric nervous system. As a crude mixture, the methanolic extract of *Loranthus acacia* should be further fractionated to investigate it’s A-typical relaxant activity on rabbit jejunum preparation.
which may be attributable to many other constituents of the mixture, that may contain a local anesthetic, or direct muscle relaxant activity (Mebeverine like activity) and/or direct hyperpolarization of smooth muscle cells by activation of Potassium channels (Kidney et al, 1993).

On isolated guinea pig ileum preparation the extract was devoid of any activity and couldn’t antagonize the Histamine induced contraction of the preparation.

Prior administration of the methanolic extract markedly potentiated the Acetylcholine induced contracture on isolated toad rectus abdominis muscle preparation, while the extract alone was without any activity on the same preparation.

In another preparation, the blockade of acetylcholine contracture by d-tubocurarine was effectively reversed by the methanolic extract of Loranthus acacia, an effect that was comparable with that of the Anticholinesterase Neostigmine. The methanolic extract demonstrated its activity in the isolated toad rectus abdominis muscle preparation (skeletal muscle) and was not shown in isolated guinea pig ileum (a smooth muscle) in line with the fact that some anticholinesterases such as Neostigmine and Pyridostigmine tend to affect neuromuscular transmission more than the autonomic system, where as Physostigmine and organophosphates show the reverse pattern (H.P. Rang, M.M. Dale and J.M. Ritter, 2000).

On isolated perfused rabbit heart preparation, the methanolic extract showed a dose dependent inhibition of the force of contraction without affecting the rate of contraction, this effect vanish upon wash out of the
perfusate. The same pattern of inhibition of the force of contraction was shown by the methanolic extract on the isolated guinea pig atrium preparation and notably no effect on the rate of contraction. Prior administration of atropine sulphate to the isolated guinea pig atrium preparation couldn’t reverse the methanolic extract negative inotropic effect on the preparation, while prior administration of the methanolic extract markedly potentiated Acetylcholine induced inhibition of the force of contraction. This activity showed by the methanolic extract of *Loranthus acacia* on the heart may be attributable in part to Anticholinesterase property of the extract through activation of M receptor, as M receptors occur in the heart (Cardiac), and also on the presynaptic terminals of peripheral and central nervous systems. M receptor activation is responsible for the vagal inhibition of the heart, as well as presynaptic inhibition in the central nervous system and periphery (H.P. Rang, M.M. Dale and J.M. Ritter, 2000).

Generally Acetylcholinesterase inhibitors leads to effects on the heart that mimic the effect of vagal nerve activation, the fall in cardiac output is attributable to brady cardia, decreased atrial contractility and some reduction in ventricular contractility, the latter effect occurs as a result of prejunctional modulation of sympathetic activity (inhibition of nor-epinephrine release) as well as inhibition of postjunctional cellular sympathetic effects (Achilles J. Pappano & Bertram G. Katzung, 2001).

As the expected activity of an Anticholinesterase inhibitor is a negative chronotropic as well as inotropic effect of the cardiac muscle, the pattern showed by the methanolic extract may be due to the fact that inhibition of adrenergic stimulation of the heart arises from the capacity of Acetylcholine to modulate or depress the myocardial response to
chatecholeamines as well as from a capacity to inhibit the release of Norepinephrine from synaptic nerve endings. The former effect can be explained in part by the inhibitory effect of Muscarinic agonists on Adenylyl Cyclase activity and by the activation of counteregulatory processes, such as activation of receptor-operated K+ currents. (Joan H. Brown & Palmer Taylor). The fresh whole plant is used folklorically to enhance wounds healing (El-Gazali et al, 1997), this activity which was proved by its Antimicrobial activity did not correlated to its Anticholinesterase activity we have found.

In relation with the above mentioned activities of the methanolic extract, many other plants exhibited different activities of which Park et al (1996) reported that *Evodia rutaecarpa* Bentham showed a strong inhibitory effect on Acetylcholinesterase in vitro and an anti-amnesic effect in vivo, it inhibited Anticholinesterase in a dose-dependent and noncompetitive manner.

Gilani A.H. et al (2004) reported the presence of Cholinomimetic and Acetylcholinesterase (AChE) inhibitory constituents in betel nut, the most commonly used drug in the world after Tobacco, Ethanol and Caffeine. This study provides first evidence for the presence of AChE inhibitory constituents in betel nut, though additional direct muscarinic stimulatory effect were not ruled out. The essential oil of *salvia lavandulaefolia* at two dosage levels was administered orally to rats for five days by (Perry-NS et al, 2002) they found that *S. lavandulaefolia* oil inhibited cholinesterase in vitro, also has an in vivo effect and this may help explain its traditional use for ailing memory.
Air dried leaves of *Nymphaea lotus* (L) methanolic extract showed a dose dependent stimulation of the isolated rabbit jejunum preparation which was refractory to Atropine antagonistic activity implicating a non-Acetylcholine induced contraction. Instead Cyproheptadine (non selective 5-HT blocker) effectively blocked the contraction induced by the extract. The small intestine was found to be less sensitive to 5-HT than the uterine muscle and the fundus strip. (Cohen et al, 1985), but it was also found to be blocked by a non-selective 5-HT blockers such as Lysergic acid diethylamide(LSD) (Gyermek, 1961). 5-HT might have ultimately exerted its stimulant effect on the intestinal smooth muscle by enhancing calcium influx (Mark et al, 1982).

Recent studies showed that the motility of gastric and intestinal smooth muscle may be either enhanced or inhibited via at least six subtypes of 5-HT receptors, the stimulatory effect occurs at nerve endings on longitudinal and circular enteric muscle, at postsynaptic cells of the enteric ganglia, and by direct effects of 5-HT on the muscle cells (5-HTα in intestine and 5-HTβ in stomach fundus) (Dhasmana et al, 1993). Craig and his team, (1990) reported that activation of 5-HT receptors in this system causes increased acetylcholine release and thereby mediates a motility-enhancing or prokinetic effect of selective Serotonin agonists such as Cisapride. As Cyproheptadine may block both 5HT and Histamine activity, the extract was shown to stimulate isolated guinea pig ileum preparation in a manner comparable to that produced by histamine but unlike Histamine it couldn’t be blocked by Chlorpheneramine maleate. This 5-HT like activity was confirmed on isolated Rat fundus strip preparation as here the methanolic extract exhibited a contraction peak similar to that of 5-HT, and both the extract and 5-HT was effectively blocked by Cyproheptadine. The increase in sensitivity of the
fundus strip to 5-HT confirmed the findings of Vane and his co-workers (1957) during their search for a more sensitive preparation for assaying Serotonin. This sensitivity might be due to a different receptor, in the fundus which unlike that of the uterus, was resistant to the blocking effect of ketanserine(Cohen et al,1985).

On isolated toad rectus abdominis muscle preparation the extract per se showed no activity and it couldn’t potentiate or antagonized Acetylcholine induced contracture.

In addition to the confirmed antimicrobial activity of the methanolic extract of *Nymphaea lotus* and its contents of flavonoids reported by (Awatif A. Elegami,et al.,2003) and by (Fossen et al,1999), our findings of a 5-HT like activity give a strong support to the folkloric use of the plant extract for piles and dyspepsia diseases,(Braun &Massey,1929).

The methanolic extract of *Anogeissus leiocarpus* (DC.) showed a dose dependent inhibition of the isolated rabbit jejunum preparation, intestinal tone and the frequency and amplitude of spontaneous contractions are also reduced. Tolazoline did not block this relaxant activity while propranolol completely blocked the inhibition; propranolol interacts with $\beta_1$ and $\beta_2$ receptors with equal affinity, lacks intrinsic sympathomimetic activity and does not block $\alpha$-adrenergic receptor. The extract per se was without effect on both isolated guinea pig ileum and toad rectus abdominis muscle preparations, which may help to exclude any autonomic and somatic contractile activity of the extract. The extract neither antagonized nor potentiated the Carbachol induced contraction on the isolated guinea pig ileum preparation. Guinea pig ileum preparation has no inherent tone in vitro and the inhibitory effect of adrenergic agonists can only be observed by showing physiological antagonism of a contractile response elicited by, for example, histamine or acetylcholine. (Ian kitchen, 1984)
The extract does not affect (in normal dose) Acetylcholine contracture on toad rectus abdominis muscle preparation.

On guinea pig atrium preparation the extract decreased markedly the frequency of contraction and was devoid of any activity on the force of contraction. The extract neither had any activity on isolated guinea pig tracheal preparation nor affected histamine induced contraction of the preparation, this test was performed as the adrenergic agonists affect respiration primarily by relaxing bronchial muscle, it has a powerful bronchodilator action and a striking therapeutic effect as a physiological antagonist to substances that cause bronchoconstriction (Brian B. Hoffman and Robert J. Lefkowitz, 1995). On rat uterus preparation the extract showed relaxant activity of the muscle that blocked the Acetylcholine induced contraction of the preparation, which indicate the response of the uterus to Cholinergic agonists and the physiological antagonism of the responses by adrenaline (Ian kitchen, 1984). Prior administration of Propranolol was without effect in preventing the extract-induced relaxation. The uterus is considered to be sensitive to adrenergic agonists, but liable to show excessive spontaneous activity, which is diminished by lowering the temperature and decreasing the calcium in the ringer solution (Gaddum et al, 1949). Akande and Yamamoto, (1998) demonstrated that the elements accounted for 26 to 74% of the ash content. Calcium and potassium concentrations were highest while Phosphorus, Aluminum, Sodium, Magnesium and Fluorine were found in appreciable amounts, these elements may participate in the irregular activities of this plant. On the other hand the crude mixture character of the extract may prevent any activity from demonstrating
itself frankly. The $\beta$-adrenergic activity, which emerges in part of this study, is in line with the folkloric use of this plant for relieving cough.

conclusion
In this study we conclude that the methanolic extract of *Loranthus acacia*, in addition to its proven antimicrobial properties, possess an anticholinesterase activity. Confirmation of this activity was done by comparing it with that of Neostigmine on isolated toad rectus abdominis muscle preparation.

The second plant *Nymphaea lotus* extract, a part from its antimicrobial property, demonstrated a 5-HT like activity confirmed by its effect on isolated rat fundus strip preparation. This activity was in line with some of its folkloric uses for dyspepsia and for piles. *Anogeissus leiocarpus* methanolic extract showed a β–adrenergic activity on isolated rabbit jejunum preparation which conform with the claimed folkloric use for relieving cough. Using different specialized adrenergic preparations, we couldn’t identify the β₂–adrenergic selectivity of the extract. This may be attributable to the nature of the extract as a mixture of many entities that mask the claimed property.
Recommendations

1-The three plants extract need a biologically guided fractionation to isolate and characterize the active fractions.

2-Loranthus acacia and Nymphaea lotus should be tested on CNS models to explore their central activities compared to their peripheral activities.

3-Toxicological studies should be performed on Loranthus acacia to establish its safety on other major organ systems (Kidneys, Liver etc).
REFERENCES


Calapai G; Crupi A; Fienzuoli F; Inferrera G; Ciliberto G; Parisi A; De Sarro G; Caputi AP.(2001). Interleukin-6 involvement in antidepressant action of Hypericum perforatum . Pharmacopsychiatry.34 suppl 1:S8-10.
Calapai G; Crupi A; Fienzuoli F; Costantino G; Inferrera G; Campo GM; Caputi AP (1999). Effects of Hypericum perforatum on levels of 5-hydroxytryptamine, noradrenaline and dopamine in the cortex, diencephalon and brainstem of the rat. J Pharm Pharmacol. 51 (6): 723-8.


Das A; Shanker G; Nath C; Pal R; Singh S; Singh H. (2002). A comparative study in rodents of standardized extracts of Bacoba monniera and Ginko biloba: anticholinesterase and
cognitive enhancing activities. Pharmacol Biochem Behav. , 73: 4; 893-900.


Heo HJ; Yang HC; Cho HY; Hong B; Lim ST; Park HJ; Kim HK; Kim EK; Shin DH. (2000). Inhibitory effect of Artemisia asiatica alkaloids on acetylcholinesterase activity from rat PC12 cells. Mol Cell. 30; 10(3):253-62.

Heo HJ; Hong SC; Cho HY; Hong B; Kim HK; Kim EK; Shin DH. (2002). Inhibitory effect of zeatin, isolated from Faitoua
villosa, on acetylcholinesterase activity from PC12 Cells. Mol Cells., 13: 1, 113-117.


Jambor-j; skrzypczak-L (1991); Flavonoids from the flowers of Nymphaea alba L. Acta-Societatis-Botanicorum-Poloniae. 60: 1-2, 119-125., 127-132


Kasture VS; Deshmukh VK; Chopde CT (2002); Anxiolytic and anticonvulsive activity of Sesbania grandiflora leaves in experimental animals. Phytotherapy research. 16(5):455-460


Magnus (1904). Pflugers Arch ges Physiol. 102,123.


Sanogo-R; Crisafi-G; Germano-MP; Pasquale-r-de; Bisignano-G; Capasso-F; Basso-F; Evans-FJ; Mscolo-N, (1998). Evaluation of Malian traditional medicines: screening for antimicrobial activity. Phytotherapy research. 12: 1, 154-156.


