PHARMACOGNOSTIC EVALUATION OF THE LEAVES AND GALLS OF *GUIERA SENEGALENSIS* J.F. Gmel

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PHARMACOGNOSY.

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ZARIA - NIGERIA.

1993

DECLARATION

I hereby declare that the work reported in this thesis was carried out and written by me under the joint supervision of Dr. M. Shok and Professor P.P. Rai in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. It has not been presented in any previous application for higher degree. The work of other investigators have been acknowledged and referred to accordingly.



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CERTIFICATION

This thesis entitled "PHARMACOGNOSTIC EVALUATION OF THE LEAVES AND GALLS OF GUIERA SENEGALENSIS J.F. GMEL" by YUSUF I. ABUBAKA meets the regulations governing the award of the degree of MASTER OF SCIENCE (Pharmacognosy) of AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my late SISTERS; Rakiya, Maimuna, Fatsuma, Halima and Umma, may Allah pardon and give them Firdaus (Amin). And my PARENTS; Mairama and Alh. Abubakar.

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I also acknowledge the assistance of the Principal and entire staff of School of Leather Technology, Zaria for allowing me to use the facilities in the School's Chemical Laboratory, Mudi S. Yusuf of Department of Chemistry, Bayero University, Kano for the provision of some laboratory reagents and solvents, and assistanceship provided by Ahmadu Bello University, Zaria. The effort of Mr. H.O. Onyeachusim - a taxonomist at Forestry Research Institute, Ibadan for confirmation and classification of the plant is recognised. I appreciate the assistance of Dr. J.A. Onaolapo, my colleagues, Miss H. Ibrahim, Mrs. R.O. Bolaji, Technical Staff of Department of Pharmaceutics and

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ABSTRACT

Guiera senegalensis J.F. Gmel. (Comoretaceae) is a shrub in dry Savannah of Sudan Zone. It is also widely distributed in Northern Nigeria. The specific feature of this plant is presence of galls on the branches of its stem, An insect Acaciothrips ebneri, Karny. and a mould fungus of the Fusarium spp. were found to be associated with the galls and these may be the cause of this pathological condition (gall).

A detailed microscopical study of the anatomical sections of the fresh leaf and gall as well as of their powdered forms was carried out and unique features noted. The qualitative chemical tests indicated the presence of free and combined reducing sugars, saponins with steroidal and triterpenoidal aglycones, flavonoids and alkaloids in the galls. Ellagitannins and condensed tannins were common to both the leaf and gall, and gallotannins unique to the gall only. Resins were detected in the gall. Paper and Thin layer chromatographic studies of the tannins and sugar extracts of the leaf and the gall revealed the presence catechol, phloroglucinol,tannic acid and glucose. A fairly high tannin content was estimated both in the leaves and the galls. The tannin estimated by hide powder method (hot leaching) gave 13.8% w/w dry weight and *19.2%* w/w dry weight yield for the leaves and galls respectively.

A systematic evaluative studies of the crude drug were carried out. The moisture content (11.3% w/v and 8.0% w/v); total ash (6.3% w/v and 4.4% w/v); acid insoluble ash values (1.7% w/v and 2.3% w/v); alcohol soluble extractive values (27/.7% w/v and 18.5% w/v) and water soluble extractive values (16.8% w/v and 18.8% w/v) were obtained for the leaf and gall respectively.

The aqueous and water soluble extractives of methanol extracts of the roasted and unroasted galls exhibited some antimicrobial effects against Streptococcus pyogens, Staphylococcus aureus and Escherichia colt,while Candida-albicans and Pseudomonas aeruginosa were less sensitive. The a queous and water soluble extractives of the methanol extracts of the unroasted galls were more active. These antimicrobial properties may explain the basis for the use of the galls in treatment of skin diseases and wound infections by traditional healers in Northern Nigeria.

finally, the various results and data derived from this study would be useful in preparation of pharmacopoeial monograph of G. senegalensis.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Preliminary examination of crude drugs will often give indication as to whether they will satisfy the more exacting standards which may be applied to them. Characteristic features of drugs could be obtained by systematic study of their morphology (Shellard, 1958). However, the evaluation of a drug requires not only that it be identified in both its entire and powdered forms by means of its macromorphological and cytomorphological features but must also be subjected to evaluation procedures which will indicate their acceptability by criteria other than their morphology (Brain and Turner, 1975a). The plant under investigation is Guiera senegalensis which finds wide application in traditional medicine for treatment of various skin diseases and wound infections in Northern Nigeria (Abubakar Personal Communications, 1991).

1.1.2 The Family Combrataceae

Combretaceae is a family of 20 genera and 600 species; comprising of tropical and subtropical trees and shrubs, usually rich in tannins. Six genera which include Guiera, Terminalia, Combretum, Quisqualis, Anogeissus and Myrobolans are found in West Africa (Trease and Evans, 1983a).

Members of the family may be recognised by their alternate entire panicles, axillary spikes or head of small actinomorphic flowers and interior ovaries forming winged fruits (Nielsen , 1965). At least two genera are common to each vegetation zone in West Africa. The genus of interest here being Guiera.

1.2 Some Genera of the Family Combretaceae

i. Terminalia (250 sps)

The fruits of Terminalia chebula are rich in tannin and the members of the genera are used in tanning and medicine.

ii. Combretum (250 sps)

a. Combretum butyrosum yields a butter like substance.

b. Combretum micranthum yields an alkaloid-combretin which has a diuretic property.

iii. Anogeissus (11 sps)

Anogeissus latifolia yield the gum known as ghattin

1.3 Guiera senegalensis

The plant G. senegalensis is a shrub and member of the family Combretaceae, order Myrtiflorae. It is found particularly in the Sudan zone where it

anti-inflammatory, analgesic and antiemetic properties (Etkins, et al. 1982).

iii. G. senegalensis has reputation as preventive for leprosy, particularly given to the new born and the child of lepper in Sokoto (Nwude, N. 1986a).

iv. G. senegalensis is employed in the treatment of symptoms underlaying ulcer, as hypotensive and antisyphilic agent (Irvine, 1961).

v. Syrup prepared from the leaves is used in the symptomatic relief of cough, and its efficacy was comparable to codeine linctus (Nwude, N. 1986b).

vi. In Zaria, Nigeria,a hot water extract of the powdered leaves is taken by women after child birth as general tonic, energy restorer and to increase lactation (Gaya, 1978a; Irvine, 1961).

vii. The decoction or powdered plant is mixed with fresh milk and given to paediatrics for treatment of infected diarrhoea and skin rashes, the residue is often applied topically to affected parts (Personal communication, 1991).

viii. Traditional healers consulted particularly in Zaria Kaduna State, Gaya and Wudil towns of

Kano State, Hadejia, Jigawa State of Nigeria revealed that the powder of roasted galls of G. senegalensis made into ointment with animal fat is used for treatment of wounds and skin diseases.

ix. In the laboratory aqueous and methanolic water soluble extracts of the leaves were found to inhibit the growth of Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Candida albicans which are the common causative agents in wound and skin diseases, and gastrointestinal disorders (Sokomba, et al, 1983).

1.4.2 Economic Uses

In some parts of West Africa, the plant is used as a fodder for domestic animals (Oliver, 1960). The roots and stems are used as chewsticks and as fire wood (Irvine, 1961). The leaves are used as tonic and as digestive for cattle, and are supposed to increase their milk yield. The dried leaves and twigs are burned around camp of domestic animals to fumigate them against biting insects and other flies and to prevent chills. The leaves of G. senegalensis are used as seasoning for gruel in Northern Nigeria (Etkins, 1988).

grows abundantly in abandoned farm lands and it is the only specie found in tropical West Africa. A typical specie of dry locality where rainfall is small and not well distributed over the year and retention of the soil is slight (Hutchinson and Dalziel, 1954; Oliver, 1960).

The plant is indigenous in Nigeria and to the Cameroon. It is marketed as 'Sabara' in Zaria, Sokoto and Kano. The plant becomes commonly infested with galls which enter into local medicinal preparations and are sold in northern Nigerian markets. Leaves and stems have been found to contain traces of alkaloids and tannins (Burkill, 1985).

Vernacular names:

Hausa	:	Sabara
Fulani	:	Goleki
Shuwa Arab	:	Kabeish
Kanuri	:	Kasheshi
Sudan Arab	:	Obeish or Globeish (Dalziel, 1955).

Habitat: : Abundant sandy wastes and on abandoned farms also on poor soil in degraded savannah forest.

Locality : It is indigenous to Senegal, Nigeria and Cameroon.

Distribution : Senegambia to Nigeria and the Sudan.

Synonym : Guiera senegalensis Lam.

The plant has grey foliage, the leaves being opposite and covered on the back with black glands. The small flowers are in dense greenish yellow heads, about 1.5cm in diameter and are protected before opening by four bracts. The fruiting head is characteristic with several slender curving fruits, 4.5cm long covered with long silky hairs crowned by a persistent calyx (Neilsen, 1965).

1.4.0 Uses of Guiera senegalensis

1.4.1 Medicinal Uses

G. senegalensis is widely used in traditional medicine. Some of the important medicinal uses of this plant are described below:

i. The Hausas of Northern Nigeria use the plant for treatment of fever. And the leaves and roots were found to be effective against malarial parasites (Etkins, 1988).

ii. The leaf infusion was clinically demonstrated to be effective in the treatment of acute gastroenteritis and dysentry. The extract has

1.5 Phytochemistry og Guiera senegalensis

Paris in 1942 found alkaloids, true tannins and catechins in the leafy twigs of G. senegalensis. In the analysis of fodder materials, the crude extracts of G. senegalensis was found to contain proteinous materials (96% w/w) (Irvine, 1961).

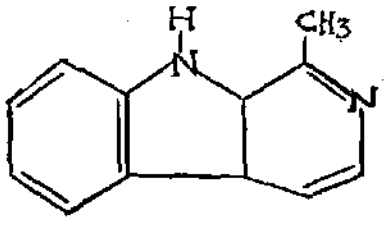
Mucilage, tannins, saponins and alkaloids were separated and identified by thin layer chromatography. Spectroscopy disclosed numerous mineral contents; magnesium, calcium and strontium are particularly abundant so is silicon. Especially characteristic are large amounts of lead and iron, mostly in the leaves. Also present are copper, zinc, nickel, cobalt, chromium and tin in trace amounts (Koumare et al., 1968). The presence of traces of alkaloids in G. senegalensis as indicated by Paris (1942) and Koumare et al (1968) were confirmed by Combier et al (1977) who isolated the alkaloids harman and eleagnine from the root. This is the first instance of indole alkaloids being isolated from Combretaceae. These two alkaloids occurred also in other plants. Harman - $C_{12}H_{10}N_2$ (Fig. 1) having a bitter taste was first isolated from the bark of Arariba rubra (Gray, 1954). Eleagnine - $C_{12}H_{14}N_2$ was isolated from the bark of the Eleagnus genus - Eleagnus angustifolia. It is sparingly soluble in water and insoluble in ethanol and chloroform (Menshikov, 1950). Eleagnine can be converted to

harman by simple sequence of dehydrogenation (Barton and Ollit, 1979).

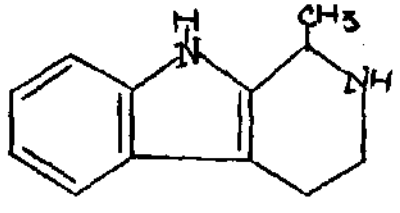
However, the presence of other alkaloids have been reported in some genus of the family Combretaceae by other workers. Combretin (Fig. 2) by Lehman in 1943 and turicine(Fig. 2) by Balensaid et al. 1946 were isolated from the leaves of Combretum micranthum. Combretin was found to have diuretic activity (Henry, 1948).

The tannins reported by Koumare et al. (1968) were found to be both condensed and hydrolysable tannins. However, they have not been structurally established.

Fig. 1: Structures of Harman and Eleagnine



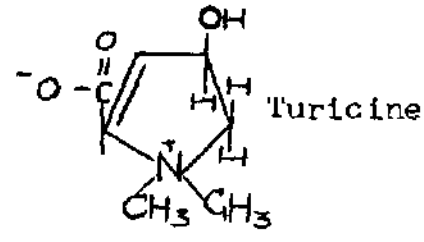
Harman



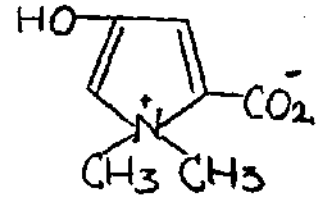
Eleagnine

This may be partly due to the fact that it is difficult to obtain tannins in pure form because many tannins are altered easily during isolation processes and most have high molecular weight, which are difficult to separate and purify. Very few tannins have been isolated in crystalline form (Ramstad, 1959b). Paris in 1942 isolated a white crystalline substance from Combretum micranthum (Combretaceae) that was soluble in alcohol, acetone, ethylacetate, diethylether and insoluble in chloroform and petroleum ether. On fusion with alkali it gave phloroglucinol and protocatechnic acid. The name combrecatechin was proposed for the substance (Irvine, 1961).

Fig. 2: Structures of Turicine and Combretin



Turicine



Combretin

1.6 Galls

A gall is a pathological formation developed on many plants' parts usually roots, leaves and stem in response to a variety of external parasitic attacks by insects, fungi or bacteria (Ramstad, 1959a).

1.6.1 Mechanism of formation

When any living part of a plant is injured the surrounding cells undergo active division and the wounded surface becomes covered with a new growth (Fritsch and Salisbury, 1949). The mechanism of control of the growth is not known but probably represents another example of the influence of growth regulating substances on the physiological activities of the plant cells (Benard and Donald, 1952).

In most cases gall formation is caused by larvae which develop from eggs placed by mother insect on the surface of the plant part. The tissue surrounding the larva starts proliferating due to enzyme containing secretions produced by the larva which by rapid conversion of starch into sugar stimulate cell division. As starch disappears from the neighbourhood of the larva, shrinkage occurs and central cavity is formed where the insect fully matures. The gall, if not previously collected and

dried, the mature insect boresits way out. During these changes, the colour of gall passes from bluish-grey through olive green to almost white (Trease and Evans, 1983b).

Galls are valuable because they contain tannin, a bitter substance used in the tanning of leather. If galls are left on the tree for one or more growing seasons, the tannin content decreases and quality becomes inferior (Ramstad, 1959a).

1.6.2 Types of galls

Galls are obtained from various sources, some of which are described below.

i. Allepo gall or Mecca gall: This is collected from Oak tree, Quercus infectoria (Fagacea) in the regions between the Mediterranian sea and Pakistan. The gall is formed on the Cambium region of the young shoot. Allepo gall is a major source of commercial tannins (Ramstad, 1959a).

ii. Chinese galls: This is obtained from Rhus semialata (Anacadiaceae) growing in the Far East. The gall is formed on the leaf stalk. The Chinese gall is an important source of medicinal tannins.

iii. Galls of G. senegalensis: These are formed on the young shoots of G. senegalensis. The plant grows abundantly in farmlands in subtropical West Africa especially around Northern Nigeria (Nielsen, 1965).

iv. English Oak gall: Formed by Adleria kollari on Quercus robur, contains about 15-20% tannins (Trease and Evans, 1983).

1.7 Tannins

The term tannin was first used in 1796, and refers to chemical substances which have the ability to convert hides and skins to leather by forming insoluble compounds with proteinaceous substances (Ramstad, 1959c). The ability of tannins to precipitate proteins is the cause of their astringency (White, 1959).

Tannins are widely distributed in the plant kingdom. Tannins are present in many crude drugs in high concentrations and localised in specific parts. A number of tannin drugs were and are used in folk medicine (Ribereau-Gayon, 11972a).

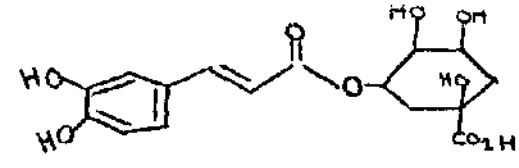
Two well differentiated structural types have been identified, these are:-

1. Hydrolysable tannins - These consist of the carbohydrate core, the hydroxyl groups which are estrified by gallic acid on one of its hydroxyl groups. As their name suggests, they are readily hydrolysed either chemically or enzymatically by tannase.

2. Condensed tannins - These are formed by condensation of catechins and are resistant to hydrolysis (Ribereau-Gayon, 1972b).

Another class of tannins has been recently recognized. This is psuedotannins. These are compounds of lower molecular weight than true tannins, and do not respond to Goldbeater's test as compared to true tannins but have similar reactions to those of tannins (Trease and Evans, 1983c). These are of varied chemical constitution for example flavonoids and certain compounds containing depside linkages. An example is chlorogenic acid, (Fig. 3) a depside of caffeic and quinic acid (Balbaa, 1976).

Fig. 3: Structure of Chlorogenic Acid



1.7.1 Hydrolysable tannins

Hydrolysable tannins are esters of sugar and phenolic acids or their derivatives. These tannins are subdivided into Gallotannins which on hydrolysis yield gallic acid, (Fig. 4) as the only phenolic moiety with a sugar which is usually glucose and the Ellagitannins which under the same condition of hydrolysis give gallic acid or galloly-gallic acid, one or more of its derivatives of which ellagic acid (Fig. 5) is the most important (Ribereau-Gayon, 1972c).

Fig. 4: Structure of Gallic Acid

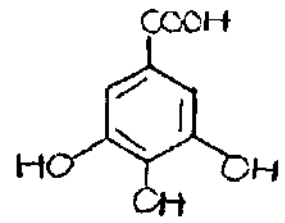
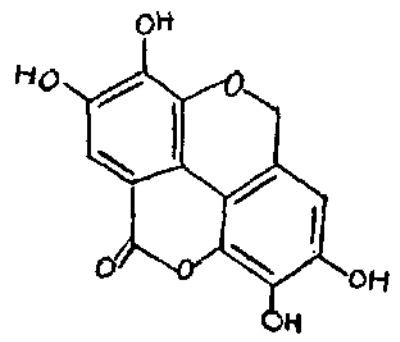


Fig. 5: Structure of Ellagic Acid



Gallic acid is found in many wood plants, bound as gallotannin but it is very reactive. More common on acid hydrolysed plant extract is its dimeric condensation product, ellagic acid, from ellagitannins present in direct extracts (Siekel and Hillis, 1970).

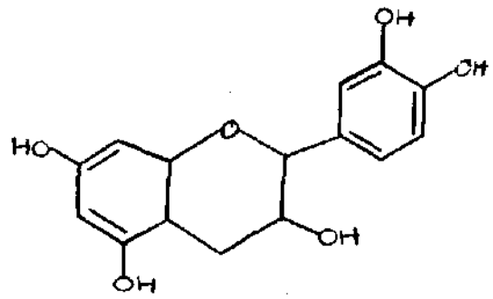
The chief commercial type of gallotannin is tannic acid from plant galls and is actually a mixture of free gallic acid and various galloyl esters of glucose whose exact composition varies according to source (Tyler et al., 1981).

1.7.2 Condensed tannins

Condensed tannins are commercially more important than hydrolysable tannins, they are mixture of several polymers of catechins (Fig. 6).

Many aspects of the properties remain to be elucidated, so far no condensed tannin has yet been isolated in a pure state (Ribereau-Gayon, 1973d).

Fig. 6: Structure of Catechin



The condensed tannins lack sugar in their molecule. In the presence of strong acids and oxidative agents, they are converted into red and brown substances referred as phlobaphenes, which are insoluble in most solvents. Phlobaphenes are normally present in tannin drugs and are known as tannin red (Ramstad, 1959).

1.7.3 Properties of tannins

Tannins are readily soluble in water (especially hot) and in other polar organic solvents but are practically insoluble in nonpolar solvents like benzene, chloroform, ether and petroleum ether (Trevor, 1976).

Tannins are non-crystallisable compounds. The essential property of tannins is their ability to combine with proteins and other polymers such as cellulose or pectin in solution. The ability to combine with protein is the cause of their astringency and forms the basis of the therapeutic application of tannins (Claus et al, 1970). Some of the important chemical properties of tannins are listed below.

(i) Tannins form dark blue or greenish soluble compounds with ferric salts.

(ii) Tannins form deep red colour with potassium ferricyanide and ammonia solutions.

(iii) Tannins are precipitated by salts of copper, lead and tin, and also by strong aqueous potassium dichromate.

(iv) In alkaline solution many of the tannin derivatives absorb oxygen.

(v) Tannins precipitate alkaloids from solution.

1.7.4 Occurrence of Tannins in the plant kingdom

Tannins are distributed widely in the plant kingdom. In fact almost every family of plant have species that contain tannins. Tannins are found in appreciable quantities in specific plant parts; leaves, fruits, bark or stem and galls (Claus *et al.*, 1970).

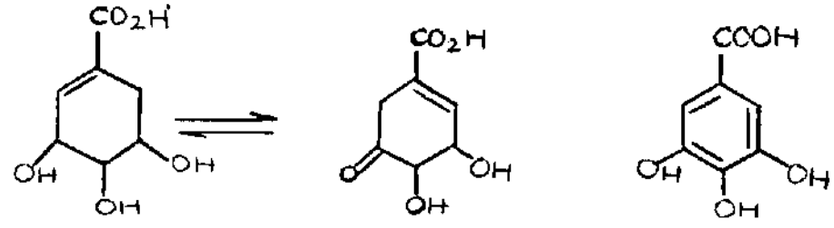
Tannins occur in vacuoles of plant cells. In some cases tannins are localised in special cells of the tissue i.e. tannin idioblasts. Tannins accumulate preferably in old tissues (Ramstad, 1959a). The individual plants and families that have been documented to contain tannins are: *Uncaria gambir* (Rubiaceae), *Pterocarpus marsupium* (Leguminosae), *Krameria triandra* (Leguminosae) and *Eucalyptus* spp. It is also found in abundance in the Gymnospermae, Salicaceae, Fagaceae, Polygonaceae, Rosaceae, Mimosaceae, Papillionaceae, Myrtaceae and Rubiaceae (Ramstad, 1959).

1.7.5 Biosynthesis of tannins

Tannins being complex in structure, little information is available as with regard to their biogenesis. However, biochemical pathways leading to various individual components of tannins are known.

(i) Gallic acid - hydrolytic product of hydrolysable tannins.

A confirmed pathway as in higher plants is from shikimic acid i.e. viz:- (Fig. 7).



Shikimic acid | 5-dehydro shikimic acid | gallic acid

Fig.7 Biosynthesis of gallic acid from shikimic acid

b. An alternative pathway which is apparently of lesser importance is operative in some plants and involves β-oxidation of various phenylpropanoid precusors. Exampie from phenylanine (Fig. 8).

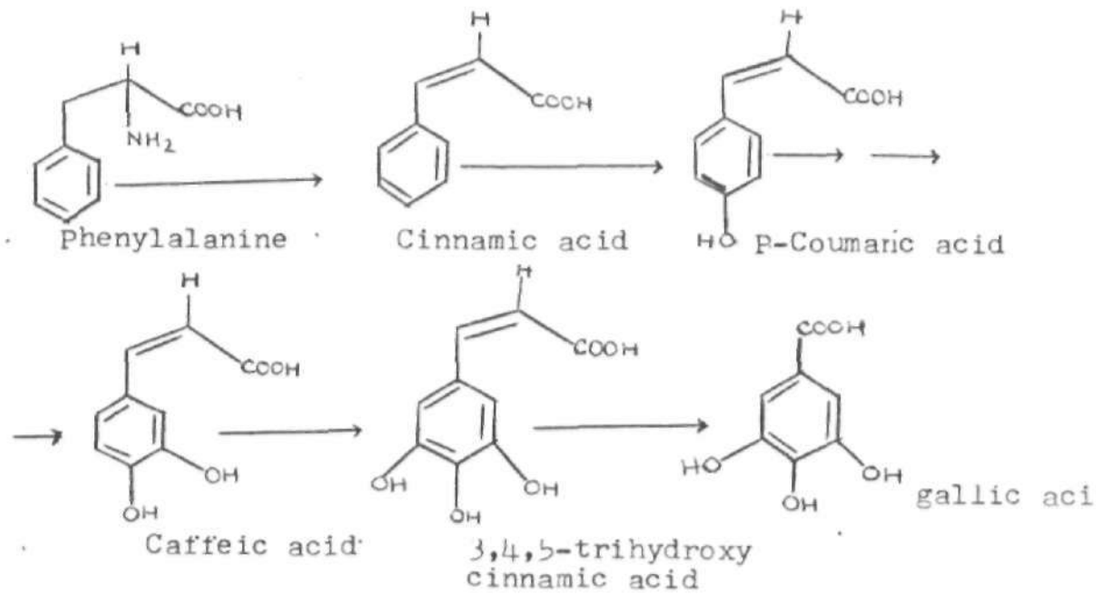


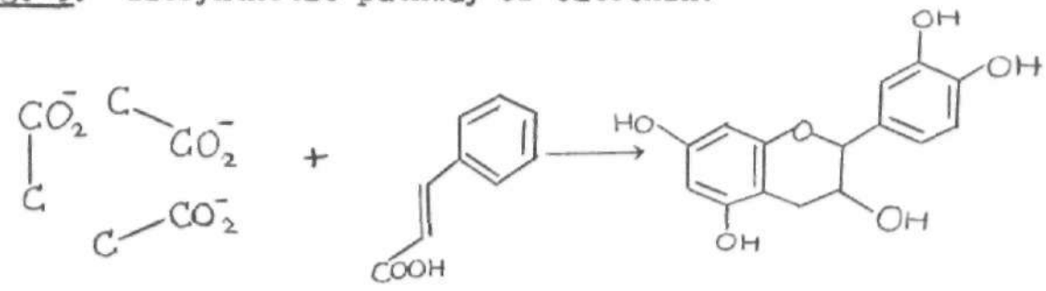
Fig. 8. Biosynthesis of gallic acid from phenylalanine

(ii) Catechin, a flavan-3-ol derivative is considered the phenolic precursor of condensed tannins, probably results from combination of acetate and cinnamic acid (Fig. 9).

Such simple polyphenol and flavonoid units, together with other closely related compounds and sugars, apparently undergo various condensation and

polymerisation reactions catalysed by local enzyme system to produce complex tannin molecule (Claws et al., 1970).

Fig. 9: Biosynthetic pathway of catechin.



3 Acetates | Cinnamic acid (equivalent) | Catechin

(Claus et al., 1970)

1.7.6 Uses of tannins

(i) Tannins find wide uses as astringents in gastro-intestinal tract and on skin abrasions. They are employed in the treatment of burns, where the protein of exposed tissues are precipitated forming mildly antiseptic, protective coat under which the regeneration of new tissues may take place (Tyler et al., 1981).

(ii) Used for tanning hides and skin to leather.

(iii) The deeply coloured compounds formed with iron salts have been used in many inks on a commercial scale.

(iv) Used as dyes and in cytological laboratories as stain.

(v) Based on their precipitation property, tannins are used in the laboratory to test for the presence of gelatin, protein and alkaloids.

(vi) Tannins are employed as antidotes for alkaloidal and arrow poisoning.

(vii) Albumin-tannate, a drug used by ostomates to reduce odour is prepared from tannic acid.

(viii) Gallic acid is used for preparation of bismuth subgallate - a developer in photography.

1.7.7. Present sources of tannins

The important sources of medicinally and commercially useful tannins can be summarised as follows:-

(1) Medicinal tannins.

i. Galls - Chinese galls obtained from Rhus semialata (Anacardiaceae).

ii. Oak gall from Quercus infectoria (Fagaceae)

iii. Gambir - Extract of Uncaria gambier (Rubiaceae).

iv. Kino - Exudate of Pterocarpus marsupium (Leguminoseae)

v. Rhatany - Roots of Krameria triandra (Leguminoseae).

(2) Industrial tannins

- Wattle bark (South Africa)
- Mangrove bark (West Africa)
- Oak bark (Europe and North America)
- Spruce bark (Europe)
- Hemlock bark (U.S.A. and Canada)
- Eucalyptus bark (Australia, Africa)
- Pine bark (Worldwide)
- Quebracho (South America)
- Chest nut (Europe and U.S.A.)
- Catechu (India, Burma and Pakistan)

(Ramstad, 1959).

The world annual production of tannin materials was estimated 30 years ago at about 1.5 billion kilograms, corresponding to about 500 million kilograms of pure tannins (Ramstad, 1959a). It is expected that the production of tannin materials has increased many folds since then.

1.8 Aims and Objectives of the Study

The plant G. senegalensis J.F. Gmel. as earlier stated in the text is widely distributed and grows abundantly in Northern Nigeria (Neilsen, 1965). It is used by the herbalists for the treatment of gastro-intestinal tract disorders, wound and skin diseases (Gaya, 1978).

The grey galls are sold in local markets and are claimed to be effective for sores on children's head (Irvine, 1961). The powdered roasted galls when made into ointment, have been found to be used for treatment of skin diseases in parts of Kano State. Furthermore galls from other plants serve as astringent and have been used in ointments and suppositories for the treatment of haemorrhoids. The gall is the main source of tannic acid (Martindale, 1989).

Tannins from other sources have been employed in medicine and for other commercial purposes (Tyler et al., 1981). Examples are:- the tanning industries, cytological laboratories and in the manufacture of inks (Ramstad, 1959a). Combier et al. (1977) have shown that the leaf and root of G. senegalensis contains tannins among its phytochemical constituents.

Specifically, the aims and objectives of this project are:-

- To carry out macromorphological and cytomorphological studies of the leaves and galls of G. senegalensis J.F. Gmel.
- To perform the qualitative chemical tests for detection of different constituent groups such as alkaloids, tannins, saponins and flavonoids.

- To determine the types of tannins and estimate the tannin content in the leaves and galls using various methods.
- To determine the type of free and hydrolysable sugars in the leaves and galls of the plant.
- To determine the antibacterial and antifungal activity of the galls if any.
- To establish the pharmacopoeial standards such as moisture contents, extractive and ash values for the galls and leaves.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.0 MATERIALS AND METHODS

2.1.0 Plant Identification and Collection

The plant G. senegalensis was identified in the field using keys and descriptions given in the 'Woody Plants' of Ghana (Irvine, 1961); 'Introduction to Flowering Plants of Tropical West Africa' (Nielsen, 1965a) and 'Medicinal Plants in Nigeria' (Oliver, 1960). The plant samples were then compared with authentic herbarium specimen placed in the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

2.2 Preparation of Herbarium Specimens

The plant sample was collected in the morning and was arranged to give complete view between blotting papers and inserted between more sheets of paper placed on a flat card board. Another card board was placed on top and this was weighed down with two bricks. The outer papers were changed every other day until the sample of G. senegalensis was completely dry. The dried sample was removed and placed on a white cardboard paper cut to herbarium size. The specimen was fied to the paper by use of cellotape.

An information card was prepared noting the general locality, botanical and vernacular names, habitat, date, time of collection, collector's name

and local uses. The card was affixed on the herbarium sheet (Walters, 1963). A voucher herbarium specimen was deposited with a taxonomist - Mr. H.D.Onyeachusim of Forestry Research Institute, Ibadan. Samples of the plant were collected in January 1991 from the natural habitat (Plates 1 - 3).

2.3 Drying and Storage

The galls and leaves were removed from the whole plant and air-dried at room temperature. The dried parts were powdered by pounding in mortar with pestle separately. The powders were packed in air-tight containers and labelled appropriately. The processed materials were stored in a cool dry place until required for the microscopical, phyto-chemical and microbial studies.

2.4 Identification of Galling Agents in G. senegalensis

The fresh galls with the contents were taken to Entomology Section of Department of Crop Protection, Institute of Agricultural Research, Ahmadu Bello University, Zaria for the identification of the galling agents.

2.5 Materials

- Pestle and mortar
- Sieve No. 40



Plate 1: *G. senegalensis* growing in its natural habitat



Plate 2: Fresh cuttings of the twigs bearing the galls and leaves of G. senegalensis.



Plate 3: Fresh galls of G. senegalensis

- Compound microscope (Wild 11 Switzerland)
- Camera lucida (Wild 11 Switzerland)
- Slides and cover slips
- 250ml Beakers
- Measuring Cylinder
- Bunsen burner
- 250ml Flat bottomed flask
- 250ml conical flasks
- Nickel and porcelien crucibles
- Filter paper ordinary
- Ashless filter paper
- Funnel
- Separatory funnel
- Oven (Gallenkamp)
- Chromatographic paper (Whatman no. 1)
- Air blower
- Fume cupboard
- Water bath
- Razor blade
- Photographic camera (Canon, Japan)
- U.V. light
- Capillary tubes
- Spray bottle and gum
- T.L.C. plates coated with silica gel
- Thistle funnel
- Thermometer
- Plates

- Cup borer no. 4
- Sintered filter plate
- Spectrophotometer (Milton Roy Spectronic 101 plus)
- Procter extractor (assembled)
- Mechanical shaker (Gallenkamp)

2.5.1 Solvents and reagents

The reagents used were all of laboratory grade, and in all cases water used referred to distilled water. Reagents used were as follows:

- Sodium acid phosphate
- Concentrated sulphuric acid
- Concentrated hydrochloric acid
- Ammonia
- Reference solutions of
  1% Tannic acid
  1% Catechol
  1% Phloroglucinol
- Chloroform
- 95% Ethanol
- Benzene
- Acetone
- Petroleum ether (60 - 80°)
- Methanol
- Lead acetate (10% w/v)
- Lead sub-acetate
- Alcoholic ferric chloride (spray reagent solution)

- Ferric ammonium citrate
- Vanillin
- Formaldehyde
- 5% Gelatin
- Sodium nitrite
- n-Butanol
- Resorcinol crystals
- Acetic acid
- Acetic anhydride
- Fehling solutions A and B
- Alkaloidal reagents:-

  10% tannic acid

  Wagner's (solution of iodine in pot. iodide).

  Hager's (saturated solution of picric acid).

  Mayer's (potassium mercuric iodide solution)

  Dragendorff's(potassium bisthmus iodide solution)
- Molisch's reagent (10% solution of naphthol in alcohol).
- Ruthenium red
- N/50 iodine
- Lime water
- 5% Copper acetate
- Aniline phthalate spray reagent (0.93g aniline and 1.66g O-phthalic acid dissolved in 100ml n-butanol, saturated with water) (Stahl, 1969).
- Standard hide powder (Denk Pharma. W. Germany)

- p-Anisaldehyde - sulphuric acid spray reagent (1ml concentrated sulphuric acid added to 0.5ml anisaldehyde in 50ml acetic acid, freshly prepared) (Stahl, 1969).
- Sand (Calais sand) (BDH Chemicals Ltd, Poole, England).
- Heavy kaolin powder (May and Baker Chemicals Ltd, Dagenham, England).
- Chrome alum solution (3% w/v chromium potassium sulphate in water).

2.6 Macroscopical Examinations and Organoleptic Properties of Leaves and Galls of *G. senegalensis*

The general macroscopical features and organoleptic properties were noted for the gall and leaf drugs. Origin, fracture, fracture surface, size, odour, taste and colour were noted (Herbert and Ellery, 1948; Trease and Evans, 1983).

2.7 Chemomicroscopical and Microscopical Examination of the Leaves and Galls of *G. senegalensis*

2.7.1 Chemomicroscopical examinations

In order to differentiate the tissues and observe for presence of substances such as cellulose, tannins, lignin, oils, starch, calcium oxalate and calcium carbonate, the various anatomical sections as well as the powder of the leaves and galls were treated with appropriate chemical reagents on microscope slides and observed under the microscope (Iyenger and Nayak, 1975).

(a) Test for lignin

The powdered gall and leaf and their sections were heated for two minutes with a mixture of phloroglucinol and conc. hydrochloric acid (1:1 v/v). Tissues like xylem and sclerenchyma because of lignification took up red colour. The intensity of red colouration is an indication to the extent of lignification (Trease and Evans, 1983d).

(b) Test for mucilage

The various sections and powdered samples of galls and leaves were treated separately with ruthenium red; mucilage in tissues stains pink or red.

(c) Test for cellulose

The powdered samples of galls and leaves were mounted in chlor-zinc-iodine. A blue colour indicates the presence of cellulose (Trease and Evans, 1983).

(d) Test for starch

The powdered galls and leaves as well as the anatomical sections were mounted on a slide in water, then irrigated with N/50 iodine. Presence of starch is indicated by blue or blue-black colouration (Iyenger and Nayak, 1975).

(e) Test for fixed oil

Gall and leaf powders as well as the anatomical sections were mounted is Sudan IV separately. Presence of oils in any of the tissues is indicated by pink-colour in the parenchyma and epidermal cells (Trease and Evans, 1983).

(f) Test for tannins

The powdered galls and leaves, and the anatomical sections were mounted in ferric chloride solution. A blue-black or greenish colour indicates the presence of tannins (Ramstad, 1959e).

(g) Test for calcium oxalate crystals

The powdered galls and leaves, as well as the anatomical sections were cleared by warming with chloral hydrate solution. The calcium oxalate crystals appear as bright structures. On treatment with concen-treated sulphuric acid, the crystals were slowly dissolved (Brain and Turner, 1975a).

(h) Test for calcium carbonate

The powdered galls and leaves on a white tile were treated with dil. sulphuric acid. Presence of calcium carbonate was indicated by effervescence with rapid evolution of gas (Brain and Turner, 1975a).

2.7.2 Microscopical examination

The powdered leaves and galls as well as the transverse and longitudinal sections of the fresh leaves and galls were subjected to microscopical examinations as follows: A thin anatomical section of the sample was taken on a clean slide and mounted in a few drops of chloral hydrate solution. The slide was slightly warmed for 30 seconds. Over heating was avoided as this could cause formation of chloral hydrate crystals. The section was carefully covered with cover slip to exclude air bubble creeping, after the section was sufficiently cleared with chloral hydrate. The slide was mounted in dil. glycerol and observed under a microscope (Iyenger and Nayak, 1975). The section when mounted in glycerol remained fresh for a long time.

The same procedure above was repeated on the powdered samples of leaf and gall for the observation of structural features.

2.8 PHYTOCHEMICAL SCREENING OF POWDERED GALLS AND LEAVES OF G. SENEGALENSIS

2.8.1 Test for Carbohydrate

(a) Extraction

Three grams of powdered galls were boiled in 50ml distilled water on a hot plate for 3 mins. The mixture was filtered while hot and the resulting filtrate cooled and used for the following tests.

(b) Molisch's test - General test

To 2ml of extract in a test-tube was added few drops of Molisch's reagent, then a small quantity of conc. sulphuric acid was allowed to run down the side of the tube at an angle of 45^{o} to form a lower layer. A purple to violet colour at the interface indicates presence of carbohydrate. The mixture is then shaken gently and allowed to stand for two minutes and then diluted with 5ml water. A dull violet precipitate further indicates presence of carbohydrate (Trease and Evans, 1983f).

(c) Test for Sugars

(i) Barfoed's test for monosaccharides

To 1ml of dilute solution of extract in a test tube was added 1ml of Barfoed's reagent and mixture heated in a water bath for 4 mins. A red precipitate indicated the presence of monosaccharides (Trease and Evans, 1983f).

(d) Fehling's test

(i) Standard test for free reducing sugars

To 2ml of the water extract, 5ml of a mixture (1:1) of Fehling solution I (A) and Fehling solution II (B) was added and mixture boiled in a water bath for five mins. A brick-red precipitate indicates the presence of free reducing sugars.

(ii) Standard test for combined reducing sugars

1ml of the water extract was hydrolysed by boiling with 5ml of dilute hydrochloric acid. This was neutralised with 10% dil. sodium hydroxide solution for the cuprous oxide to be precipitated. The Fehling's test was repeated (as in d.i). A brick red colour indicates the presence of combined reducing sugar

(iii) Selivanorff's test (standard test for ketoses)

To a small volume of the water extract was added few crystals of resorcinol and an equal volume of conc. hydrochloric acid and heated in a water bath. A rose colour indicates the presence of ketoses example fructose (Trease and Evans, 1983f).

(iv) Test for pentoses

To a small portion of the water extract, was added an equal volume of dilute hydrochloric acid containing little phloroglucinol. The mixture was heated in a water bath. A red colour indicates the presence of pentoses.

2.8.2 TESTS FOR GLYCOSIDES

(a) Test for cyanogenetic glycosides

A small amount of the powdered galls/leaves was taken in a test-tube and distilled water was added just cover the powder. A prepared moist sodium picrate paper was suspended at the neck of the test tube by means of a cork. The tube is placed in a water bath and allowed for one hour. A brick-red colour on the picrate paper indicates the presence of cyanogenetic glycosides (Trease and Evans, 1983).

(b) Test for anthraquinone derivatives

(i) Borntrager's tests

For the presence of free anthraquinones. 0.5g of powdered galls/leaves is taken in a dry test tube, to this 10ml of chloroform was added, shaken and heated for about five minutes. The extract is filtered while hot and allowed to cool. To the filterate was added equal volume of 10% ammonium solution and shaken. Bright pink colour in the (upper) aqueous

layer indicates the presence of free anthraquinones (Sofowora, 1982).

(ii) Test for presence of free and/or combined anthraquinones

0.5g of the powdered galls/leaves was boiled with 10ml of 10% hydrochloric acid for 2 to 3 mins. The extract was filtered while hot and allowed to cool. To the filterate, an equal volume of chloroform was added. The tube inverted several times, care is taken to avoid vigorous shaking. The solution was transferred to a separatory funnel and the two layers are allowed to separate. The lower chloroform layer was drained into a clean test tube and 10% ammonium added and shaken. When the two layers separated, a bright pink colour in the aqueous layer indicated the presence of free and/or combined anthraquinone glycosides (Trease and Evans, 1978).

(iii) Test for anthraquinone derivatives in a reduced form which are not easily hydrolysed

0.5g of powdered galls/leaves was boiled with 10ml of 10% $FeCl_3$ and 5ml of dilute hydrochloric acid for 5 minutes. The mixture was filtered while hot and allowed to cool. The filterate was shaken with equal volume of chloroform in a separatory funnel. The chloroform layer was transferred into another test tube containing 5ml of 10% ammonia. A bright pink colour in the upper layer (aqueous) indicates

the presence of anthraquinone which are not easily hydrolysed example C-glycosides (Trease and Evans, 1983).

(c) Test for saponin glycosides

To a small quantity of powder of sample was added 10ml of 95% ethanol and heated to boiling on a water bath. The mixture is filtered while hot and allowed to cool. 2.5ml of the filtrate was diluted with 10ml distilled water in a test tube. The test tube was stoppered with a cork and shaken vigorously for 30 seconds then allowed to stand for 45 mins. A honey-comb froth indicates the presence of saponins (Sofowora, 1982a).

(d) Test for saponins (steroidal and triterpenoidal) - Haemolytic test

0.2g of the powdered material was extracted with 10ml warm water, filtered and filtrate retained. 2ml of 1.8% sodium chloride solution was added to two test tubes. To one of the test tube (t1), 2ml of distilled water was added and the other (t2) 2ml of extract was added. The concentration of sodium chloride in each case is now isotonic with blood-serum. Blood was obtained by pricking the thumb at the base of nail and blood was drawn into a small pipette. Five drops of the blood was added to each of the test tube (t1 and t2). The tubes were stoppered and inverted gently

to mix the contents. Haemolysis in test tube containing extract (t2) and not in the control (t1) indicates presence of saponins (Brain and Turner, 1975b).

(3) Test for sterols and terpenes

(a) Extraction

5g of the powdered sample was extracted by maceration with 50ml of 95% alcohol. The extract was filtered and the filtrate is evaporated to dryness in a beaker. The residue is dissolved in 10ml of chloroform (anhydrous) and re-filtered. The filtrate was divided into two equal portions which were used for the following tests.

(i) Salkowski's test for steroidal ring

The first portion of the (3a) above was taken in a test tube, 2ml of concentrated sulphuric acid was added carefully to form a lower layer.

A reddish brown ring at the interface indicates the presence of a steroidal ring (Sofowora, 1982a).

(ii) Lieberman-Burchard's test for terpenes

To the second portion of the filtrate from (3a) was added 1ml of acetic anhydride with mixing. 1ml of concentrated sulphuric acid was added down the side of the test tube. The formation of a reddish violet colour at the interface of the two liquids and a green colour in the chloroform layer indicates presence of terpenes.

2.8.3 Test for Flavonoids

(a) Extraction

2g of the powdered galls was completely detanned with acetone and the acetone was decanted. The remaining acetone was completely evaporated on a water bath. The residue was mixed in warm water and mixture was filtered while hot then allowed to cool. The filtrate was used for the following tests (Brain and Turner, 1975b).

(b) Lead acetate test

To 5ml of the detanned water extract, was added 2-3 drops of lead acetate solution. A buff coloured precipitate indicates the presence of flavonoids (Trease and Evans, 1983h).

(c) Sodium hydroxide test

5ml of detanned water extract was taken in a test tube and an equal volume of 10% sodium hydroxide was then added. A yellow solution indicates the presence of flavonoids. On addition of dilute hydrochloric acid, the solution becomes colourless.

(d) Ferric chloride test

2ml of the detanned water extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added.

A green or blue colour indicates the presence of phenolic nucleus (Brain and Turner, 1975).

(e) Shinoda test

0.5g of the powdered galls/leaves was boiled in ethanol in a test tube on a water bath for 5 minutes, filtered and cooled. Four pieces of magnesium fillings were added followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids (Geissman, 1962).

2.8.4 Test for Alkaloids

10g of the powdered gall was taken in a small beaker and strong ammonia solution was added in a quantity sufficient to just moisten it and mixture allowed to stand for about 10 mins after through mixing was done. Sufficient quantity of mixture of chloroform and ethanol (1:1) was added just to soak and suspend the powder. The mixture was allowed to stand for 20 minutes with occasional stirring with a glass rod. The mixture was filtered through a plug of cotton wool in a filter funnel. The marc was washed twice with 2ml chloroform and the washings were added to the filterate. The combined filtrate was concentrated to dryness on a water bath. Care was taken to avoid over heating. The residue was cooled and dissolved in 5ml chloroform. The chloroform

extract was transferred to a separatory funnel and was shaken with 3ml sulphuric acid. The two layers were allowed to separate, the chloroform (lower) layer was drained off and discarded. 3ml of chloroform was further added, shaken and drained off and discard until the acid layer was colourless.

The acid layer was made alkaline with strong ammonia and extracted with 3ml of chloroform. The extract was retained and evaporated to dryness. The residue was dissolved in 3ml of ethanol and the following tests were carried out after neutralising with dilute sulphuric acid (Trease and Evans, 1983).

(b) General tests

To 0.5ml of ethanol extract residue obtained from above (2.7.4a) was added 2-3 drops of the following reagents.

- Wagner's reagent
- Hager's reagent
- Dragendorff's reagent
- 10% tannic acid solution
- Mayer's reagent.

Most alkaloids are precipitated in most or all of the above reagents. False positive result which could occur as proteins were present and could be precipitated was eliminated by process of heating during the course of extraction (Trease and Evans, 1983i).

2.8.5 Test for resins

(i) Test for resins

0.5g of powdered sample was mixed with 10ml acetic anhydride and was added 1ml of concentrated sulphuric acid. A purple to violet colour indicates the presence of resins.

(ii) 10ml of petroleum ether extract was made from 0.5g of the powdered sample and filtered into a test tube. An equal volume of copper acetate solution was added and mixture was shaken vigorously and allowed to separate. A green colour indicates the presence of resin (Trease and Evans, 1983j).

(ii) Test for oleo-gum resin

0.1g of the powdered sample was triturated with 0.5g sand and transferred into a test tube containing 3ml ether and shaken well. The mixture was evaporated into a thin film on a water bath. In the fume cupboard, bromine vapour was passed over the residue. A violet colour indicates the presence of oleo-gum resin (Trease and Evans, 1983j).

2.8.6 Tests for Tannins

(a) Extraction

3g of the powdered galls/leaves were boiled in 50ml of water for three minutes on a hot plate.

The mixture was filtered while hot and resulting filtrate was used to carry out the following tests for tannins.

(b) Ferric chloride test

1ml of extract was diluted with 4ml water and few drops of 10% ferric chloride were added. A bluish black colour indicates presence of hydrolysable tannins and greenish colour indicates presence of condensed tannins. (Trease and Evans, 1983C)

(c) Lead subacetate test

To 1ml of the extract was added 2-3 drops of lead subacetate solution. A coloured precipitate indicates the presence of tannins.

(d) Ferric ammonium citrate test

To 1ml of water extract was added 5 drops of 0.25% ferric ammonium citrate solution. To the mixture was added 0.5g solid sodium acetate to adjust the solution to pH 8 using pH indicator paper. The mixture was boiled in a water bath and filtered while hot. A purple, violet or black precipitate which is insoluble in hot water indicates presence of tannins (Balbaa, 1976).

(e) Bromine water test

A few drops of bromine water were added to 1ml of the water extract in a test tube. Condensed tannins give a buff coloured precipitate, hydrolysable tannins give none at all.

(f) Test for phloroglucinol nucleus

15ml of alcoholic extract of galls/leaves were made by boiling 0.5g in 10ml of ethanol and filtered. 5ml was taken in an evaporating dish and placed on a water bath, 2ml of vanillin reagent were then added. The mixture was evaporated to dryness. A drop of concentrated hydrochloric acid was added. A crimson colour indicates the presence of phloroglucinol which frequently occurs with catechol.

(g) Gelatin test

To 2ml of the water extract was added an equal volume of 0.5% gelatin solution containing 10% sodium chloride. A precipitate indicates the presence of tannins (Trease and Evans, 1983c).

3.8.6.2 Distinguishing tests for tannins

(a) 5ml of the water extract from 2.7.6a was boiled with 5ml 40% formaldehyde in 10% hydrochloric acid solution for 30 mins. A precipitate was formed, the mixture was filtered and the

filtrate is retained. The precipitate was insoluble in hot water indicates the presence of catechins. The filtrate was then divided into 3 portions.

(b) To the first portion of the filtrate above (3.7.6.2a) was added few crystals of sodium nitrite, a green colour indicates the presence of ellagi-tannins.

(c) To the second portion of filtrate obtained from above (3.7.6.2a) was added dropwise 5ml lime water. A blue-grey colour of precipitate indicates the presence of gallotannins.

(d) To the third portion from above was added two drops of 10% ammonium solution, the mixture was heated on a flame and exposed to air. A green colour indicates the presence of pseudotannins for example chlorogenic acid.

2.9 CHROMATOGRAPHIC ANALYSES

2.9.1 Chromatographic analysis of tannin extracts

3.0g powdered galls or leaves was boiled with 50ml of distilled water for three minutes on a hot plate. The mixture was filtered whilst hot. The resulting filtrate was concentrated, allowed to cool and was used for chromatographic separation. The

above procedure was repeated but using mixture of water and 95% ethanol in the ratio 1:1 as the solvent of extraction (Trevor, 1976).

Reference samples

- 1% w/v of the following standard samples were prepared in distilled hot water.
- Tannic acid
- Catechol
- Phloroglucinol

Conditions for paper chromatography

Chromatographic paper	:	Whatman No.1 (20cmx20cm)
Solvent system	:	n-Butanol : acetic acid : water (4:1:5).
Technique	:	Ascending method
Visualisation	:	(i) U.V. 365 nm. (ii) Spray with 5% alcoholic ferric chloride solution (Balbaa, 1976; Trevor, 1976).

Conditions for thin-layer chromatography

Adsorbent	:	Silica gel G (0.25mm) (Air dried and activated at 100°C for 30 minutes).
Solvent system	:	n-butanol : acetic acid : water (BAW) (4:1:5).
Technique	:	Ascending method

Visualisation : (i) U.V. 366nm.

(ii) Spray with 5% alcoholic ferric chloride solution (Trevor, 1976).

2.9.2 Chromatographic Analyses of Sugar Extracts

(i) Extraction of sugars

3g of powdered galls/leaves was extracted with 50% of a mixture of water and 95% ethanol (1:1) by heating on a water bath for 10 minutes. The extract was mixed well with 1g of activated charcoal to remove phenolic compounds (Ribereau-Gayon, 1972e). The mixture was filtered through glass wool and concentrated on a water bath. The clear aqueous extract is spotted on Whatman No.1 paper (Harbone, 1973).

(ii) Analysis of hydrolysed extract

3g of the powdered galls/leaves was extracted with 50ml mixture of water and 95% ethanol (1:1) by heating on a water bath for 10 minutes, filtered while hot and allowed to cool. 10ml of the fitrate were taken in a test tube and hydrolysed by boiling with 15ml 1M sulphuric acid. Excess acid was removed by adding 5% barium carbonate solution. The resulting mixture was filtered, and the filtrate was mixed with 0.5g activated charcoal and re-filtered. The clear filtrate was spotted on a Whatman No.1 paper.

(iii) Reference solutions

1% w/v of the following standard samples were made in 10% solution of isopropanol to prevent bacterial contamination.

- Sucrose
- Fructose
- D-Glucose
- Xylose
- D-Glucuronic acid
- Galactose
- Galacturonic acid

Paper chromatography

System 1:

Chromatographic paper	:	Whatman No. 1 (20cm x 20cm)
Solvent system	:	Chloroform : Ethanol (60:40)
Technique	:	Ascending method
Visualisation	:	U.V. 365 nm
	:	Anisaldehyde - sulphuric acid reagent and heated at 105°C until spots appeared distinctly.

System 2:

Chromatographic paper	:	Whatman No. 1 (20cm x 20cm)
Solvent system	:	n-Butanol : acetic acid : water (4:1:5).
Technique	:	Ascending method
Developing time	:	18 hours

Visualisation : U.V. 365nm

: Aniline hydrogen phthalate spray reagent and heated at 105°C until maximum colour intensity is attained.

2.10 ESTIMATION OF TANNIN IN LEAF AND GALL OF G. SENEGALENSIS

2.10.1 Estimation of tannin by gravimetric method

2g of finely powdered leaf/gall was extracted by boiling with 100ml water for one hour. The mixture was filtered while hot. Another 100ml of water was added to the residue and heated to boiling. Filtration was repeated and filtrate (extracts) combined. 30ml of 5% copper acetate solution was added gradually with stirring to the combined filtrate (extract) while boiling. Precipitate (copper tannate) formed was collected in an ashless filter paper and washed with water till free from copper acetate as indicated by colourless filterate which was discarded. The precipitate (with ashless paper) was ignited in a pre-weighed platinum crucible. Complete oxidation of the residue was achieved by addition of few drops of concentrated nitric acid. The crucible and content were heated to constant weight at 105°C. The tannin content (in percentage) was calculated using the following formula:

1g of copper oxide (CuO) = 1.305g of tannin (Balbaa, 1976).

2.10.2 Estimation of tannin content by hide powder method

(a) Extraction

Procter extractor was used for the extraction of the powdered leaf and gall. Hot and cold extractions were carried out as follows:

(i) Hot water extraction

The beaker was half filled with caleis sand which had been purified by washing thoroughly with dilute hydrochloric acid, then with water and dried. A thistle funnel, covered with a piece of muslin cloth, was attached to an L-shapped capillary tube having a tap at the outlet. The thistle funnel was placed in the sand in an inverted position. The beaker was then filled with distilled water, the tap at the outlet of the capillary tube was opened and using the mouth, water was drawn through the capillary by sucking. The water passed through the sand into the thistle funnel and out through the outlet. Water was allowed to flow freely for a few minutes while simultaneously filling up the beaker with more distilled water. When a smooth flow was achieved, the tap was closed and the supply to the beaker was also stopped. 20g of dry powdered gall/leaf of G. senegalensis was gradually poured into the beaker and left to soak overnight. The beaker and its content was placed in a water bath. The first 75ml of the extract were collected at room

temperature while the next 300ml were collected at a temperature of 50°C and a final volume of 625ml were collected at boiling point, making a total volume of 1 litre of the extract for the hot (leaching) extraction. Extracts were collected while keeping the level of water in the beaker constant as stated earlier. The whole leaching procedure was carried out over a period of four hours.

(ii) Cold water extraction

The procedure as described for hot water extraction was repeated for the cold extraction (leaching). The cold extraction was however performed at the room temperature.

(iii) Total solids

50ml of the extract (gall or leaves) were measured accurately with a pipette into a tared evaporating dish, evaporated to dryness on a water-bath and transferred into the oven. The extract was dried for 1 hour 30 minutes at 105°C, cooled in a desicator and weighed. Drying continued at 30 minutes interval until a constant weight was obtained. The difference between the final weight and the initial weight gives the weight of the residue. The total solids was then calculated with reference to the initial weight of the

G. senegalensis leaf/gall powder soaked and is expressed as a percentage.

(iv) Total solubles

About 70ml of the extract was filtered using filter paper and 50ml of the filtrate was accurately pipetted into a pre-weighed tared crucible evaporated to dryness then transferred into an oven and dried to constant weight at 105°C and total solubles determined as above (Society of Leather Trades Chemists, 1965).

The following formula was used for the calculation:-

Total solids/solubles = Z/20 x 1000/50 x 100

Where Z = weight of the residue.

(b) Non-tans

(i) Preparation of chrome-tanned hide powder

7.2g of hide powder was weighed into a conical flask and 72ml of distilled water added. The mixture was left to stand for 1 hour before 7.0ml of 3% chromium potassium sulphate solution were added, with frequent stirring and allowed to stand overnight. The mixture was filtered using muslim cloth. The cloth was drained and squeezed and the cloth with the powder were placed in a beaker followed by 93.5ml of water. This was mixed thoroughly and the cloth washed with water taking care not to lose any powder. The mixture was left

standing for 15 minutes. It was then drained and squeezed. This process was repeated three times. The product was the chrome-tanned hide powder (Society of Leather Trades Chemists, 1965).

(ii) Non-tans determination

100ml of unfiltered extract/tannic acid was added to the chrome-tanned hide powder in a conical flask. 20ml of distilled water was added to the mixture which was shaken vigorously by hand and then with a mechanical shaker at 50 rpm for 30 minutes. The mixture was filtered using a muslin cloth supported by a funnel; drained and squeezed. 1g of kaolin powder was added to the filtrate which was then refiltered through a 15cm filter paper. Filtration was repeated until a clear solution was obtained.

50ml of the filtrate was taken in a pre-weighed tared crucible and evaporated to dryness on a water bath. The evaporating dish (crucible) with residue was then transferred to an oven and heated to constant weight at 105^{o}C for 1 hour. At all stages, the evaporating dish was allowed to cool in a desicator before taking the weight (Society of Leather Trades Chemists, 1965).

2.10.3 Estimation of tannins by spectrophotometric method

(a) Materials:

- Hide powder R.S. (Denk Pharm. W. Germany)
- Phosphotungstic acid solution T.S. (BDH Chemicals Ltd, Poole, England).
- Sodium carbonate (380g/l)
- Pyrogallol R. (reference) (May and Baker Laboratory Chemicals, Poole, England).
- Spectrophotometer (Milton Roy Spectronic 101 plus).

(b) Preparation of drug extract

20g of powdered gall that was previously passed through mesh size nos. 40 was weighed into a 250ml conical flask containing 150ml water and the mixture heated over boiling water-bath for 30 minutes. The flask and contents were cooled in running tap water and the mixture transferred into 250ml volumetric flask and diluted to 250ml with water. The solids were allowed to settle and the supernatant filtered through a filter paper 12cm in diameter. The first 50ml of filtrate was discarded and the remainder used for the assay (African Pharmacopoeia, 1986).

(c) Estimation of total tannins

5.0ml of the filtrate (drug extract) above (2.9.3a) was diluted to 25.0ml with water. 2.0ml of

this mixture was mixed with 1.0ml phosphotungstic acid solution and 17.0ml of sodium carbonate (380 g/l) TS. The extinction at 750nm exactly after addition of sodium carbonate TS was determined in a 1.0cm cell using water as the compensation liquid and recorded as E_1.

(d) Tannins not bound with hide powder

10.0ml of the drug extract was added 1.0g hide powder RS and shaken well for one hour. The mixture was filtered and 5ml of the filtrate was diluted to 25.0ml with water. To 2.0ml of the solution was added the same quantities of reagents stated above and extinction under the same conditions as for E_1 determined and recorded as E_2. The procedures was repeated but using the powdered leaf.

(e) Preparation and determination of reference solution

Accurately weighed 50.0mg of pyrogallol was transferred to a 100ml volumetric flask and diluted to 100.0ml with water. 2.0ml was mixed with same quantities of the reagents stated earlier and extinction E_3 determined under same conditions.

The percentage of tannin was calculated from formula:

$$4.2 \times 3.125 \times \frac{(E_1 - E_2)}{W \times E_3}$$

Where W = weight of the drug taken (African Pharmacopoeia, 1986).

2.11 QUANTITATIVE EVALUATION OF POWDERED GALLS AND LEAVES OF G. SENEGALENSIS

2.11.1 Determination of moisture content of powdered galls and leaves by "Loss on drying method"

A tared evaporating dish was heated at 105°C to constant weight and its exact weight was noted. 3g of the powdered gall/leaf was accurately weighed into the dish. The evaporating dish and its content was placed in an oven at 105° C and dried to constant weight (achieved by checking the weight at 30 minutes interval after an initial drying for one hour). The total loss in weight was determined by subtracting the constant weight of the porcelein dish from the combined weight of the porcelein and its content.

The percentage of the moisture content was then determined with reference to the initial weight of the powdered gall/leaf. Two other determinations were done and average of these was taken as the moisture content of the drug (B.P., 1980).

2.11.2 Determination of extractive values

(i) Alcohol-soluble extractive value

5g powdered gall/leaf was accurately weighed into a 250ml stoppered conical flask. 100ml of 90% ethanol was added and stoppered firmly. The flask was shaken on a mechanical shaker for 6 hours and then allowed to stand for 18 hours.

The extract was filtered quickly by suction filtration. The weight of a clean, heated and cooled flat bottomed evaporating dish was accurately determined. 20ml of the filtrate was taken into the evaporating dish. The filtrate was evaporated to dryness. The residue was dried to constant weight at 105°C in an oven and final weight determined.

The weight of the residue was determined by substracting the weight of the evaporating dish from the final weight (total weight of the evaporating dish and residue). The alcohol extractive value was then calculated with reference to the initial weight of the powdered drug and expressed in percentage.

i.e. alcohol extractive value = $\frac{c - b}{a} \times 5 \times 100$

Where

a = weight of powder

b = weight of empty evaporating dish

c = weight of evaporating dish and residue.

Three different determinations were done and the average of these was taken as the alcohol-soluble extractive value (B.P., 1980).

(ii) Water-soluble extractive value

The procedure in 2.10.2 (ai) was repeated but using 0.25% chloroform in water instead of 90% ethanol. Chloroform was added as a fungustatic. Three different

determinations were made and the average of these was taken as the water-soluble extractive value (B.P. 1980).

3.11.3 Determination of ash values

(i) Determination of total ash

A nickel crucible was heated at 105°C to constant weight in an oven and its accurate weight was determined after cooling in a desicator. 2g of the powdered gall/leaf was accurately weighed into the crucible. The crucible and its contents were gently heated over a bunsen flame until it was moisture free and was completely charred. The heat was gradually increased until most of the carbon had vapourised and was heated strongly such that the inorganic ash could be seen (residue was free from carbon) - almost white. The ash was cooled, weighed and weight noted. The heating and cooling continued until the weight of residue was constant. The weight of ash was obtained by subtracting the weight of crucible from the final weight (of crucible + residue or ash).

i.e. $(b - a)g$ = wt. of residue

where a = weight of empty nickel crucible

b = weight of crucible and residue.

The ash value (in percentage) of the powdered gall/leaf was calculated with reference to the initial

weight of the powdered gall/leaf.

$$\text{i.e. Ash value} = \frac{b - d}{2} \times 100$$

Three different determinations were carried out and the average of these was taken as the ash value. The ash was retained for experiment 2.10.2b(ii) below (B.P. 1980).

(ii) Determination of acid insoluble ash

The nickel crucible and ash obtained from 2.10.2b(i) above was transferred into a beaker containing 25ml 2M hydrochloric acid. The beaker and its contents were boiled for five minutes and filtered through an ashless filter paper. The washings were repeated three times, each time passing the washings in a manner as to allow the collection of the residue at the tip of the cone of filter paper. The funnel along with filter paper and contents were dried in an oven at 105°C.

The weight of clean heated and cooled nickel crucible was accurately determined. The filter paper with its contents was folded into a small cone and transferred into nickel crucible (pre-weighed). The crucible was gently heated with a bunsen flame until the filter paper was completely ashed and then heated strongly for a few minutes. The crucible and contents were cooled and final weight was noted. The weight of

the residue Z(ash) was then calculated by subtracting the weight of crucible from the total weight of crucible and residue. Also the acid insoluble ash value with reference to the initial weight of the drug was calculated.

$$C \text{ (weight of ash in grams)} = b - a.$$

$$\text{Acid insoluble ash value} = \frac{b - a}{2} \times 100$$

Where a = weight of empty nickel crucible

b = weight of crucible and residue

2.12 Preliminary Antimicrobial Studies of the Galls of G. senegalensis

2.12.1 Materials

(a) Media: Media used were nutrient agar, nutrient broth, Sabouraud's broth and agar.

(b) Test organisms:
Escherichia coli, Staphylococcus aureus, Streptococcus pyogens, Candida albicans and Pseudomonas aeruginosa. These organisms were subcultured from typed organisms.

2.12.2 Preparation of extracts and test organisms

Fresh galls were collected, cleared off insects, eggs and larvae, and dried at 40°C. The galls were

divided into two portions. The first portion was powdered while the second was roasted. 10g of the powdered unroasted gall was placed in conical flask containing 100ml distilled water, shaken with mechanical shaker for three hours and decanted into a beaker. The same quantity of powdered . was similarly extracted with 100ml methanol and evaporated to dryness and residue redissolved in 100ml distilled water. The aqueous extract was sterilised by aseptic filtration using sterile Millipore membrane filter. The same procedure above was repeated for roasted gall powder.

The culture media were prepared according to the manufacturer's specifications and sterilised by autoclaving.

20ml each of nutrient agar and Sabouraud's agar were poured into sterile plates. Twenty four hours nutrient broth culture of each of the organisms was prepared and diluted with sterile broth to give a 10^6 viable count per ml. The plates were innoculated by even spreading of culture of the organism to ensure a uniform growth using fresh sterile cotton swab for each organisms.

2.12.3 Testing for antimicrobial activities using cup-plate agar method

Zone of inhibition method was used for evaluating the antimicrobial activity of the extracts as this

method enables the use of uniform quantity of extract.

Four holes of about 9mm diameter each were made in the innoculated plates with sterile cork borer no.4 and each hole was filled aseptically with 0.1ml extract (A, B, C or D), using sterile micropipettes. The plates were allowed a pre-diffusion time of one hour and then incubated at 37°C for 18 hours except for Sabouraud's agar which were incubated at 25°C for 18 hours. The sterility of the aqueous extract was determined by innoculating 5ml of each of the extract in a sterile tube and incubated at 37°C for 18 hours (Duguid et al., 1980). The procedure was repeated to produce eight replicates. Samples used were A, B, C and D. The organisms used were Staph. aureus, Strept. pyogens, E. coli, Ps. aeruginosa and C. albicans.

Key:

A : Aqueous extract of the roasted galls.

B : Water soluble methanol extractives of the roasted galls.

C : Aqueous extract of unroasted galls.

D : Water soluble extracted of methanol extractives of the unroasted galls.

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 Collection and Identification of Plant Material

The plant sample which was colled at its natural habitat along Shuwarin Road, Gaya, Kano State, fitted the morphological description of G. senegalensis (Combretaceae) described in the "Woody Plantsof Ghana" (Irvine, 1961), "Introduction to Flowering Plants of Tropical West Africa" (Nielsen, 1965a) and "Medicinal Plants in Nigeria" (Oliver, 1960).

3.2 Preparation of Herbarium Specimen

The identity of the plant was further confirmed by comparison of the prepared herbarium specimen with those available at the Department of Biological Sciences, Ahmadu Bello University, Zaria. Voucher specimen was also sent to the Forestry Research Institute, Ibadan for authentication and was given a voucher specimen number FHI 104312 by the taxonomist - Mr. H.D. Onyeachusim.

3.3 Drying and Storage

Drying was carried out under shade at room temperature in order to protect thermolabile constituents. The sample was dried to prevent chemical changes due to enzymatic degredation and to prevent microbial attack during storage.

The dried samples were powdered and sieved using number 40 mesh size sieve. Powder is convenient for storage and solvents can penetrate during extraction. The powders were packed in separate containers and closed tightly to exclude air.

3.4 Galls of G. senegalensis

Gall is a pathological formation on many plant parts and is caused by either bacteria, insects or fungi (Ramstad, 1959a). Presence of gall which is a specific feature was noticed on the stem branch of the G. senegalensis.

3.4.1 Identification of gall causative agents

The process of identification is essentially an attempt to match an unknown to a known entity, "the known" being either a specimen or a description or both (Palmer et al., 1989b).

The insects and larvae were found together in the fresh gall and were identified as Acaciothrip ebneri, Karny (Thripidae) by comparing with a description and specimen availabe at the Entomology Section of Department of Crop Protection, Institute of Agricultural Research, Ahmadu Bello University, Zaria. The larvae were white with poorly defined segments and reduced antennae (Fig. 9). The Thripidae have been reported to deposit their eggs in the tissue of

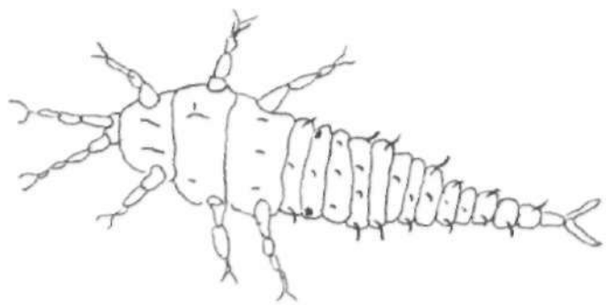


Fig. 10 Microscopical feature of larval stage of *Acaciothrips ebneri*, karny. Mag. x40

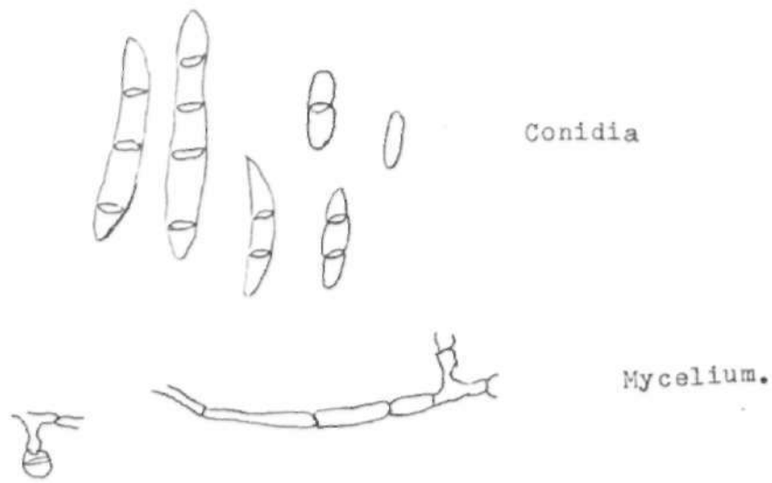


Fig. 11. Feature of a Mould fungus of *Fusarium* spp Mag. x400

green plants (Palmer et al., 1989a). Similarly a mould fungus was identified from the inner surface of the gall. This was achieved by culturing on Sabouraud's glucose agar at room temperature and specimen was mounted in cotton blue lactophenol and observed under high power magnification (Trevan et al., 1990). The result revealed two distinct features:

(i) Sickled shaped and long fusiform multinueleate conidia with crosswalls which were borne on the tips and along the sides of the hyphae (conidiosphores).

(ii) The conidiosphores are clustered, forming characteristic stalked bodies of cylindrical form (Fig. 10). This fungus was of the Fusarium specie.

The Fusarium spp. are wide spread. The specific identification of these fungi is often difficult because two nomenclatures were frequently used (Moreau and Moss, 1979). The Fusarium spp. are found in soil and cause diseases in plants. They are capable of utilising cellulose and starch as the sole source of carbone more readily (Skinner et al., 1947).

About half of specie of Thrips fed on fungi, mostly hyphae while others feed on spores and plant tissues (Palmer and Mound, 1983). A visible direct feeding damage may involve streaking of leaves or galling (Ananthakrishna, 1984). The occurrence of Acaciothrip ebneri together with the fungus of Fusarium spp. in the gall could therefore not have been a coincidence, since the latter serves as source of food to the former and the Fusarium spp. utilise cellulose and starch readily available in the plant as source of carbon. Therefore the gall of G. senegalensis is caused by Acaciothrips ebneri, Karny and fungus of the Fusarium spp.

3.6 Macroscopical examination and organoleptic characters of G. senegalensis

The following features were noted:

Galls

Origin:	Derived from stem
Surface:	Inner - Brown
	Outer - Bluish green
Fracture:	Short
Fracture surface:	Smooth
Taste:	Very astringent
Size:	Small, medium and large
Shape:	Irregular
Odour:	Pungent

Leaf

Lamina shape:	Ovate
Margin:	Entire
Apex:	Obtuse
Base:	Symmetrical
Texture:	Smooth
Colour:	Grey-green
Size:	3.0cm by over 2cm
Odour:	Pungent and comporaceous

3.7.1 *Chemomicroscopical and Microscopical Examination of the Leaf and Gall of G. senegalensis*

(a) Test for lignin

The rounded bodies (stone cells) and fibres in the sample of powdered galls gave red colour with phloroglucinol and concentrated hydrochloric acid indicating lignin. Trichomes were however not lignified. Scattered lignified tissue elements were observed in the samples of the longitudinal and transverse sections of the gall.

The fibres in the samples of the powdered leaves gave positive test (red colour) for lignin but they were, however, lightly lignified.

(b) Test for mucilage

Pink colour was observed in some cells of the leaf and gall in the samples. This indicates the presence of mucilage. This is in comformity with the findings of Koumare et al. (1968), who reported the presence of this type of carbohydrate in the leaves of G. senegalensis.

(c) Test for cellulose

Blue colour was observed on the walls of the cell elements in the powdered samples of leaves and the galls.

(d) Test for starch

Blue-black colouration was observed in the parenchyma cells and in free state in the sample of the galls. Starch was indicated in the sections of the galls. However, this cell inclusion was absent in the powder and anatomical sections of the leaves.

(e) Test for fats and oils

Distinct red colour was observed in both the anatomical sections and powdered leaves but pink colour was observed in the anatomical and powdered sample of the galls. Hence presence of oils and fats was indicated.

(f) Test for tannins

An intense blue-black colour turning to green colour was observed in both anatomical and powdered samples of the leaves and galls. A high concentration of tannin was present in the cork cells and parenchyma cells of the inner and outer layers of the anatomical sections through the gall.

(g) Test for suberin

Red colour around the periderm and cork cells of the anatomical section and powdered samples of the galls on warming with Sudan IV (in alcohol). Suberin was however absent in the anatomical section of the leaf. Suberin is a polysaccharide found mainly in cork cells of barks and galls (Wallis, 1958).

(h) Test for calcium oxalate

Single and twin prisms of calcium oxalate crystals were observed as shiny structures in free state and in parenchyma cells and as sheath on fibres of the powdered and anatomical sections of the leaves.

Rosette and prisms of calcium oxalate crystals were identified as bright structures in the anatomical section and powder of the galls.

The crystals in the samples were slowly dissolved on addition of 20% sulphuric acid.

(i) Test for calcium carbonate

Effervescence occurred with evolution of gas on a white tile on addition of dilute sulphuric acid. This confirms the presence of calcium carbonate that were observed as crystoliths in the microscopical examination of the samples of the powdered leaves and galls.

(j) Test for anthraquinones

Brownish colour was observed in anatomical sections of the samples of the leaves and the galls, indicating the absence of anthraquinones.

(k) Test for proteins

Red to violet coloured patches were formed on warming the samples of the gall with Millon's reagent indicating the presence of proteins. Presence of proteins in other parts of this plant was reported by Irvine (1961) in the analysis of G. senegalensis as fooder for animals.

The results of the chemomicroscopical examinations are summarised in Table 1.

Table 1: Summary of Chemomicroscopical Examination Of The Leaves and Galls of G. senegalensis.

Constituent	Leaf	Gall
Lignin	±	+++
Mucilage	±	±
Cellulose	±	±
Starch	-	+++
Fats and oils	±	±
Tannins	+++	+++
Suberin	-	±
Proteins	++	++
Calcium oxalate	++	+++
Calcium carbonate	±	±
Anthraquinones	-	-

Key:
- ± Present in trace quantity and in few samples.
- ++ Present in small quantity.
- +++ Present in large quantity.
- - Absent.

3.7.2 Microscopical examinations

(a) Leaf

Microscopical examination of the leaf showed the presence of anisocytic stomata, numerous non-lignified unicellular covering trichomes with cystolith of calcium carbonates, elongated epidermal cells, prisms of calcium oxalate of variable sizes and phloem fibres. Reticulate and spiral vessels were common features, however, tracheids and sclereids were absent. Oil in the spongy mesophyll cells was observed (Fig. 12).

Transverse sections through the leaf showed clear differentiation into cuticularised lower and upper epidermises. Thick layer of parenchyma was evident with prisms of calcium oxalate. Stomatal openings were present and palisade cells were observed at the lower epidermis. However, unicellular covering trichomes with swollen bases were on both epidermises. Xylem and phloem elements were organised in rows (Fig. 13).

The features observed in the leaf are indicative of its morphological group, however, special features worth noting are presence of oil droplets in the spongy mesophyll cells, rare form of anisocytic stomata cystolith of calcium carbonate at the swollen base of covering trichomes and prismatic form of calcium oxalate crystals.

Fig. 12 Structural Features of the Powdered Leaf
(Mag. x 400)

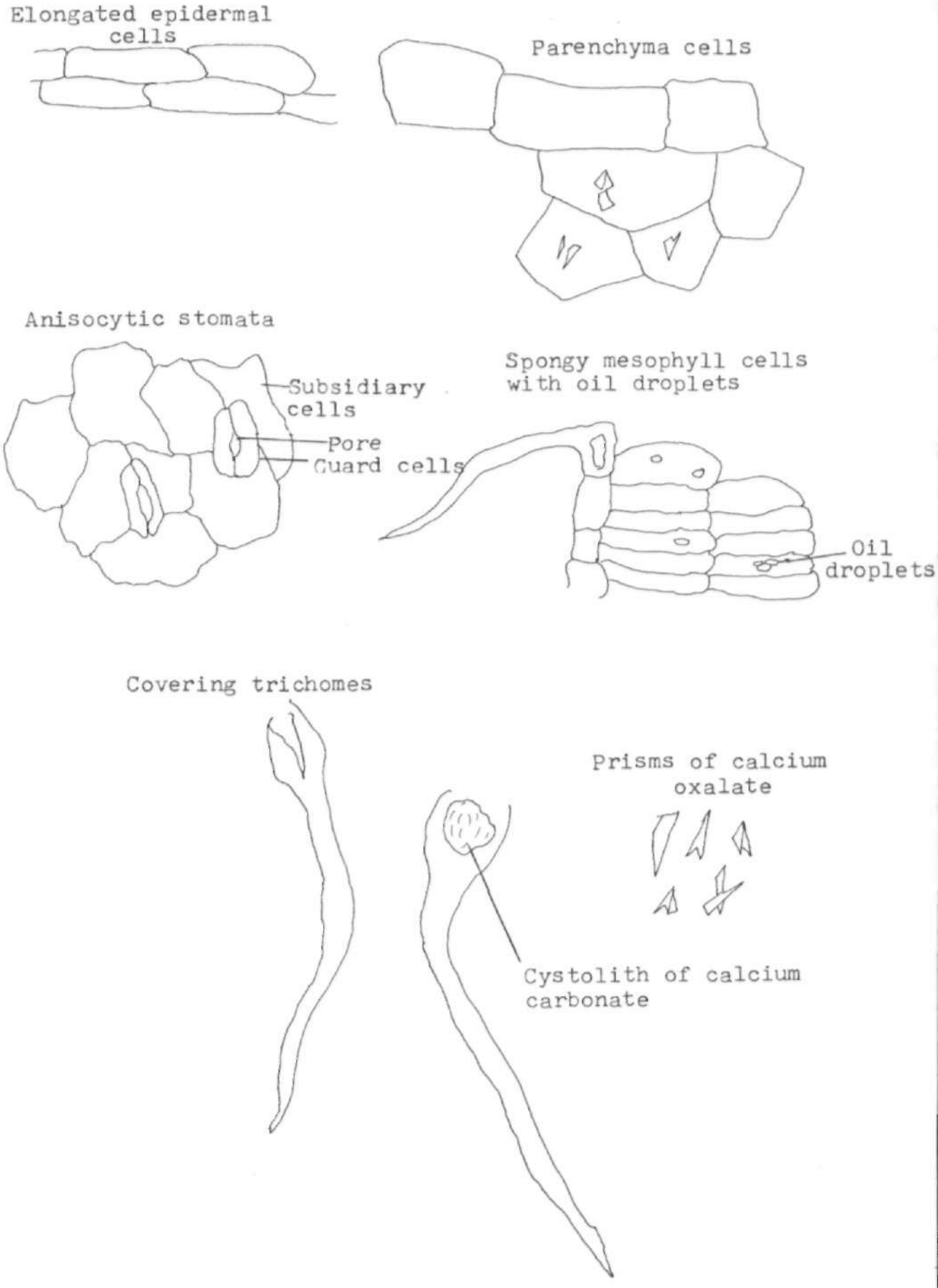


Fig. 12: Continued

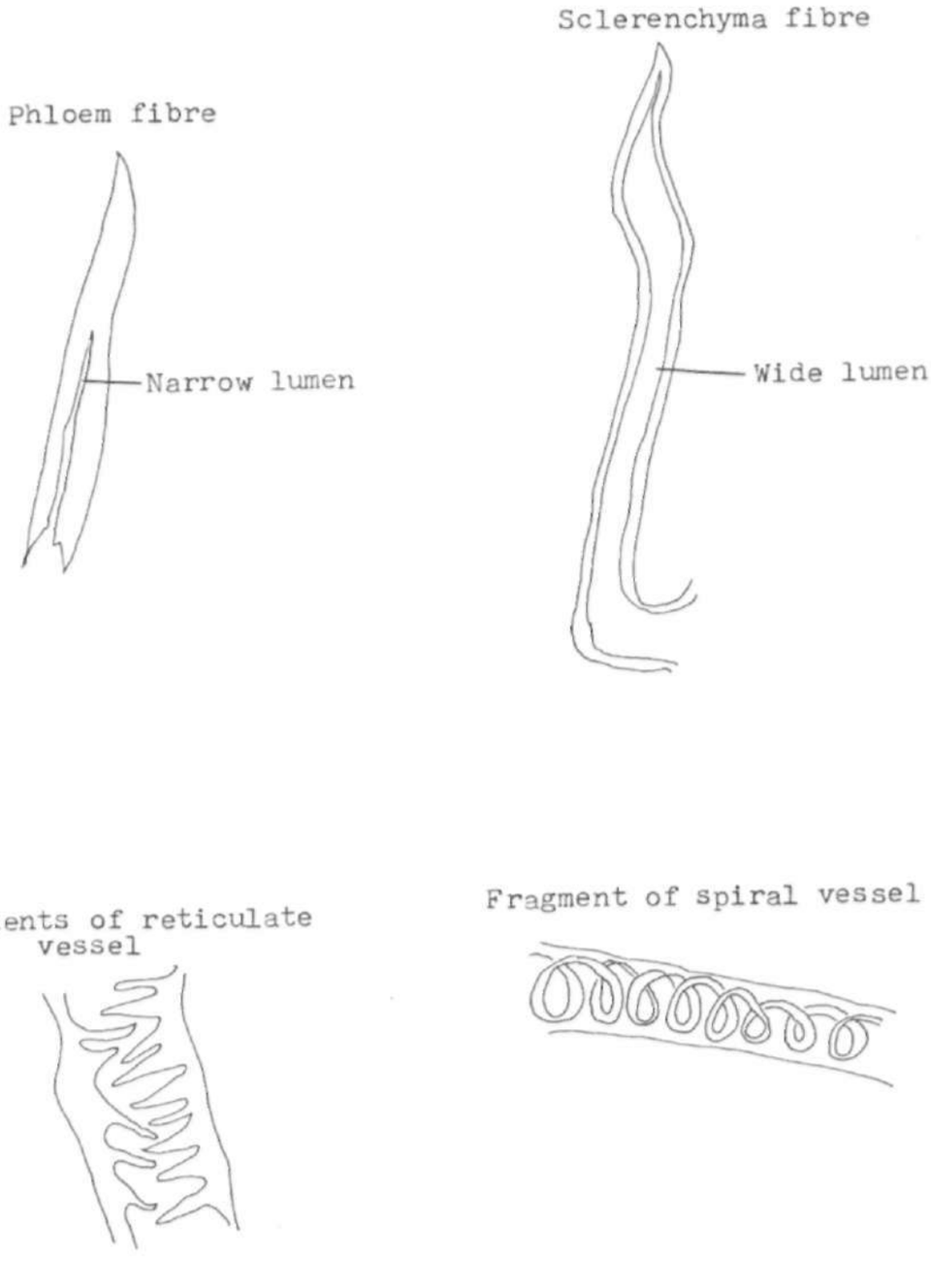
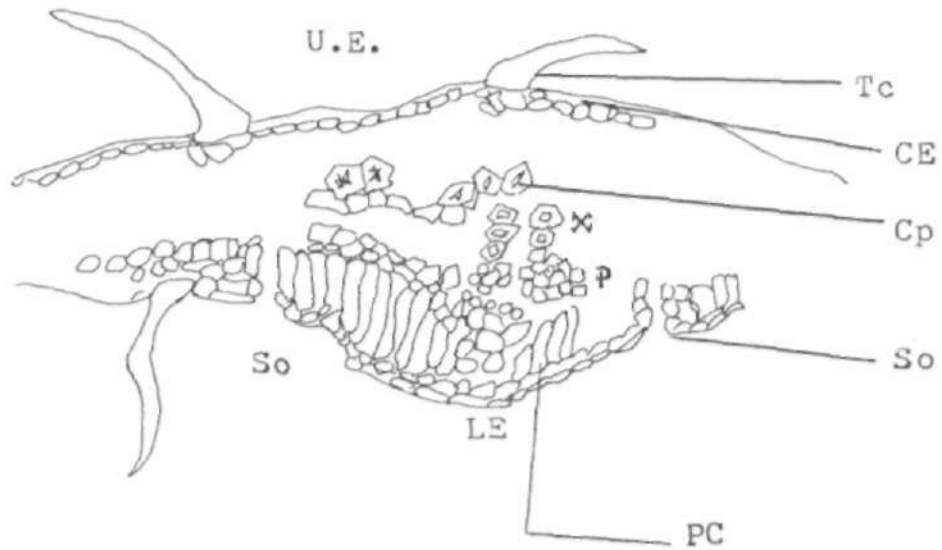


Fig. 13: Diagramatic T.S. of the leaf



Key:
- X : Xylem
- P : Phloem
- So : Stomatal openings
- Tc : Covering trichomes
- UE : Upper epidermis
- LE : Lower epidermis
- CE : Cuticularised epidermis
- PC : Palisade Cells
- Cp : Calcium oxalate

The cytomorphology of the leaf is therefore that of dicotyledenous leaf. But the presence of rare anisocytic stomata at the lower epidermis, presence of numerous covering trichomes on both epidermises, highly cuticularised epidermis and thick layer of parenchyma cells suggest special features of the leaf and adaptation to its habitat-poor soil in degraded savanna forest of sub-tropical West Africa.

(b) Gall

Microscopical examination of the powdered gall revealed numerous non-lignified unicellular covering trichomes, cork cells in transverse view, numerous lignified stone cells with narrow cavity, rectangular and irregular sclereids, sclerenchyma fibres having wide lumen with sheath of calcium oxalate and twisted phloem fibres. Rare pitted tracheids, fragments of reticulate, annular and spiral vessels were also observed. Crystals of calcium oxalate were present (Fig, 14). These could form significant diagnostic features of the powdered galls.

The outer zone of the transverse section (T.S) of the galls is composed of smooth flattened epidermis, unicellular covering trichomes and cork cells (Fig. 15). The inner zones of the T.S. is of disorganised

Fig. 14 Microscopical Features of the powdered Gall of G. senegalensi
(mag. x 400)

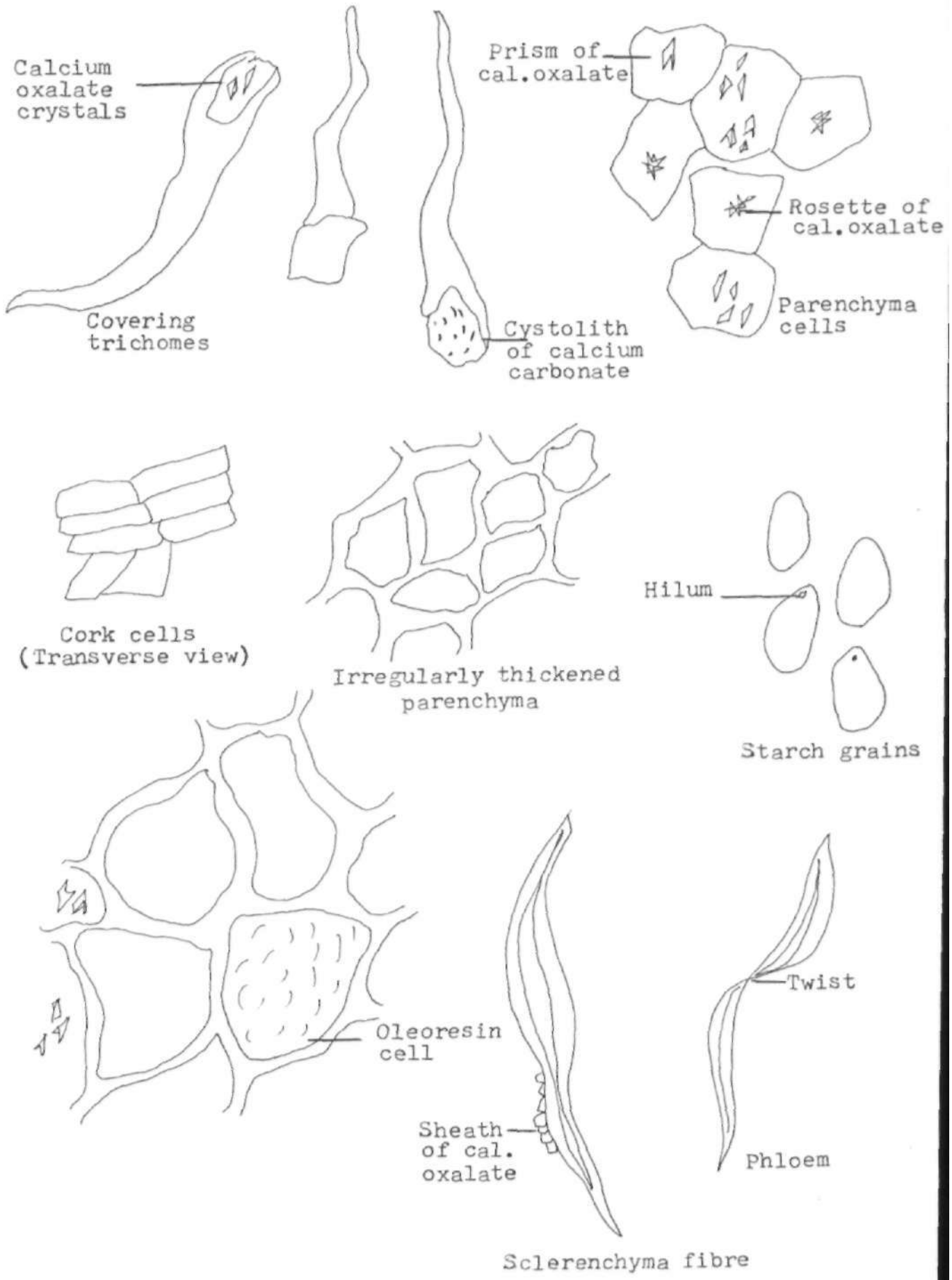


Fig. 14 Continued

Fragments of Xylem vessels



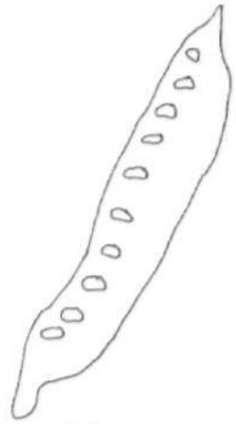
Reticulate



Spiral

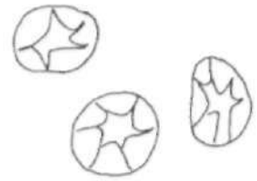


Annular

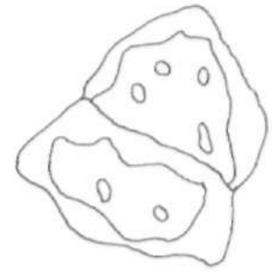


Tracheid

Sclereids



Stone cells



Irregular pitted sclereids



Rectangular pitted sclereids

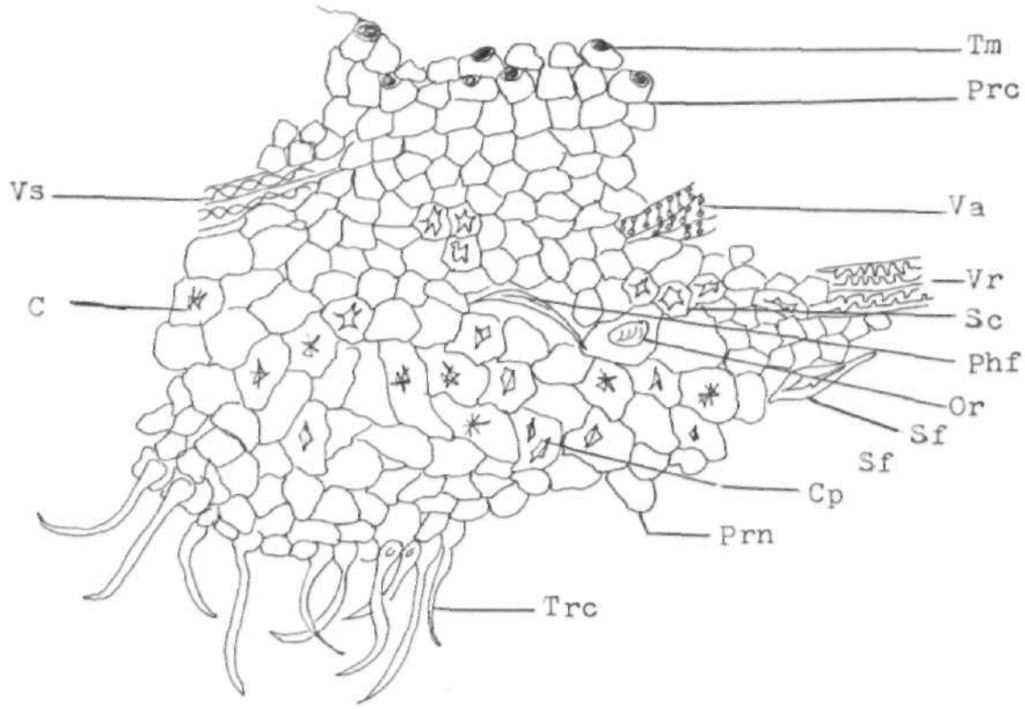
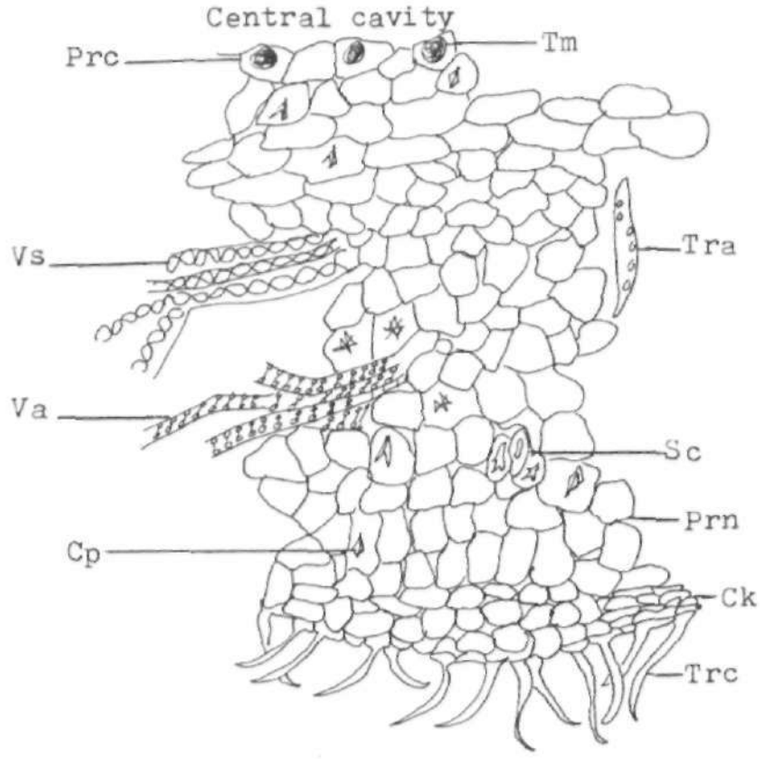


Fig. 15 : Tranverse Section of the fresh gall of G. senegalensis (X 400)

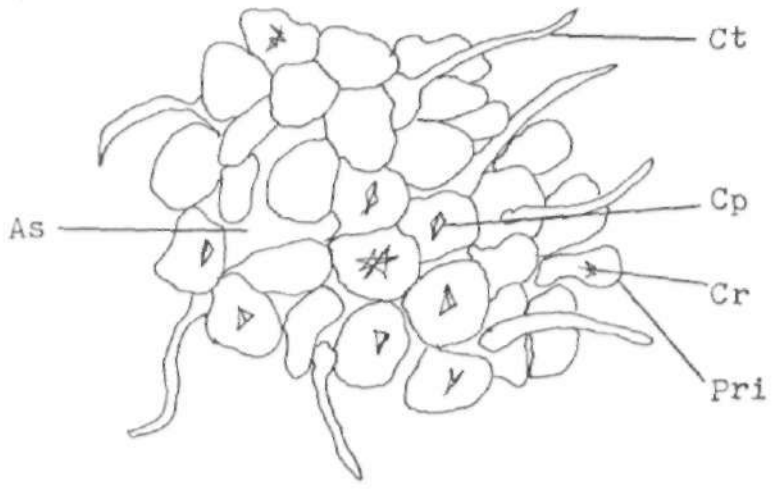


Key

Tm = Tannin masses
Prc = Thick-walled parenchyma
Prn = Thin-walled parenchyma
Sc = Sclereids
Sf = Sclerenchyma fibres
Va = Annular vessel
Vs = Spiral vessel
Vr = Reticulate vessel
Tra = Tracheid
Trc = Covering trichome
Cp = Prisms of cal. oxalate
Cr = Rosette of cal. oxalate
Or = resin cell
Phf = Phloem fibre

Fig. 16 : Longitudinal section of the fresh gall of G. senegalensis (X 400)

Fig. 17. Epidermal Layer of a Young Fresh Gall of G. senegalensis



(X 400)

Key: Ct = Covering trichomes
As = Air spaces
Cr = Rosette of cal. oxalate
Pri = Irregularly shaped parenchyma
Cp = Prism of cal. oxalate

parenchyma cells while the longitudinal section (L.S) presented organised and arranged parenchyma. The parenchyma cells of the inner zone of the anatomical sections (T.S and L.S) are thick walled, containing tannin masses, oleoresin cells rosette and prismatic forms of calcium oxalate crystals. Lignified stone cells were a common feature (Fig, 15 and 16). Disorganised, partially lignified numerous residual vessels and few phloem fibres were observed. The vessels were short in transverse section, long, lignified annular and spiral coupled with pitted tracheids in longitudinal section. The observed non-continuous residual vessels could explain the ceasation of growth of the aerial part of the plant when the gall must have matured and dried.

The outer epidermal layer of a young gall showed presence of chlorenchyma cells which on clearing with chloral hydrate and irrigating with 95% ethanol, showed irregular parenchyma cells containing prisms of calcium oxalate with frequent air spaces.

Numerous unicellular covering trichomes were a common feature while the glandular trichomes were absent. Stomata were absent in the galls (Fig. 17). The epidermal layers of a young branch stem below and above the gall were examined for presence of stomata.

They (stomata) were found to be absent thus confirmed the absence of stomata in the gall of G. senegalensis.

3.7.3 Description of microscopical features of the powdered leaf and gall of G. senegalensis

(a) Leaf

Cell	-	Epidermal	
Shape	-	Polygonal and elongated	
Anticlinal wall	-	Straight	
Thickening	-	Cellulose	
Stomata			
Type	-	Anisocytic	
Frequency	-	Rare	
Location	-	Lower epidermis	
Trichomes	-	Covering	
Position	-	Upper and lower epidermises	
Frequency	-	Numerous	
Form	-	Unicellular	
Surface	-	Smooth	
Cell wall	-	Not lignified	
Base	-	Swollen	
Xylem			
Type	-	Vessel	Tracheids
		Present	Absent
Frequency	-	Frequent	

Wall	-	Partially lignified
Apex	-	None
Thickening	-	Reticulate and spiral
Size	-	Fragmented

Fibres

Size	-	Fragmented
Frequency	-	Not frequent
Wall	-	Thickened and lignified
Apex	-	Tapering/acute
Lumen	-	Wide

Calcium oxalate

Form	-	Prisms
Location	-	Free and in parenchyma cells
Frequency	-	Frequent
Arrangement	-	Single and twin

Calcium carbonate

Form	-	Cystolith
Location	-	Swollen base of covering trichomes.

(b) Gall

Cork cells	-	Present
Shape	-	Straight elongated
Walls	-	Lignified

Parenchyma			
Shape	-	Polygonal and irregular	
Anticlinal wall	-	Straight, wavy	
Thickening	-	Cellulose	
Trichomes	-	Covering	
Frequency	-	Numerous	
Form	-	Unicellular	
Surface	-	Smooth	
Cell wall	-	Not lignified	
Base	-	Swollen with cystolith of calcium carbonate	
Xylem		Vessel	Tracheids
Frequency	-	Frequent	Rare
Wall	-	Lignified	Lignified
Thickening	-	Spiral, reticulate and annular	Pitted
Appex	-	-	Acute
Lumen	-	-	Absent
Secretory cells			
Type	-	Oleoresin cells	
Occurrence	-	as idioblast in parenchyma	
Size	-	100u	
Frequency	-	Rare	
Shape	-	Round/circular	

<u>Sclerenchyma</u>		Fibre	Sclereids
Size	-	Large	Medium
Frequency	-	Rare	Numerous
Walls	-	Lignified	Lignified
Lumen	-	Wide	Narrow, pitted
Shape	-	-	Round, rectangular and irregular
Apex	-	Acute	-
<u>Calcium oxalate</u>			
Size	-	15-20u	
Form	-	Prism and rosette	
Location	-	Free, in parenchyma and as sheath with fibres	
Frequency	-	Numerous	
Arrangement	-	Single and aggregate	
<u>Starch grain</u>			
Size	-	60-75u	
Shape	-	Oval	
Hilum	-	Pointed	
Striations	-	Absent	
Aggregation	-	Single	
Frequency	-	Numerous	
Location	-	Free and in parenchyma cell.	

3.8.0 Phytochemical Screening of the Powdered Leaves and Galls of G. senegalensis

The literature perusal has revealed that the leaves, twigs and roots have been previously screened for phytochemical constituents.(Irvine, 1961; Koumare et al., 1968; Combier et al., 1977). Hitherto, no phytochemical studies on the galls have however been reported. In this work the galls as well as the leaves have been subjected to various qualitative chemical tests and the results also are discussed below. A summary of the chemical constituents detected in the leaves and galls of G. senegalensis is presented in Table 2.

3.8.1 Test for carbohydrates

Most carbohydrates including sugars are wholly or partially soluble in cold or hot water. Therefore water was used as the solvent for extraction, consequently the water extracts of the leaves and galls were used to carry out the tests for presence of various classes of carbohydrate, monosaccharides, reducing sugars, pentoses and ketoses.

The literature has shown that the plant contain mucilages. In addition sugars have been found to be the core of various glycosides and tannins.

Table 2: Phytochemical Constituents of the Leaves and Galls of G. senegalensis

Group constituents	Leaf	Gall
Carbohydrate	Present	Present
Monosaccharides	+++	+++
Reducing sugars	++	++
Reducing sugars in combine form	++	++
Ketoses	++	++
Pentoses	-	-
Glycosides		
Cyanogenetic	-	-
Anthraquinones	-	-
Saponins	++	++
Sterols	+	+
Terpenes	+	+
Cardiac glycosides	-	-
Flavonoids	+++	+++
Alkaloids	+	+
Resins	-	±
Tannins		
Condense	+++	+++
Pseudotannin (chlorogenic acid)	+	+
Hydrolysable: Ellagitannins	++	++
Gallotannins	-	++

Key: - Absent
± Presence in small quantity and in few samples.
+ Present in small amount
++ Present in high amount
+++ Present in very high amount.

(a) Molisch's test - General test for carbohydrates

On addition of few drops of Molisch's reagent to the leaf extract followed by 1ml concentrated sulphuric acid down the side of the test tube at an angle of 45°C, a dull violet colour was observed which indicated the presence of carbohydrate.

The aqueous extract of the galls gave positive test with Molisch's test.

(b) Test for sugars

1. Barfoed's test for monosaccharides

Red precipitate was formed on warming the aqueous extract of the gall with 4ml of Bardoed's reagent. Similar result was obtained on repeating the above test on the aqueous extract of the leaves. This indicated the presence of monosaccharides. Monosaccharides are found as free sugars in higher plants and are the component of glycosides and polysaccharides (Harbone, 1973).

2. Fehling's test

i. Standard test for reducing sugars

A brick red precipitate was observed on boiling 2ml of each of the extracts with 5ml each of mixture of Fehling solutions I and II, indicating the presence of free reducing sugars in the leaves and galls.

ii. Standard test for combined reducing sugars

A brick red precipitate was observed on warming the hydrolysed extracts with Fehling's solutions I and II indicating the presence of reducing sugar in combine form.

(b) Standard test for ketoses (Salivenorff's test

A rose colour was slowly formed on heating the 2ml each of extracts on water bath with few crystals of resorcinol and an equal volume of concentrated hydrochloric acid. This is the positive test,for example glucose. Fructose and glucose form osazones. An immediate formation of rose colour indicates fructose while glucose forms osazone slowly, that is, some minutes on standing (Brain and Turner, 1975). Thus the ketose sugar present in the galls may be glucose, since the osazone formation was slow.

(c) Standard test for pentoses

Dark green and green colours were produced instead of red colour on addition of equal volumes of dilute hydrochloric acid containing a little phloroglucinol to 1ml of the water extracts of the galls and leaves respectively, indicating the absence of pentoses in the extracts of the leaves and galls of G. senegalensis.

The results have indicated the presence of reducing sugars in free and combined state in the water extracts of the leaves and galls. On further investigation the sugars present are the ketoses (example glucose). Presence of free and combined reducing sugars in the galls is in agreement with the fact that sugars are found on stems and underground organs (Harbone, 1973). And the gall of G. senegalensis is formed on the stem branch . The reducing sugar in the combine form could be in association with tannins, flavonoids and saponins in glycosidic formation. These classes of compounds have been found in the plant (Koumare et al., 1968). The sugar in the galls of G. senegalensis present may be glucose as the results have thus indicated.

3.8.2 Test for glycosides

Glycosides are compounds with diverse chemical nature, therefore there is no common test for this group of compounds. However tests that are selective to particular type of glycosides were employed to identify the type of glycosides present in the leaves and galls of G. senegalensis.

Glycosides are present in almost all plants but the types vary from plant to plant (Trease and Evans, 1983).

(a) Test for cynogenetic glycosides

The moist yellow sodium picrate paper did not change when suspended in a test tube containing the leaf extract and warmed on a water bath for 1 hour. Thus indicating the absence of cyanogenetic glycosides.

Negative result (no change of colour) was obtained as well when the test was repeated on the water extract of the galls. The result have thus confirmed the absence of cyanogenetic glycosides in this plant.

(b) Tests for anthraquinone derivatives

i. Test for the presence of free anthraquinones

Light brown colour was observed in the (upper) aqueous layers. Presence of free anthraquinones that are not in glycosidic linkage with sugars is indicated by formation of bright pink colour at the upper layer (Sofowora, 1982). The result above indicated the absence of free anthraquinones.

ii. Test for presence of free and combined anthraquinones

Bright pink colour was not observed in the aqueous layers indicating the absence of free and or combined anthraquinones in the samples of the leaves and the galls.

iii. Test for presence of anthraquinones derivatives in a reduced form which are not easily hydrolysed

Light and dark brown colours were observed in the aqueous layers in the test carried out on the extracts of the galls and leaves respectively. This indicated the absence of anthraquinones that are not easily hydrolysed.

Bright pink colour in the aqueous layer indicates the presence of anthraquinones which are not easily hydrolysed, for example, C-glycosides (Trease and Evans, 1983g).

Anthraquinone derivatives were found to be absent in all the tests carried out on the leaves and galls of G. senegalensis. Also they were not detected when chemomicroscopical examinations were carried out on the anatomical sections and powder samples of the leaves and galls. Anthraquinones have not been reported in the phytochemical studies carried out by various workers (Irvine, 1961; Koumare et al., 1968).

(c) Test for saponins

These are glycosides of both triterpenes and sterols and have been detected in over seventy families of plants. They are surface active agents with soap-like properties and can be detected by

their ability to cause foaming and to haemolyse blood cells. The search in plants for saponins has been stimulated by the need for readily accessible sources of sapogenins which can be converted in the laboratory to animal sterols of therapeutic importance. Saponins are of economic value especially for their toxicity to cattle and their sweet taste (Harbone, 1973).

1. Frothing test

A heavy honey comb froth which lasted for 60 minutes was observed in the aqueous extracts of the leaves and galls. This indicated the presence of saponins in the samples.

2. Test for saponins - Haemolytic (steroidal and triterpenoids)

Haemolysis occurred in the two test tubes containing extracts of leaves and galls of G. senegalensis and none in the control tubes. This indicated the presence of saponins.

3. Test for sterols and triterpenoidal ring

(i) Salkowski's test for steroidal ring

A reddish brown ring at the interface was observed indicating the presence of steroidal ring.

(ii) Liebermann-Burchard's test for terpenes

A reddish violet colour at the interface of the two liquids and a light green colour in the chloroform layer were observed. This indicated the presence of terpenes.

The frothing, haemolytic and chemical tests have confirmed the presence of steroidal and triterpenoid saponins in the galls of G. senegalensis. This class of compounds have been reported by Koumare et al. (1968) as one of the phytochemical constituents in the leafy twings of G. senegalensis.

3.8.3 Test for flavonoids

Flavonoids occur as aglycones as well as glycosides and are widely distributed in nature and frequently found in their highest concentration in association with essential oils. They are the largest group of naturally occurring phenolic compounds. The flavonoids are generally water soluble and are insoluble in organic solvents. Their aglycones are sparingly water soluble but fully soluble in ether (Brain and Turner, 1975). The flavonoids are present in all vascular plants but some classes are more widely distributed than others (Harbone, 1973). Some members are physiologically active, for example, rutin, rutenane (Harbone, et al., 1975).

The following tests were carried out for the identification of flavonoids in the leaf and gall of G. senegalensis.

(i) Lead acetate test

Heavy buff coloured precipitate was observed on addition of three drops of lead acetate solution to each of the two test-tubes containing detanned water extracts of samples of leaves and galls respectively indicating the presence of flavonoids.

(ii) Ferric chlorides test

The presence of phenolic nucleus was indicated in the detanned extracts of the leaves and galls, this was when green coloured solution was formed on addition of few drops of 5% ferric chloride solution.

(iii) Sodium hydroxide test

Yellow solution (positive test) was observed on performing the sodium hydroxide test on the detanned water extracts of samples of leaves and galls. The solutions became colourless on addition of dilute hydrochloric acid, indicating the presence of flavonoids.

(iv) Shinoda test

This test is applied as a confirmatory test for flavonoids. Red and pink colours were observed in the test tubes containing extracts of the galls and leaves of G. senegalensis respectively. This confirmed the presence of flavonoids in the galls.

The presence of this class of compounds has been observed in the leafy twigs of the plant as reported by Irvine (1961) and also by Koumare et al. (1968).

3.8.4 Test for alkaloids

Various tests for alkaloids were carried out using the following standard alkaloidal reagents:

i. Wagner's reagent: Light brown and brownish precipitates was observed with the extracts of the leaves and galls respectively.

ii. Mayer's reagent: White and creamy white precipitates were formed with addition of Mayer's reagent to the two test tubes containing the methanolic extracts of the leaves and galls respectively. This indicated the presence of alkaloids.

iii. Positive test (yellow colour) was observed on addition of few drops of picric acid solution to the ethanolic extracts residue of leaves of leaves and the galls.

iv. 10% tannic acid produced buff coloured precipitate when added in drops to the ethanolic extracts residue of the leaves and galls.

v. Orange and orange yellow precipitates were observed on addition of few drops of Dragendorff's reagent to the extracts of leaves and the galls of G. senegalensis respectively.

All the alkaloidal tests were positive, indicative of the presence of alkaloids in the samples of the leaves and galls of G. senegalensis.

Alkaloids in trace amounts were revealed by Paris in 1942 as reported by Irvine (1961), in the leaf twigs; Koumare et al. (1968) separated and identified the alkaloids in the roots by thin layer chromatography. Combier et al. (1977) confirmed the presence of the alkaloids in the roots and subsequently two types were isolated from the root.

The preliminary phytochemical screening has thus indicated the presence of alkaloids in the galls conforming with the results revealed by many workers. Therefore it is suggested that further work should be extended towards the isolation and structural analysis of these group of compounds in the gall to ascertain the similarity or otherwise with those identified in the leafy twigs and roots of this plant (G. senegalensis).

3.8.5 Test for resins

Resins are amorphous products of a complex structure, they are usually formed in schizogenous or schizolygenous ducts or cavities and are end products of metabolism and are used in pharmacy (Tyler et al., 1970).

i. Test for resin

Positive result (purple turning to violet colour) was obtained on mixing the powdered gall with acetic anhydride followed by addition of 1ml concentrated sulphuric acid.

A negative results was obtained for the leaves. The result obtained indicates the presence of resins in the gall.

ii. Test for oleo-resin

A positive result (violet colour) was observed in the galls indicating the presence of oleo-resins in the samole of the galls. Oleo-resins were absent in the leaves.

The results have thus confirmed the presence of resins in the galls. Oleo-resin cells have been identified in some samples of the powdered galls in the microscopical studies. Oleo-resin cells are common features in galls (Wallis, 1958). However this class of compounds were absent in the leaves of G. senegalensis.

3.8.6 Tests for tannins

Various tests were carried out on the water extracts of the powdered leaf and gall of G. senegalensis. The following results were obtained.

i. Ferric chloride test

An initial green turning to bluish green colouration was observed on treating the water extract of the gall with few drops of 5% ferric chloride solution. Similar observation (initially green turning to blue) was made on repeating the test on water extract of the leaf, indicating the presence of tannins which could be hydrolysable, condensed types or both.

ii. Lead sub-acetate test

The water extract of the leaf gave yellow buff coloured precipitate whereas the gall water extract gave buff coloured precipitate with lead-subacetate reagent. This indicated the presence of tannins. Tannins are precipitated by heavy metals.

iii. Bromine water test

Buff coloured precipitate was observed with the water extracts of the leaf and gall. This indicated the presence of condensed tannins. Hydrolysable tannins do not give this (Balbaa, 1976b).

iv. Test for phloroglucinol nucleus

An alcoholic extract containing phloroglucinol nucleus when treated with a drop of concentrated hydrochloric acid develops crimson colour. Phloroglucinol occurs frequently with condense tannins.

The water extracts of the leaf and gall produced crimson coloured solution indicating the presence of the phloroglucinol nucleus.

v. Ferric ammonium citrate test

Violet coloured precipitate is formed when an aqueous extract containing tannins is treated with a few drops of ferric ammonium citrate solution (Trease and Evans, 1983).

The water extract of samples of leaf and gall gave positive result (violet precipitate) with ferric ammonium citrate solution indicating presence of tannins.

vi. Gelatin test

One of the best known and standard test for tannins is their precipitation of gelatin. Only true tannins precipitate gelatin (Trevor, 1975).

0.5% solution of each of water extract of leaf and gall added to an equal volume of 0.5% gelatin produced dirty white precipitate.

3.8.6.2 Distinguishing tests for classes of tannins

The following tests were designed to distinguish different classes and sub-classes of tannins found in nature and as applied to G. senegalensis.

(a) Formaldehyde test for catechins

An extract containing catechins when treated with 40% formaldehyde in 10% hydrochloric acid for 30 minutes gives a precipitate which when filtered is insoluble in hot water.

A light precipitate was observed with leaf water extract. The gall extract produced a heavy precipitate. The precipitates were insoluble in water. The filtrates

from above La and Ga were retained for the following tests. The results have indicated the presence of catechins in both the leaf and gall of G. senegalensis and it may be concluded that catechins could be more in the galls than in the leaves because of the quantity of precipitate. The filtrate above La and Ga were each divided into three portions.

(b) Sodium nitrate test for ellagitannins

To the first portion of leaf filtrate from above (La) was added few crystals of sodium nitrate; a green colour was observed, indicating the presence of ellagitannins in the leaf. The same test carried out on the gall filtrate (Ga) obtained from above produced a green colour. The results have thus indicated the presence of ellagitannins in the leaf and gall of G. senegalensis.

(c) Lime water test for gallotannins

Grey or light brown colour or precipitate produced on addition of lime water to an aqueous extract indicates the presence of gallotannin (Balbaa, 1976). The second portion of the filtrate (La) did not show any colour change when treated with lime water. This indicated the absence of gallotannins in the leaf.

However, repeating the test on the third portion of the gall water extract produced a (Ga) grey precipitate indicating the presence of gallotannins in the gall. Gallotannins are the chief components of galls although are of limited distribution in plants. Therefore from the results obtained, it may be inferred that gallotannins are some of the phytochemical constituents of the gall of G. senegalensis.

(d) Test for presence of the chlorogenic acid

A bright green solution was gradually produced on treating the third portion of the filtrate (La) from the leaf with 10% ammonia solution and exposing to air. This indicated the presence of chlorogenic acid. A repeated procedure on the filtrate from the gall extract (Ga) gradually developed a green precipitate indicating the presence of chlorogenic acid probably in high concentration.

An extract containing chlorogenic acid, which is an example of a pseudotannin, when treated with a few drops of 10% ammonia solution and exposed to air gradually develops a green colour (Trease and Evans, 1983).

Ellagitannins, chlorogenic acid and phloroglucinol were common to both the leaf and gall. Gallotannins were however present in the gall only. From these

results, it could be concluded that the type of tannins present were condensed, hydrolysable and pseudotannins.

Tannins have antiseptic property (Tyler et al., 1981). This may justify the local use of water extract of the leaf for the treatment of various gastrointestinal tract disorders like acute gastro-entritis and dysentry, wounds and other skin infections (Irvine, 1961). Also the use of the gall in form of ointment for the treatment of various skin infections and sores on children's head is thus justified (Abubakar, personal communication,1991; Irvine, 1961).

3.9.0 Chromatographic studies of leaf and gall extracts of G. senegalensis

Chromatographic methods of analyses furnish information on the homogeneity, molecular size and structure of many compounds including carbohydrates and provide useful data such as Rf values which are used in the identification of the compounds in question. These separation methods in addition provide alternative ways for the isolation of carbohydrates, their derivatives, tannins and other chemical constituents (Pigman and Horton, 1980).

3.9.1 Chromatographic studies of the tannin in the leaf and gall extract of G. senegalensis

Thin layer and paper chromatographic studies of the leaf and gall of G. senegalensis were carried out (solvent system: n-butanol : Acetic acid : water 4:1:5, and spray reagent: alcoholic ferric chloride solution).

Thin layer chromatography of the aqueous alcoholic (1:1) extract of the leaf and gall revealed three spots each with hRf 44, 74, 81 and 67, 74 and 96 respectively (Table 3). Two spots from the leaf extract (hRf 74, 81) correspond with phloroglucinol (hRf 74) and catechol (hRf 81). Two spots of the gall extract (hRf 74, 96) correspond with phloroglucinol and tannic acid (hRf 96) in terms of spot colour and mobility. Aqueous gall extract revealed two spots (hRf 68 and 81). The spot (hRf 81) compared to catechol.(hRf 81) A spot with hRf 44 was observed in the aqueous leaf extract. Two spots which were consistent (hRf 44 and 68) in the leaf and gall did not correspond to any of the reference tannins.

The paper chromatography of the aqueous alcoholic leaf and gall extracts revealed three spots with hRf 95 and hRf 80 and 95 respectively (Table 4). All the three spots compared with tannic acid (hRf 95) and catechol (hRf 80) in terms of spot colour and mobility. The aqueous leaf and gall extracts did

Table 3: hRf values and colour characteristics in Thin Layer chromatography of tannins in leaf and gall extracts of *G. senegalensis*

Extract/References	hRf	Colour Responses Day light	UV (365nm)	After spray with ferric chloride reager
Phloroglucinol	74.0	Not Visible	no floures-cence	Brown
Catechol	81.0	"	-	Blue Black
Tannic acid	96.0	"	Bright Fluorescence	Orange
Gall aqueous-alcohol Extract 1	67.0	"	-	Yellow
2	74.0	"	-	Light brown
3	96.0	"	-	Light orange
Gall aqueous extract 1	68.0		Fluorescence	Yellow
2	81.0		-	Blue
Leaf aqueous-alcohol Extract 1	55.0	Not Visible	-	-
2	74.0	"	-	Yellowish brow
3	81.0	"	-	Blue black
Leaf aqueous extract	44.0	"	Fluorescence	Orange Yellow

Solvent system: n-butanol: acetic acid: water (4:1:5)

Table 4: hRf values and colour characteristics of tannins in leaf and gall extracts of *G. senegalensis* (paper chromatography)

Extract/References	hRf	Colour Responses Day light	UV (365 nm)	After spray with ferric chloride reagent
Phloroglucinol	72.0	Not Visible	Not Visible	Brown
Catechol	80.0	"	-	Blue black
Tannic acid	95.0	"	Bright Fluorescence	Orange
Gall aqueous-alcohol Extract 1	44.0	"	-	No colour spot
2	80.00	"	-	Brown
3	95.00	"	-	Orange
Gall aqueous extract	-	-	Fluorescence	No spot
Leaf aqueous alcoholic extract 1	40.0	Not Visible	Fluorescence	Yellow
2	95.0	"	Bright Fluorescence	Orange
Leaf aqueous extract	44.0	"	Fluorescence	Orange Yellow

Solvent system: n-butanol: acetic acid: water (4:1:5)

not produce any spot that correspond to any of the reference tannin samples. Several investigators have applied chromatography to tannin mixtures using solvents containing a good proportion of water and this seemed to be the most useful (Trevor, 1976). Hence the use of mixture of alcohol and water (1:1) as solvent of extraction in this study. Results (Tables 3 and 4) have shown that this solvent mixture gave a good resolution. Chromatographic analyses of the leaf and gall have confirmed the presence of tannins (tannic acid, phloroglucinol and catechol as some of the tannin that are present) in G. senegalensis. Phytochemical tests in this project had earlier revealed the presence of these components of tannins in the leaf and gall of G. senegalensis.

3.9.2 Chromatographic studies of sugar extracts of leaf and gall of G. senegalensis

Paper chromatographic studies of the aqueous alcoholic extract of the gall have revealed the presence of a brown spot that flouresced, (solvent system: n Butanol : Acetic acid : Water 4:1:5; spray reagent: aniline phthalate) wi h hRf 27.3 which correspond to standard glucose in terms of colour and mobility hRf 27.3 (Table 5). The aqueous alcoholic leaf extract under the same

Table 5 : Rf values and colour characteristics of various sugars in gall and leaf extracts of *G. senegalensis*

Aqueous - alcoholic extracts	Solvent system	Rf	Spray Reagent	Colour Reaction	UV (366nm)	Day light
Leaf						
Spot 1	n-Butanol-Acetic acid water(4:1:5)	28.0	aniline phthalate	Brown	white fluorescence	Not visible
Spot 2		45.6	"	Bright White	voilet fluorescence	"
Gall						
	n-Butanol-Acetic acid water 4:1:5	27.3	"	Brown	white fluorescence	Not visible
Standard						
Fructose		31.8	"	Yellowish brown	fluorescance	
Xylose	"	34.5	"	Red	"	
Glucose	"	27.3	"	Brown	White fluorescence	Not Visible
Galactose	"	24.5	"	Brown	fluorescence	
Galat uronic Acid	"	25.5	"	Orange Yellow	fluorescence	
Sucrose	"	23.5	"	"	"	"
Glucuronic Acid	"	29.5	"	"	"	"
Leaf						
Spot 1	n-Butanol-Acetic acid water(4:2:4)	52.0	Aniline phthalate	Light brown	White fluoresence	"
Spot 2		79.9	"	Bright white	voilet fluoresence	"
Gall						
		62.5	"	Light Brown	White fluoresence	"
Standard						
Fructose	"	55.7	"	Yellowish brown	No fluoresence	"
Glucose	"	62.5	"	Brown	White fluoresence	"
Galactose	"	61.5	"	"	No fluoresence	"

Table 5 Cont'd

Aqueous - alcoholic extracts	Solvent system	hRf	Spray Reagent	Colour Reaction	UV (366nm)	Day light
Leaf	Chloroform:Ethanol 60:40					
Spot 1		36.8	Anisaldehyde sulphuric acid	pink	white fluorescence	Buff colour
Spot 2						
Gall		36.8	"	"	"	"
Glucose		36.8	"	"	"	"
Fructose		48.1	"	purple	fluorescence	colourless
Galactose		29.0	"	"	"	"

chromatographic conditions revealed two spot with hRf 28.0 and 45.6. The spot with hRf 28.0 compared with glucose (hRf 27.3) in terms of spot mobility and colour reaction. The second spot (hRf 45.6, violet flourescence) did not correspond to any of the reference compounds (Table 5). The result indicates that the sugar in the leaf may be glucose.

Varying the percentage composition of the solvent system but using the same spray reagent,the aqueous alcoholic gall extract revealed a light brown spot (hRf 62.5) that flouresced at 366nm, corresponding with glucose in terms of spot colour and spot mobility (hRf 62.5). The aqueous alcoholic leaf extract revealed two spots with hRf 62.0 and 70.9 respectively (Table 6). The light brown spot (hRf 62.0) compared to glucose with respect to spot colour and mobility (hRf 62.5). The second spot (white flourescence, hRf 70.9) did not correspond to any of the reference sugars, however it compared with the second spot of the hydrolysed extract of the leaf on same chromatographic conditions (solvent system 4:2:4; spray reagent aniline phthalate) in terms of spot colour and under U.V. (366nm) light.

The hydrolysed extracts of the two samples (leaf and gall) Table 6 revealed a spot each (hRf 62.5) corresponding to glucose hRf 62.5 in terms of spot

Table6 : hRf values and colour characteristics of sugar hydrolysates in gall and leaf extracts of G. senegalensis

Hydrolysed extracts	Solvent system	hRf	Spray Reagent	Colour Reaction	UV (366nm)	Day light
Gall	n-Butanol:Acetic acid: water (4:1:5)	27.3	Aniline phthalate	Brown	white fluorescence	Not visible
Leaf						
Spot 1	"	39.0	"	"	"	"
Spot 2	"	46.0	"			
Gall	n-Butanol-Acetic acid water (4:2:4)	52.5	"	"	"	"
Leaf						
Spot 1	"	62.5	"	Yellowish -brown	"	"
Spot 2	"	70.9	"	white	violet fluorescence	"
Gall	Chloroform-Ethanol (50:40)	36.8	Anisaldehyde sulphuric acid	pink	white fluorescence	Buff colour
Leaf	"	36.8	"	"	"	"

Paper chromatography

colour, mobility and appearance in U.V. (366nm) light. However a second spot (hRf 70.9) in leaf extract did not correspond to any of the reference sugars.

Replacement of the solvent system with chloroform : ethanol (60:40); spray reagent: anisaldehyde-sulphuric acid, the aqueous alcoholic extracts of the leaf and gall, the hydrolysed aqueous alcoholic extracts of the two samples revealed a pinkish spot each (hRf 36.8) that correspond to glucose in terms of spot mobility (hRf 36.8) and colour reaction respectively.

The results of the paper chromatographic studies are shown in Tables 5 and 6. From these results, glucose was identified as the only sugar in the free state and in combination with other hydrolysable compounds like tannins, saponins, flavonoids, etc. Phytochemical investigation of the gall has indicated the presence of glucose and tannins. Therefore the presence of glucose is in conformity with two facts; that glucose is the usual free sugar in higher plants and that sugar, most importantly glucose is an integral unit of tannins (Harbone, 1973; Ramstad, 1959).

3.10.2 Hide powder method

A standard hide powder method was employed (Society of Leather Trades Chemists, 1965). The material was extracted by process of leaching using cold and hot water. Tannic acid was used as a standard and the results are presented in Table 8.

Total solids

The percentage total solids is the total amount of solids present in theextract with reference to the initial weight of material used. These values were higher in hot leaching than the cold leaching. Since the samples were obtained from the same source, it was expected that with heat application more solids will be leached out. The same explanation is applicable to total solubles representing the amount of water soluble substances present in the extract (Table 8). Application of heat during leaching increases the amount of tannin compounds in solution as demonstrated by Ogundokun (1991).

Non-tans

The percentage of non-tans is the amount of substances that are not absorbed by the hide powder; tannins are absorbed by the hide powder. Therefore the amount of tannins can be deduced by finding the

colour, mobility and appearance in U.V. (366nm) light. However a second spot (hRf 70.9) in leaf extract did not correspond to any of the reference sugars.

Replacement of the solvent system with chloroform : ethanol (60:40); spray reagent: anisaldehyde-sulphuric acid, the aqueous alcoholic extracts of the leaf and gall, the hydrolysed aqueous alcoholic extracts of the two samples revealed a pinkish spot each (hRf 36.8) that correspond to glucose in terms of spot mobility (hRf 36.8) and colour reaction respectively.

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3.10 Quantitative estimation of tannin in the leaf and gall of G. senegalensis

The estimation of the potency of a preparation is an invaluable aid in its use and is usually done by setting lower limit in active ingredient level and this is achieved by determining the content of the active ingredient. Where constituents can be easily isolated, a simple gravimetric procedure can be used. However, where this is not possible some indirect estimations must usually be made (Brain and Turner, 1975).

3.10.1 Gravimetric method

The tannin content determined by gravimetric method for the leaf and gall of G. senegalensis is presented in Table 7.

The gravimetric method is based on the precipitation of tannins in solution by copper sulphate as copper tannate which on incineration yields copper oxide. 1g of copper oxide is equivalent to 1.305g tannins (Balbaa, 1976).

From the result it could be seen that the galls contain high quantity of tannins (16.2% w/w) when compared with that of the leaf (10.6% w/w).

3.10.2 Hide powder method

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Non-tans

The percentage of non-tans is the amount of substances that are not absorbed by the hide powder; tannins are absorbed by the hide powder. Therefore the amount of tannins can be deduced by finding the

difference between the percentage total soluble and percentage non-tans, tannins being water soluble.

Higher percentage of tannin was obtained by the hot leaching compared to the cold leaching (for both the leaves and galls). The gall was found to contain higher percentage of tannins. From the results it is evident that the hide powder method gave higher yield than the gravimetric method. This can be attributed to the better extraction (that is, 12 hours leaching) and also propably due to the sensitivity of the method.

The tannin content of G. senegalensis is comparable with some of the official sources of tannins, for example English gall (15.0 - 20.0%) and Thea sinensis (10.0 - 24.0%). The English galls from Quercus infectoria finds wide use in the treatment of haemorrhoids and as main source of tannic acid (Martindale, 1989). Therefore it may be inferred that the galls of G. senegalensis could be used as substitute to some official sources of tannins for example the English galls and in the treatment of haemorrhoids. Also the amount of tannins could justify the local use of the galls for treatment of wounds and skin infections for example sores on children's head, since tannins are known to have antiseptic property (Trease and Evans, 1983).

Table 7: *Estimation of tannins in the leaf and gall of G. senegalensis by gravimetric method.

	Leaf powder	Gall powder
Percentage range (% w/w)	10.20 - 11.05	15.66 - 16.66
Average (mean) (% w/w)	10.60 ± 0.35	16.20 ± 0.41

Table 8: Estimation of tannins in the leaf and gall of G. senegalensis by Hide powder method

	Cold leaching (% w/w)		Hot leaching (% w/w)		Tannic acid (% w/w)
	Leaf	Gall	Leaf	Gall	
Total solids	17.5	24.0	19.5	27.5	97.5
Total solubles	14.2	20.0	16.5	25.8	85.2
Non-tans	2.2	4.5	2.5	6.6	10.4
Tannins	20.0±0.09	16.5± 0.06	13.3± 0.05	19.2± 0.07	74.8±0.04

*Each value represents a mean of three determinations in three replicates.

3.10.3 Estimation of tannin in the leaf and gall by spectrophotometric method

The tannin content of the leaf and gall have been estimated by spectrophotometric method, and were calculated to be 15.46% and 21.14% for the leaf and the gall respectively. Results are presented in Table 9.

The spectrophotometric and the hide powder methods are based on the ability of the tannin to convert hide powder to leather. However, they differ in that the former measures absorbance as a function of concentration whilst the latter makes use of percentage dry weight. The percentage content estimated by spectrophotometric method is highest when compared to gravimetric and hide powder methods even though the samples were collected at the same time and from the same source. This could be attributed to the fact that there may be other substances that absorb in the same U.V. region which may have increased the absorbance resulting in apparent higher tannin content.

3.11.0 Quantitative evaluation of powdered galls and leaves of *G. senegalensis*

The evaluation of a drug requires that it be identified in both its entire and powdered forms by means of its macromorphological and cytomorphological characters. Whilst these diagnostic features

enable the nature and origin of the material to be stated, it could well be that faulty collection or possibly incorrect or extended storage have altered the content of constituent of the drug to a point where it is no longer acceptable.

Table 9: Estimation of tannins in the leaves and galls of G. senegalensis by spectrophotometric method.

Sample Taken (W) 20g	E_1	E_2	E_3	%	Average
	0.650	0.034	0.026	15.5	
Leaf	0.645	0.035	0.026	15.4	15.46 ± 0.05
	0.650	0.035	0.026	15.5	
	0.895	0.046	0.026	21.02	
Gall	0.889	0.045	0.026	21.20	21.14 ± 0.08
	0.890	0.046	0.026	21.20	

*All absorbances (extinctions) were determined at max = 750nm.

Sample calculation:

Leaf

$$\% \text{ tannin content} = 4.2 \times 3.125 \times \frac{E_1 - E_2}{W \times E_3}$$

W = Weight of drug taken = 20g
E_1 = 0.650
E_2 = 0.034
E_3 = 0.026

$$\therefore \quad \% = 4.2 \times 3.125 \times \frac{0.650 - 0.034}{20 \times 0.026}$$

$$4.2 \times 3.125 \times 1.18 = 15.5\%$$

Key:

E_1 = Extinction (absorbance) of the total tannin in the drug extract.

E_2 = Extinction of the tannins in the extract not bound with hide powder.

E_3 = Extinction of reference solution (pyrogallol) used as control.

Drugs must therefore be subjected to evaluation procedures which will indicate their acceptability by criteria other than their morphology (Brain and Turner, 1975).

The following evaluative parameters were determined on the leaf and gall powders of *G. senegalensis*.

3.11.1 Determination of moisture content

The moisture content of the air-dried powdered leaves and galls were determined to be 11.3% w/w and 8.2% w/v respectively (Table 10). These appeared to be within acceptable limit, as most drugs could contain 10-20% w/w moisture yet their constituent are not affected while for some drugs their quality deteriorate if as much as 10% is present and in such cases enzymatic action could be responsible (Shellard, 1958).

3.11.2 Extractive values

Results are as presented in Table 10. The extractive values of the leaf and the gall indicate that the water soluble extractive value for the gall (19.8% w/v) is more than that of the leaf (16.8% w/v) indicating higher quantity of such extractives in the gall. Further more the results have shown that cold and hot leaching processes gave higher percentage of water soluble substances in both the leaf and gall. This could be attributable to better extraction (overnight soaking and 12 hours continuous leaching). On the other hand the alcohol-soluble extractives for the leaf were found to be invariably higher (25.7%) but that of the gall is comparable to the water soluble extractive. The water and alcohol soluble extractives signify the presence of fair amount of constituents that are leached into water and alcohol respectively.

Therefore it would appear that the use of water as medium and vehicle in the traditional preparation for the treatment of gastrointestinal disorders and skin diseases in the northern part of Nigeria may be valid. Leven et al. (1979) reported that certain plant constituents like saponins, flavonoids and tannins which have been detected in the aqueous extracts of the leaf and gall have antibacterial activity against

some organisms. These organisms have been found to be common causative agents of wound infections, gastrointestinal disorders and skin diseases (Cruickshank et al., 1973).

3.11.3 Determination of ash values

The ash value is intended to measure the amount of residual substance not volatilised when drug is incinerated (African Pharmacopoeia, 1986). And total ash represents ash from plant tissue (physiological ash) and ash from extraneous matter (non-physiological ash) for example sand and earth (Brain and Turner, 1975). *Results show that the leaves and galls have total ash values of 6.30 and 4.43 respectively (Table 10). From the results it can be seen that the acid insoluble ash values for the leaf and gall (1.50 and 2.30) when compared with total ash of the same parts indicated that the samples contain fairly large amount of contaminating soil (extraneous matter) although it is evident that natural ash is significant representing elements taken during growth and present in the plant in the tissues mainly as calcium carbonate and oxalate. These have been found to be in large amount in the leaf and the gall.

Table 10: Moisture content, ash and extractive values of G. senegalensis leaf and gall powder.

Parameters	Powder	
	Leaf	Gall
Moisture content (% w/v)	11.30	8.00
Ash values (% w/v)		
- Total ash	6.30	4.43
- Acid-insoluble ash	1.73	2.30
Extractive values (w/v)		
- Alcohol-soluble	25.70	18.50
- Water soluble	16.83	19.83
- Cold leaching	17.50	24.00
- Hot leaching	19.80	27.50

* Each value represents a mean of three separate determinations.

3.12.0 Investigation of the antimicrobial properties of the galls of G. senegalensis

Results were recorded in millimeter as diameter of zones of inhibition. An organism is considered susceptible to the effect of an extract if a zone of inhibition was observed.

There was no growth in the incubated tubes each of which contain the aqueous and water soluble extractives of methanol extracts alone in each case.

From the results obtained, it can be seen that all the extracts showed activity against the tested organisms. Streptococcus pyogens, Staphylococcus aureus and Escherichia coli were more sensitive to the effects of both extracts of roasted and unroasted galls. While Pseudomonas aeruginosa and Candida albicans were least susceptible. The organisms are more susceptible to the extracts of unroasted galls in comparison to those of the roasted galls. The methanolic extracts of roasted and unroasted galls exhibited higher activity. However, Staphylococcus aureus was found to be more sensitive (18.5mm) to the aqueous gall extract. The diameter of the inhibition zones for the standard organisms are shown in Table 11.

The absence of microbial growth in the incubated tubes containing aqueous roasted and unroasted gall extracts alone showed that they were sterile. The inhibition zones produced against Staph. aureus, Strept. pyogens and E. coli suggest the presence of antimicrobial principles in the aqueous and water soluble extractives of methanol extracts of the gall of G. senegalensis. The reduced activity of the roasted gall extracts as compared to the unroasted gall extracts suggested the undesirability of the roasting of the gall as practised by people and traditional healers around Wudil and Gaya towns of Kano State, Nigeria.

The greater antimicrobial effect of the water soluble extractives of methanol extract indicates that the antimicrobial principles were extracted into methanol in higher concentration. The low values of the zones of inhibition in the case of P. aeruginosa and C. albicans show that these organisms are less sensitive to the antimicrobial effects of the extracts compared to the other organisms.

The organisms were selected based on the fact that they are most common causative agents of skin diseases, wound infection and gastrointestinal disorders (Cruickshank, et al. 1973). Certain plant constituents such as alkaloids, flavonoids, tannins and saponins have been associated with antimicrobial properties (Leven et al., 1979; Balbaa et al., 1978). Tannins have also been found to be active against microbial toxins and can weaken vaccines. Persimmon tannin for example, inactivated bacterial toxins such as Clostridium tetani, Diphteria, Staphylococcus alpha and Bordetella pertusis toxins, it can also inhibit growth of virus (Fukami and Okonogi, 1979). Phytochemical studies of this plant have indicated the presence of flavonoids, saponins, alkaloids and tannins in the twigs and roots of the plant (Koumare et al., 1979). The presence of these compounds have been observed also in the galls in

this study. Therefore, the observed effects may be due to some of these constituents. The result is in agreement with antimicrobial studies carried out on the leaf by Sokomba et al. (1983), stem and roots by Le Grand et al. (1988).

The fact that the aqueous extracts and water soluble extractives of methanol extracts of the gall of G. senegalensis have been observed to exhibit antimicrobial properties against the tested organisms correlated well with the use of the gall in the traditional treatment of infections by herbalists in northern Nigeria (Irvine, 1961; Personal communication, 1991).

Table 11: Antimicrobial activity* of the extracts of gall of G. senegalensis.

Organisms	Roasted galls		Unroasted galls	
	Aqueous extract (A)	Aq.methanolic extract (B)	Aqueous extract (C)	Aq. methanoli extract (D)
	Diameter of Zones of Inhibition (mm)			
Staphylococcus aureus NCTC 6571	10.0	14.5	18.5	15.0
Streptococcus pyogens NCTC 8198)	12.7	13.5	17.2	17.5
Escherichia coli (NCTC 10418)	10.0	14.0	11.5	12.2
Psuedomonas aeruginosa (NCTC 6750)	10.0	9.0	10.0	12.5
Candida albicans (Standard)	10.0	10.6	11.0	12.0

*Each figure represents a mean of four separate determination each in two replicates

CHAPTER FOUR

4.0 CONCLUSION AND RECOMMENDATIONS

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

Pharmacognostic evaluation of the leaves and galls of G. senegalensis has been carried out. A mould fungus of the Fusarium specie and an insect Acaciothrip ebneri, Karny were identified as the gall formation causative agents.

Tannins were estimated using various methods. Gravimetric method revealed 16.2% w/w and 10.6% w/w while 13.8% w/w and 19.2% w/w were obtained by Hide powder method for the leaf and the gall respectively, and spectrophotometric method indicated 15.46% w/w and 21.14% w/w for the leaf and the gall respectively.

The aqueous and water soluble extractives of methanol extract of the gall exhibited some anti-microbial activity against Staph. aureus, Strept. pyogens, E. coli and Ps. aeruginosa. It would seem as if roasting reduces the potency of the gall as the active constituents might have been partly destroyed by heat, therefore roasting the galls as practised locally prior to use is disadvantageous. The results derived from this study justify the use of the parts of this plant in traditional medicine in Kano and Jigawa States of Nigeria.

It is suggested that some of the results and data particularly those of microscopical features and pharmacopoeial values could be useful in preparation of monograph of G. senegalensis J.F. Gmel.

4.2 Recommendations

The scope of this study is a very wide one in fact some aspects of this work could serve as lead for further study. Specifically it is hoped that further work should include the evaluation of the phytochemical constituents in the gall at different seasons of the year for possible variability. Isolation and chemical characterisation of the types of flavonoids, saponins, alkaloids and tannins present in the plant especially the gall as well as comparable antimicrobial studies with standard agents should be pursued as the leaf, roots (Sokomba et al., 1983, Le Grand et al., 1988 and Mahmoud, 1993) and gall from this study have been shown to be potential sources of antibacterial and antiviral agents.

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