THE USE OF EXTRACTS FROM ACACIA AND MANGROVE FOR THE PRESERVATION OF ANIMAL TISSUES

BY

GANIYU ISIAKA ADEKUNLE

USMANU DANFODIYO UNIVERSITY,

SOKOTO.

NOVEMBER, 1992.

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GANIYU. I. ADEKUNLE
(B.Sc., M.Sc. (BENIN))

A THESIS WRITTEN IN THE DEPARTMENT OF CHEMISTRY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph. D) OF THE USMANU DANFODIYO UNIVERSITY, SOKOTO.

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CERTIFICATION

This thesis by GANIYU ADEKUNLE has met all the requirements for the award of the Degree of Doctor of Philosophy of Science of the Usmanu Danfodiyo University, Sokoto and is approved for its contribution to knowledge.

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DEDICATION

This work is dedicated to my beloved mother, Madam Atinuke Amoo who answered the call of God by the time I needed her most. May her Soul Rest in Perfect Peace, AMEN.

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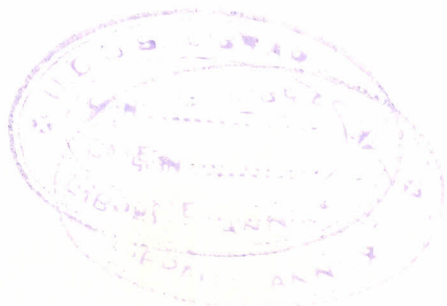

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my supervisors, Dr. (Mrs) F.W. Abdulrahman, Head of Department of Chemistry and Dr. A.N. Eboatu, former co-ordinator of faculties of Science and Agriculture and now Honourable Commissioner for Commerce, Industry and Technology, Anambra State of Nigeria, for their financial help, interest, comments and advice before, during and after my work.

My thanks go to Dr. A.A. Nduji (External Supervisor), former Head of Department of Industrial Chemistry Laboratory, National Research Institute for Chemical Technology (NARICT), Zaria, now Associate Professor, Department of Food Technology, I.M.T. Enugu, and Mr and Mrs Gboyega Olawale of NARICT, Zaria for their immense contributions to my laboratory work and their support throughout my stay in Zaria.

I am grateful to all members of staff of Leather and Leather Products Laboratory, NARICT, Zaria who by their help, jokes and critisms made my Laboratory session quite enjoyable.

My sincere gratitude to some friends indeed- Gboyega Olawale, Tunde Oyebode, Alh. Usman Dantoro, Jelili Adebisi, Dr. Rotimi Oyesola, Raufu Hamdala Book-shop, Dare Deremi, Jide Ibironke, Republic Igbodo,

Kayode Odujobi, Captain Mike Gbadebo, Engineer Jerry Ojediran, John Alabi, Ayo Fatogun, Charles Afolabi, Tayo Ajana, Tirimisiyu Ajadi, Akin Ogundipe, Audu Friday, Gbadebo Isaac, Aremu Kadir, Segun Fatona, Aminu Umar, Segun Sanni, Biola Omotosho, Leye, Oke, Dr. A. I. Ukatu, Dr L. Ugwu and my uncles especially uncles Sanmi Falade, Niyi Omodehin, Toyin Illufoye, Tayo Odujobi and Jide Deba who have contributed to the success of my thesis.

Finally, I wish to express my appreciation to my brothers and sisters Olaitan Amoo, Gboyega Amoo, Kemi Amoo, Aina Odujobi, Bukola Ajao, Oreoluwa Illufoye, Bola and Demola Omodehin, Segun Ahmed, Shehu and Maryam Adegboye, Nike Illufoye, Lola and Kayode Falade.

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ABSTRACT

The oldest system of tanning relies on the chemical action of vegetable materials containing tannin or tannic acid on the protein constituents of skin or hides.

The tannins belong to the polyphenols, the catechol tannins and the pyrogallols. The non-tannins consist of bodies which have similar chemical nature to the tannins.

The dried plant samples were ground, and sieved. The powdered plant samples were soaked in distilled water overnight and were then extracted.

The extracts were analysed for:

(i) total solids (ii) total solubles (iii) non-tannins. The tannins contents were calculated by difference. The moisture contents were determined by weighing and drying, to constant weight, a known weight of finely ground materials. The pH was obtained with the aid of pH meter.

The Cow organs/tissues were fixed in each of the extracts for seven months before embedding them in wax. The mounted slides were stained and photographed.

Acacia contains the highest tannin 49.67% followed by red mangrove 32.24% and white mangrove 18.09%. From the effect of the plant extracts on the organs/tissues tested, acacia plant preserved best followed by white mangrove and red mangrove respectively.

The tannin extracts stabilized the proteins, of

the organs/tissues tested. This will reduce the demand on formalin and other preservative agents, and hence help to conserve our foreign exchange.

CHAPTER ONE

1. INTRODUCTION

1.1 Historical Accounts

Vegetable tanning, dating from 3000 B.C. is usually employed for heavy leather.[1] The system relies on the chemical action of vegetable materials containing tannins on the protein constitutent of skin.[2]

Leather production is one of World's oldest known crafts and tannin - bearing pods of *Acacia nilotica* were found by Schiaparelli with part processed and finished leather in a 5,000 year old tanyard site in upper Egypt.[3] By about 6,000 B.C., tanning with vegetable tannins was common throughout the Mediterranean and the technique spread throughout the world, reaching America after the European penetration of that continent.

In addition to their applications in leather manufacture and dyeing, tannins are used in the clarification of wine and beer, as a constituent to reduce viscosity of drilling mud in oil well, and in boiler water to prevent scale formation. Due to its astrigent properties, tannin has been used in the treatment of tonsillitis, pharyngitis, harmorrhoids, skin eruptions, diarrhoea, intestinal bleeding, and as antidote for metallic, alkaloidal, and glycosidic poisons, with which it forms insoluble precipitates.[2] Water soluble tannins form dark blue and dark green solutions with iron salts, a property utilized in the manufacture of ink.[2] In plants, tannins

are secreted to seal off the affected area of plants in response to injury, or insects or disease attacks on the plant. They deactivate both viruses and extracellular enzymes by insolubilising them as they will any typical protein molecules.[3] Tannins play a part in assisting plants to resist fungal attack.[3] Tannin extracts from Acacia nilotica are locally used for the cure of pile and stomach disorder.[4]

1.2 Objective

Objective of this work is to ascertain if vegetable extracts from known tannin-bearing Nigerian plants could be utilized in the preservation of biological (animal) specimens. Tanning provides protection against the action of bacteria as well as that of autolytic enzymes. Granting that it is the collagen fibres that are more utilized in the skin tannage, it has been argued that there should be no chemical reason why other polypeptides (proteins) cannot react with these tanning materials. Therefore it is expected that treatment of plant extracts can stabilize other body proteins- hence preservation. Many tannin-bearing plants occur in Nigeria. They include the Acacia, Mangroves, Eucalyptus etc. Some of them have been used in skin tannage.

1.3 The Chemistry of Tannins

Vegetable tannins are polyphenolic substances which are responsible for the tanning action. The non-tannins do not take part in the tanning process directly but they are important as modifiers of the tannin action e.g. they give body to the leather, and disperse the tan aggregates.[5]

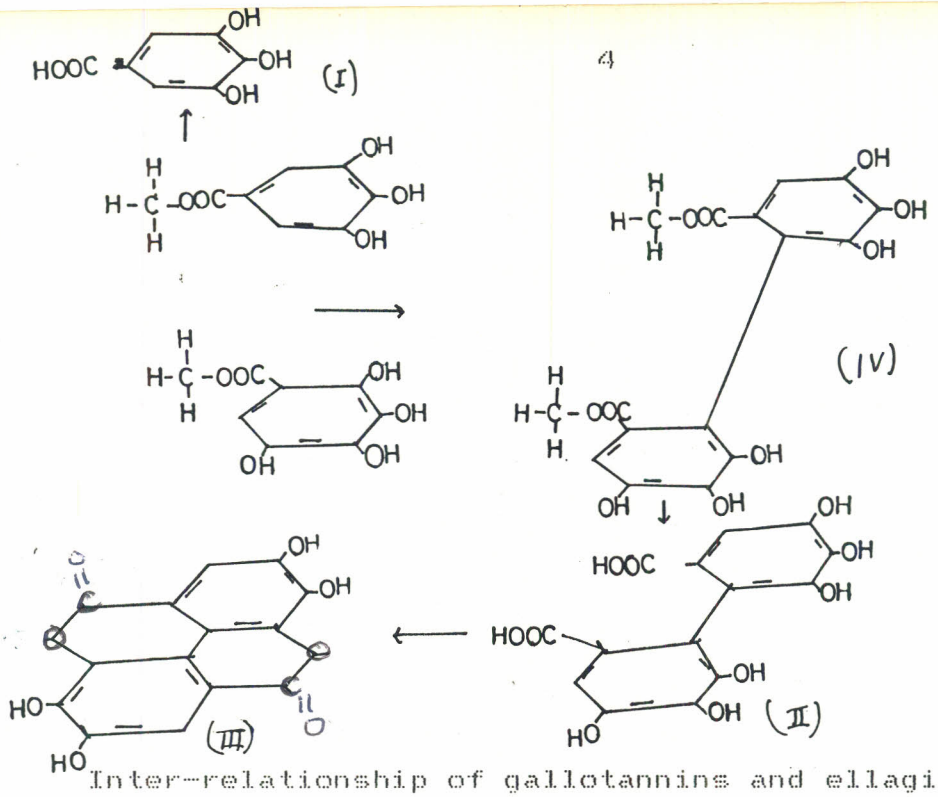
Vegetable tannin extracts are produced by simple water extraction of a wide variety of plants. They are used in water and if appplied to hides and skins in organic solvents, no tannage occurs. For a phenolic substance to exhibit a tanning property it must have a molecule large enough to cross - link adjacent collagen chains, a large number of phenolic groups to achieve combination at several points. The lower limit of size is defined by the work of Russell et. al [6-8] showing that the capacity to tan begins to develop when three galloyl groups are attached to a glucose molecule and is well evident in various pentagalloyl sugars.

The vegetable tannins are classified into the the hydrolysable and condensed tannins as suggested by Freudenberg [9] and based on the structural types. The main distinctions between the two groups arise from their action towards hydrolytic agents, particularly acids. The hydrolysable tannins which have a polyester structure are readily hydrolysed by acids (or enzymes) into sugars, polyhydric alcohols, phenols, carboxylic acids. Depending on the nature of the carboxylic acid, a subdivision into gallotannins and ellagitannins is also usually made. [10]

Inter-relationship of gallotannins and ellagitannins

On hydrolysis, the gallotannins give gallic acid (I) and the ellagitannins give hexahydroxydiphenic acid (II) which is isolated as its stable dilactone ellagic acid (III) or acids derived by the chemical transformations (such as oxidation, reduction and ring fission) of hexahydroxyphenic acid. The alcoholic portion of the hydrohysable tannins is d - glucose, although there also exist several authenticated cases of other sugars and guinic acid acting as the core.[10]

The condensed tannins do not readily break down with acids; instead they undergo progressive polymerization under the action of acids to yield the amorphous phlobaphenes or tannin reds. The derivation of the tannins temselves is a matter of some conjecture, although most workers agreed that they are formed by condensation or polymerization of monomeric flavan - 3-4 diol or flavan-3-ol precursors.



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1.4 Hydrolysable Tannin Extracts

The members of this group which are of importance in terms of their uses are:

(1) Tannic acid - A phis chinensis galls on leaves of Rhus semiala (Anacardiaceae)

(2) Turkish tannin - cynips tinctoria galls on twigs of Quercus infectoria (Fagaceae).

(3) Sumac extract - Leaves of Rhus coriaria (Anacardiaceae).

(4) Tara extract -pods of caesalpinia spinosa (Leguminosae)

(5) Valonia extract - acorn cups of Quercus Aegilops (Fagaceae).

(6) Myrobalan extract - fruit of Terminalia chebula (Combretaceae).

(7) Divi-divi extract - pods of (caesalpinia) coriaria (Leguminosae).

(8) Algarobilla extract - pods of Caesalpinia brevofolie (Leguminosae).

(9) Oak extract- bark and wood of various Quercus SPP. (Fagaceae).

(10) Chestnut extract - bark and wood of castanea saliva and dentala (Fagaceae).

1.4.1 The Galotannins or Tannic acid

The chemistry of these extracts shows that they consist of "gallotannins" and "tannic acid" which can be degraded by acid or enzyme to yield only gallic acid and glucose.

The classical work of Fischer [12] and his collaborators led to the formulation of tannic acid as

penta -m - digalloyl - glucose and Turkish tannin as pentagalloylglucose. Fischer synthesized pentagalloyl and penta - m - digalloyl glucose and found them amorphous, like his natural products.

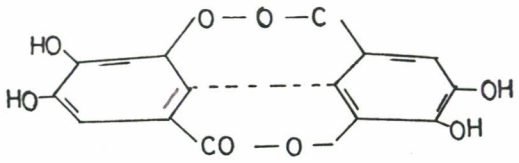
Karrer et. al.[13] and Karrer[14] fractionated tannic acid and Turkish tannin by precipitation with aluminium hydroxide and concluded that tannic acid was a pentagalloyl glucose molecule of the type envisaged by Fischer, with varying proportions of the galloylhydroxyl groups further esterified with gallic acid. The glucose molecule was envisaged as fully substituted and as carrying a small proportion of m - digalloyl groups, and possibly a few higher linear ester groupings (e.g. trigalloyl forms.)

Two dimentional paper chromatography of tannic acid extracts from chinese galls (White et. al.[15]) showed five components. White[16] and King and White[17] identified three of these compounds as gallic acid (21 percent), m - digallic acid (7 percent), and probably pentagalloylglucose (3 percent) and suggested another might be a trigallic acid (about 1 percent). However, Haworth et. al.[18] found that esterase treatment of the same gallotannin sample gave glucose as the only suger and King and White[19] suggested that the oligosaccharide core is inapplicable and that the true structure of gallotannin is yet to be determined.

1.4.2. The Ellagitannins

The hydrolysable tannin extracts are often referred to as the ellagitannins, because their solutions deposit on standing, the crystalline dilactone, ellagic acid. This must

be viewed as a derivative of gallic acid and is obviously important in relation to the structure of the tannins present in these extracts. Acid hydrolysis of the extracts increases the amount of deposition, and the characteristic



Ellagic acid

bloom on the surface of leather tanned with ellagitans.

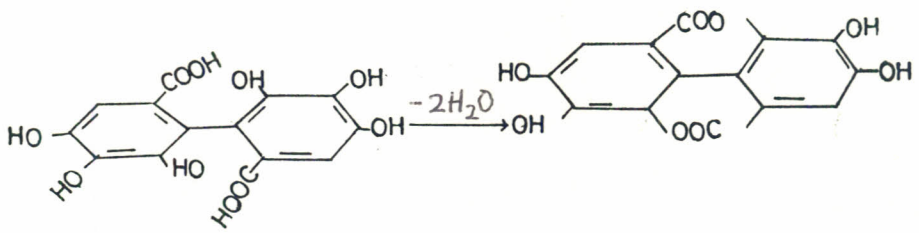
Ellagic acid was first described by Braconnet and chevreul[20] and the accepted formula was put foward by Graebe.[21] It is synthesized from gallic acid[22] and is characterised by its insolubility in water. Kurby et. al.[23] showed by paper chromatography that ellagic acid existed in the free condition in several hydrohysable extracts.

In myrobalan extract the bloom deposited by the extract contained not only gallic acid but also the crystalline chebulinic acid.[24] Freudenberg found this substance hydrolysed with acids to give glucose, gallic acid and acid of unknown structure called "split acid" but later renamed chebulic acid by Schmidt et. al.[25] on hydrolysis of chebulinic acid.

Schmidt and Nieswandt[26] isolated from myrobalan extract a crystalline product called chebulagic acid, while Schmidt and Lademann[27] found the same substance in the carboxylic acid fraction of divi-divi extract.

Schmidt et. al.[28-29] isolated from myrobalan and divi-divi extracts a crystalline substance termed "corilagin."

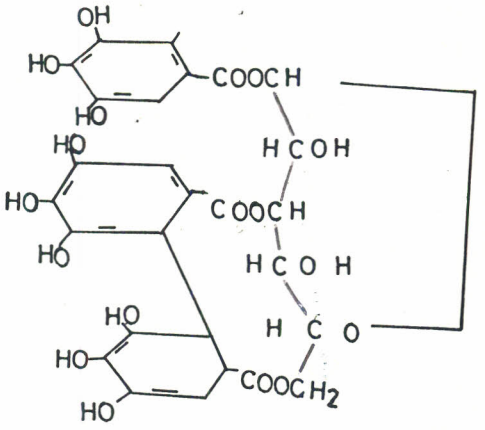
Schmidt and Schmidt[29] observed that careful hydrolysis of chebulagic acid yield corilagin and chebulic acid while Schmidt et. al. reported that the hydrolysis of methylated corilagin gave hexamethoxydiphenic acid instead of the ellagic acid formed by the hydrolysis of the original corilagin. This indicated that hexahydroxydiphenic acid is present in corilagin in such a manner that it could be liberated by acid hydrolysis.



hexahydroxy diphenic acid ellagic acid

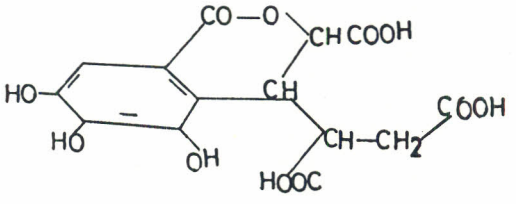
It was also found possible to split off only 1 gallic acid group from corilagin, leaving a hexahydroxydiphenoyl glucose as the other reaction product. Schmidt et. al.[30] formulated corilagin as 1 galloyl -3-6- hexahydroxydiphenoyl-p-glucose with the diphenic acid ester linked to the 3 and 6 - OH groups of the glucose molecules.

This formula was verified by Schmidt et. al.[31]



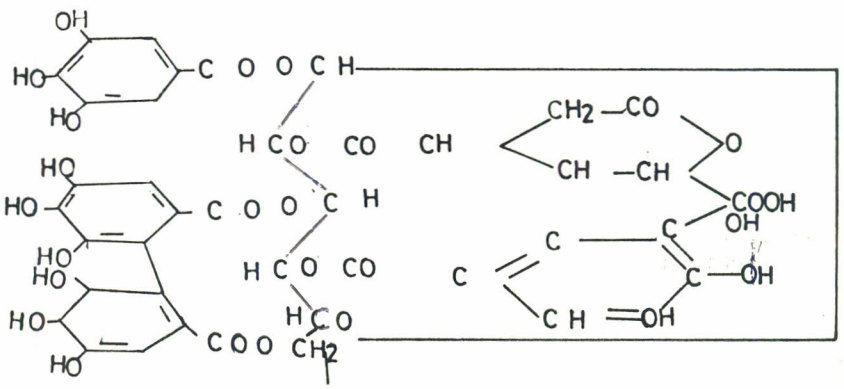
Corilagin

Meanwhile Schmidt et. al.[32] and Schmidt and Menyer[33] had established the structure of chebulic acid as a tricarboxylic acid containing a galloyl group whose carboxyl group was hidden by lactone formation'.



Chebulic acid

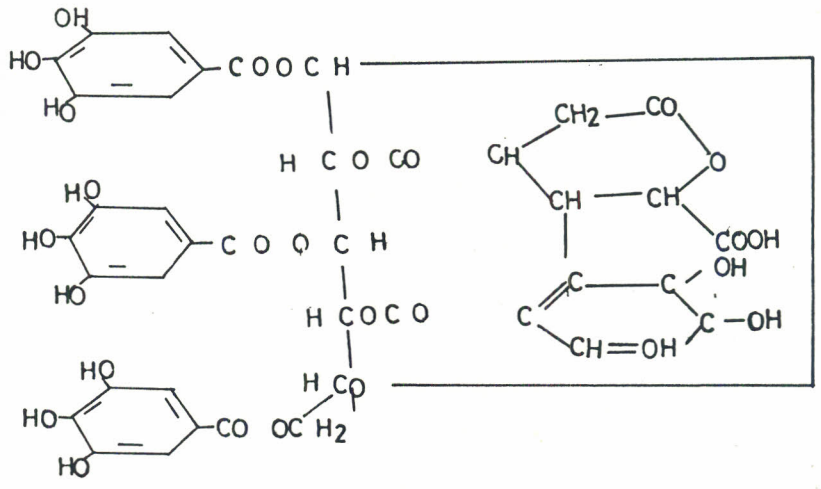
Supporting evidence for this formula has been given by Grimshaw et. al.,[34] Haworth and Silva,[35] and by Haworth et. al.[36] Since chebulagic acid gave corilagin and chebulic acid on careful hydrolysis, it was now obvious that in chebulagic acid also the 1 - position of the glucose was occupied by gallic acid and the 3, 6 positions by the diphenic acid. Schmidt[37] therefore suggested that the chebulic acid was attached in the 2 and 4 positions of the glucose molecule existing as a lactone, differing in structure from the structure it changed to when split off by hydrolysis. The structure for chebulagic acid is:



Chebulagic acid

Similarity between chebulinic and chebulagic acids is that the former on hydrolysis carried 2 H-atoms and gave 3 moles of ellagic acid instead of a mole of each of ellagic acid and gallic acid given by chebulagic acid.[37] Schmidt suggested, therefore, that in chebulinic acid the glucose 3 and 6 positions are occuplied by two galloyl groups instead

of the hexahydroxydiphenic acid found in chebulagic acid. Schmidt et. al[38] indicated that mild hydrolysis of chebulinic acid gave chebulic and gallic acids and a digalloylglucose while Schmidt and Schach[39] established the structure of the latter as 3, 6-digalloylglucose - a fact supporting



the above structure

1.5 Condensed Tannin Extracts

The most important tannin extracts of this group are:

1. Gambier extract - leaves of twigs of uncaria gambier (Rubiaceae).
2. Catechu (Burma cutch) extract - wood of Acacia catechu (Leguminosae).
3. Quebracho extract - wood of schinopsis lorentzii, balansae (Ancardia-ceae).
4. Wattle extract - bark of Acacia mollissima (Leguminosae).
5. Tizerah extract - wood and roots of Rhus pentaphylla (Anacardiaseae).
6. Urunday extract - wood of Astronium balansae (Anacardiseae).
7. Mangrove extract - (Borneocutch) - bark of various Rhizophoraceae spp.

8. Hemlock extract - bark of Tsuga canadensis (Pinaceae).

9. Spruce extract - bark of Picea abies (Pinaceae).

10. Larch extract - bark of larix decidua.

11. Tea tannins - leaves of camellia sinensis (Theaceae).

1.5.1 The Hypothesis of Tannin Structure

(a) The catechin hypothesis of condensed tannin structure put formward by Freudenberg[9] claiming that the condensed tannins are polymers of catechin;

(b) The flavipinacol hypothesis of Russell,[40-42] a dimeric structural variant of the catechin hypothesis.

(c) The leucoanthocyanidin hypothesis whereby many tannins have been chemically identified as leucoanthocyanidin provides a new interpretation of condensed tannin chemistry.[83]

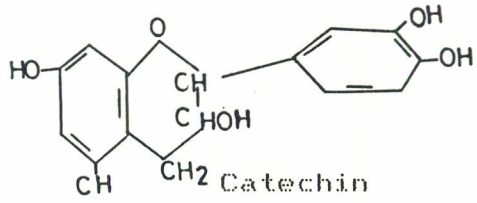
(d) The Forestal observation of Theodore et. al.[43-48] postulated that tannin extracts are complex in composition and that each contains appreciable number of polyphenolic substances- many of which are tannins in their own right. The behaviour of an extract is dependant on this complexity; each individual polyphenol in an extract may play a part in tanning, and there can be no question of a single structural formula for any extract.

1.5.2 The Catechin Hypothesis

The chemistry of the condensed tannin extracts revolved around the catechin hypothesis of Freudenberg,[9] Perkin and Everest.[49] Freudenberg adopted the term "catechin" as a collective name for those substances in plant extracts which

resembled catechin in yielding phloroglucinol and protocatechnic acid on alkali fussion, and in giving amorphous insoluble products - phlobaphenes or reds on heating with mineral acids. His argument was that most flavonoid substances are based on phloroglucinol linked to catechol by a ring system involving a 3-carbon chain, and since a similar skelecton characterized the anthocyanidins and catechins, it must also be present in the condensed tannins. These, since their reactivity resembled that of catechin, must be polymers of catechin, not of the related flavonoids or anthocyanidins present in the extracts.

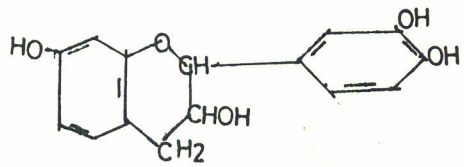
The evidence for the importance of catechin in condensed tannin extracts is confined largely to gambier and Burma cutch extracts. Meyer[50] demostrated the existence of catechin and gallocatechin in oak and chestnut barks.



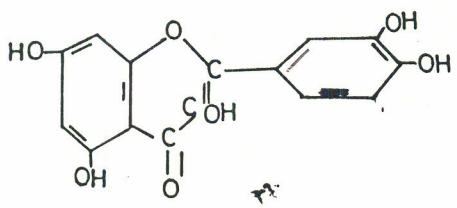
Catechin

The only substance which could readily be isolated in crystalline form from any of these extracts was catechin; and it was observed in significant amount only in gambier and Burma cutch. The compound is highly reactive and readily converted to an amorphous product capable of tanning hides. Catechin existed in six isomeric forms: D- catechin, L- catechin, - catechin, D- epicatechin, L-epicatechin, and -epicatechin and various inter-conversions of these

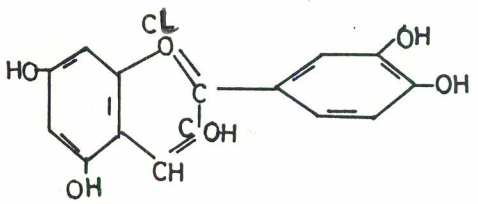
products could take place on the basis of the formula



In accordance with this formula, catechin yielded phloroglucinol and protocatechnic acid of condensed tannin extracts. It is found in association with other substances such as flavonols and anthocyanidins, which possess similar carbon skeletons built up of a phloroglucinol nucleus linked to a catechol nucleus via a six-membered pyran ring e.g. quercetin and cyanidin.



Quercetin



Cyanidin Chloride

In gambier and Burma cutch extracts, as reported by White *et. al.*[15] catechin is the constituents. In the gambier extracts it forms about 50 percent of the water soluble content.

Hathway,[51] and Hathway and Seaking[52-54] showed that catechin can undergo polymerization during autoxidation at a neutral pH and that the process involves quinone polymerization. A similar polymer was obtained bypolyphenol oxidase treatments and both polymers were similar in properties, to "phlobatannins" isolated from gambier and

Burma cutch extracts, suggesting that head-to-tail quinone polymerization of catechin is the likely mechanistic basis of its participation in the formation of tannin extracts of the cutch and gambier types.

The presence of catechin in gambier and Burma cutch suggests that these extracts should be regarded as constituting a subgroup of their own. The pholobaphene test for condensed tannins, i.e. the formation of an insoluble red precipitate on boiling an extract with mineral acid, can be used to distinguish different subgroups of the condensed tannins, if gambier and Burma cutch are accepted as a subgroup by virtue of their high catechin content. The eucalptus and mangrove extracts can be considered as a second subgroup, since the red colour of their phlobapheneS can be shown chromatographically (Hills, King and White [55] [17]) to be due to the fact that as much as 10 percent of anthocyanidins is formed when these extracts are heated with mineral acid.

1.5.3. The Flavpinacol Hypothesis

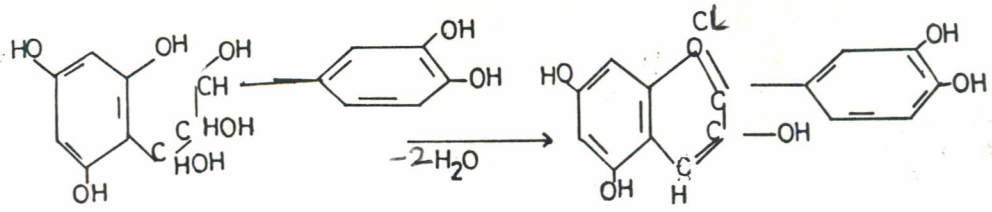
The flavpinacol hypothesis of condensed tannin structure postulated by Russell [40] illustrates the danger of identifying tannins by chemical test only. Russel commented that catechin was the only 3 -hydroxyflavanol then known to exist and suggested that 4 - hydroxyflavans might equally be of importance in tannin chemistry. He claimed that 4 - hydroxyflavanol prepared by the reduction of the corresponding chalcones dimerized to products resembling

the phlobaphenes in reactivity. In the case of 4,7,3, 4 - tetrahydroxy-flavanol, he gave 18 instances of identical behaviour of this substance and of hemlock tannin extract in response to chemical tests.[56] Russell and Todd then stated that "phlobatannins must be constituted in the same way" and later claimed that because hemlock tannin extract gave protocatechnic acid and pyrogallol on alkali fusion, hemlock tannin was a flavpinacol hydroxylated on the pattern of bis (7,8,3 , 4 - tetrahydroxy). flavpinacol. Ressell et. al[57] compared the ultraviolet absorption spectra of a number of their polyhydroxyl -flavpinacol with those of vegetable tannin extracts and showed both had absorption maxima in the region 2,800 to 2, 900 A.

1.5.4 The Leucoanthocyanidin Hypothesis

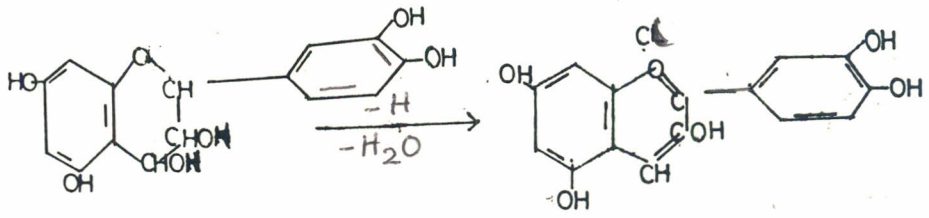
The general hypothesis of condensed tannin structure is that which suggests the condensed tannins are leucoanthocyanidins. Bate and smith[58] reported that many of the substances in the leaves and petals had been identified as tannin by tests which demostrated them to be polyphenols and "leucoanthocyanidins."

Rosenheim[59] postulated the existence in such tissues of colourless anthocyanidin precursors which he called "Leucoanthocyanidins" and these hypothetical substances had a C , C , C trio structure giving rise to anthocyanidins through elimination of water e.g.



Triol Cyanidin Chloride

Bate-Smith[58] applied the phlobaphene and the vanillin-HCl tests for catechins in flowers of woody plant species and reported a positive reaction to both tests and demostrated that where the phlobaphene test was positive the red colour was due to the formation of anthocyanidins. Catechin gave a cream-coloured product in the phlobaphene test and not a red product as had been claimed.[9] He suggested that the phlobaphene test was more of catechin and that a positive test resulted from the conversion of leucoanthocyanin to anthocyanidins under the influence of mineral acids. He suggested that leucoanthocyanins with a 3,4- diol structure are capable of such conversion.[58]



Flavan - 3,4-diol Cyanidin Chloride

Bate-Smith,[60-61] Bate-smith and Lerner[62] demostrated using paper chromatography the presence of leucoanthocyanins in the leaves of many woody plant species and concluded that

their presence in the dicotyledons is a primitive character associated with a tree-like or woody habit of growth. They pointed out that the leucoanthocyanins give many of the diagnostic chemical reactions of tannins and that their distribution is highly congruent with that recorded for tannins in the botanical literature.

King and Bottomley[63] isolated from the heartwood of Acacia melanoxylon an amorphous 7,8,3',4'-tetrahydroxyflavan-3,4-diol called "melacidin." They claimed that its discovery revealed the nature of these anthocyanidin-tannin precursors offering alternatives to the Flavpinacol hypothesis of condensed tannin structure. King and Clark-Lewis[64] having confirmed the structure of their flavan-diol by synthesis, claimed that it gave an anthocyanidin colour with hydrochloric acid. King and Bottomley[65] reported that melacacidin combined the properties of a leucoanthocyanin and a phlobatannin and stated "the behaviour of melacacidin with acids implied that the hitherto imperfectly defined phlobatannins are also derivatives of flavan-3,4-diol." Finally, King and Clark-Lewis[66] reported the conversion of melacacidin to anthocyanidin, thus crude tannin which is similar to melacacidin can be regarded as leucoanthocyanidin.

Forsyth[67] isolated from cacao bean a leucocyanidin which gave cyanidin, epicatechin, (-) catechin, and unidentified polyphenols on heating with mineral acid. King and Bottomley[65] suggested that the cyanidin formation is an oxidation process and that if it could "take place through a

compensating reduction of the flavandiol to catechin the reason for the tannin reactions of the leucoanthocyanidins become obvious.[65] This presumably involves the assumption that phlobaphenes are formed from catechin which, in turn, arises from leucoanthocyanidins as a reduction balancing the oxidation which produces cyanidin.[68] Swain provided supporting evidence for the flavan-3-4-diol structure of the leucoanthocyanins and confirmed that their conversion to anthocyanidins involves oxidation.

Kieser et. al.[69] provided further data showing that the tannin content of pear juice as determined by permanganate titration consisted mainly of a complex leucoanthocyanin.

Rauer et. al.[70] favoured the flavandiol hypothesis of leucoanthocyanin structure; Hillis[71-72] considered these developments from the view point of leather tannin chemistry with regards to the extent to which leucoanthocyanins precursors of tannins are responsible for the red colour which develops in vegetables - tanned leather.

Hillis[72] examined the possibility that leucoanthocyanins might be one of the first forms of polyphenolic compounds synthesized in the plants and might be the precursors of the anthoxanthidins, anthocyanidins, catechins and tannins. He assumed that if this were so, leucoanthocyanins should be formed in areas of intense metabolism, and in accord with this he found the greatest concentration of these substances in the growing tips of young leaves, in the cambial region

of various eucalyptus species being examined.

Roux[73] observed that treatment of quebracho extract with HCl - propanol under pressure gave fisetinidin and suggested that corresponding leucoanthocyanin might be present in S. lorentzii heart wood. Roux[74] claimed that this reagent and the use of p-toluene sulphonic acid as a diagnostic spray reagent for paper chromatograms, indicated "the presence of leucoanthocyanins in relatively high concentration in both wattle and quebracho extracts."

King and White[75-76] made a comprehensive study of the polyphenol content of the fresh leaves, twigs, barks, sapwood and heart-woood of the three quebracho species (S. balansae lorentzii, heterophylla) and reported that the sapwood contained significant amounts of leucocyanidin.

1.5.5 The Forestal concept

Arata[77] obtained both phloroglucinol and protocatechnic by fusing quebracho extract with alkali. Nierenstein[78] obtained resorcinol and claimed that alkaline degredation of the brominated extract gave isovanillic acid (Monomethyl protocatechnic acid) and that anthracene could be obtained by distilling the phlobaphenes with zinc dust. Perkin and Gunnell[79] identified as flavanol fisetin a product first obtained by Arata, showed that gallic acid was present in the hydrolyzed extract, and suggested that ellagic acid might also be present.

Joblonski[80] and Einbeck and Einbeck and Joblonski[81] confirmed the existence of resorcinol nuclei in quebracho extract by isolating typhnic acid (Trinitro resorcinol) on

oxidation with nitric acid. Einbeck and Joblonski[82] observed that the oxidation also produce 3,5- dinitro- resorcylic acid from the ethyl acetate soluble portion of the extract. This shows that atleast some of the resorcinol nuclei were linked by a meta-positioned carbon atom to whatever other structure were present.

Alkali fusion of the Wattle extract produced resorcinol, while potassium permanganate oxidation of the methylated product gave veratric acid and trimethyl gallic acid confirming Einbeck and joblonski's[83] findings that the extract contained both catechol and pyrogallol nuclei.

1.6 PRINCIPLES OF VEGETABLE TANNING

Vegetable tannins are obtained by water leaching vegetable barks, leaves, woods, nuts or roots either warm or cold. Such extracts contain tannins, sugars, saccharides, flavones, gums etc. All plants yield tannins but the choice today is limited to those with high tannin content, which can be harnessed economically.[83-84]

The catechol tans are polyphenolic based on catechol structure and may contain a few carboxyl groups giving colloidal dispersions at pH 5.0. The more soluble ionised molecules have a dispersing action on the larger micelles. At pH above 5.0 they are strongly ionised, which reduces the dipole moments and molecular aggregation thus increasing the solubility while the colour changes to dark brown. In acid solutions the ionisation is depressed, dipole aggregation increases, colloidal precipitation occurs and

the colour changes to pale brown. Technically they give a firmer, harder leather and large amounts can be fixed on the protein fibre giving increased weight or yield of leather per unit of protein.

Pyrogallol (hydrolysable tans) are esters of gallic acid or ellagic acid with glucose, which can be hydrolysed by alkali or enzymes, yielding insoluble deposits of ellagic acid (known as bloom). The mechanism of vegetable tanning is complex. It consists of ionic attraction between the catonic collagen and the anionic tan micelles. The smaller, more ionised micelles react readily. The fixation is readily attributed to dipole or H-bond fixation to peptide and other groups on the protein. Under appropriate conditions, e.g. low pH, high concentration or time, further aggregation of tan occurs on the already fixed tans.

The protein fibres thus become coated with colloidal deposits and the tans replace bound water on the protein. On drying the tanned leather, they inhibit the collapse of protein fibres and dipole or H-bond cross-linking between adjacent protein molecules. On ageing tan fixation becomes stroger. Tan fixation is partly reversible by elution with water at high temperatures or pH values but a proportion of

the tan is very resistant.

1.7 METHODS OF TANNING

1.7.1 Vegetable tanning:- is carried out in a series of pits in which the hides/skin is immersed in an aqueous solution of

the vegetable tans. A counter-current system is used whereby the new hides go into a much used old liquour for a day and progress through a series of 8 pits to a fresh liquour of about 5.5% tan and pH 3.5. This is followed by 3 to 6 weeks for sole leathers in stronger liguour of 15% tan and pH 3.4 followed by hot liquours of 18% tant at 40 C followed by a bleach pit of 19% tan containing 1% bisulphite to improve the colour. They are rinsed free from surface tan, and drained. This process can be speeded up by agitation of the system by use of rocker frames and circulators.[85]

1.7.2 Synthetic tans (Syntans)

These have different chemical structures and are made water soluble by the introduction of a sulphonic acid group. They are highly ionized and have a strong ionic attraction for the protein's basic group with a consequent dehydrating effect. Syntans with high secondary valency forces will have a more pronounced leathering effect and give a thicker leather (replacement syntans) while those with a greater protion of sulphonic groups give a thinner less flexible leather (auxiliary syntans).[106]

1.7.3 Mineral Tanning

The basic salts of chromium zirconium and aluminium are used in mineral tanning in the leather industry.

Their initial fixation is on the acid groups of the protein where they displace water molecules and form cross-links between adjacent acid groups, stabilising the wet skin

structure. Thus on drying, the shrinking of the protein molecules is impeded and there will be less chance of cross link formation between protein groups due to the presence of mineral salt.

However the dehydration effect of these tannings and the quantity fixed is less with vegetable tanning and therefore shrinkage and hardening on drying is more pronounced.
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1.7.4 Aldehyde Tanning

Formaldehyde and other aldehydes combine with the basic groups of the protein to form cross links with basic groups on adjacent molecules in the wet protein. Small amounts of aldehyde are sufficient to produce a significant effect. This modification in the wet protein structure impedes the shrinkage phenomena on drying, and the formation of cross links between hydration sites.

1.7.5 Dehydration Methods

These are methods of rendering the skin soft when dry as the effect is lost if the skin is re-wetted.

Thus treatment of the wet skin with high salt concentrations will dehydrate the skin protein, so that on drying shrikage of the fibres and subsequent formation of cross links is impeded.

Properly salted skins dry out to give white and flexible product which have the appearance tannage. The effect is lost if the salt is washed out. Removal of water with a solvent, for instance washing the wet skin in an excess of acetone, will produce the same white leather.

A white flexible, dry product is produced by "Freeze

drying" whereby the wet skin is frozen and subjected to vaccuum. This allows the fibres to remain flexible and shrink together.

The physical properties of a given leather will depend on the type of tanning given to it, the quantity fixed and the drying technique used. A further modification can be made by the application of oils.

The penetration of the tannins through the thick skin is important to get the required uniformity of distribution.

1.8 FACTORS AFFECTING TANNING OPERATIONS

The factors affecting tanning operations are pH, concentration, and temperature. These factors affect the colloidal properties of the tannin medium and the chemical reaction of the tannin molecules.

1.8.1 Effect of pH

The weak acid nature of the tannin molecules will aid in the increase in charge on the particles with increase in pH, and the colloidal particles will disperse as the vegetable tannin compounds become more soluble. The pH of the solution will also affect the colour of the extract. The greater solubility at high pH will generally result in a darker colour and less cloudiness than at lower pH.

1.8.2 Effect of Concentration

The higher the concentration, the greater the fixation of the vegetable tanning material by the hide protein. High concentrations of vegetable tanning materials may result in a large partticle size of tannin which will cause

precipitation and thus block the penetration of extract into the skin.

1.8.3 Effect of Temperature

The higher the temperature, the greater the dispersion of vegetable tanning materials and the greater the rate of reaction of the vegetable tanning materials with the hide protein.

1.9 INDUSTRIAL USES OF TANNIN EXTRACTS

As a result of the increasing knowledge of the chemistry of tannin extracts, their potential value as raw materials for industrial purposes other than the leather industry has been realized. Certain extracts, particularly wattle and quebracho are produced in large scale, and their low cost relative to other sources of mono; di-, and trihydric phenols makes them of particular value to industry. Some of their industrial uses are:

1.9.1 Tannin Extracts in Plastics, Resins and Adhesives

Due to their polyphenolic nature, the tannin extracts are potential alternatives to the phenols used in the plastics and related industries. Wattle and quebracho extracts have received most attention and have been successivefully incorporated into phenolformaldehyde-type plastic moulding-powder phenols and as accelerating agents capable of enhancing reactivity in the same way as the more costly resorcinol or phloroglucinol.

Thermosetting tannin extract-formaldehyde-based adhesives can be made and they display a reactivity intermediate between that of phenol-formaldehyde and

resorcinol-formaldehyde-based adhesives. Tannin-containing barks are also used as extenders in moulding powders, providing a phenol-containing alternative to the normal wood fillers.[83]

Ion-exchange resins can be made from tannin-formaldehyde resins, the normal extracts providing products which are of decided interest in view of their content of o-dihydroxyphenol groups and the pronounced chelating action they display. The sulphited tannins provide resins carying sulphonic acid groups, and these have been commercially produced. Ion-exchange resins capable of chelation can also be produced from tannin-epoxy derivatives and are stable.[83]

1.9.2 Tannin Extracts and Corrosion

Both condensed and hydrolysable tannin extracts have been used to prevent corrosion in low - and medium pressure steam boilers, and to mobilize the scale laid down in such boilers by hard waters and in preventing caustic craking in steam boilers.[87] In most cases soda ash is blended with a mixture of tannin extracts selected to deal with specific condition of water treatment as determined by analytical examination of the water concerned. The dosage is closely related to the hardness of the water used for steam generation and to the nature of hardness. The oxygen corrosion problem is met by the alkaline treatment, the tannin extracts absorbing oxygen in proportion to the catechol or pyrogallol groups content.

1.9.3 Preservation of Fishing Nets and Textiles

Tannin extracts are used to preserve fish netting, the process prevents attack by cellulose - decomposing moulds and bacteria[83]. The techniques involve initial impregnation of the nets by soaking in hot solutions of tannin. Several such treatments may be given, and the extract is fixed to the net fibre by further treatment with hot dichromate solutions. In the case of nets for continuous use, the tannin extract treatment is repeated every few months and the dichromate treatment once a year[83].

An alternative and preferred method of fixation involves treating the tanned net with ammoniacal copper sulphate solution[88]. Various tannin extracts have been used but the best are the Burma cutch, Borneo cutch (Mangrove), quebracho, or wattle extracts. The preservation of woolen articles such as blankets is used by Japanese manufacturers.

1.9.4 Antivirus and Antifungal Actions of Tannin

Many plants accumulate tannins in tissues which are no longer the seat of active metabolish, particularly in barks and heartwoods, and White[16,89] has reviewed the evidence suggesting that this accumulation serves to protect plants from fungus and virus attack. Tannins are not significantly toxic to fungi and bacteria, but, at concentration levels above approximately 1%, they prevent spore germination and growth of various microorganisms and inhibit the activity of most viruses. If the inhibited organisms are removed from the inhibitory environment, metabolic activity recommences.

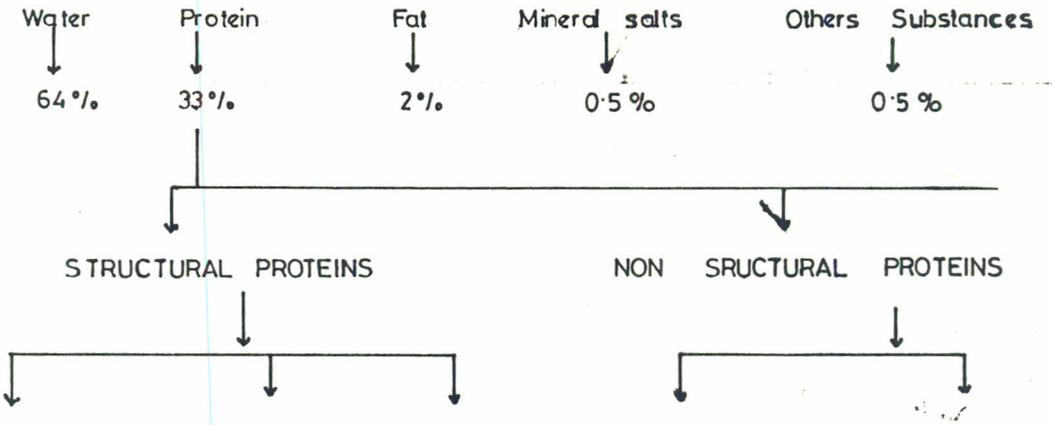
A variety of published work show that tannins inactivate viruses or invasive extracellular enzymes by insolubilizing them in the same way as they insolubilize hide and convert it to leather.

The effect of tannins in preventing spore germination and enabling plants to resist fungi has been demostrated by many workers[90–96], while the inhibition of virus by a simple tanning action is cleared from the work of many workers.[97–103]

1.10 STRUCTURE OF HIDES AND SKINS[104]

Fresh hides and skins consist of water, protein, fatty materials and some mineral salts. Of these, the most important for leather making is the protein. This protein consists of many types. The important ones are collagen which on tanning, gives leather and keratin, which is the chief constituent of hair, wool, horn and epidermal structures.

The approximate composition of a fresh hide is as follows:-



1.11 MECHANISM OF VEGETABLE TANNING

1.11.1 Contribution of Collagen to Tanning.[90]

Proteins are polyamides, formed by the bonding of an amino group from one amino acid molecule with the carboxylic acid group of another, with the elimination of water resulting in a peptide

```
    O      H
    I      I
( - C   -  N  -    ) linkage.
```

A representation of a protein molecule is:

```
  O   H         O   H         O   H         O   H
  II  I         II  I         II  I         II  I
- C - N - CH - C - N - CH - C - N - CH - C - N -
          I             I             I
          R             R             R
```

where R_1, R_2, R_3, etc are the amino acid side chains. They represent a wide variety of possible groups.

(a) The peptide groups of the helical backbone are available for hydrogen bonding with the tannin molecules.

(b) Hydroxyl groups from the side chains of hydroxylproline, serine, threonine and tyrosine are available for donor or acceptor hydrogen bonding.

(c) Amino groups are available from arginine, lysine, and histidine residues for donor or acceptor hydrogen bonding as well as the electrostatic salt linkage resulting from the charged $-NH_3^+$ forms.

(d) Carboxyl groups are available from aspartic and glutanic acid residues for donor or acceptor hydrogen bonding as well as electrostatic salt linkage resulting

from the charged carboxyl (-coo-) form.

(e) Polarizable portions of the collagen structure can permit van der waals forces to contribute to collagen-tannin interraction.

1.11.2 Contribution of Tannins to Tanning [105]

(a) Certain of the tannins possess carboxyl groups which at the pH of tanning, would exist in the unionic form (-(-cooH) since they are fairly weak acids.

(b) The phenolic hydroxyl groups are characteristic functional groups in the vegetable tannins, and they would be expected to be in the unionized phenolic form at the normal pH of tanning.

(c) All of the known functional groups in the vegetable tannins are capable of participating in hydrogen bonding reactions as well as interracting by van der waals forces as already stated.

1.11.3 Process of Mechanism of Vegetable Tanning

The chemistry of vegetable tanning has been extensively studied and is already available in the literature (5-82). It has also been established that the major mechanistic pathways include:-

(a) Physical deposition of the tannins on the collagen fibres. This introduces mechanical impedance to inter - fibre slippage as well as imparts a filling effect.

(b) Multipoint hydrogen - bonding of phenolic hydroxyl groups of tannin materials with the peptide carbonyl oxygen.

(c) The binding of the tannin materials to the protein by

electrostatic bonds known as van der waals forces.

For example the hydrogen - bonding is illustrated as shown below:-

Carbonymoiety Hydroxyl Hydrogen

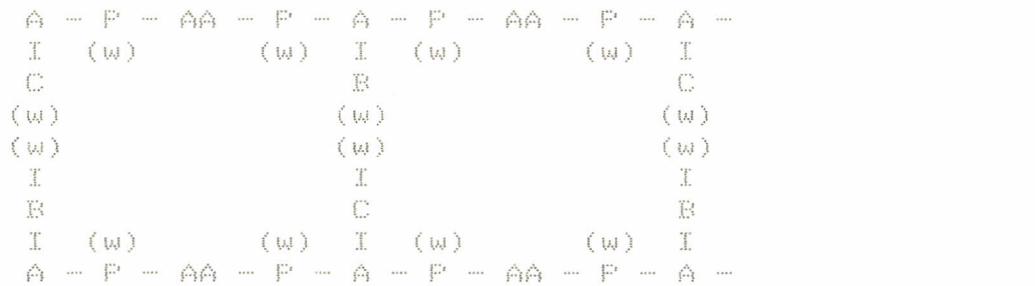
of polypeptide group of bonding

chain tannin molecule

Amine moiety Hydroxyl Hydrogen

of polypeptide group of bonding

chain tannin molecule



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Schematic diagram of two polypeptides

AA - Amino acids
AC - Free acid groups
AB - Free basic groups
P - Peptide groups
W - Water molecules.

1.12 HISTOLOGICAL STUDY
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THEORY

Some cells can be satisfactorily studied by placing them on slides for staining and for microscopic observation (e.g. Wright's stained blood smears). However, for most cytological work it is neccessary to cut tissues into thin, translucent slices only a few microns thick. Sectioning is facilitated by freezing the tissue or by embedding it in a supporting medium such as paraffin, celloidin, or plastics.

The first step in the preparation of histological materials is fixation. Different chemicals are used as fixatives e.g formalin, alcohol, Bouin's fluid, zenker's fluid, etc. and by rapid freezing. Fixation stabilizes tissues, preventing post-mortem change, begins a hardening which facilitate sectioning, and promotes affinity of certain tissues elements for particular dyes. In fixation, proteins are crosslinked or denatured and rendered insoluble; lipids and carbohydrates may or may not be preserved, depending on the nature of the fixation. For example, fats are removed from tissues immersed in alcohols. Therefore, it has often been neccessary to use different techniques (technical procedures) to study the various constituents of a cell. It is significant, however, that methods for examining sectioned tissues by electron microscopy permit study of many cell constituents in a single light microscopic preparation.

For sectioning, the tissue is commonly infiltrated with paraffin which does not mix with water. The paraffin will

not penetrate into tissues until water is removed. Dehydration is accomplished by passing the tissues through a series of graded alcohols, up to 100%. Since paraffin is also insoluble in alcohol,[108] The latter must be replaced by an agent miscible with both alcohol and paraffin, e.g Xylene or cedar wool oil. These agents render the tissue translucent. The tissue is then placed in melted paraffin, which replaces the clearing agents; this step is referred to as infiltration. The tissue is then embedded in paraffin by allowing the latter to harden and then the material is ready for sectioning and staining. Sections are usually 3 to 10 um thick. Celloidin is an alternative embedding medium for cutting large objects (e.g. brain) and for hard and brittle material (e.g. cartilage). Plastics, particularly epoxy resins, are employed as the embedding medium for both light and electron microscopy. They produce less tissue and cellular damage than paraffin and allow the production of thinner sections about 0.02 um thick

It is obvious that structure seen in sections may be altered by chemical fixation, dehydration, or the mebedding process. The description of feature in the fixed cells has brought forth the objection that they are not true features of the living cell, but artifacts of technique. The answer to such objection must always be the consistency of the findings obtained by different technical procedures.

The freeze drying technique seems in some instances to cause less alteration of the living tissues than do the standard methods. In this technique fresh tissues are

preserved by placing them in a liquid such as isopentane at

- 170 C with liquid nitrogen. The frozen tissue is dehydrated in a vacuum and may thus be embedded without previous chemical fixation and dehydration in alcohols. This method is particularly useful for studying the localization of certain enzymes which are destroyed by the standard methods.

In the frozen section technique, a piece of tissue is placed directly on the stage of a special microtome equiped with an outlet for compressed carbon dioxide gas which cools the stage and freezes the tissues sufficiently for the cutting of sections. This methods is used in clinical work for sectioning biopsy material when speed is important. In cytological work the freezing method is particularly useful for studying the lipid content of cells because it avoids the use of fat-solvent dehydrating and clearing agents. The method may be used for either fresh or fixed material; in the former case, it is useful for studying cell enzymes which are destroyed by chemical fixation.

The list of chemicals used for staining is longer than those used for fixation. Most stains are classified acids or bases. They are neutral salts with acid and basic radicals. When the colouring property is in the acid radical of the neutral salt, the stain is spoken of as an acid dye, and the tissues which stain with the dye are called acidiophilic. Eosine is an acid dye with such general usage that the terms eosinophilic and acidiophilic are often used.

In some cases, it is clear that basophilic substances which attract basic dyes are themselves acids, as in the staining of nucleic acids with methylene blue. Special methods and stains are used to demostrate different structures, but the reaction between tissue and dye is often poorly understood.

1.13 Acacia Trees

Acacia trees are common in Sokoto State, just as the rubber and palm trees are in the rain and swamp forests of the south. Acacia trees are found in the Sudan and Sahel Savannah of the north. The importance of this tree to the leather industry cannot be over emphasised. The barks, pods leaves and even the roots are useful in the manufacture of leather because of their tannin contents. The polyphenols of Acacia plant form cross-links with protein fibres resulting in full, supple and resilient leather.

Acacia is a popular tanning material produced locally and is used by the rural and mechanized tanneries for the production of vegetable tanned crust leather in Nigeria. Acacia nilotica pods have been known as a source of tannins for a long time.

Different names have been given to this material by different tribes of Nigeria such as:

1. Hausa — Bagaruwa
2. Yoruba — Bonni
3. Kanuri — Kangar/Kangor
4. Fulani — Gabdi

The percentage tannin contents varies with the location of the tree. It is a complex mixture of both hydrolysable and condensed tannins. This is a sure indication of the good leather making characteristics of the material. It is partially soluble in water and provides a high degree of tannage. Acacia pods have high tannin contents comparable with the other known vegetable tanning materials such as encalyptus, mimosa and myrobalans.

The tree varies in size from mere shrubs to tall trees of about 80 feet in height according to the environmental conditions.

The pods are collected in December - February. The pods which are from 4 - 7 inches long contain 7-11 seeds in each.

1.14 Mangrove Trees

Like the Acacia trees the mangrove trees are in abundance in the south, particularly riverine areas. There are two types; White mangrove (Languncularia racemosa) and Red mangrove (Rhizophora recemosa). Of these two types, Red mangrove is common. Little is known about the use of mangrove trees as tanning materials in Nigeria.

Like Acacia trees, mangrove trees vary in size from mere shrubs to tall trees.

CHAPTER TWO

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2. EXPERIMENTAL

2.1 MATERIALS

(a) Plant Samples:-

Sample A: Acacia plant (*Acacia nilotica*)

Grains
Seeds
Stem
Branches
Leaves

Sample B: Red Mangrove (*Rhizophora racemosa*)

Stem
Branches
Roots
Leaves

Sample C: White Mangrove (Languncularia recemosa)

Stem
Branches
Leaves
Roots

(b) Animal Sample: (Cow organs/tissues)

Skin
Kidney
Liver
Stomach
Intestine
Muscle
Lungs
Pancreas
Spleen
Brain

2.2 REAGENTS

(a) Crome alum ($CrK(SO_4)_2 . 12H_2O$ solution (3%))
(b) Gelatin salt
(c) Hide powder
(d) A.R. Chloroform ($CHCl_3$), BDH, 95% purity
(e) A.R. Absolute alcohol (C_2H_5OH), J.B., 99.86% purity.
(f) A.R. Xylene ($C_6H_4(CH_3)_2$), M & B, 90% purity
(g) Haematoxylin and Eosin stains.

3.2 Preparations of Reagents

(a) Crome alum solution (3%) - 3g of chromium potassium sulphate A.R. was dissolved in distilled water to make 100cm Solution.

(b) Gelatin salt - 1g of photographic gelatin and 10g of pure sodium chloride were dissolved in 100cm^3 of distilled water.

2.4 EQUIPMENT AND APPARATUS

(a) Procter extractor (Improvised)
(b) "Apex" model 116A cutter mill
(c) B.S. Sieve mesh 12 (B.S. No 410/1943)
(d) B.S. Sieve mesh 25 (B.S. No 410/1943)
(e) Mitocrome.
(f) Camera - Jenaval contrast, with model MF-AKS 24x36 automatic photographic equipment.

2.5 EXTRACTION OF TANNINS FROM SOLID PLANT MATERIALS.

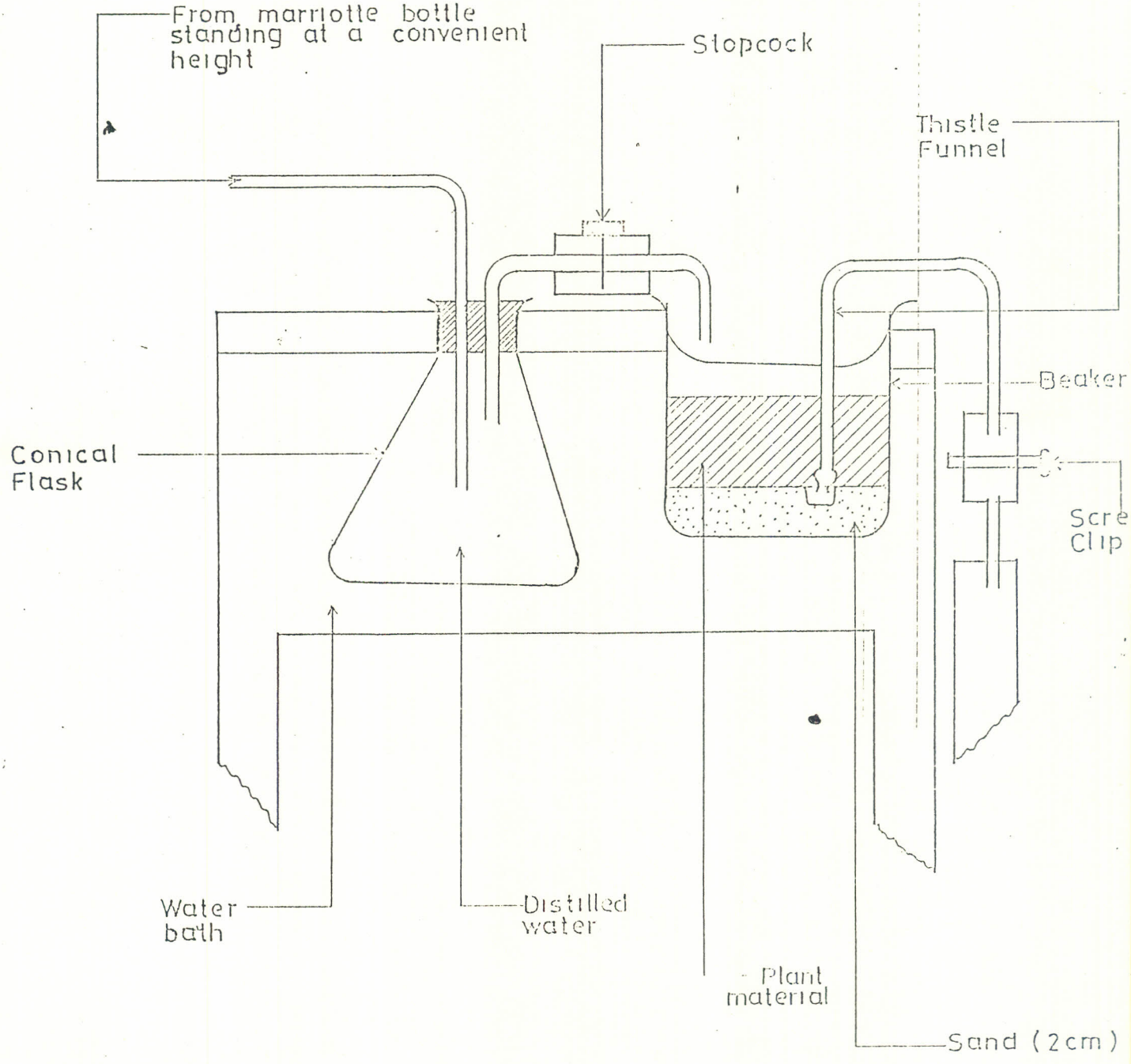
(a) Preparation of Materials

The plant samples were collected from Sokoto (Acacia plant) and Lagos (Red mangrove: Red (M) and White mangrove: White (M)). They were sun dried and ground in a mill until particles passed through a B.S. sieve mesh 12 (B.S. No 410/1943).

(b) Extraction of Tannins

The dried powder plant samples were soaked in distilled water overnight and were extracted using procter extractor

Fig. 2.1: Schematic design of Proctel Extractor



(Fig 2.1). Collection of extract from the extractor was controlled by regulating the opening of the taps at 2dm^3 in four hours.

After collecting the first 150cm^3 the temperature was raised to 50^{o}C and a further 750 cm^3 was collected. The the temperature was raised to boiling point as rapidly as possible and a further 1100cm^3 was extracted to make the volume to 2dm^3. The tannin concentration of the extracts obtained is 4g/dm^3.

2.6 ANALYSIS OF EXTRACTS

2.6.1 Determination of Total Solids

50cm^3 of the homogenised tannin extract was pipetted into an evaporating dish and evaporated to dryness on a water bath. The residue was dried at 105 C in a vacuum oven to constant weight.

2.6.2. Determination of Total Solubles

The tannin extract was fitered through a filter paper. The first 150cm^3 of the filtrate was rejected and the filtration continued. 50cm^3 of the optically clear filtrate was evaporated to dryness on a water bath and dried as for total solids.

2.6.3 Determination of Non-tannin

(1) The preparation of chrome-tanned hide powder

7.28g of hide powder was digested with 70cm^3 of distilled water for one hour. 7cm^3 of chrome alum solution was added to each beaker of hide powder, stirred frequently for 2 hours and was allowed to stand overnight. The powder

was transferred to a filter cloth, drained and squeezed. The cloth containing the powder was placed in a beaker, opened out in a bag fashion and $105cm^3$ of distilled water was poured on to the powder. The powder and water were mixed together and digested for 15 minutes. The cloth and powder were lifted out, drained and squeezed to 75% moisture content by weighing the water content of the powder. The powder was digested three more times in the same manner with distilled water.

(ii) The Non-tannin Determination

$100cm^3$ of tannin extract was added to the digested powder to remove the tannin content of the extract. The contents were shaken vigorously for 15 seconds and tranferred to mechanical shaker for 10 minutes. The powder containing the tannin and solution containing the non - tannins were transferred to a filter cloth, drained and squeezed to obtain the solution (filtrate). 1g of kaolin was added to the filtrate, mixed and filtered through a 15cm pleated filter paper. The filtration was repeatedly carried out until a clear solution was obtained. The filtrate was tested with gelatin - salt for turbidity. $50cm^3$ of the cleared filtrate was evaporated to dryness on the water bath and later dried in a vacuum oven at 105 C to constant weight.

2.6.4 Determination of Moisture

2g of the finely ground material was accurately weighed and dried to constant weight at 105 C in a vacuum oven for 24hrs.

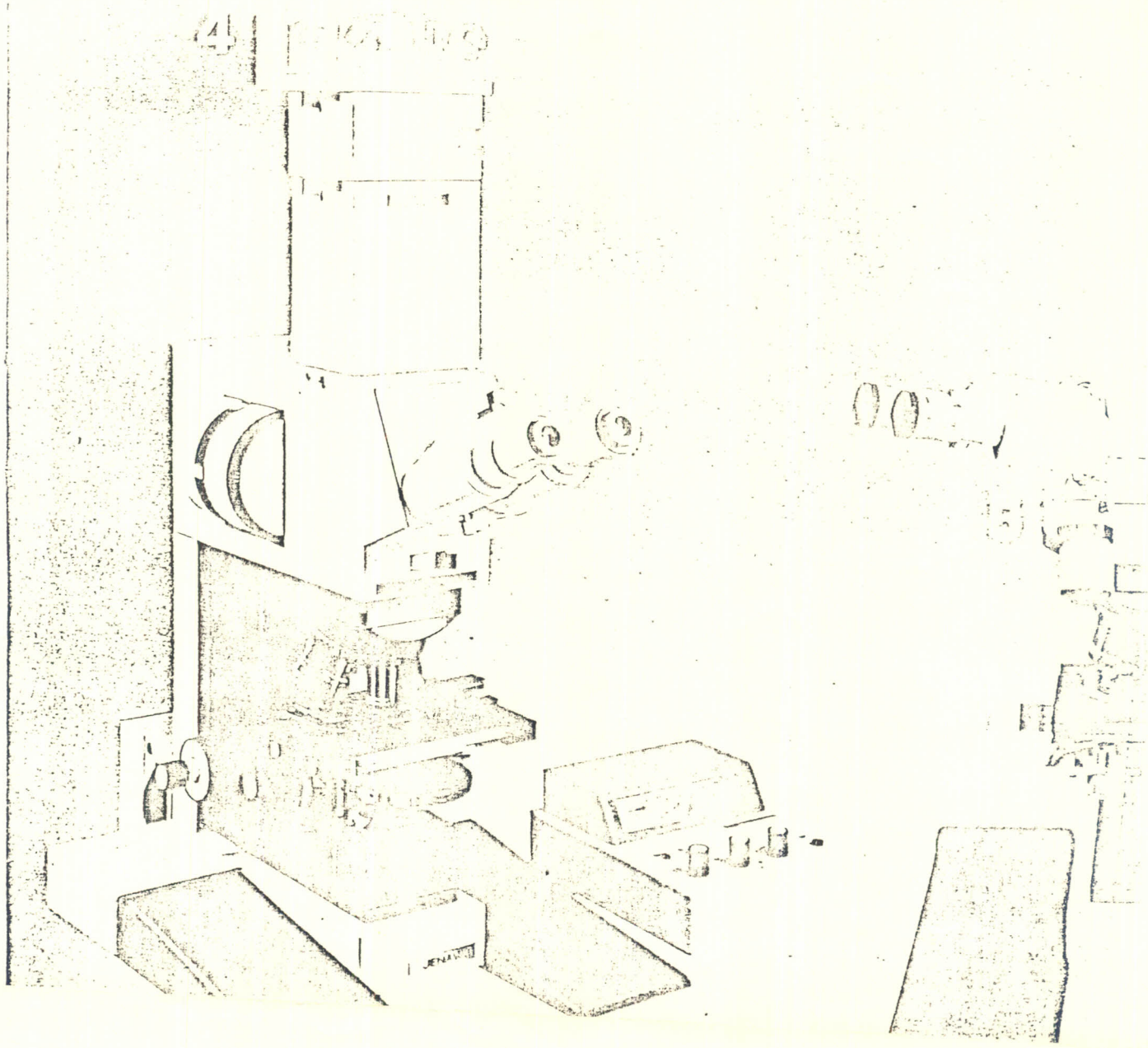
2.6.5 Determination of pH

The pH of the extracts were measured using a pH meter. The meter was first calibrated with the standard buffer solutions of pH 4 and 9, and distilled water. Then the combined electrode was dipped into the extracts to measure their pH.

2.7. HISTOLOGICAL TECHNIQUES [110,111]

The cow organs/tissues were collected from abattoir, cut into pieces (0.8g) immediately and immersed in different plant extracts (4g tannin/dm^3). The organs were kept in the different extracts (fixatives) for seven months, removed and embeded in the wax before they were mounted on the slides using normal histological techniques before being stained.

The photographs of the slides were taken using Jeneval contrast with model mf - AKS 24 x 36 automatic photographic equipment.



CHAPTER THREE

3. RESULTS AND DISCUSSION

3.1 RESULTS

The results obtained from the experiments are presented in Tabel 2.1

CALCULATIONS

The various entries in Table 2.1 were calculated using the following formulae:-

$$\% \text{ Total Solids} = \frac{\text{Residue} \times 20 \times 100}{X} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\% \text{ Total Solubles} = \frac{\text{Soluble Residue} \times 20 \times 100}{X} \quad \ldots\ldots\ldots\ldots (2)$$

$$\% \text{ Non-tannins} = \frac{\text{Residue} \times 20 \times 100}{X} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\% \text{ Tannins} = \% \text{ Total Soluble} - \% \text{ Non-tannins} \ldots\ldots\ldots\ldots (4)$$

$$\% \text{ Moisture} = \frac{Wo - Wf}{Wo} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where

Initial weight of sample (in grams) = X

Stock Solution = $1000cm^3$

Amount taken for analysis = $50cm^3$

Multiplication factor = $\frac{1000cm^3}{50cm^3} = 20$

Weight of sample before heating = Wo

Weight of sample after heating = Wf

RESULTS: TABLE 2.1, TOTAL ANALYSIS OF PLANT MATERIALS

PLANT MATERIALS	% TOTAL SOLIDS	% TOTAL SOLUBLES	% NON-TANNINS	% TANNINS	% MOISTURE	pH
1 ACACIA HUSK	44.44	41.29	14.14	27.15	6.84	4.70
2 ACACIA SEED	33.38	30.89	20.46	10.43	9.40	5.10
3 ACACIA STEM	25.61	19.79	5.18	14.61	5.04	3.90
4 ACACIA BRANCHES	13.30	12.74	3.69	9.05	6.50	4.35
5 ACACIA LEAVES	25.65	25.64	13.88	11.76	7.47	6.50
6 RED (M) STEM	19.84	18.67	4.33	14.34	11.00	5.10
7 RED (M) BRANCHES	11.69	11.70	4.18	7.52	9.08	4.39
8 RED (M) LEAVES	24.45	23.01	12.69	10.35	5.25	5.70
9 RED (M) ROOTS	20.48	19.74	4.53	15.21	6.10	4.35
10 WHITE (M) STEM	9.84	9.49	2.43	7.06	5.62	4.15
11 WHITE (M) BRANCHES	11.79	10.58	4.31	6.27	5.46	3.62
12 WHITE (M) LEAVES	19.99	18.54	12.02	6.52	5.67	4.35
13 WHITE (M) ROOTS	12.18	11.02	4.28	6.74	6.21	3.76

TABLE 2.2, LEVEL OF PRESERVATION

	AGRAIN	ACASED	ACASTE	ABRAIN	ALAVES	RESTM	REDMBRAIN	REDMLAVES	RODOTS	WHITEM	WHITBRAIN	WHITLAVES	WHITROOTS	FORMALIN
KIDMEY	1	6	0	4	6	6	0	6	1	2	6	0	6	10
PANCREAS	10	0	0	10	10	10	10	0	0	0	10	0	10	10
INTESTINE	10	0	8	0	8	8	0	8	6	0	0	6	0	10
MUSCLE	10	10	0	8	0	6	0	6	8	10	0	10	10	10
STOMACH	6	0	6	6	0	10	0	0	0	0	10	0	10	10
LUNGS	2	6	6	0	6	6	6	0	0	2	6	2	0	10
SKIN	0	0	0	6	0	0	10	0	0	10	0	10	0	10
SPLEEN	6	0	0	0	0	6	0	0	0	6	0	0	6	10
LIVER	0	10	10	10	10	2	2	0	2	2	1	1	1	10
AVERAGE	5.0	2.4	3.3	4.9	4.4	6.0	2.0	2.2	1.9	2.4	3.7	3.2	4.8	10.0

KEY

PRESERVATION VERY GOOD	= 10
PRESERVATION GOOD	= 8
PRESERVATION FAIRRLY GOOD	= 6
PRESERVATION FAIR WITH DISTORTION	= 4
PRESERVATION POOR	= 2
PRESERVATION VERY POOR	= 1
NO PRESERVATION	= 0

Table 2.3 Sum - average level of preservation and percentage average of tannin contents of the plant materials.

Plant Materials	Sum - average level of preservation	Percentage average of tannin content
Acacia Plant	20.00	14.60
Red Mangrove Plant	12.10	11.85
White Mangrove Plant	14.10	6.65

Fig 3·1: Histogram of percentage total solids

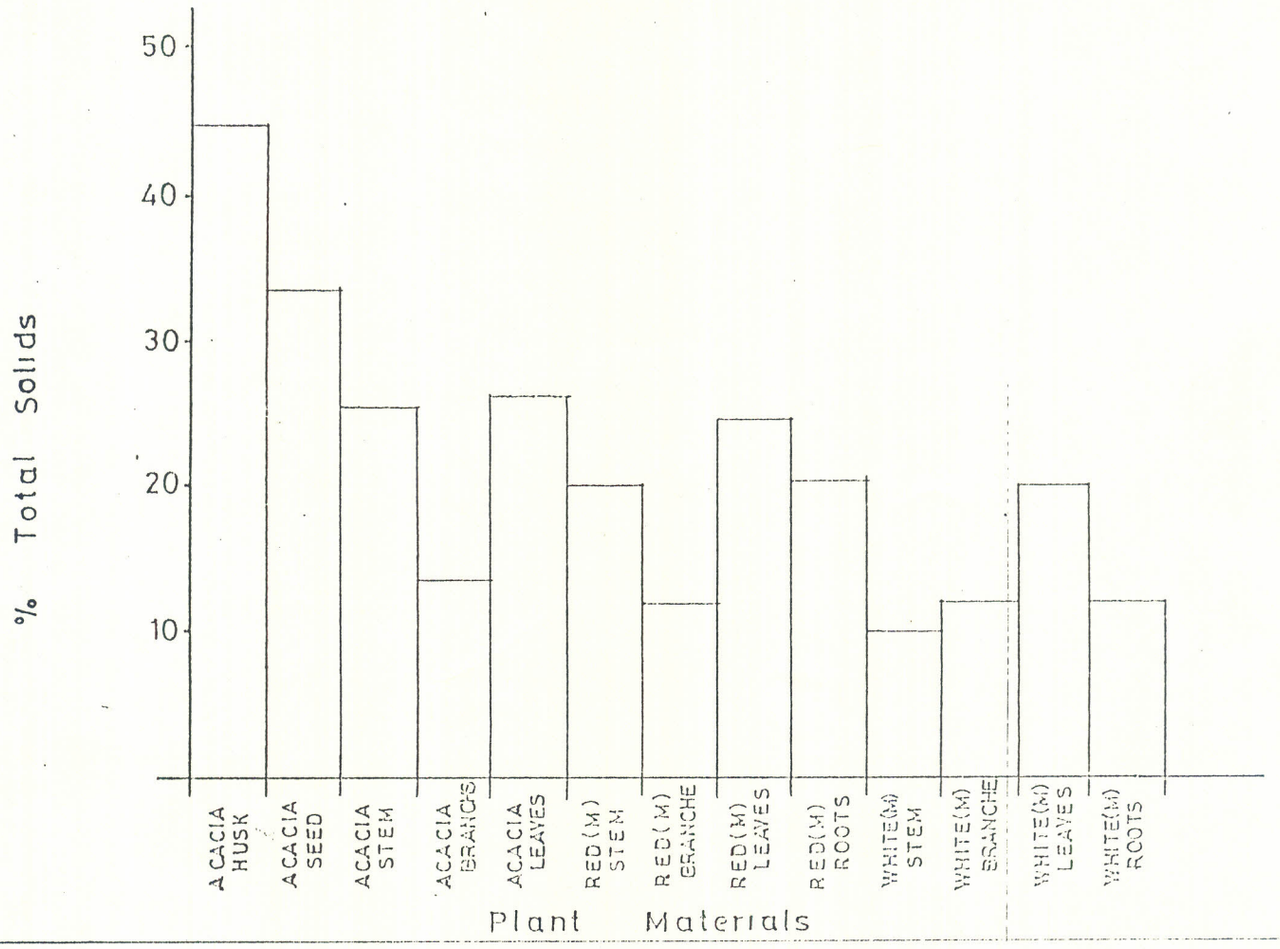


Fig 3·2: Histogram of percentage total solubles

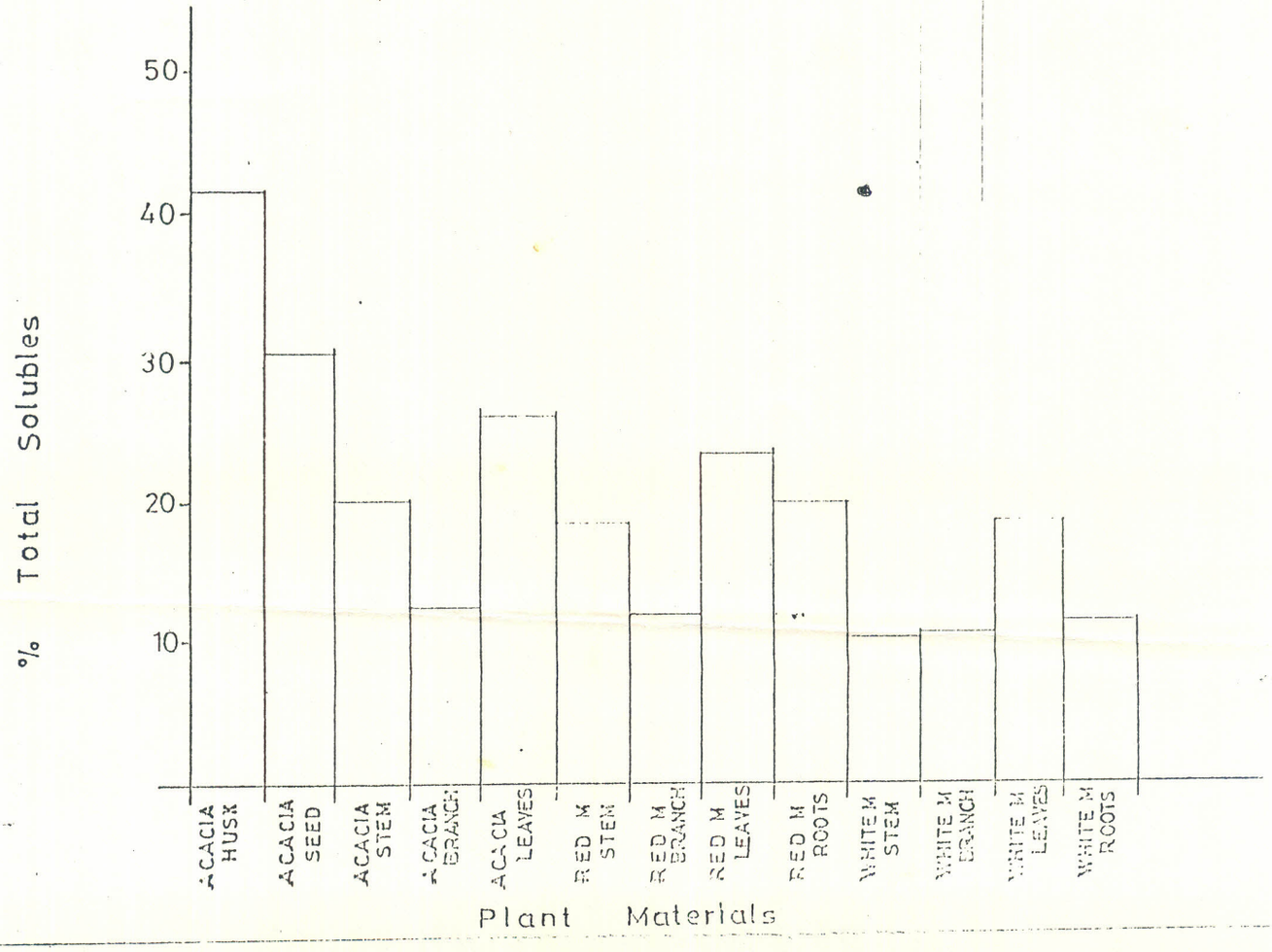


Fig3·3: Histogram of percentage non-tannins.

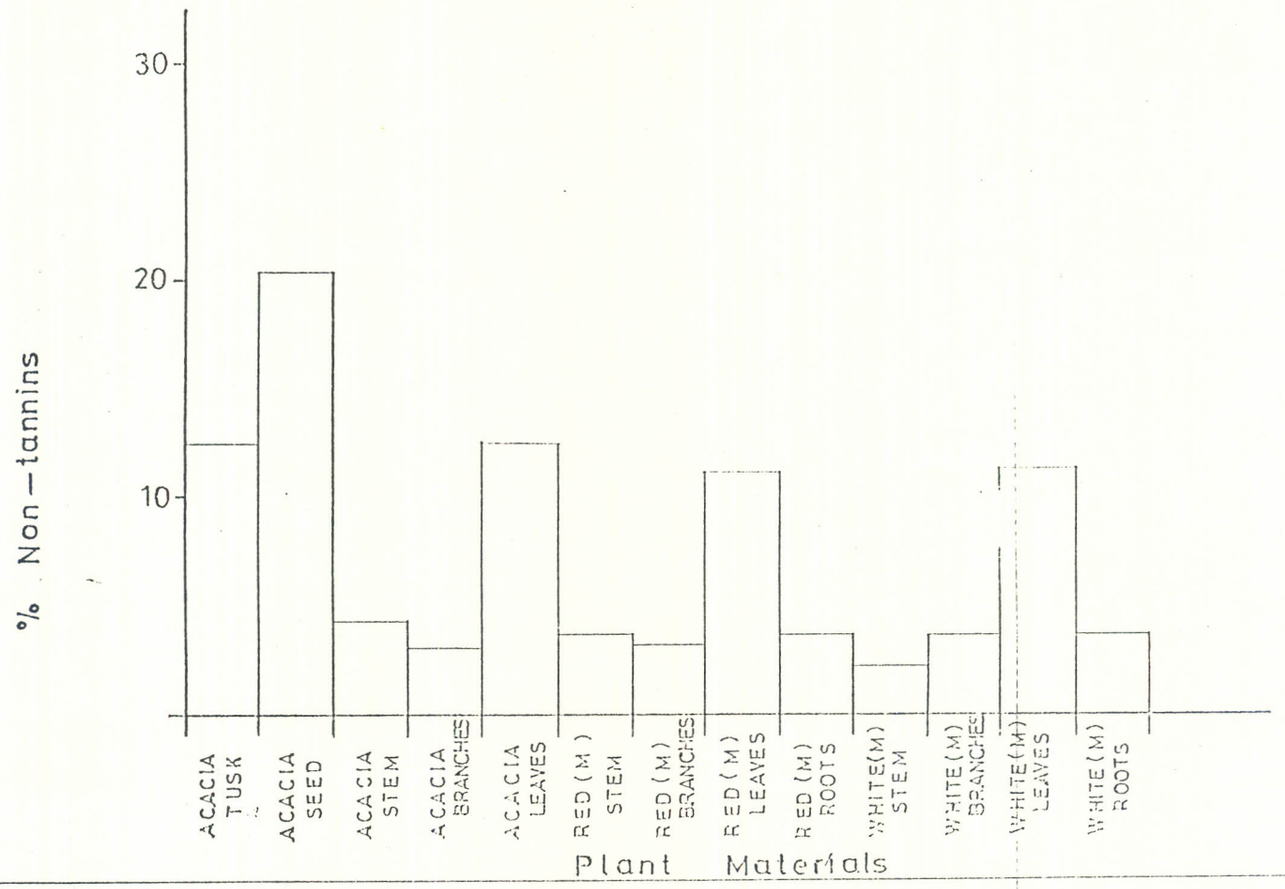


Fig3·4 Histogram of percentage tannin.

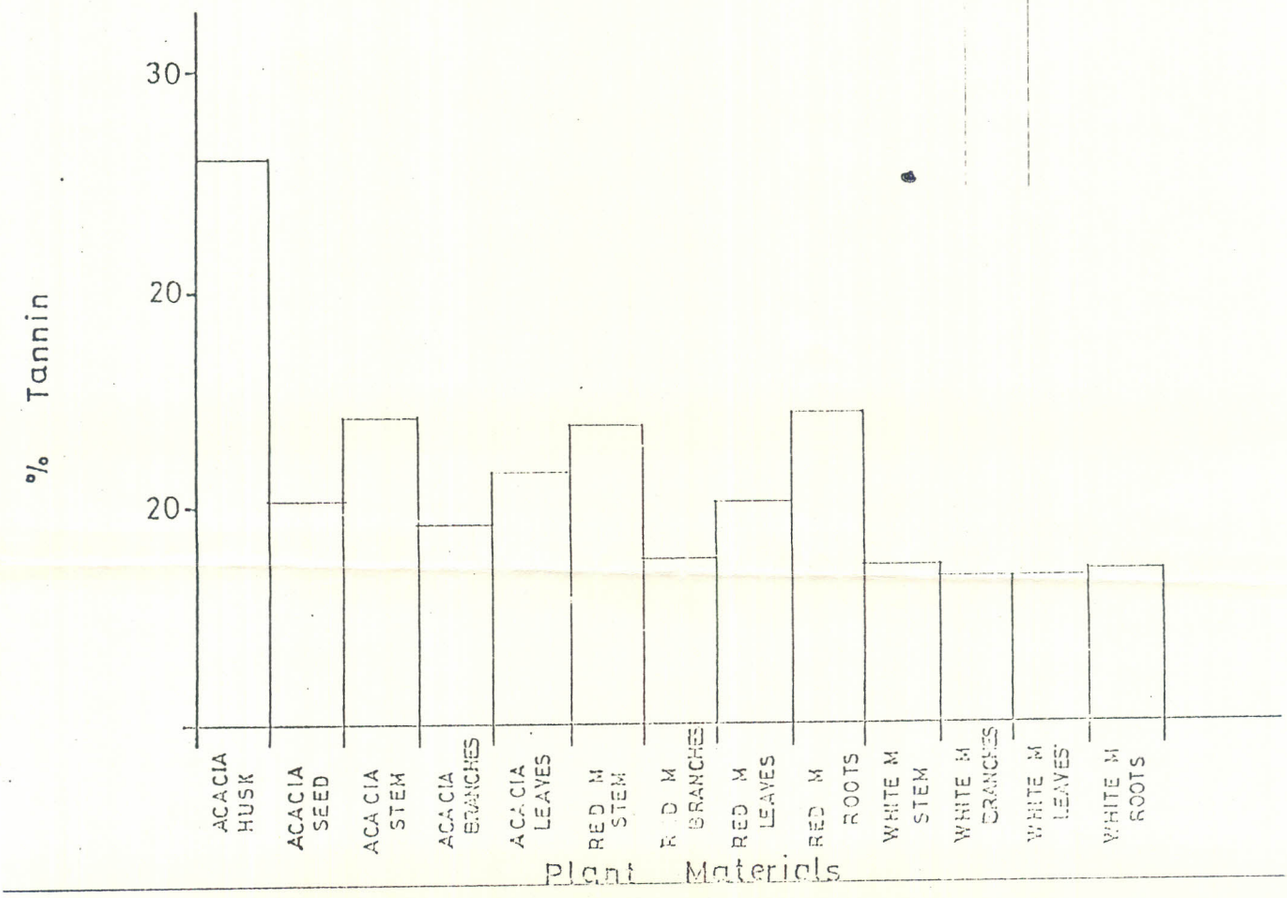
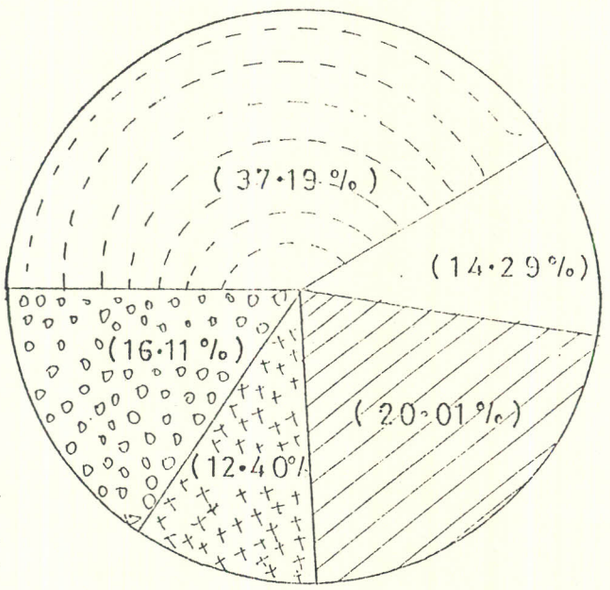


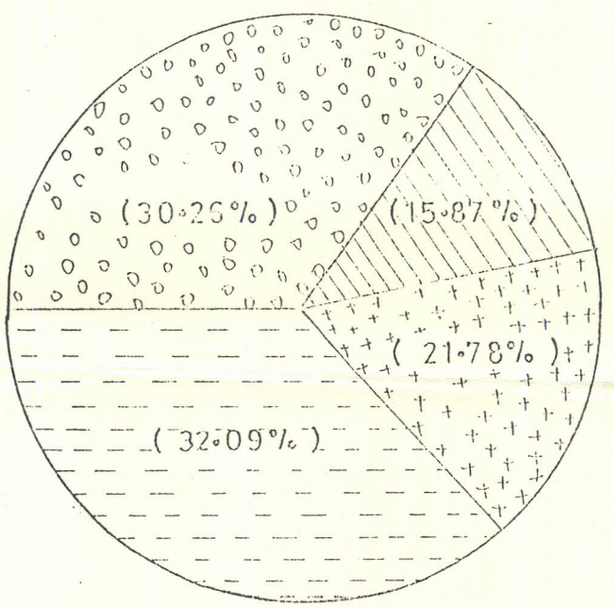
Fig 3·7 Distribution of tannin content of Acacia.



KEY

	Acacia Husk
	Acacia Seed
	Acacia Steem
	Acacia Branche
	Acacia Leaves

Fig 3·8 Distribution of tannin content of red mangrove.



KEY

	Red (M) Stem
	Red (M) Branch
	Red (M) Leaves
	Red (M) Rocks

Fig 3·5 Histogram of pH

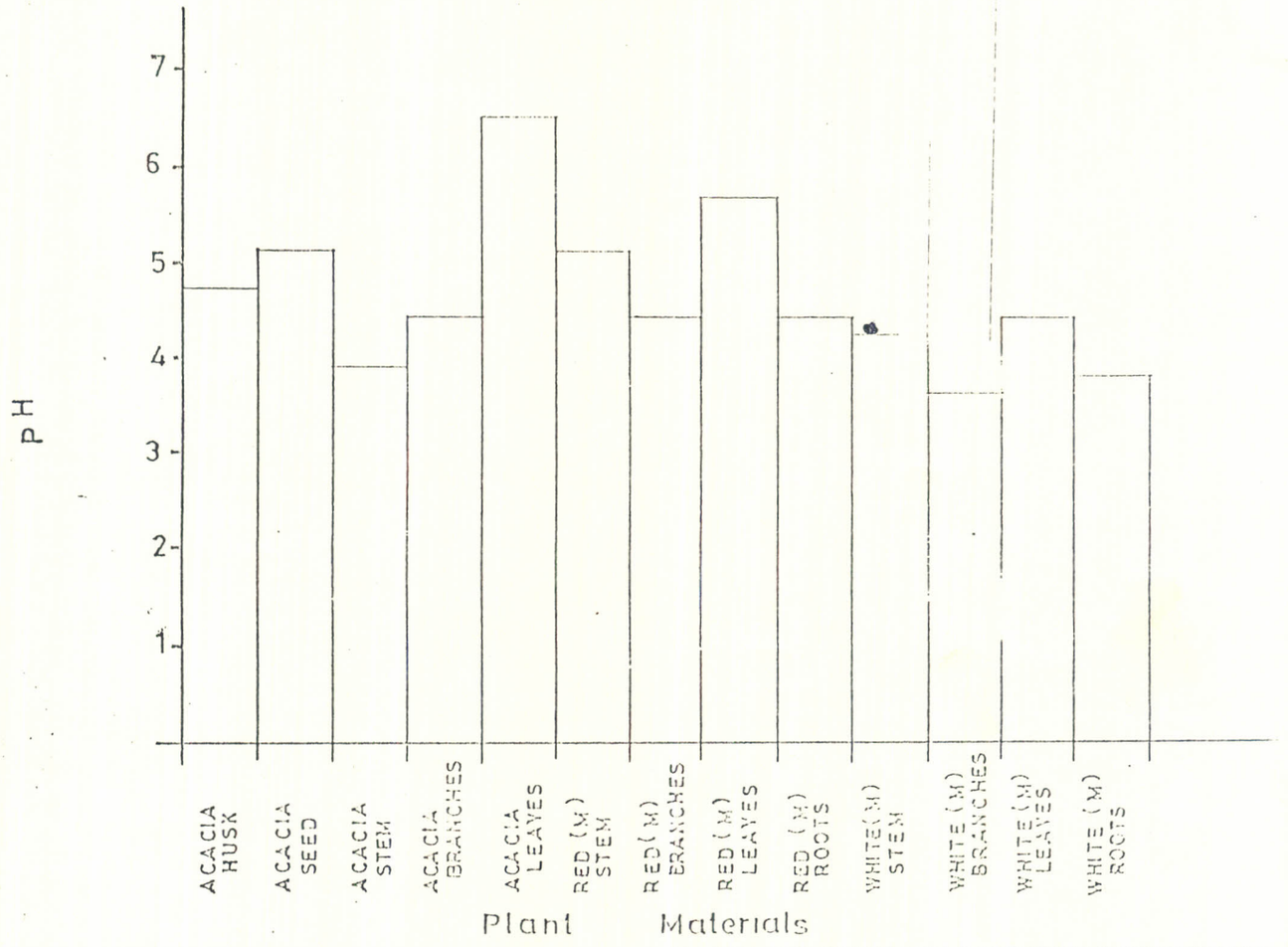


Fig 3·6 Histogram of percentage moisture

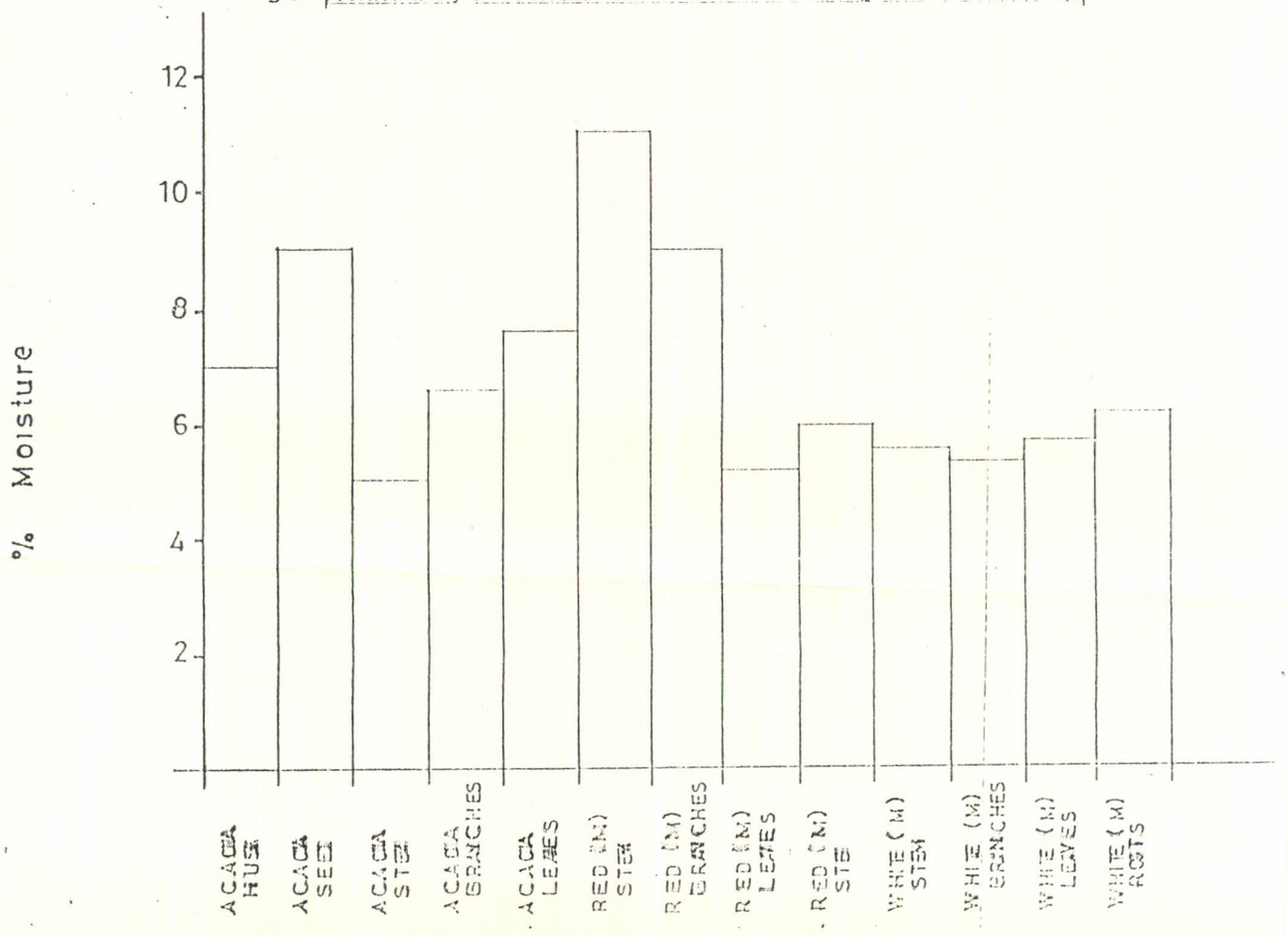


Fig 3·9 Distribution of tannin content of white mangrove

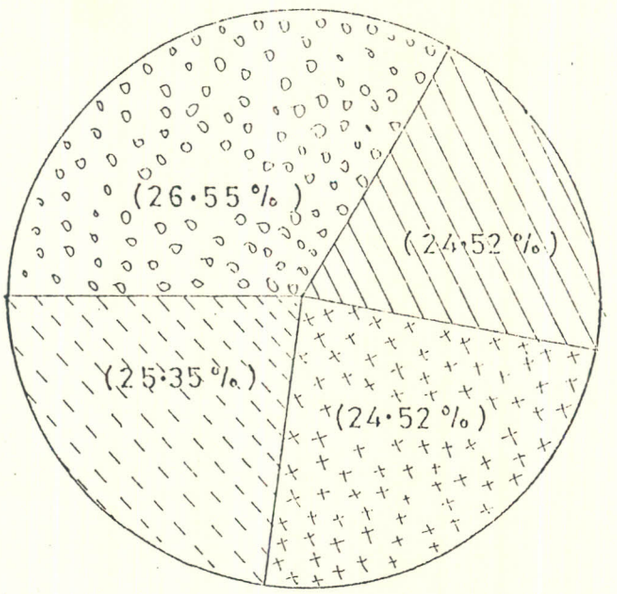


Fig 3·10 Relationship of the total tannin content of the plant samples

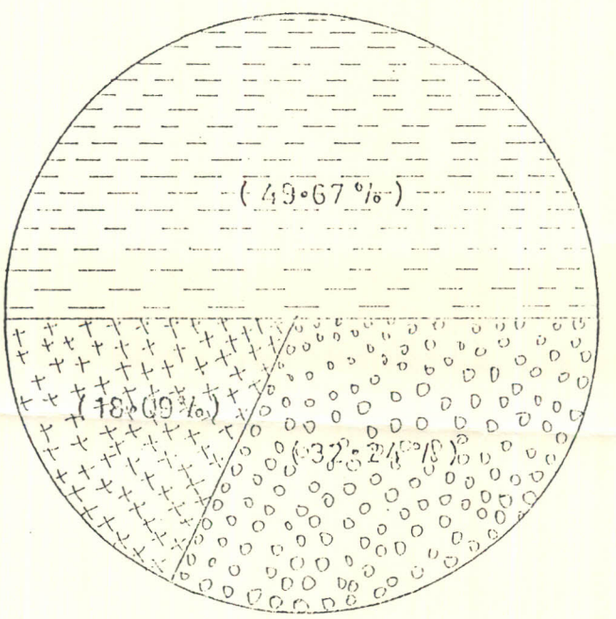
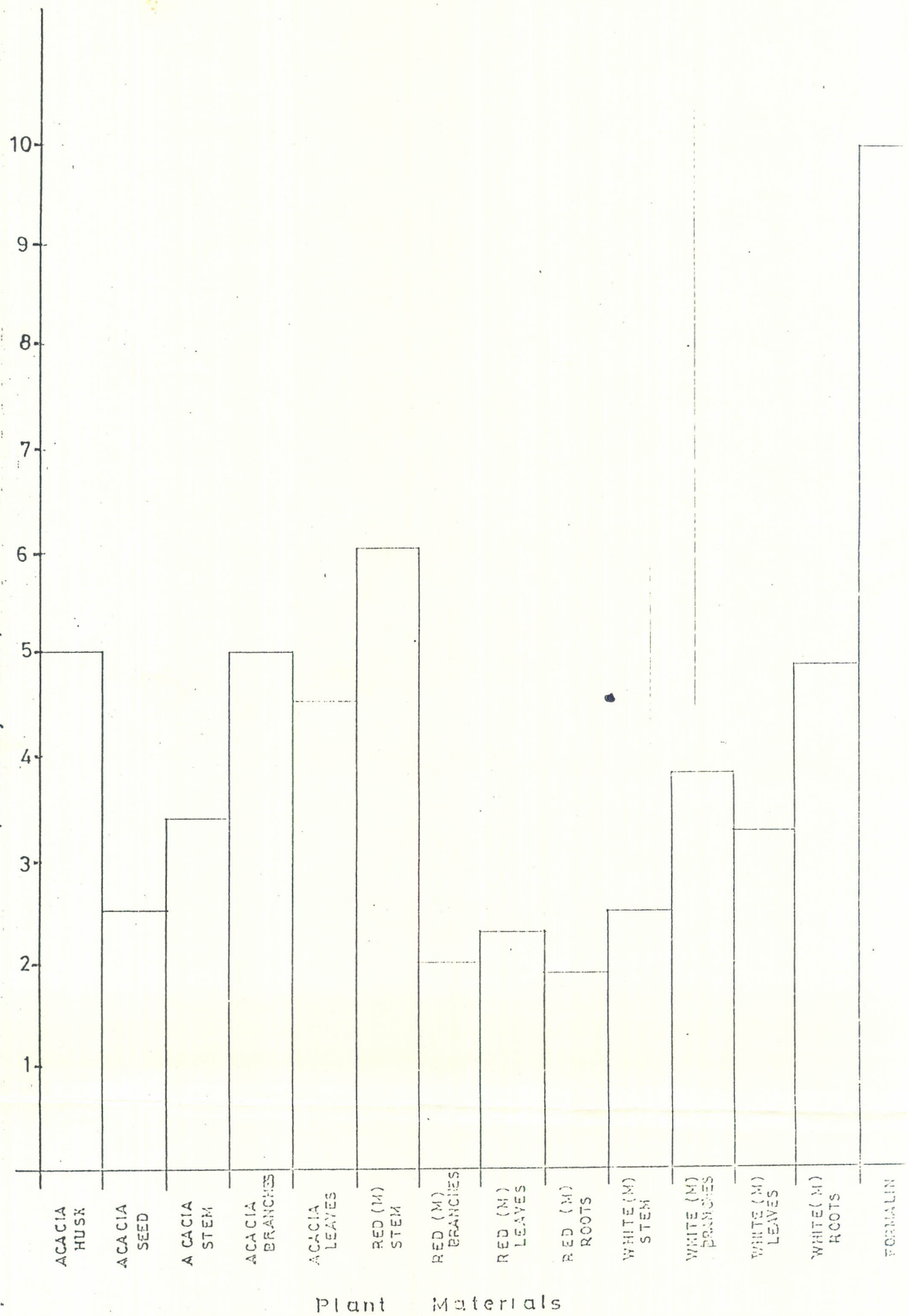


Fig 3:11 Level of preservation :



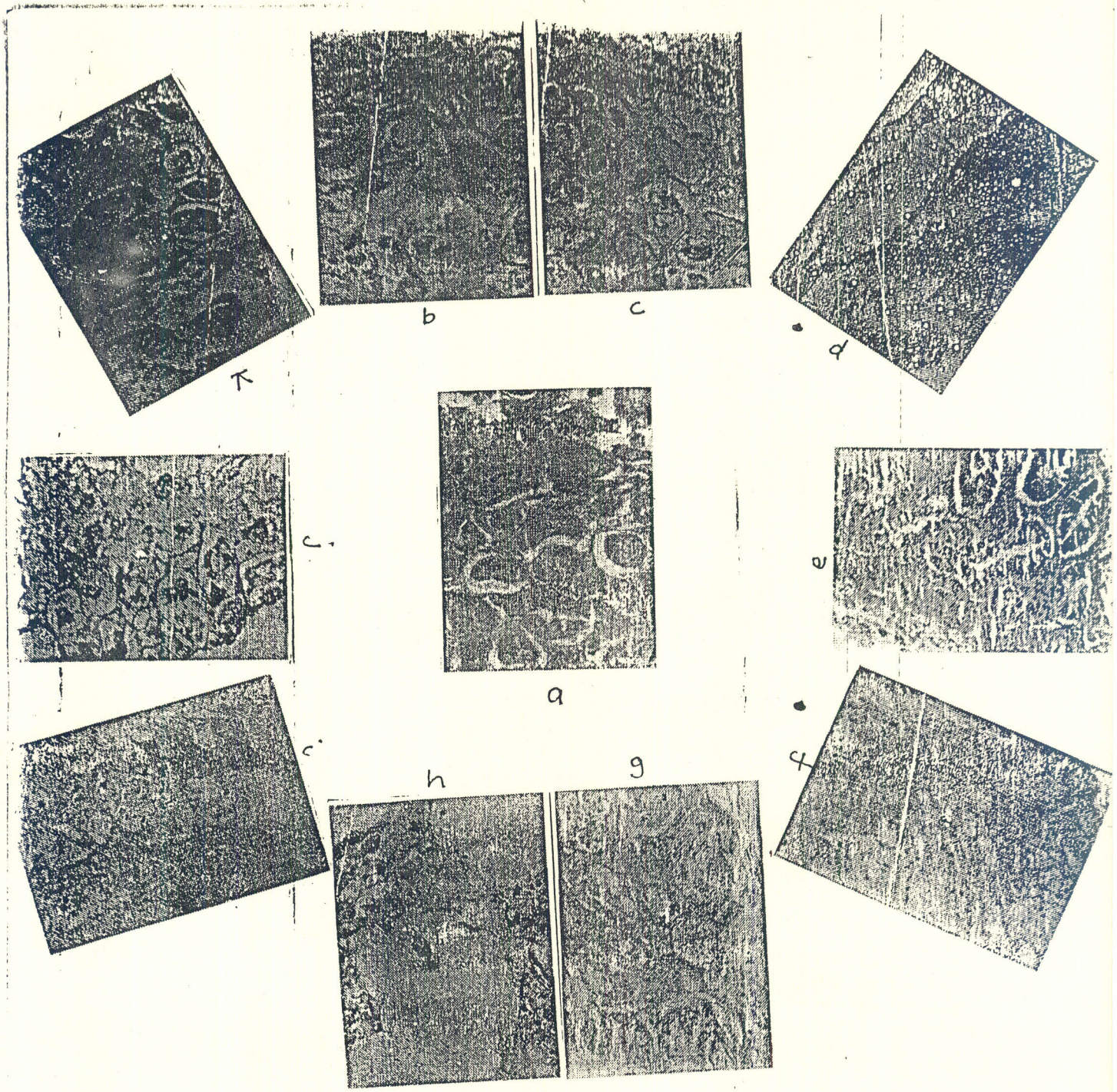


Fig. 3.12

a = KIDNEY + FORMALIN

b = KIDNEY + RED(M) STEM

c = KIDNEY + RED(M) LEAVES

d = KIDNEY + RED(M) ROOTS

e = KIDNEY + WHITE(M) STEM

f = KIDNEY + WHITE(M) BRANCHES

g = KIDNEY + WHITE(M) ROOTS

h = KIDNEY + ACACIA HUSK

i = KIDNEY + ACACIA SEED

j = KIDNEY + ACACIA BRANCHES

k = KIDNEY + ACACIA LEAVES

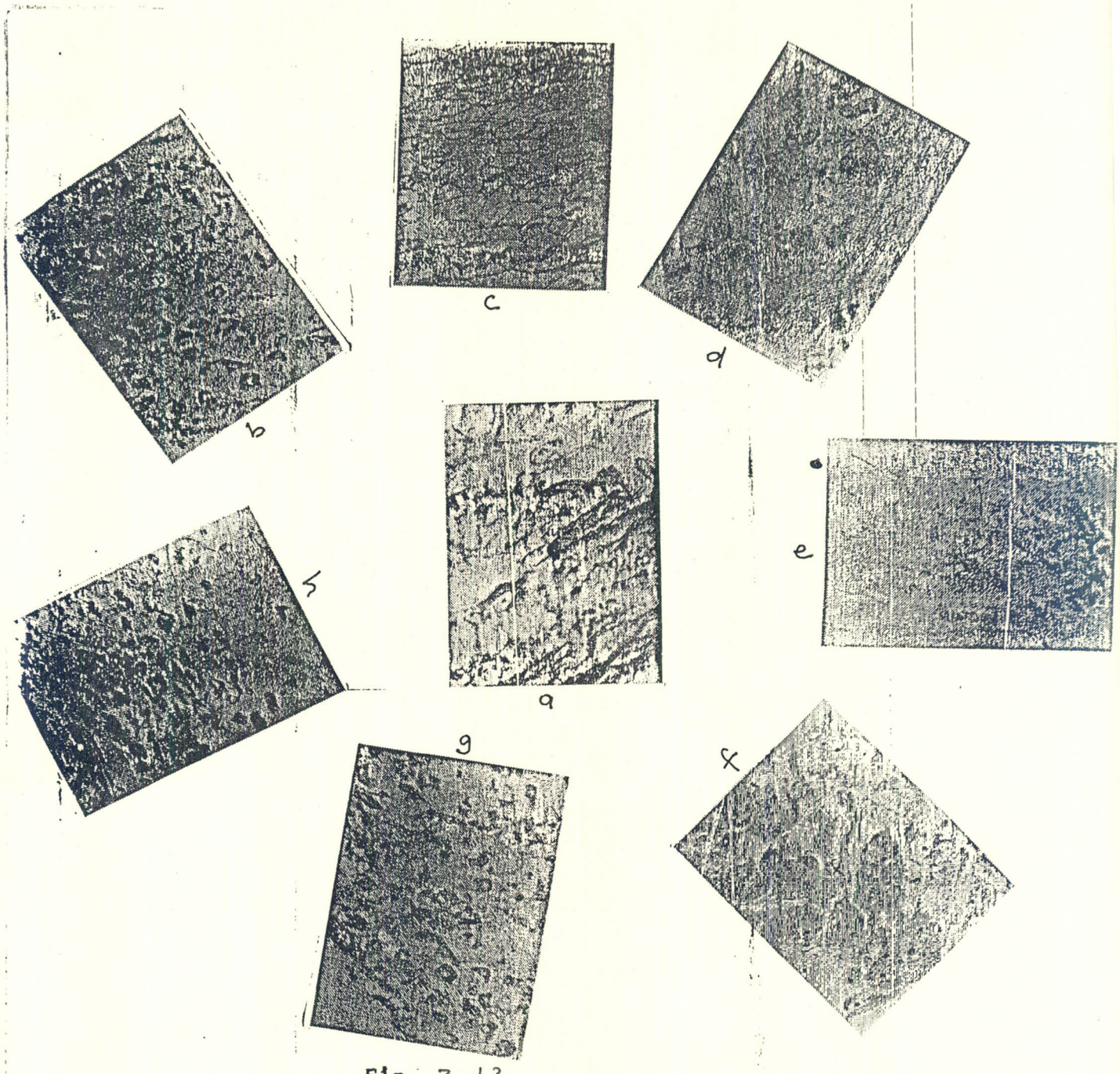


Fig. 3·13

a = PANCREAS + FORMALIN

b = PANCREAS + RED(M) STEM

c = PANCREAS + RED(M) BRANCHES

d = PANCREAS + WHITE(M) BRANCHES

e = PANCREAS + WHITE(M) ROOTS

f = PANCREAS + ACACIA BARK

g = PANCREAS + ACACIA BRANCHES

h = PANCREAS + ACACIA LEAVES

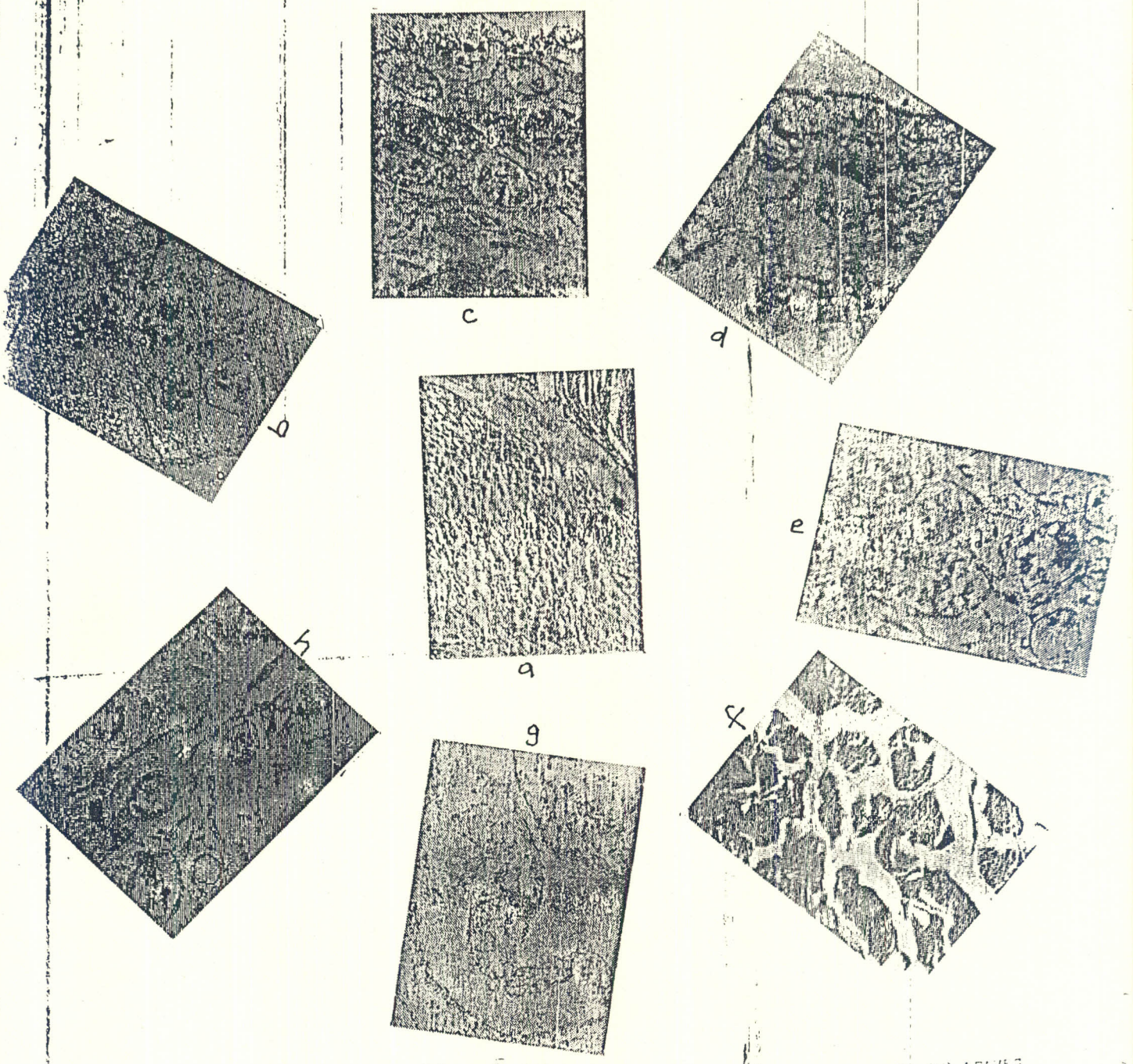


Fig. 3.14

a = INTESTINE + FORMALIN

b = INTESTINE + RED(M) LEAVES

c = INTESTINE + RED(M) ROOTS

d = INTESTINE + RED(M) STEMS

e = INTESTINE + [illegible] (M) LEAVES

f = INTESTINE + ACACIA HUSK

g = INTESTINE + ACACIA STEM

h = INTESTINE + ACACIA LEAVES

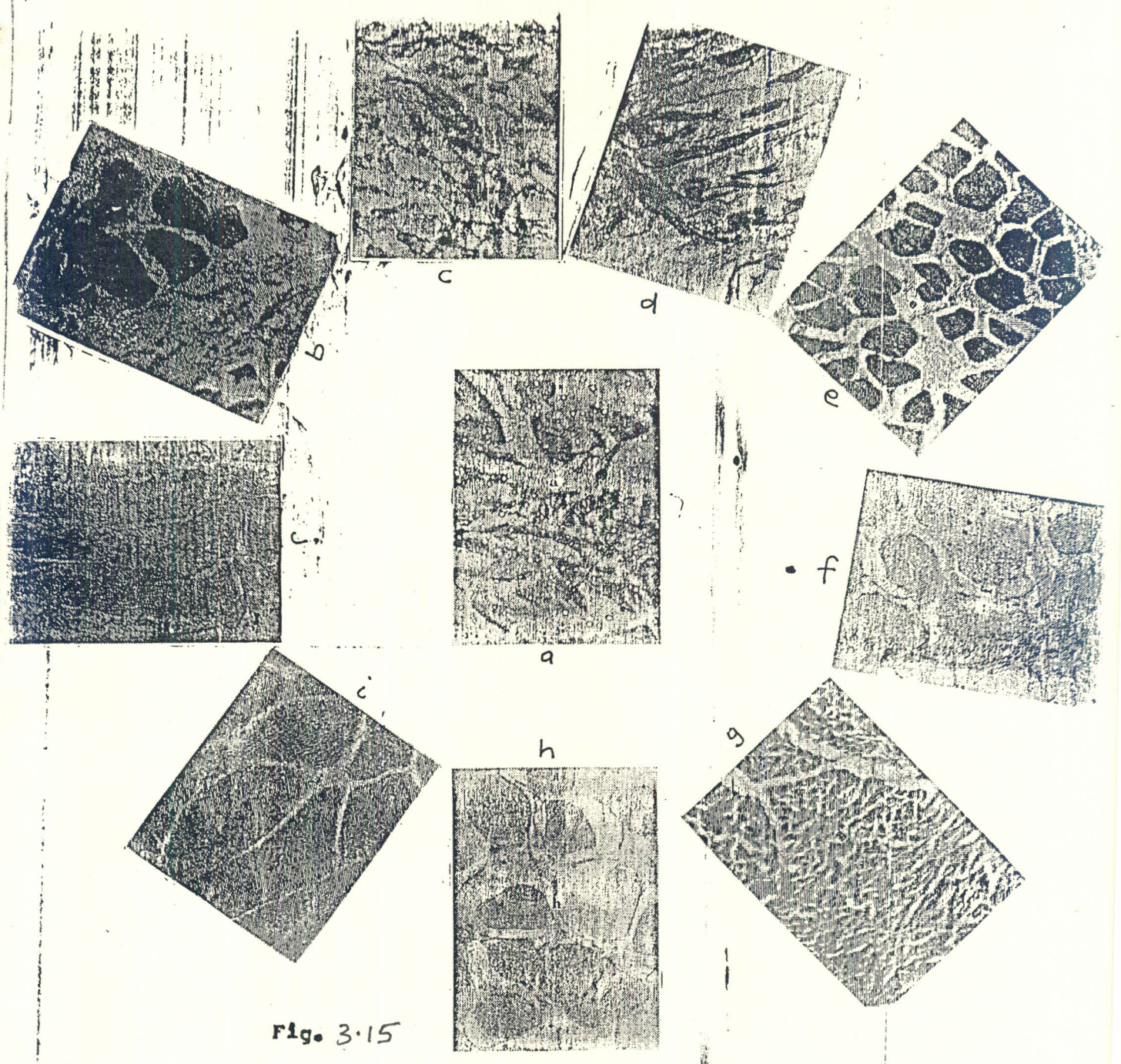


Fig. 3.15

a = MUSCLE + FORMALIN

b = MUSCLE + RED(M) STEM

c = MUSCLE + RED(M) LEAVES

d = MUSCLE + RED(M) ROOTS

e = MUSCLE + WHITE(M) STEM

f = MUSCLE + WHITE(M) LEAVES

g = MUSCLE + WHITE(M) ROOTS

h = MUSCLE + ACACIA HUSK

i = MUSCLE + ACACIA SEED

j = MUSCLE + ACACIA BRANCHES

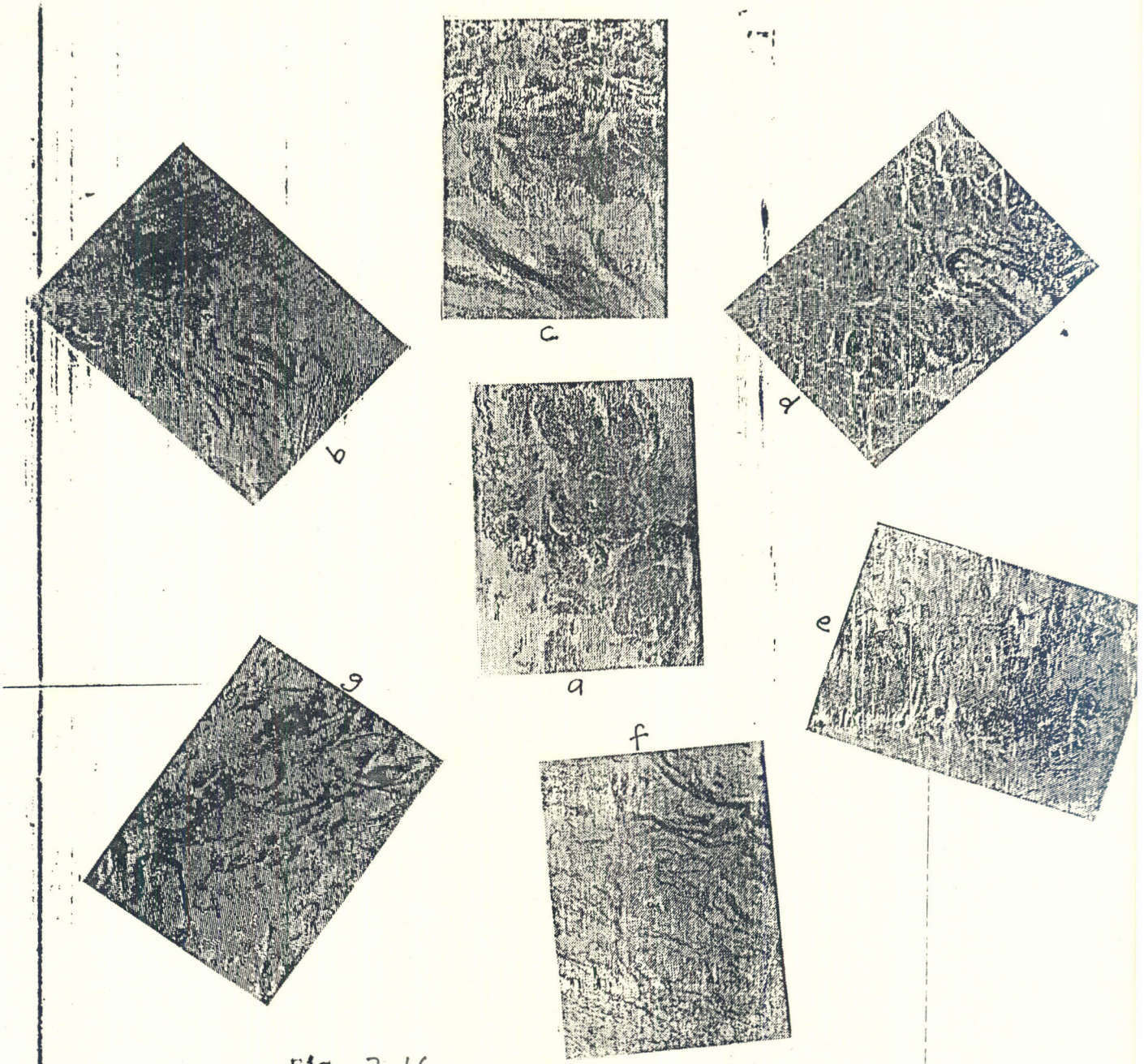


Fig. 3.16

- = STOMACH + FORMALIN
- = STOMACH + RED(M) STEM
- = STOMACH + WHITE(M) BRANCHES
- = STOMACH + WHITE(M) ROOTS

e = STOMACH + ACACIA HUSK

f = STOMACH + ACACIA STEM

g = STOMACH + ACACIA BRANCHES

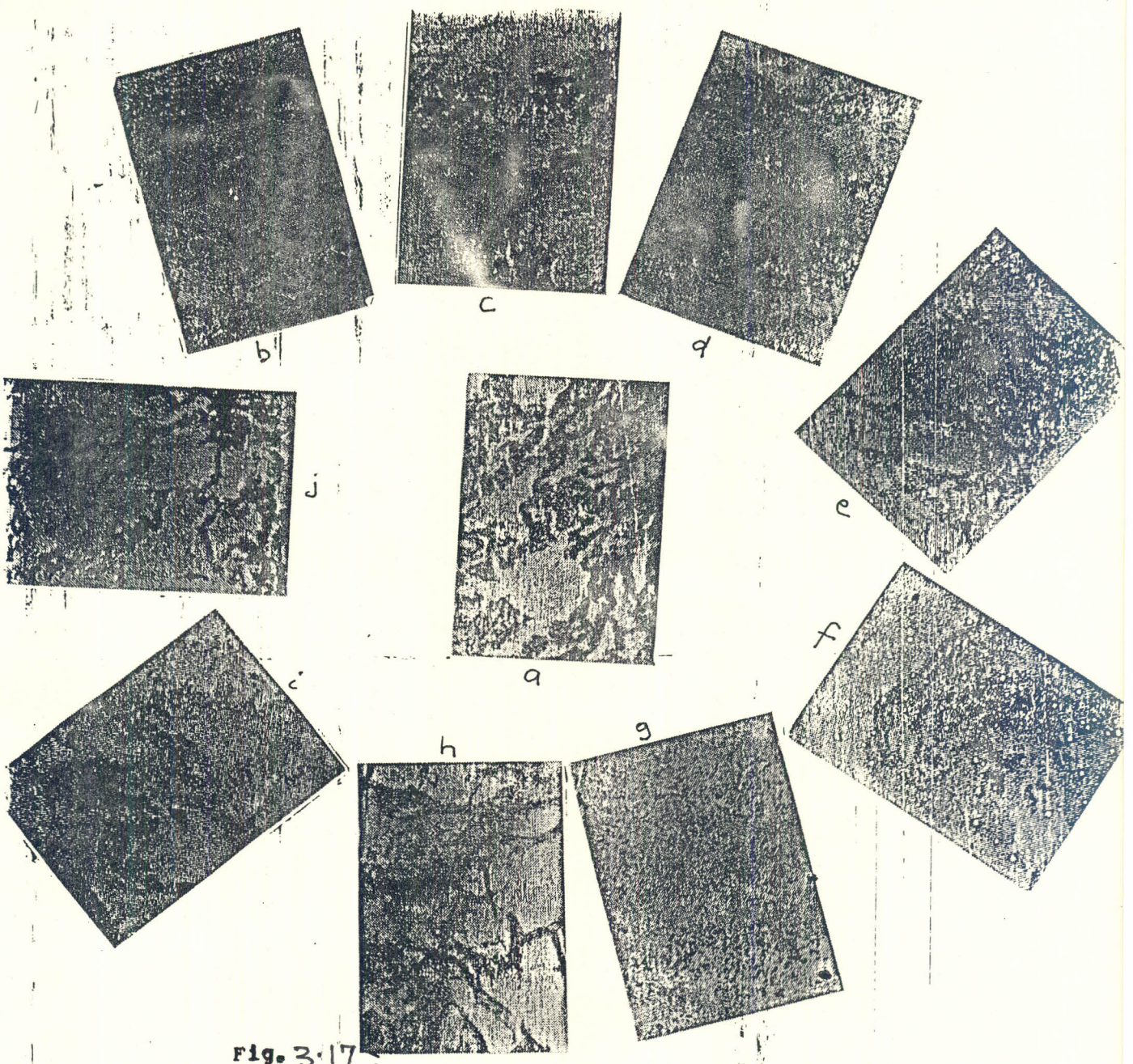


Fig. 3.17

a = LUNGS + FORMALIN

b = LUNGS + RED(M) BRANCHES

c = LUNGS + RED(M) STEM

d = LUNGS + WHITE(M) STEM

e = LUNGS + WHITE(M) BRANCHES

f = LUNGS + WHITE(M) LEAVES

g = LUNGS + ACACIA BUSK

h = LUNGS + ACACIA SEED

i = LUNGS + ACACIA STEM

j = LUNGS + ACACIA LEAVES

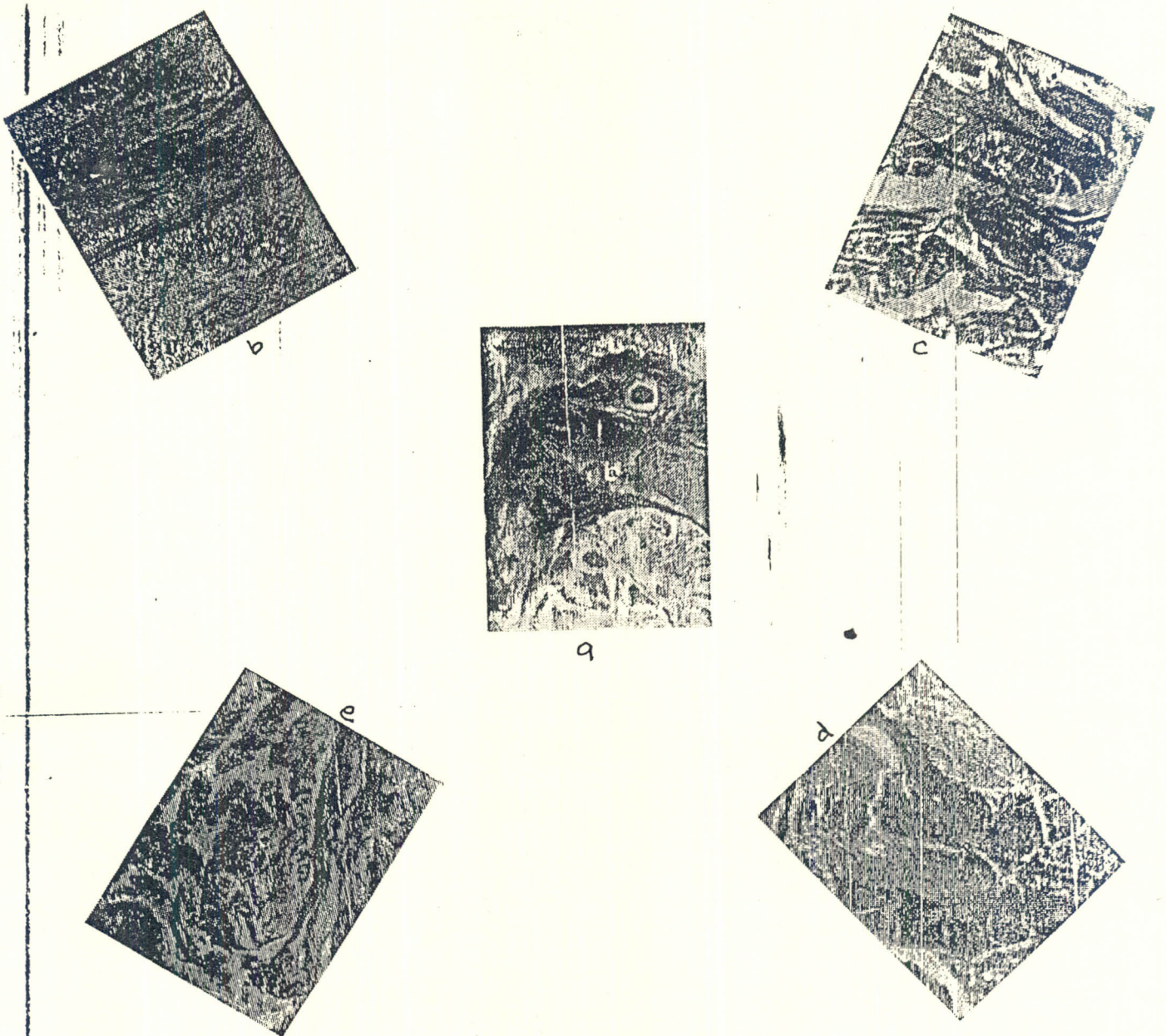


Fig. 3.18

a - SKIN + FORMALIN

b - SKIN + RED(M) BRANCHES

c - SKIN + WHITE(M) STEM

d - SKIN + WHITE(M) LEAVES

e - SKIN + ACACIA BRANCHES

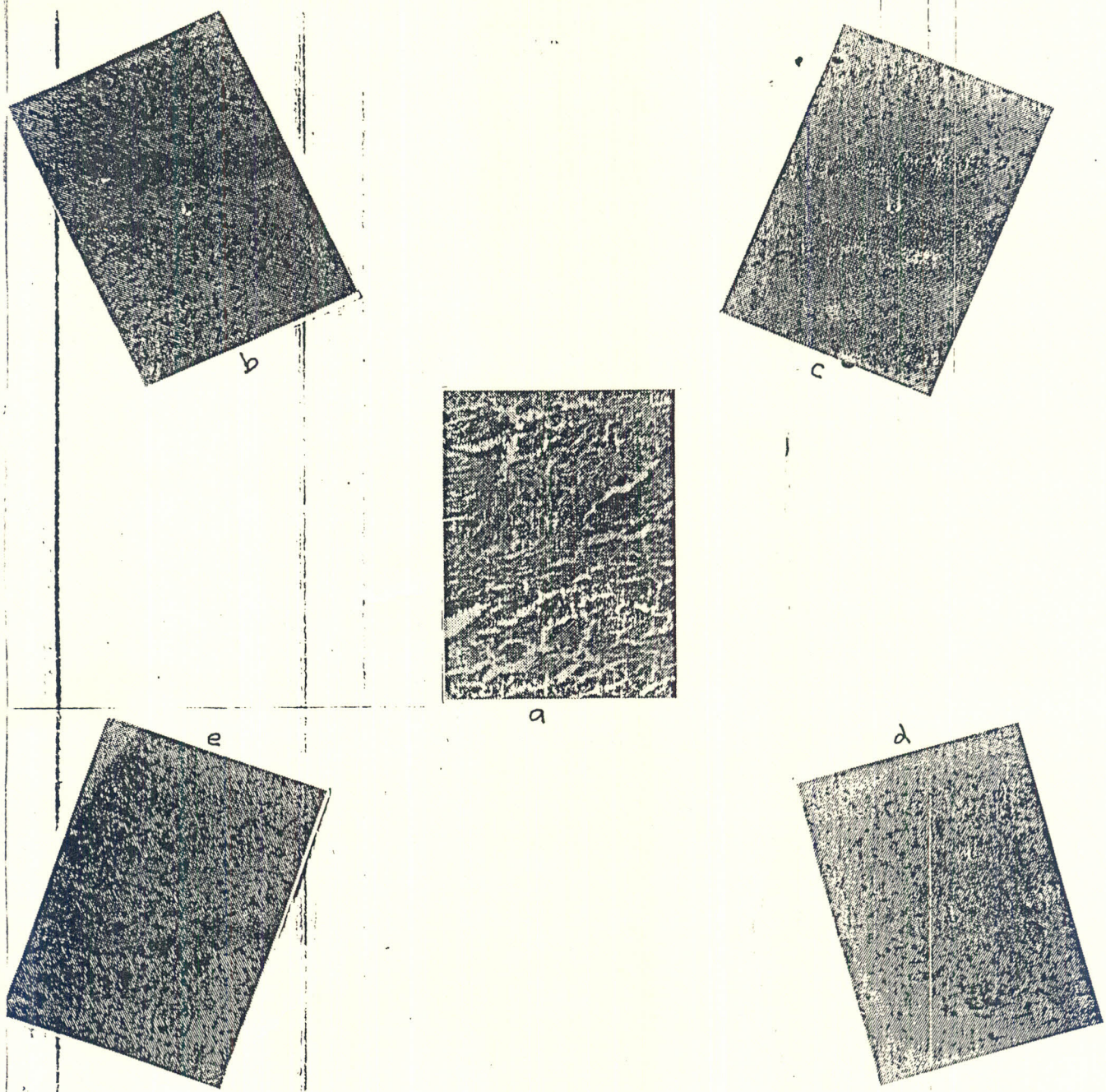


Fig. 3.19

a = SPLEEN + FORMALIN

b = SPLEEN + RED(M) STEM

c = SPLEEN + WHITE(M) STEM

d = SPLEEN + WHITE(M) ROOTS

e = SPLEEN + ACACIA HUSK

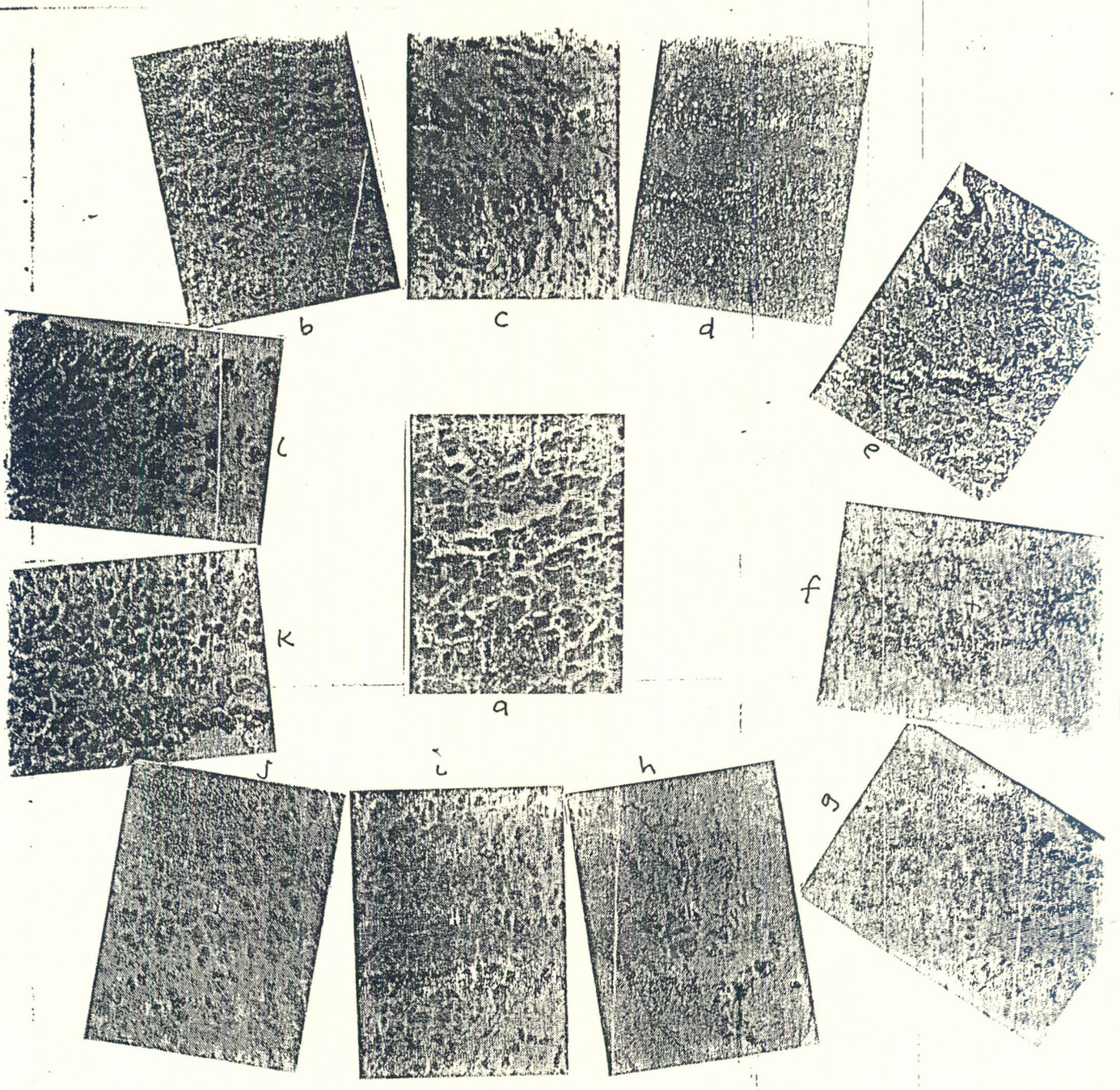


Fig. 3.20

a = LIVER + FORMALIN

b = LIVER + RED(M) STEM

c = LIVER + RED(M) BRANCHES

d = LIVER + RED(M) ROOTS

e = LIVER + WHITE(M) STEM

f = LIVER + WHITE(M) BRANCHES

g = LIVER + WHITE(M) LEAVES

h = LIVER + WHITE(M) ROOTS

i = LIVER + ACACIA RED

j = LIVER + ACACIA STEM

k = LIVER + ACACIA BRANCHES

l = LIVER + ACACIA LEAVES

3.2 DISCUSSION

Figures 3.1, 3.2 and 3.3 show the histograms of percentages of total solids, total solubles and non - tannins respectively.

From the results in Table 2.1 and figure 3.4 it is observed that Acacia nilotica, Red mangrove and White mangrove contain appreciable amounts of tannin in their different tissues.

Moreover, tannin is known to occur widely in the vegetable kingdom. In the temperate zone, Hemlock, Oak, Quebracho etc. are tannin-bearing plants. This work has confirmed that these tropical plant species contain tannin in appreciable quantities. The variations in the tannin content with respect to the plants and their tissues are hardly surprising as organic compounds such as flavones, alkaloids, terpenes are known to concentrate in different tissues of a given plant. These variations may be due to geographical as well as nutrient distribution.

Table 2.2 and figure 3.11 show the degree of preservation recorded with the different plant extracts, as well as with the extracts from different parts of each plant. The histological studies revealed that plant extracts preserved the animal tissues as effectively as formalin. Though there are variations in the degree of preservation of different extracts this may be due to the type of tannins present in each of the extracts. It may also be due to the differences in the rate as well as level of penetration of the tannins in each of the extracts. The variations can also

occur from the nature or type of animal tissues under consideration. The concentrations of tannins, non-tannins, or the pH of each of the extracts used can also result in varying degrees of fixation.

From the results depicted in figure 3.12 the Red mangrove stem and leaves, White mangrove branches and root and Acacia seed branches and leaves preserved the kidney fairly well. The glomeruli and capsules were intact but with less distortions. White mangrove stem, Red mangrove root and Acacia grains fixed this organ poorly, with the disintegration of capsules and glomeruli.

In Figure 3.13, all the plant extracts preserved the pancreatic system very well, Red mangrove branches, White mangrove root, and Acacia leaves retained the parenchyma cells well in animal tissues. Similarly, for Red mangrove stem, White mangrove branches and Acacia grains and branches, the connective tissues did not suffer any distortion. These extracts preserved the pancreas in a better form than formalin.

It is apparent from Figure 3.14 that Acacia grains, stem and leaves, Red mangrove root, and White mangrove leaves fixed the intestine as effective as formalin.

As can be seen in Figure 3.15 muscle fibre bundles are not putrefied in these extracts after several months. Finer muscle details such as striation are still visible.

It is shown in Figure 3.16, that most of the plant extracts stabilized the stomach tissues very well.

From the results discribed in Figure 3.17, all the

plant extracts preserved the lungs fairly well.

Figure 3.18, shows that Red mangrove branches, White mangrove stem, leaves and Acacia branches generally preserved the skin.

In figure 3.19, Red mangrove stem, White mangrove stem, root, and Acacia grains kept the spleen connective tissues and lymphocytes from decomposition.

Also from Figure 3.20, Acacia seed, stem, branches and leaves fixed the liver cells very well. The remaining plant extracts poorly preserved the hepatocytes of the liver with remarkable distortion.

CHAPTER FOUR

4.1 CONCLUSION AND RECOMENDATION

From the work described in this thesis, the following conclusions are drawn:-

1. all the plants studied, viz, Acacia, nilotica, Red and White mangroves contain varying degrees of tannin and/or other fixing agents in their different tissues such as the stem, root, branches, leaves and seeds (for acacia only).
2. these fixatives (plant extracts) are extractable in cold water.
3. these plant extracts containing 4g/dh of tannin preserve animal (cow) tissues and organs in such a way that after 7 months there is no evidence of putrefaction.
4. histological studies indicated that these organs and tissues are preserved intack.
5. the degree of preservation depended not only on the nature of the organs or tissues, but primarily on the part of the plant from which the extract is made.
6. the ability to preserve these organs and tissues also varies from one plant extract to another.
7. though the concentration of the tannin is neccessary for the level of preservation, it is perhaps possible that the chemical nature of the tannin/non-tannin present may have a role.
8. in most cases, it is found that formalin, a traditional fixative for animal tissues/organs performed better

than these vegetable extracts. However further work is needed to fully understand this. Other factors such as the concentration of the extracts, temperature, pH, the nature of the tissues/organs (including the presence of adipose tissues) etc. should be studied.

9. in some cases (such as in acacia grains extract) it was observed that the tissues preserved by the vegetable extracts are coloured. This is significant in the sense that for histological studies further staining may not be necessary.

10. however it must be emphasised that these plant extracts, when left for long form particles called "bloom" and also grow fungi.

11. during preservation exercise some tissues such as brain get dissolved in the plant ectracts. This observation is difficult to explain. Perhaps it may be due to the large number of globular protein in the brain tissue.

13. the texture of all the animal tissues fixed in the plant extracts are softer than those preserved in formalin. This shows that there is no shrinkage of these tissues in the extracts which is an advantage over formalin where there is shrinkage.

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