ASSESSMENT OF LEATHER QUALITY PRODUCED BY OPTIMIZING LIMING AND INCREASING REACTIVITY OF TANNINS OBTAINED FROM *CAESALPINIA CORIARIA* AND *PARKIA CLAPPERTONIANA*

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN ANALYTICAL CHEMISTRY

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DECEMBER, 2017

DECLARATION

I declare that the work in this thesis entitled "Assessment of Leather Quality Produced by Optimizing Liming and Increasing Reactivity of Tannins obtained from *Caesalpinia coriaria* and *Parkia clappertoniana*" has been carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged by way of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Irimiya Akawu

___________________	___________________	___________________
	Signature	**Date**

CERTIFICATION

This thesis entitled **Assessment of Leather Quality Produced by Optimizing Liming and Increasing Reactivity of Tannins obtained from *Caesalpinia coriaria* and *Parkia clappertoniana*** by Irimiya AKAWU meets the regulations governing the award of the degree of Doctor of Philosophy in Chemistry of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my beloved late Daddy, Akawu Gado Gidan Tagwai.

ACKNOWLEDGEMENTS

My Sincere and deepest gratitude to Almighty God for His sustenance and guidance through my period of studies in Ahmadu Bello University, Zaria. My hearty thanks go to my supervisors Prof. V.O Ajibola, Prof. C.E. Gimba, Dr E.D Paul and Late Dr. I.K. Adamu, for their expertise and constructive suggestions towards the success of this project.

I would like to express my wholehearted thanks to my parents Late Mr. Akawu Gado, Mrs. Rakiya Akawu and my Disciplers Dr. & Mrs. Moses Galadima, Dr. & Mrs. Mohammed Jibril and the entire brethren for their kind support and prayers towards me. I would also like to thank the Nigerian Institute of Leather and Science Technology (NILEST), Samaru-Zaria for the permission to undergo a PhD study and the Department of Chemistry, Ahmadu Bello University, Zaria.

Special thanks go to Mr. C.C Maju the Head of Department of Leather Technology and Dr. J.I Tagang the Director of Leather Technology, NILEST for their inputs and supply of relevant materials for this research and Justin Thomas for editing this thesis and Mr. Emmanuel Akawu, Mr.& Mrs. B. Obaje and Humphrey Nchom for their moral and financial support.

I also acknowledge the contributions of Dr. E. N. Oparah, Prof. (Mrs.) E.B. Agbaji, Dr J Habila, Prof. D.S Yawas, Pastor S.A. Ekwuribe, Mr. S. F. Tanko, Mr. C. Emeka, Mr. A.Laban, Mr. A. Levi, Mr. A. Enoch, Mr. I. Ashiru, Mr. A. Yusuf, Mrs. H. Abebe, Miss H. Danladi, Mr.A. Simon, Mr. J. Yantai, Mal. A. Adamu, Mr. Okon, Mr. Y. Arung, Mr.I. Awan, Mr. A. Elkana, Mr. M. James, Mr. P. Ayodele and Sis. N. David.

Finally, I am indebted to show my special thanks to my very dear wife, Mrs. Patience Akawu and my sons (Kyomnom Israel, Bethel Kpeiknom and the unborn), Pastor Azariah Akawu and Pastor Iliya Netson for being there for me. Love you all.

ABSTRACT

Tannin chemically stabilizes hides and skins against bacterial attack, transforming the raw material into leather. In the search for alternative sources of tanning materials, only a few of these materials have shown true value as commercial products in the vegetable tannin field. The negative effect of trivalent chromium both to the tanners and the environment has increased the search for alternative tanning materials which could be eco-friendly.Pods of *Caesalpinia coriaria* (DD) and husk of *Parkia clappertoniana* (PC) tannins were extracted using Procter apparatus and Soxhlet apparatus. The crude extracts of both DD and PC were purified using gel permeation chromatography and modified using sodium bisulphite and pancreatic trypsin .The modified tannins were characterized using UV-VIS, FT-IR, TLC, sorptive capacity and binding ability. The modified tannin and 16different tannin ratios of DD and PC were used in the tanning of goat skin. The result of the qualitative and quantitative assessment placed DD to be hydrolysable tannins with 47% pure tannins and PC to be condensed tannins with 42.33% pure tannins. This shows that the two plants have more than 10 % threshold tannin content required for commercial extraction. The TLC of methanolic extract of DD and PC using glacial acetic acid indicated the components of the tannins to be 3,4,6,tri- O- Galloyl-D-glucose, tannic acid ,pyrogallol and gallic acid for DD, and catechol, gallic acid for PC. The ultraviolet visible absorption spectra for the modified PC and DD was found to be lower than that of the unmodified. A peak for modified PC was found to be at 460 nm while the unmodified was at 480 nm. The use of sodium bisulphite to modified PC and DD shows a good effect by reducing the large molecular weight of PC and increasing the reactivity of PC and DD. The use of pancreatic trypsin was found to be suitable for PC. PC extract has the best properties in terms of binding and sorption by 45.50% and 95% respectively at 10% sodium bisulphite followed by

DD extract which has 15.33% and 46% binding and sorptive capacity respectively at 10 % sodium bisulphite. On the contrary, DD showed no significant change as the concentration of pancreatic trypsin increased. Also, the binding capacity of PC increased from 12% to 25% with the increase in the concentration of pancreatic trypsin from 2% to 8%,whereas DD showed no significant change. The pH (4.03) of DD could inhibit enzyme activity. The FT-IR spectra of modified sample of DD gave similar absorption bands with the unmodified except with the removal of three bands (1624.5, 965.4 and 670.9 cm^{-1}) which arose from C = O, C = CH and Ar-H bending out of plane. Also, PC spectra gave similar bands except the presence of C – H stretching in aromatic methoxyl groups (2926.0; 84.933 cm^{-1}) in the modified which was not found in the unmodified. This could be attributed to the break of bond or hydrolysis of the high molecular weight of the tanning by the reagent (sodium bisulphite).The liming process was optimized using Box Behnken design The quadratic polynomial model was used to characterize the liming process. The plots showed that these independent variables significantly influenced the liming conditions. In this work, it was established that the best condition for the liming process was found to be: 1.5 %, lime concentration, 20 hr and 300 % water. The quality of the leathers was determined according to the International Union of Leather Technologists and Chemist Society (IULTCS) official methods. The values obtained, met the standard requirement for vegetable leathers. A careful selection of the percentage liming used in the liming process and Fatliquoring of the tanned leather with vegetable tannins such as DD and PC with modification using sodium bisulphite and pancreatic trypsin could be used to produce a quality leather and serve as a substitute for chrome tanning or as complementary, since it is possible to get Shrinkage Temperature of 90 ^{o}C.

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Definitions and Glossaries of Terms

Bating:	A processing step that follows liming. Enzymes are used to impart softness, stretch and flexibility to the final leather.
Beamhouse:	The early stages of processing in the tannery where fleshing, liming, unhairing, bating and pickling are done.
Bloom:	Insoluble residues formed when hydrolysable tannins (ester-based plant polyphenols) are hydrolysed during tanning.
Condensed-Tan:	Vegetable tannin with flavonoid structure – also called catechol
Deliming:	The step after liming; when the pelt is prepared in readiness for the subsequent step (bating) at pH 8-9. The commonest reagent is ammonium salt. Chloride is preferred to sulphate, but carbon dioxide is increasingly used.
Divi-divi:	*Caesalpinia coriaria*
DSC:	Differential scanning calorimetry: a technique for measuring the thermodynamic parameters associated with a reaction or transition by determining energy absorption or energy produced as the temperature rises at a specific rate.
Flaying:	Process of removal of the hide or skin from the animal carcass.
Fleshing:	Process of removing any adipose tissue on the flesh side of the skins and usually done after liming.
Grain burst:	A physical test that indicate a point at which the grain surface of the leather burst after distention
Grain crack:	A physical test that indicate a point at which the grain surface of

the leather cracks after distention.

Grain: Indicates the outer hair side of a hide or skin after the hair and epidermis have been removed

Gums: The high molecular weight fraction of vegetable tannins, > 3000 Da: complexes of carbohydrates with polyphenols. They are undesirable components because of their reaction on the pelt surface.

Hide: Pelt of a large animal, e.g. cattle.

Hydrolysable tan: Vegetable tannin based on saccharide esterified by polyphenol – also called pyrogallol tannin.

Insoluble: The amount of materials present in any vegetable tanning materials that are not soluble in water.

Leather tanning: Numerous steps involved in converting animal hide and skins into finished leathers.

Leather: Hide or skin with its original fibrous structure more or less intact, tanned to be imputrescible (British Standard Glossary of Leather Terms, British Standards Institution, BS 2780:1983). The definition excludes any material made from waster tanned bye products, such as shavings reconstituted into polymer matrix; these materials are often designated Leather board or by other name connoting leather.

Non-Tans: Low molecular weight fraction of vegetable tannin extracts, < 500 Da. They are unreactive as tanning agents, but useful in the tanning process for solubilising the tannins.

Pelt: Hide or skin that is being processed in the beamhouse.

Pickling:	Acidification of pelt in brine for preservations or preparation prior to tanning.
Soaking:	First stage in processing, primarily to rehydrate preserved skin. Usually involves a preliminary dirt soak, one or more main soaks and rinsing.
Syntan:	Synthetic tanning agent: polymerized aromatic hydroxyl compounds, designed to perform various functions depending on the structure, including assisting chemical processes and acting as solo tanning agents; like vegetable tannins.
Tannin content:	The proportion of tannins in the vegetable tanning materials.
Tanning materials:	Any materials that are used in leather production and they include woods, barks, fruits and leaves from various plants.
Tanning strength:	Ratio of tannins to soluble non-tannins and indicate the ability of the tanning liquor to produce good leathers.
Tanning waste materials:	Parts of plants e.g. barks, wood, fruits and leaves that are disposed to the environments after extraction of tannins.
Tanning:	Conversion of putrescible organic material skin into a stable material capable of resisting biochemical attack.
Tans:	Usually applied to vegetable tannins; the molecular weight fraction 500 – 3000 Da. of vegetable tannin extracts distinguished from the 'non-tans' and the 'gums.'
Total solids:	The amount of solid materials contained in any tanning materials. This is generally obtained by deducting the moisture content from the total weight of the materials.
Total soluble:	The total amount of water soluble materials present in any

vegetable tanning Materials.

Shrinkage temperature:is the most common measurement of hydrothermal stability; an increase in T_S indicates a consequence of tanning. Sometimes, this term is used interchangeably with 'denaturation temperature and occasionally with 'melting temperature.'

Unhairing: Removal of hair, usually by chemical means, but biochemical treatments are known. In this context, the general term unhairing include removal of wool or bristle.

Vegetable tan: Polyphenolic mixture of plant origin; may be in the form of ground plant material, but now more usually refers to dried extracts of plant material.

Wet Blue: it was because it is not usually dried before progressing to post tanning and it is blue because of the nature of the chrome complexes bound to the collagen.

Wet White – Non-Chrome tanned leather or chemically stabilized pelt, capable of being split and shaved.

LIST OF ABBREVIATIONS

Abbreviations:

AN:	*Acacia nilotica*
BIS:	Bureau of Indian standards
DD:	*Caesalpinia coriaria*
DSC:	Differential Scanning Calorimetry
FT-IR	Fourier Transform Infrared Spectroscopy
IULTCS:	International Union of leather technologist and chemist society
IUP:	International Union for physical testing
NT:	Non- tannins
PC:	*Parkia clappertoniana*
SLTC:	Society of Leather Technologist and Chemist
TS	Tensile Strength
UNIDO:	United Nations Industrial Development Organization
min	minutes
Ts	Shrinkage Temperature

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Raw hides and skins are converted to leather in a process called tanning (Mahdi *et al.*, 2009). Tanning chemically stabilizes hides and skins against bacterial attack and convert the raw material into leather (Leticia *et al.,* 2015). The conventional use of tannins as agents for converting animal hides and skin to leather is one manifestation of the most evident activity of the tannins, which is their ability to interact with and precipitate proteins, including the proteins found in animal skin. There are two types of Tanning: mineral (Chromium) and vegetable tannings. Chromium is commonly used as mineral tanning agents (Commision, 2003).One of the desirable qualities of chrome tanning over vegetable tanning is the high speed of protein fixation leading to the high shrinkage temperature of the resultant leather and an extra ordinary dyeing suitability.

Chrome tanning is the most widely used by the leather industry, because it makes leathers with excellent physical and organoleptic properties. Approximately 90 % of the tanning process is carried out with chromium (III) salts. However, only a fraction of chromium salts used in the tanning process (60%) reacts with the hides and the skins. The rest of the salt (40%) remain in the tanning exhaust bath and are subsequently sent to effluent treatment plant where the chromium salts end up in the sludge (Mwinyihija *et al.,* 2006). Leather wastes containing chromium are considered hazardous waste and must be placed in special landfills(Marsal *et al.,*2012, as cited in Spier *et al.,* 2015).

On the other hand, vegetable tanning has always been one of the most important alternatives to Chromium tanning as an environmental friendly system and it is considered the "green tanning agent" becauseof its biodegradation (Jianzhong*et al.*,2009,Spier *et al.,* 2015). According to Faxing *et al.* (2005) and Bi (2006), vegetable tanned leather has excellent fullness, mouldering properties, wear resistance, air permeability and solidness; hence, properties as mentioned above obtained from Chrome tanning may also be achieved by preparation and modification of the vegetable tannins. Vegetable tanned leather is used in making heavy leather such as furniture leather, garment leather and shoe upper leather. Worldwide, researchers are paying particular attention to the use of vegetable tanning agents to replace chrome tanning agent (Koloka and Moreki, 2011). Vegetable tanned leather is the oldest and most classic type of leather, combining the values of quality and tradition (Castiello *et al.,* 2008). To safeguard and improve these traditional leathers, Professor H.R. Procter, one of the pioneers in applying scientific principles to leather manufacture, said, “Science must follow before it can lead” (cited in Reed, 2013); there is a need to study the process that local tanners use and analyse the tanning strength of the vegetable tanning material they use (Nduru, 2015).

The principles of vegetable tanning have not changed since ancient times, still using the tanning properties of plant materials, mainly extracted from trees like oak, chestnut and mimosa, as well as quebracho and tara from Latin America. Previously, the skins were hung to macerate in vats in direct contact with the bark, roots, berries and leaves for lengthy periods of time (up to 18 months or even two years), but now, concentrated extracts are used. These have optimal tanning capacity and have allowed maceration times to be considerably reduced(Premier Vision Leather, 2017).

Vegetable tannins are polyphenolic secondary metabolites of higher plants, and are either galloyl esters and their derivatives, in which galloyl moieties or their derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores (gallotannins, ellagitannins and complex tannins), or they are oligomeric and polymeric proanthocyanidins that can possess different interflavanyl coupling and substitution patterns (condensed tannins) (Khanbabaee and Ree, 2001). Bate-Smith (1962) as cited by (Hagerman, 2002) defines tannins as "water-soluble phenolic compounds having molecular weights between 500 and 3000 giving the usual phenolic reactions and having special properties such as the ability to precipitate alkaloids, gelatin and other proteins".

There are three (3) major types of vegetable tannins: hydrolysable, non-hydrolysable (condensed) and complex tannins (Haroun *et al.*, 2013). The hydrolysable tannins are readily hydrolysed by acids, alkalis or enzymes (tannases) into a sugar or a related polyhydric alcohol (polyol) and a phenolic carboxylic acid. Depending on the nature of the phenolic carboxylic acid, hydrolysable tannins are subdivided into gallotannins and ellagitannins. Hydrolysis of gallotannins yields gallic acid while hydrolysis of ellagitannins yields hexahydroxydiphenic acid which is isolated as ellagic acid. Hydrolysable tannins are considered as one of the most potent antioxidants from plant sources (Haroun *et al.*, 2013). However, large molecular weights tannins limit penetration into the collagen fibres and lower the thermal stability or tanning effect (Leticia*et al.*, 2015). Complex tannins are the combination of both hydrolysable tannins and condensed tannins (Lokeswari and Bompalli, 2015). The condensed tannins produce leathers with high shrinkage temperature of 70 to 80 ^{o}C. Whereas, hydrolysable produce leathers with shrinkage temperature of 70 to 74 ^{o}C (Salminen *et al.*, 2011).

Collagen molecules in skin is composed of three polypeptide chains with triple helical structure, and they are summed up through hydrogen bonding to form collagen fibres(Liao, 2003). In the tanning process, the collagen reacts with the tannins.

Although, the aromatic ring of vegetable tannins contains phenolic hydroxys, the aromatic ring still keeps hydrophobicity. Studies have indicated that tannins first approach the surface of collagen fibres by hydrophobic bonding, and then combine with collagen fibres by multi-hydrogen bonding. Other components of plant extracts, including polyphenolic compounds with small molecular weight, are not able to form multi-hydrogen bonding with collagen fibres due to the limited phenolichydroxylsor lack of structure of ortho-phenolic hydroxyls (Liao, 2003). For this reason, they have relatively weaker adsorption capacity on collagen fibres. It is the distinctive molecular structures of collagen fibres and tannins that establish the foundation of adsorption selectivity of hide collagen fibres to tannins. Survey into the utilization of small phenolic compounds (ranging from 500-3000Da) such as catechin is carried out to improve penetration, sorptive capacity and binding ability with subsequent in situ enzyme-catalysis and sulphitation.

Shrinkage temperature (hydrothermal stability) is one of the properties that is routinely used to characterize collagen (Covington *et al.,* 1997). Whether nature, structurally modified or chemically modified, shrinkage temperature is the effect of wet heat on the completeness of the material usually in terms of the denaturation transition. The discerned initiation of the transition is referred to as the shrinkage temperature reflecting the observation that collagenic materials respond to wet heat by shrinkage. The main reaction between the vegetable tannins has always been thought to be by hydrogen bonds formed between the hydroxyl groups on the polyphenols and oxygen or nitrogen atoms on the protein (Teng *et al.,* 2013; Covington *et al.,* 1997). It has however been found that polyphenols also fix to amino side chains by electrostatic salt links with carboxylate or hydrogen bonding with carboxylic acid groups depending on the pH (Teng *et al.,* 2013).There is a positive correlation between the molecular weight and the binding capacity, and the sorptive ability of tanning; that is, the higher the molecular weight of the tannin, the stronger the tanning ability it will present (Luiz*et al.*, 2002; SLTC, 1999).

Tannins are widely found in parts of plants such as bark, wood, fruit, leaves, roots and growths. Tannins are found in the plant kingdom widely distributed such as wattle, oak, chestnut, mangrove, quebracho, myrobalans, sumac, Turkish gall, potentilla, Divi-Divi, locust bean, palmetto, hemlock, eucalypts, pomegranates, gallnuts from oak and sumac which contains the highest occurring level of about 50 to 75 % tannins (gallotannins). There are so many areas in which tannins can be applied, ranging from wine production to pharmaceutical applications. Phenolic metabolism in plants is complex, and yields a wide array of compounds ranging from the familiar flower pigments (anthocyanidin) to the complex phenolic of the plant cell wall (lignin). However, the groups of phenolic compounds known as tannins are clearly distinguished from other plant secondary phenolics in their chemical reactivities and biological activities.

1.2 Statement of the Research Problem

In the quest to explore more sources of vegetable tanning materials, only a few of the materials investigated are of economic importance. The Nigerian leather industry has depended mainly on imported tannins of Mimosa and Quebracho and to a lesser extent on the local plant Bagaruwa (*Acacia nilotica*). This indigenous plant has not met the needs of the leather industry in Nigeria and the result is that the importation of tannins stiffles the leather industry. Nigeria is blessed with vast vegetation and biodiversity; the problem however, is that only limited studies have been carried out on alternative sources of tannins. There is therefore the need to reappraise this area of research to search for new and sustainable local sources of vegetable tannins suited for the Nigerian leather industry and the world at large.

The Nigerian Institute of Leather and Science Technology (NILEST), Zaria has screened about one hundred (100) vegetable tannin plants and found their concentrations low except *Parkia clappertoniana, Acacia nilotica* and *Caesalpinia coriaria* (Adewoye, 1986). Apart from the general problem of low hydrothermal stability of the leather where vegetable tannins from *Parkia clappertoniana* are used, there is also the unique problem of low penetration into the collagen fibre. About 40% of Chromium used during tanning process remains in the waste and thedisposal of chromium contaminated sludge produced as by-products of the waste water treatment is becoming a widely known environmental problem in leather industry (Nduru, 2015; Moorthy and Mekonnen, 2013).

Tannery effluent containing chrome adversely affects the mitotic processes and lessens seed germination in extensively cultivated pulse crops. At high concentrations chromium is toxic, mutagenic and teratogenic. Due to the above mentioned disadvantages of chrome tanning, the tanners are now encouraged to adapt new ecofriendly methods of tanning such as vegetable tanning materials (Moorthy and Mekonnen, 2013 as cited in Nduru, 2015; Bielicila *et al.*,2005; Mole, 1993).

The combination tannages between mineral and vegetable tannins have been in use to produce semi-chrome and chrome-retan leathers (ALCA, 1957, as cited by Haron, *et al.*, 2012). Since the character of leather is determined by the first tannage, the synergy between two vegetable tannin is expected to give the leather the organic character, thus making it more eco-friendly than the chrome-retan (Borasky *et al.*, 1949 as cited by Haron *et al.*,2012). There is need to further source for raw materials for tanning without compromising the quality of the resultant leather (Teng *et al.*, 2013). Nigeria's leather industry is the second highest foreign exchange earner after the oil industry, exporting over 600 million square feet of leather in 2009, and earning about $680 million from tanned skin. The leather industry offers a huge potential for growth, even though it constitutes mainly the small and medium scale enterprises. Nigeria has one of the largest economies in sub-Sahara Africa and the fifth fastest growing economy in the world, but it is heavily reliant on oil and gas exports, which makes it very unstable because growth is dependent on prevailing conditions in the global oil market. The Central Bank of Nigeria (2010) reports that the heavy dependence on the oil sector is reflected by the fact that the non-oil sector contributed only 6.5% of GDP. Hence, in order to develop a balanced economy there is a clear need to expand the growth of the non-oil sector, one of which is the leather industry. Export figures show that the leather industry was the strongest non-oil export in 2005 with exports in excess of $160 million as reported by the Nigerian export policy (Mohammed and Wuyah, 2014).

One of the leading sectors in Nigeria driving national output, employment and export is agriculture sector. The agriculture sector of the economy, which currently accounts for about 24 % of GDP, has been acclaimed to have the potential of contributing about 65 per cent of employment generation and 50 per cent export share if its vast and enormous value-adding opportunities in agroindustry are explored. The livestock agro-industrial segment, especially the leather industry, presents an even more compelling narrative of revenue generation and industrial deepening through value and supply chain integration (https://www.thisdaylive.com/index.php/2017/07/03/).

1.3 Significance of the Study

This research is expected to develop alternative sources of tannins which are eco-friendly. Vegetable tanned leathers have the advantage of being biodegradable and fitting well with consumer needs at both performance and appearance (Teng *et al.*, 2013). Development in vegetable tannin extract could lead to the production of more versatile product base, like tannic acid, dyes and adhesive (Hemingway, 1992). The national economy would be enhanced through the reduction in importation of tannins. The cost of leather production and the effect of environmental pollution associated with chrome tanning would be reduced (Nduru, 2015). In Nigeria, *Caesalpinia coriaria* is not well known as tannin producing plant especially by the traditional tanners and, therefore, has not been developed to be used. Therefore, the low hydrothermal stability produced by the vegetable tannins can be overcome by formulating the two classes of tannins (condensed and hydrolysable tannins) together, to improve the quality of the resultant leather.The findings of this research will serve as a means for the development of protocol of vegetable tanning using alternative sources of tanning materials.

1.4 Aim and objectives

The aim of this research is to improve the leather quality produced by optimizing liming, and increasing reactivity of tannins obtained from *Caesalpinia coriaria* and *Parkia clappertoniana*. The aim would be achieved through the following objectives:

i. extraction of tannins from *Caesalpinia coriaria* (DD)and *Parkia clappertoniana*(PC);

ii. evaluation of the tanning strength of the vegetable tanning materials;

iii. modification of tannins using chemical and biological catalysis;

iv. characterization of the extracts before and after the modification;

v. preparation of different ratios of *Caesalpinia coriaria* with *Parkia clappertoniana;*

vi. optimization of the liming process of the collagen;

vii. development of a protocol for commercial tanning of leathers using the optimum conditions obtained;

viii. examining the quality of the tanned leather; and

ix. Preparation of hide powder.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of the *Parkia clappertoniana* Plant

Robert Brown, a Scottish botanist in 1826 named the widely known African locust beans as *Parkia species*, after Mongo Park, a Scottish surgeon who explored West Africa in 1790s (Oladunmoye, 2007). He gave this tree a local name 'nitta' a pantropical genus of evolutionary interest with centres of distribution in Africa, Southeast Asia and South America. It is a primitive group within Leguminosae plants consisting of about 70-77 species (Hopkins, 1983; Burkill, 1995 as cited in Oladunmoye, 2007). In 1955 Keay introduced the name *Parkia clappertoniana* to replace *Parkia africana*, arguing that Brown's use of Africana contradicts the code for botanical nomenclature because valid epithets already existed and some of the descriptions obtained from the material clearly associated with these.

Kayode (2012) reports that locust beans tree is commonly found in West Africa and its habitat is in the savannah as it is characteristic of transition areas from Sahelian to Sudanian eco-zones. Parkia's adaptation to its natural environment makes it more drought tolerant than many of the exotics grown as alternative tree resources and that, *Parkia* species, *biglobosa* have been studied in detail than any other species. Parkia is known by several vernacular names such as *nareje* in Fulfulde, *dorawa* in Hausa, *toroko* in Yoruba, *ruh* in Yendang, *wupga* in Igala, *néré* in Francophone Africa and locust bean in Anglophone Africa (Hopkins, 1983; El-Mahmood, Ameh, 2007),as cited in Kayode, 2012). This explains its socio-economic potential in various local communities. It is a native tree to sub-Saharan Africa and the natural range starting from Senegal in the west to Uganda in the east, including 19 African countries. This multipurpose tree has different ethnic uses. According to a report on 13 ethnic groups in northern Benin Republic, it has been discovered that various parts of *P. biglobosa* plant can be used for diverse purposes (Oladunmoye, 2007).

2.1.1 Scientific Classification of *Parkia clappertoniana*

One of the many plants that contain tanninsis *Parkiaclappertoniana,* which is commonly known as West African locust beans. It belongs to family *Leguminesaemimosacea* which has about 236genera.

Kingdom	Plantae
Sub kingdom	Tracheoxbionta
Super division	Spermatophyte
Division	Meognoliophyta
Class	Magnoliopsida
Sub class	Brosidae
Order	Fables
Family	Fabaceae
Genus	Parkia R. Br
Species	*Parkia clappertoniana*

2.1.2 Economic Importance of *Parkia Clappertoniana*

The *Parkia* plant serves so many purposes and hence is called a multipurpose tree. The most important use of African locust bean is found in its seed, which has both food and non - food uses. The seed mainly serves as a source of useful ingredients for consumption (Campbell-platt, 1980). Fermented seeds (‘soumbala’, ‘dawadawa’, ‘netetu’ ‘Kula’, ‘Iru’) serve primarily as condiments for adding flavor to sauces and soups served in various dishes among the diverse tribes of Nigeria. Young flower buds are added to mixed salads. Roasted seeds are used as a coffee substitute known as ‘Sudan coffee’ or ‘café nègre’. Ground seeds are mixed with *Moringa* *oleifera* L leaves to prepare sauce, and are also used to make doughnuts. The tree enriches the soil due to the presence of special nodules on the tree's root that produce nitrogen while simultaneously preventing soil erosion. The bark is rich in tanning and may be used for tanning hide, but the resulting leather is usually of moderate quantity especially with regards to colour which is often reddish uneven and darkens when exposed to light (Mustapha, *et al.*, 2012)

Native fishers have thrown the tree's fruit pod shells into water to stun fish. It is reported that in the dry season farmers have broken off branches from surrounding growth to make it easier for livestock to get at the tree's young leaves, shoots and fruit pods(Zarin *et al.*, 2016). The dried seeds have also offered animal fodder. Locusts are known to gorge on the fruit pods and strip the trees bare. The hard, reddish-brown wood has been used locally for carpentry work. It yields a high quality charcoal and has also been burnt as fuel (Sue, 2008).

This plant has so many medicinal values. The seed has been used locally for the treatments of stomach upsets and, in the Gambia, the bark has been used for relieving toothache. In African medicine the leaves and fruit pulp, were reported to account for treatments of at least 40 different ailments in African medicine (Sue, 2008). Lawal*et al.* (2016) report that *Parkia clappertoniana*) has been used locally for treating diarrhea. The antidiarrheal properties of the plant extracts are effective against multidrug resistant strains of *Shigella* and *Salmonella* species as proven through experiments performed by agar well diffusion method using standard guidelines, the findings affirmed that *Parkia clappertoniana* had growth inhibitory effect against the tested bacteria (Lawal *et al.*, 2016).

Kambou (1997) reported the use of *Parkia* plant powder to control *Strigahermonthica* and germination inhibition of 97-100% and 92% when untreated powder extract and decorticated powder of *Parkia* were used respectively. According to Field and Latinga, (1989), tannins are the main secondary exudates in *Parkia* and are toxic to animals especially in aquatic areas(Okwoche *et al.*, 2016). Lane *et al.* (1991) reports the presence of triterpenes carotenoids, tannins and polyphenolic compounds in Parkia fruits (Okwoche *et al.*, 2016). The leaves may also be pounded and used as a local soap. Boiled pods are used to dye pottery black. The ash is applied as a mordant. The seeds contain fat and essential proteins high in amino acids such as methionine and cysteine (Adama and Jimoh, 2011), feed to livestock and a source of raw materials for medicine and fodder, soil amendment, charcoal and firewood (Oni, 1997).

The use of the tree as medicine is especially pronounced in rural areas where there is little access to modern medicine. The bark is the most important for medicinal purposes, and also the leaves. Medicinal applications include the treatment of parasitic infections, circulatory system disorders, such as arterial hypertension, and disorders of the respiratory system, digestive system and the skin. The list of diseases treated by the African locust been tree include serious pain, diarrhoea, sterility, bronchitis, pneumonia, leprosy, venereal diseases, bad teeth, skin irritations, guinea worm and swellings. The crushed pods from the tree are used for fishing while leaving the nutritional value of the fish unaffected so long as they are cooked or dried. The pulp of the tree is prepared as flour used to thicken soups and broth. The fresh fruit pulp is sweet (it is made up of about 60 % sugar) and very high in carbohydrates (Alabi *et al.*, 2004). The husks and pods are reported to be good for feeding livestock (Wang *et al.*, 1996). In addition, the fruit pulp and seed residues are also used in making animal feeds (Wang *et al.*, 1996).

2.1.3 Morphology of the Plant

The tree begins to bear fruits within four to seven years after planting. The tree grows in the savannah region of West Africa, the height of which ranges from 7 to 20 m; in some exceptional cases some might reach heights of up to 30 m, with a wide-spreading umbrella-shaped crown. It performs basic functions ecologically in cycling of nutrients from deep soils, and in holding the soil particles with the aid of the roots to prevent soil erosion. The tree also serves as shade for man (Oni, 1997). It needs an altitude of about 300 metres with an average rainfall of 400 - 700 millimetres per year and an average mean annual temperature of 28 ^{o}C. It prefers well-drained, deep, cultivated soils, but can also be found on shallow, skeletal soils and thick laterites. (Database, 2016).

The African locust bean seeds are contained in branches of pods that make up the most valuable part of the plant. The pods are flat and large/irregular cluster of up to 30 seeds. It is a tree that has spreading branches with fat trunk. It has long tap roots and wide lateral roots up to 10 m spreading from the bole. It has a thick dark grey-brown and a narrow opening bark from which amber gum comes out gradually in drops when the bark is wounded. It has dark green leaves that interchange repeatedly and regularly with one another, they are bipinnate with pinnae of up to 8 to16. The tree can also grow on rocky slopes, stony ridges or sandstone hills. *Parkia clappertoniana* occurs in a diversity of zones, ranging from tropical forests with high and well-distributed rainfall to arid zones where mean annual rainfall may be less than 400 mm (Database, 2016). Because of its deep taproot and ability to restrict transpiration, it has an ability to withstand drought conditions.

Parkia clappertoniana can be seen in the savannah region of Nigeria, although they are not cultivated normally. The tree can also be found in other African, countries, including Benin, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Gambia, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Senegal, Sierra Leone, Sudan, Togo, and Uganda (Campbell-Platt, 1980).*Parkia clappertoniana* is good to the soil beneath it; this soil derives its nutrients from the dung and urine of animals that shelter under the tree's shade. It can also be used as timber for making pestles, mortars, bows, and seats (Assam *et al.,* 2012).

Parkia species are usually preserved and kept safe by the people in the particular area where they grow because of their being valuable sources of food, especially the seeds which serve as source of useful ingredients for consumption. The pods and husks have also been discovered to be good food for livestock. The various importance of *Parkia clappertoniana* make it an ideal plant to promote across Africa, as it combines in a single species two of Africa's greatest needs, which are food security and tree cover coupled with the health benefits.

Plate 2.1: ***Parkia clappertoniana*tree**

African locust bean tree fruits during the months of December to March of the year. Plate 2.1 and Plate 2.2 presents a fruiting locust bean tree. The fruits are ready for harvest in April and have many leguminous pods each with a tough pericarp. The pods contain yellow powdery pulp in which seeds are embedded (Plate 2.3).

Plate 2.2: Fruiting of *Parkia clappertoniana*

The seeds have hard, black testa making them less vulnerable to insects and rodent infestation. At maturity the pod length averages 232 mm ($173 \leq L \geq 290$ mm) with a mean diameter of about 15 mm ($10 \leq d \geq 20$ mm) (Oladele *et al.*, 1985).Mature pods of African locust bean occur in large bunches. Each pod varies between 12 and 30 cm in length and 12 – 25 mm in breadth (Adewumi, 1993).

Plate 2.3: Mature pods of *Parkia clappertoniana*

The pod has tough pericarp while the seeds have hard testa (Ogonesyan, 1988). The pod is tough and fibrous, enclosing a soft, powdery, yellowish pulp in which small seeds are embedded. Each locust bean tree yields about 25 to 52 kg of pods from which 6-14 kg of bean may be collected and has the total life span of 80-90 years (Odunfa, 1982). The morphology of the locust bean fruit is given in Figure 2.4

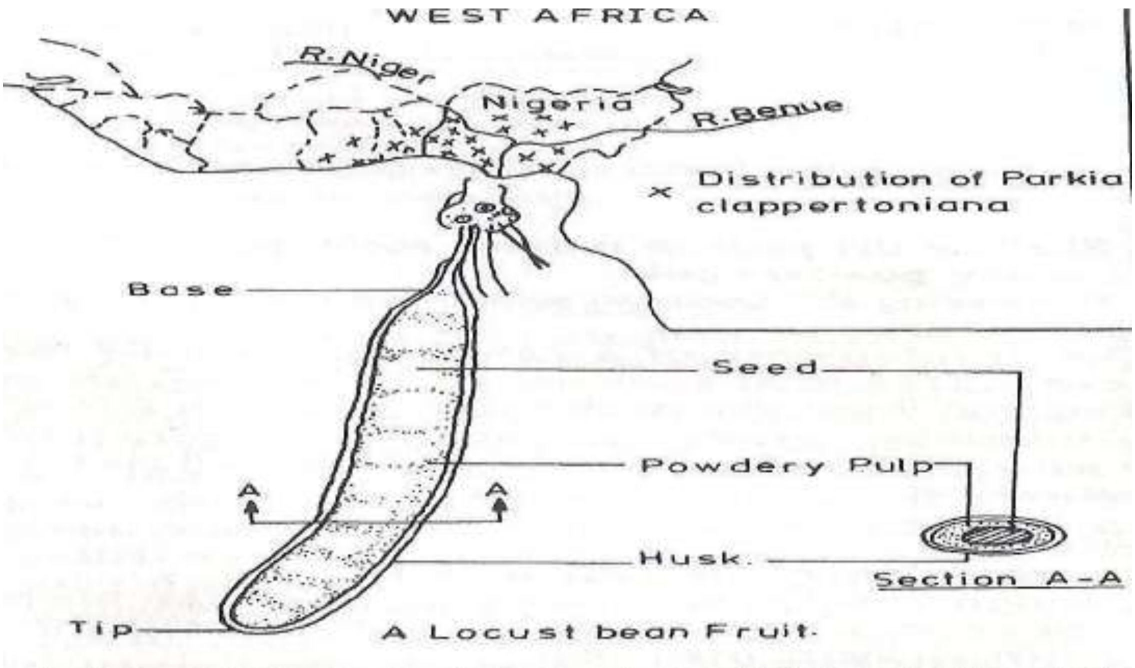


Figure 2.1: Distribution of *Parkia clappertoniana* in Nigeria and a *Parkia* fruit source: Oni (1990)

Adewoye *et al.* (1986) worked on *Parkia clappertoniana* fruit husk and reports that a fruit husk of *Parkia clappertoniana* is a promising tanning material in Nigeria. In 1988 and 1989, Adewoye and others further carried out two researches on the phenolic constituents, flavonols, flavones and tannins of *Parkia* fruit husk. They discovered that the fruit husk contains 40 % of tannin content and the tannins belong to the non-hydrolysable type (condensed) (Adaramola, *et al.,* 2013).On the other hand, the tannins in the husk of *Parkia clappertoniana* species were studied and the outer part of the plant's fruit pods was reported to have about 40% of the tannins (Mustapha *et al.,* 2012). The water solubility of the husk (under standard conditions) is about 63% and is made up of 45 and 10 % tannins and non-tans respectively.

2.2 Origin of the *Caesalpinia coriaria* Plant

Caesalpinia coriaria is a small or medium-sized leguminous evergreen tree that belongs to family Fabaceae. It is most frequently called Divi-Divi. It is distributed throughout Central America and north of South America. It was introduced in Kolkata, India during 1834 as a shade trees in urban landscapes (Chacko *et al.,* 2002). The species thrives in areas having annual rainfall of 600 mm to 2500 mm and mean annual temperature of 15-28 ˚*Caesalpinia coriaria* is a drought resistant, wind-resistant and fairly saline tolerant tree. It attains 9 m in height with a dense low spreading umbrella- like crown, somewhat resembling babul (*Acacia nilotica*) in appearance. It has short trunk and often crooked in nature. Very recently the species received very much attention due to the anti-bacterial and antioxidants properties. Apart from this, it has high tannins content, (Mohana and Raveesha, 2006; Lokeswari and Sujatha, 2011; Anandhi *et al.,* 2011). According toRamanan (2016), that *Caesalpinia coriaria* can be comfortably added in Agroforestry system because its serve as source of food to domestic animals especially cattle, horse and sheep.

The fruits and seeds characteristics have been considered as something that helps in the reproductive biology of plants. Floral phenology and reproductive biology are the basis for the breeding programs and to regulate the genetic structure (Ramanan, 2016).

Plate 2.4: Fruiting *Caesalpinia coriaria*

2.2.1 Classification of the Plant

Caesalpinia coriaria is a leguminous tree or large shrub native to the Caribbean, Mexico, Central America and Northern South America. Its common name include Divi-Divi, Casclalote, Guaracabuya, Guatapana, Nacascol and Watapana (Aruba) (Crossman *et al.*, 2006).

The *Caesalpinia coriaria* is as follows:

Botanical name:	*Caesalpinia coriaria* wild
Family:	Fabaceae-pea family/leguminosai, caesalpinioideae
Genus:	*Caesalpinia* L. –nicker
Species:	*Caesalpinia coriaria* (Jacq) wild –dividivi
Order:	Fabates
Kingdom:	Plantae – Plants
Sub kingdom:	Tracheobionta – vascular plants
Super division:	Spermatophyte – seed plants
Division:	Magnoliophyta – flowering plants

Caesalpinia coriaria does not often reach its maximum height of 9.144 m for the reason that its growth is disturbed by the trade winds that prevail in the exposed coastal sites where it commonly grows. In other environments it grows into a low dome shaped tree with a clear canopy space. Leaves are bipunnate, with 5 to10 pairs of pinnae, each pinna with 15 to 25 pairs of leaflets; the individual leaflets are 7mm long and 2mm broad. The fruit is a twisted pod 5mm long (Plate 2.4). In all the species of *Caesalpinia*, Divi-Divi (*coriaria*) is of high tannins quality, effective and useful than others (Crossman *et al.*, 2006).

Plate 2.5: Pods of *Caesalpinia coriaria*

The pods of *Caesalpinia coriaria* are very rich in tannin and are used in the tanning industry (Plate 2.5). Divi-Divi is often used in rapid drum tanning of light leathers and in leather dressing. The pods are also used to prepare a blackish or bluish dye for cotton and wool and a black ink, used for example, in the decoration of traditional potteries and gourds in Central America (Perez-Tello, 1995).

2.2.2 Economics Importance of *Caesalpinia coriaria*

As earlier mentioned, very recently the species have received much attention due to the anti-bacterial and antioxidants properties, in addition to its good tannins content, fodder and avenue suitability (Mohana and Raveesha, 2006; Lokeswari and Sujatha, 2011; Anandhi, 2011). Due to its fodder and nitrogen fixing capacity it can be effectively included in agroforestry system, floral phenology and the harvesting index. It starts flowering from mid-September to October (Plate 2.4). It was observed that it has a mean of 41 flowers per inflorescence with pod set and also has a seed set of around 2-4 %. The pods attain harvest maturity in 21 weeks after anthesis, indicating a distinctive colour change from a yellowish brown to a darker brown colour. The seeds are orthodox in nature and have a 70% germination rate (Ramanan, 2016).

The study of the influence of particle size, temperature, methanol content and time on the extraction of tannins from *Caesalpinia coriaria* by pressure autoclaving method was conducted by Lokeswari and Sujatha (2011), and temperature was found to be the main factor influencing tannin extraction. A particle size range of 5-15 mm is favoured for tannin extraction (Lokeswari and Sujatha, 2011).*Caesalpinia coriaria* is virtually an inexhaustible source of bioactive metabolites within the more than 500 species distributed worldwide. Many of these species are endemic. For example, Brazil-wood (*Caesalpinia echinata*) exists only in Brazil and played an important role in the history of the country. *Caesalpinia pulcherrima* is native to Central America, with samples in various other parts of the world, and has been widely used in folk medicine, due to its emmenagogic and abortifacient action (Baldimzanin *et al.,* 2012).

Mohana and Raveesha, (2016) reveal the anti-bacterial efficacy of powdered leaf and pod extracts of *Caesalpinia coriaria* using water, petroleum ether, benzene, chloroform, methanol and ethanol as solvents. The results showed that antibacterial activity is due to the presence of phenolic and acidic fraction (Mohana and Raveesha, 2016).Other species such as *Caesalpinia sappan* and *Caesalpinia bonduc,* have been used for the treatment of inflammation and improving blood circulation, along with serving as an antimalarial, an anthelmintic for the treatment of jaundice and as a digestive. Phytochemically, several classes of compounds were isolated from plants of genus *Caesalpinia*, mainly flavonoids, diterpenes, and steroids. From a pharmacological point of view, these species have antiulcer, anticancer, antidiabetic, anti-inflammatory, antimicrobial, and antirheumatic activities, among others, confirming information derived from ethnopharmacological studies (BaldimZanin *et al.,* 2012).

Caesalpinia L. is a genus of plants belonging to the subfamily Caesalpinioideae of the family *Caesalpiniaceae* that consists of more than 500 species, which are mostly woody species occurring in tropical and subtropical zones

(Plate 2.5). Pharmacologically, species of this genus exhibit analgesic, adaptogenic, antiulcer, anthelmintic, antibacterial, insecticidal, antifungal, anti-inflammatory, antipyretic, antioxidant, antiproliferative, antiviral, immunomodulatory, and immune suppressive activities. Other species, such as *Caesalpinia sappan, Caesalpinia ferrea*, and *Caesalpinia bonducella* show antinociceptive effects. In an abdominal writhing test induced by acetic acid in mice, the stem extracts of *Caesalpinia sappan* significantly inhibited the number of contortions. The crude extract from fruits of *Caesalpinia ferrea*, when subjected to the same pharmacological model discussed above, produced a 51% and 88% reduction of abdominal contractions, respectively, for doses of 10 and 20 mg/kg. Comparatively, indomethacin (20 mg/kg) reduced 92% of contractions, while the oil from the seeds of *Caesalpinia bonducella* (400 mg/kg) showed an inhibition of 48.6%, and standard aspirin inhibited the number of contortions by 66.5% (Anandhi1 and Kanimozhi, 2016, Baldim zanin *et al.*, 2012).

Mohana and Raveesha (2006) reveals the efficacy of the powdered leaf and pod extracts of *Caesalpinia coriaria* using water and successively with different solvents viz., petroleum ether, benzene, chloroform, methanol and ethanol. Anti-bacterial activity assays of all the extracts against the important phytopathogenic xanthomonaspathovars, known to cause diseases in tomato, French bean and cotton, were carried out by cup diffusion method. Aqueous pod extract showed significant activity. Among the five solvents extracts tested, methanol extract of both leaf and pod were most active against all the test bacteria, followed by ethanol extract. Comparison of the inhibitory activity of the extracts with the antibiotics bacteriamycin 2000 and streptocyclineshowed that methanol and ethanol extract of both leaf and pod and aqueous extract of pod were significantly higher than that of the antibiotics tested. Phytochemical analysis of leaf and pod materials revealed that antibacterial activity is due to the presence of phenolic and acidic fraction (Mohana and Raveesha, 2006).The presence of diverse secondary metabolites (phytochemicals) has been reported from the genus *Caesalpinia* (Yongo *et al.,* 2016).

Kibria (2014) carried out a study on the anti-diabetic effect of *Caesalpinia coriaria.* Aqueous, acetone, ethanol and ethyl acetate extracts of *Caesalpinia coriaria* were prepared. The yields of the four extracts were calculated. In vitro antidiabetic activity of *Caesalpinia coriaria* was analysed by evaluating the α-amylase and α-glucosidase inhibitory potential of the *Caesalpinia coriaria*. The four extracts were further proceeded with phytochemical analysis. The quantitative assay of the phytochemical compound was estimated. From the result obtained the plant *Caesalpinia coriaria* has the potential to inhibit the α-amylase and α-glucosidase activities which is very significant in reducing the glucose level in the system (Kibria, 2014).

According to Lokeswari and Sujatha (2011), *Caesalpinia coriaria* is a substrate for Gallic acid production. It is a small thorn less tree or shrub of tropical America and West Indies and introduced into India, more than a century ago, whose seed pods are a rich source of tannin. It has been grown successfully in different parts of India, particularly Madras State and Andhra Pradesh State, and is important as source of tanning materials. The pods of these species are used for the extraction of tannin and the tannin content is as high as 40 to 42 %. It prefers a well-drained soil, becoming stiff in hard soils with high clay content, tolerating moderate to high temperatures. Flowers blossom between July and August and fruiting takes place between October to December. The seeds are small and germination percentage is about 30% (Lokeswari and Sujatha, 2011). Tannin or tannic acid is found in some quantity in most plants, with concentrations in the wood, bark, roots, leaves, fruits and galls. In Divi-Divi the major concentration is in the pods. Estimates of the tannin content of Divi-Divi pods run from 25 to 45 % up to 40 to 55 %, with a mean of about 35 to 47 %. These variations in content estimates represent differences in methods of analysis, and also actual differences of content that have been conditioned by climatic, edaphic and other environmental factors. In any case, Divi-Divi provides a cheap source of material with comparatively high tannin content. Tannase (tannin acyl hydrolase, E C 3.1.1.20) is the enzyme responsible for the decomposition of hydrolysable tannins, especially gallotannins, to glucose and gallic acid. Tannins belong to the phenolic class of secondary metabolites formed via Shikmic acid path way leading to the formation of other phenolics such as flavones, coumarin, lignins and aromatic amino acids. They have been reported to be the fourth abundant plant constituents after cellulose, hemicellulose and lignin (Lokeswari and Sujatha, 2011).

2.3 Vegetable Tannins

The tannin materials are obtained in the form of wood, bark, fruit and leaves from various plants (Standard, 2007). Some plants are preferred for tanning over others due to the level of their tannin content (Koloka and Moreki, 2011).But they can easily change their structures through oxidative, reductive and enzymatic effects. Also they give characteristic colour reactions with iron salts. They are polyphenolic compounds, able to associate and combine with protein mainly by hydrogen bonds (Yusuff *et al.,* 2013, Afsar and Sekeroglu, 2008).

Bate Smith 1962 as cited by (Liao, 2003)defined tannins as “water-soluble phenolic compounds having molecular weight between 500 and 3000 Da giving the usual phenolic reaction and having special properties such as the ability to precipitate alkaloids, gelatin and other proteins. The origin of the word tannin is derived from the practice for which these compounds were used in the leather industry (Moran, 1994).

Tannins are sometimes called plant polyphenols according to Haslam (1989), although originally the name tannin was given to the plant extracts exhibiting astringency, without knowing their chemical structures. The features distinguishing tannins from plant polyphenols of other types are basically, basic compounds, pigments, large-molecular compounds, and also anti-oxidant activities, etc. These features of tannins lead to qualitative and quantitative analytical differences between tannins and other polyphenols. Unlike the analysis of polyphenols in general, quantification of tannins is based on their binding ability(Okuda and Ito, 2011b). The classical hide powder method is based on the binding with animal skin protein, and the relative astringency RA and relative affinity to methylene blue (RMB) determinations are based on the binding with blood and methylene blue respectively, under controlled pH (Okuda and Ito, 2011).

Haslam (1996) substituted the term “polyphenol” for “tannin” in an attempt to emphasize the multiplicity. It was noted that molecular weight as high as 20,000Da have been reported, and that tannins form complexes not only with proteins, alkaloids and certain polysaccharides. It is preferable to use the term tannin, which emphasizes the character that sets tannins apart from all other phenolics, namely the ability to precipitate proteins(Hagerman, 2002). Khanbabaee and Ree (2001) define tannins as polyphenolic secondary metabolites of higherplants, and are either galloyl esters and their derivatives, in which galloyl moieties or their derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores (gallo- tannins, ellagitannins and complex tannins), or they are oligomeric and polymeric proanthocyanidins that can possess different interflavanyl coupling and substitution patterns (condensed tannins) (Khanbabaee and Ree, 2001).

2.3.1 The Vegetable Extracts Fractions

The typical fractions of the vegetable extracts consist of Non-tans, Tannins and Gums.Non-tans are the low molecular weight fraction, with a molecular weight <500.These compounds are designated non-tans to indicate that they have low tanning power; however, their presence in the extract is useful, because they contribute to solubilizing the tans and hence assist penetration into the pelt (Covington, 2009).Tannins are medium molecular weight fraction with molecular weight 500-3000. The function of the tannins hangs basically on the phenolic hydroxyl content, to provide the astringency. Lower molecular weight compounds lack astringency while higher molecular weight hinders penetration into the pelt.

Gums are the high molecular weight fraction, with molecular weight >3000.These compounds are tans and have higher molecular weight polyphenols complexes with carbohydrate species. The astringency and high molecular weight prevent penetration into pelt, causing surface reaction only. Hence, their presence is undesirable (Covington, 2009). It is well known that higher yields of extract can be obtained by using more aggressive conditions such as higher temperature: this is actually counterproductive, because the extract contains higher amounts of gums than tannins.

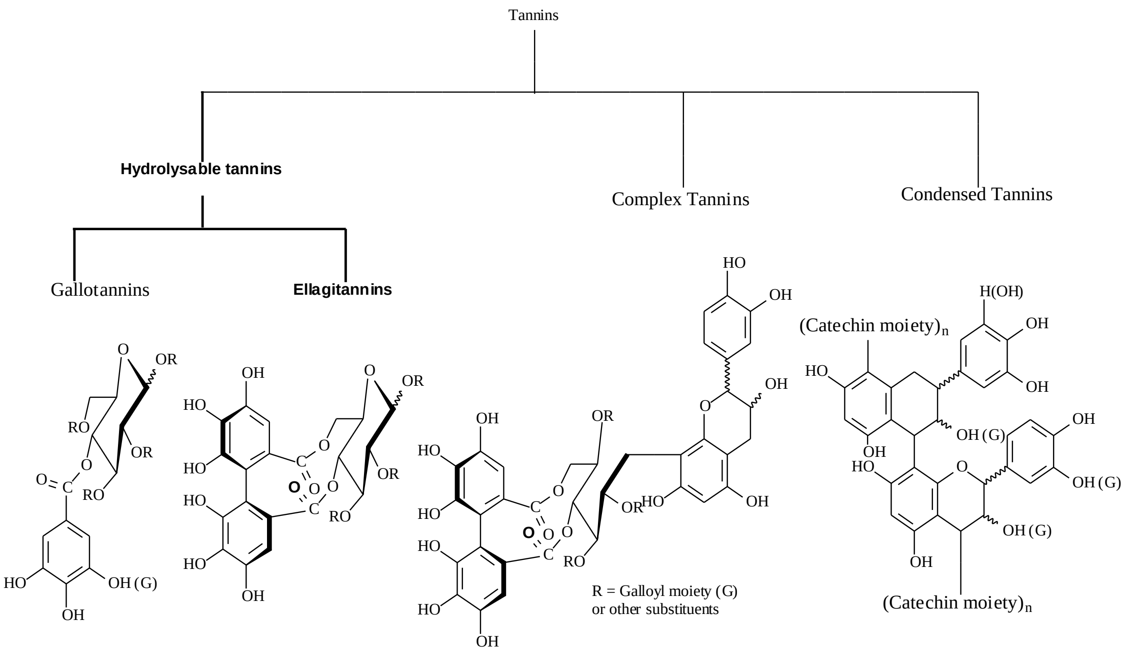
2.3.2 Uses of Tannins

Though tannins have found uses in other areas such as dye mordant and formaldehyde scavenger in ink, wood adhesives and polyurethane manufacture, and in promoting blood clotting, its use in leather tanning is very significant (Poser and Gosman, 1990 as cited by Vieira *et al.*, 2011). There are many tannin extract plants all over the world servicing the leather industry. The raw materials of these extract plants are usually sourced from different parts of vegetable plants such as leaves, barks, roots and fruit pods, among others. Some of the vegetable plants that have been utilized include wattle, quebracho, mimosa, chestnut, valonia and myrobalan. The commercial tannin extracts are produced either as liquid concentrates or powder. Leaching is the first important processing unit in tannin extract production.

The important parameters forthe solid–liquid extraction process include the choice of solvent, particle size of material being leached, and solvent to feed ratio, temperature and time employed for leaching. Water is commonly the solvent of choice because of solubility, safety, ease of separation, inertness and availability. Temperature and duration of extraction improveextract yield, but these are ultimately constrained by solvent selectivity and vapour pressure, as well as colour sensitivity and onset of hydrolysis. In the same vein, smaller particle size encourages high extraction yield but high pressure drop, grinding energy and purification cost(Mustapha*et al.*, 2012).

2.3.3 Tannin Classification

Tanninsare classified into four major groups(2.1): gallotannins, ellagitannins, condensed tannins, and complex tannins (Lokeswari and Kumar, 2015; Hagerman, 2002; Haslam, 1989).



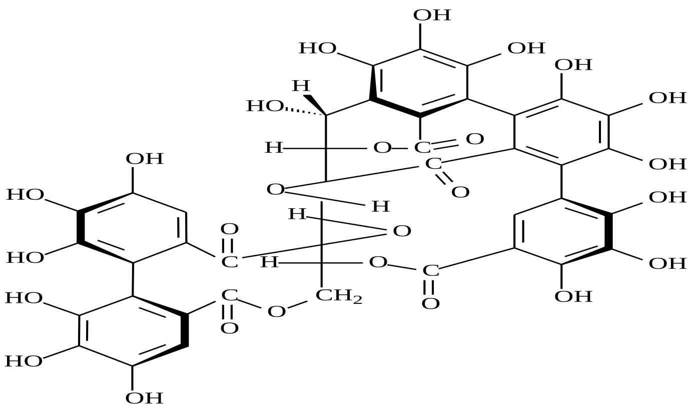
(2.1)

Tannin Classification

In addition to the commonly used classification as hydrolysable tannins and condensed tannins, Okuda and Ito (2011) went further to categorize tannins into two other types: polyphenols of constant chemical structure (Type A) and polyphenols of variable composition (Type B). This can be traced back to what Haslam's (1989) assertion that "The chemical complexity and heterogeneity of plant tannins means that they do not lend themselves to ready quantitative assessment and this has produced a confused picture of their significance, both evolutionarily and ecologically." Both types of tannins and related polyphenols account for a large part of plant polyphenols, but accurate structure-activity correlations on a molecular basis can be determined mainly for type A compounds, among which are hydrolysable tannins such as the ellagitannins and their oxidized congeners, some gallotannins, epigallocatechin gallate and caffetannins (Okuda and Ito, 2011).

2.3.4 Classification of the Tannins based on their Structural Properties

The observation that many tannins can be fractionated hydrolytically into their components, for example, by treatment with hot water or with tannases, led to the classification of such tannins as hydrolysable tannins. Non-hydrolysable oligomeric and polymeric proanthocyanidins were classified as condensed tannins. Therefore, the term hydrolysable tannins include both the gallotannins and the ellagitannins. It should also be mentioned here that there are ellagitannins that are not hydrolysable, because of a further C–C coupling of their polyphenolic residue with the polyol unit, but are nevertheless, for historical reasons, classified as hydrolysable tannins as seen in 2.2 (Khanbabaee and Ree, 2001).



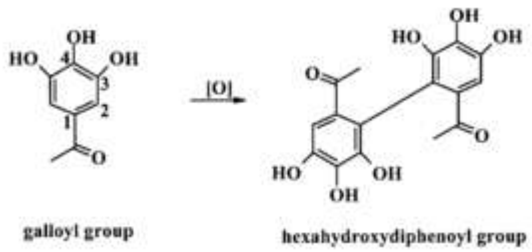
(2.2)

Typical ellagitannins with a C-glycosidic bond and with a d-gluconic acid unit

2.3.5 Hydrolysable Tannins

Hydrolysable tannins are derivatives of gallic acid (3, 4, 5-trihydroxyl benzoic acid). Gallic acid is esterified to a core polyol, and the galloyl groups may be further esterified or oxidatively cross-linked to yield more complex hydrolysable tannins (Nduru, 2015; Haroun *et al.,* 2013; Vieira *et al.,* 2011 ; Hagerman, 2002).

Hydrolysable tannins are further divided into gallotannins and ellagitannins. With more than 500 natural products characterized so far. Hydrolysable tannins, in addition, are free radical scavengers. Thus, the in vitro antimutagenic andanticarcinogenic activity of tannic acid has been previously reported (Hagerman, 1998 as cited by Haroun *et al.*, 2013). The hydrolysable tannins are usually compounds containing a central core of glucose or other polyhydric alcohol esterified with gallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins), depending on the nature of phenolic moiety liberated upon hydrolysis (2.3). These hydrolysable tannins give blue-black colour with aqueous solutions of iron salts(Lokeswari and Sujatha, 2011,Wagh, 2010).

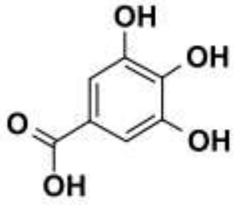


(2.3)

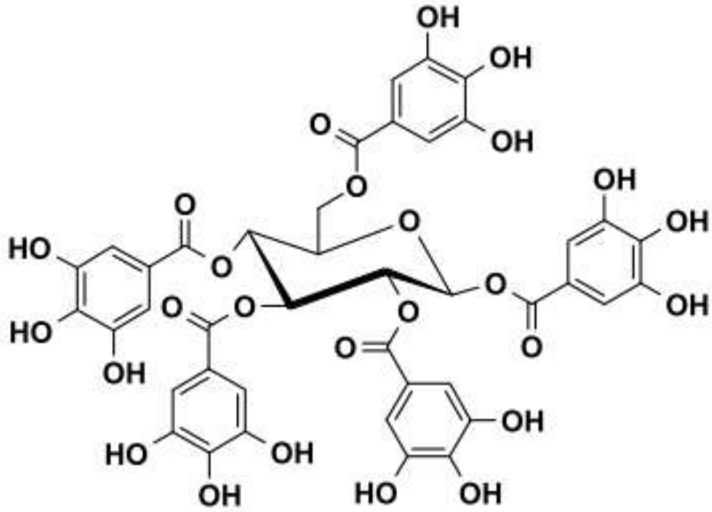
Structural fragments of Hydrolysable Tannins, Source: Tang *et al.*, 2003)

2.3.5.1 Gallotannins

The gallotannins are simple polygalloyl esters of glucose. The prototypical gallotannin is pentagalloyl glucose (β-1, 2, 3, 4, 6-Pentagalloyl-O-D- Glucopyranose). Pentagalloyl glucose (PGG) has five identical ester linkages that involve aliphatic hydroxyl groups of the core sugar (Hagerman, 2002;Keulder, 2006; Vieira *et al.*, 2011). Gallotannins are the simplest hydrolysable tannins, containing polyphenolic and a polyol residue, whereas galloyl units or their meta-depsidic derivatives are bound to diverse polyol-catechin-, or triterpenoid units (Khanbabee and Van Ree, 2001). Gallotannins yield gallic acid and glucose or quinic acid on hydrolysis. (Bhat*et al.*, 1998 as cited by Wagh, 2010). A great variety of polyol residues are possible, most of the gallotannins isolated from plants contain a polyol residue derived from d-glucose (Khanbabaee and Ree, 2001).



gallic acid



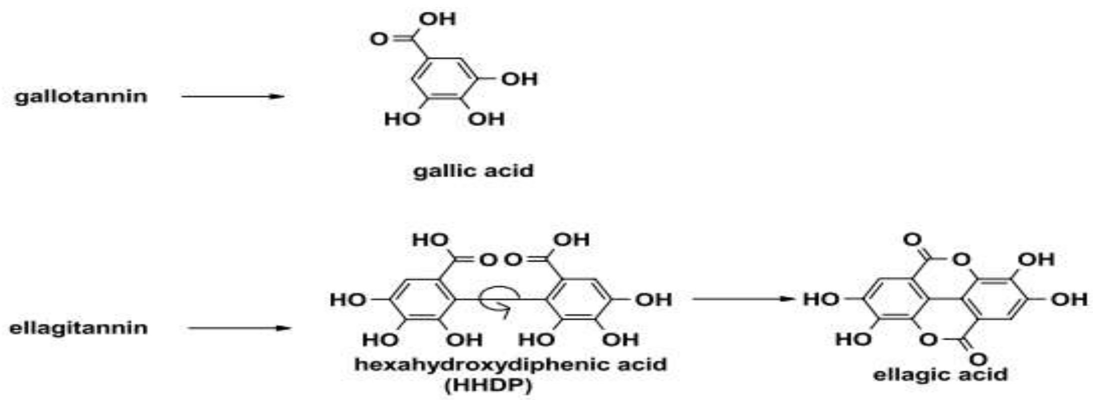
1, 2, 3, 4, 6-penta-O-galloyl-glucose **(2.4)**

Like all of the gallotannins, Pentagalloylglucose (PGG) has many isomers (2.4). The molecular weights of all the isomers of PGG are the same (940 g/mol), but chemical properties such as susceptibility to hydrolysis and chromatographic behaviour and biochemical properties such as ability to precipitate protein; are structure-dependent (Hagerman, 2002).

2.3.5.2 Ellagitannins

Ellagitannins have anticarcinogenic, antioxidant and antifibrosis properties. Anti-carcinogenic after effect of ellagitannins was apparent in several blight types including esophageal, colon, skin, breast and prostate cancers (Stoner and Gupta, 2001; Han *et al.,* 2006; Pornsiri *et al.,* 2013 as cited by Lokeswari and Bompalli, 2015). Plants such as *Caesalpinia coriaria* (Divi-Divi), *Caesalpiniabrevifolia* (Algarobilla tannin) *Terminalia chebula* (myrobalan seeds), *Castanea sativa* (Chestnut), were reports to contain ellagitannins in their bark, nuts or leaves. Wagh (2010) reported the formation of ellagitannins from gallotannins by oxidative coupling of at least two galloyl units which are C-C coupled to each other and absence of a glycosidically linked catechin unit. Ellagitannins yield ellagic acid, gallic acid and glucose on hydrolysis (Wagh, 2010).

With more than 500 natural products characterized so far, the ellagitannins form by far the largest group of known tannins. Ellagitannins are formed from the gallotannins by the oxidative coupling of at least two galloyl units (2.5), yielding an axially chiral HHDP unit (Khanbabaee and Ree, 2001). Oxidative coupling of galloyl groups converts gallotannins to the related ellagitannins. The simple ellagitannins are esters of hexahydroxydiphenic acid (HHDP). HHDP spontaneously lactonizes to ellagic acid in aqueous solution (Hagerman, 2002; Okuda and Ito, 2011; Nduru, 2015).

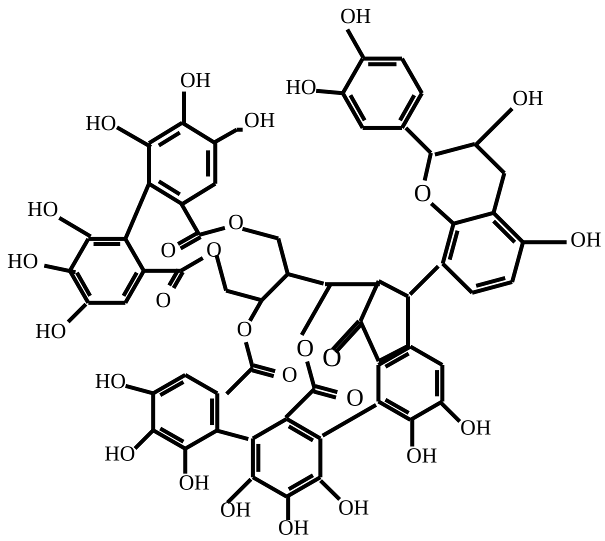


(Hagerman, 2002). **(2.5)**

Gallotannin and Ellagitannin

2.3.6 Complex Tannins

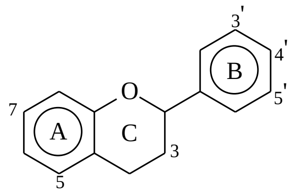
Complex tannins can be classified into flavano-ellagitannins having a flavan-3-ol, and flavono-ellagitannins having a flavonoid glucoside, each connected to C-1 of glucose in the hydrolysable tannin moiety through a carbon-carbon linkage (Okuda and Ito, 2011**,** Lokeswari and Bompalli, 2015).The structures of the complex tannins are built up from a gallotannin unit or an ellagitannin91 unit, and a catechin unit. One example from this substance class is acutissimin A (2.10) having a flavogallonyl unit (nonahydroxytriphenoyl unit) bound glucosidically to C-1, and linked via three further hydrolysable ester bridges to the d-glucose derived polyol (Khanbabaee and Ree, 2001). The complex tannins have structural characteristics of both the above two classes in the same molecules. The phlorotannins are polymeric forms of phloroglucinols (1,3,5-trihydroxbenzene) found in algal (Tang *et al.*, 2003).



(2.6)

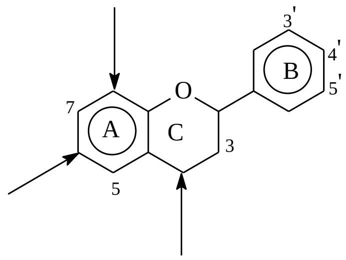
2.3.7 Condensed Tannins

Condensed tannins are polyphenolic natural products composed of flavan-3-ol sub-units linked mainly through C4–C8 (or C4–C6) bonds (2.6). The structural diversity of condensed tannins is due to the different sub-units, interflavonoid bond position, branching and the presence of non-flavonoid substituents such as gallic acid and sugars. In addition, condensed tannins also vary markedly in molar mass distribution. The extractable condensed tannin polymers in the plants may be composed of molecular species with a wide range of molar masses up to 20,000 (about 40 flavan-3-ol units). The bioactivity capacity of condensed tannins is generally recognized to be largely dependent on their structure and particularly the degree of polymerization (Hagerman, 2002, Zhang and Lin, 2008). Condensed tannins are also accepted as proanthocyanidins (PAs). They are aswell alleged flavones because they are polymers of flavan- 3-ols such as catechin or flavan-3, 4 - diols accepted as leucocyanidins (Okuda and Ito, 2011). Proanthocyanidins have been reported to be strong antioxidants. Also, they function as anticancer, anti-ulcerogenic, antiprotozoal, cicatrizing and anti-inflammatory agents(Raïssa *et al.*, 2013; Rhazi*et al.*, 2015). These oligomeric and polymeric proanthocyanidins contain two to ten or more catechin units. The flavan-3-ol units are linked principally through the 4 and 8 positions (Khanbabee and Ree, 2001). Condensed proanthocyanidins degrade in strong acid to give corresponding anthocyanidin (Wagh, 2010).



(2.7)

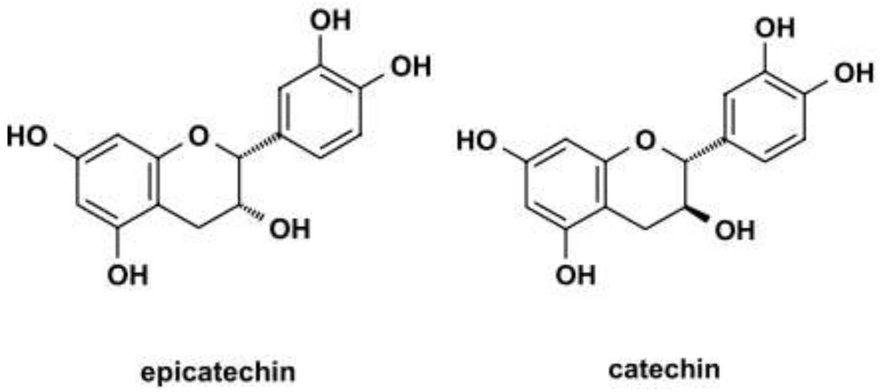
Catechin unit



(2.8)

Flavonoid ring system, showing the positions for hydroxyls and the positions for polymerization

Proanthocyanidins (condensed tannins) are polymeric flavonoids. The flavanoids are a diverse group of metabolites based on a heterocyclic ring system derived from phenylalanine (B) and polyketide biosynthesis (A). Although the biosynthetic pathways for flavanoid synthesis are well understood, the steps leading to condensation and polymerization have not been elucidated. The flavanoid skeleton, the standard letters to identify the rings, and the numbering system are shown in 2.7.

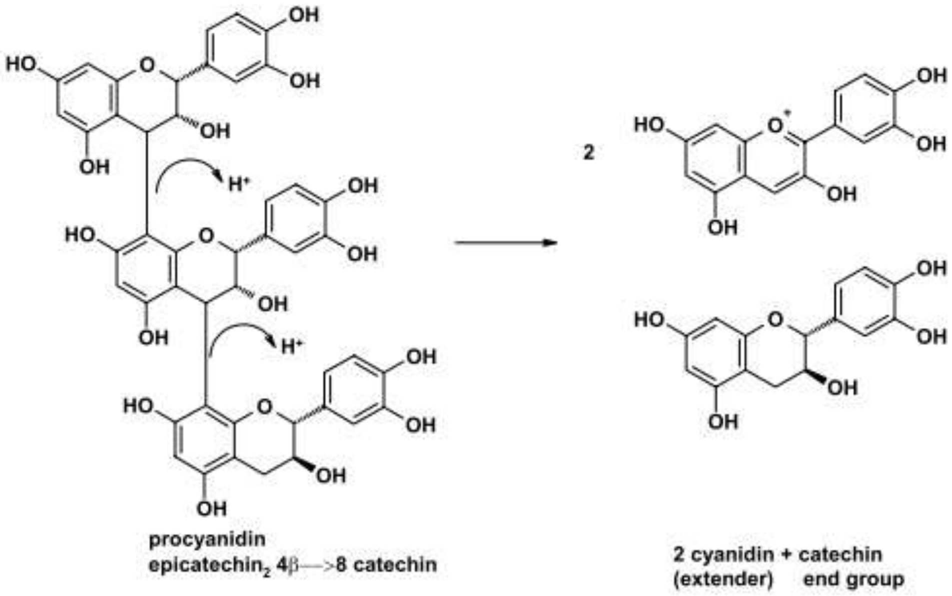


(2.9)

Flavan-3-ols

The addition of a third phenolic group on the B ring yields epigallocatechin and gallocatechin (2.8). Much less common are flavan-3-ols with only a single phenolic group on the B ring, para to C-2 (epiafzelechin, afzelechin with stereochemistry corresponding to epicatechin, catechin respectively) (Hagerman, 2002).

Although the term condensed tannins is still widely used to describe these flavonoid-based polyphenolics (2.9), the chemically more descriptive term “proanthocyanidin” is gaining acceptance (Hagerman, 2002; Zhang and Lin, 2008). Proanthocyanidins are compounds that yield anthocyanidin pigments upon oxidative cleavage in hot alcohols.



(2.10)

Proanthocyanidins compounds that yield anthocyanidin pigments upon oxidative cleavage in hot alcohols

2.4 Significance of Tannins in Plants

Bhat *et al.* (1998) report that tannins are the second most abundant group of polyphenols after lignins. They are considered as classic defence compounds of plants against herbivores. It was observed that the long lived trees have higher tannin content than short lived trees. The factors like high temperature, water stress, extreme light intensity and poor soil quality were reported to increase tannin contents in plants. The chemical basis of tannin defence was attributed to their ability to precipitate proteins. The protein tannin complex was reported to inhibit the invasion of host tissues by microbes and also inhibit microbial growth by reducing the availability of metallic ions necessary for the metabolism of microbes. Tannin complex formation with proteins and carbohydrates was documented to protect plants from herbivores due to unpalatability, and astringent (strong) sensation. The antinutritional effect exerted by the tannin protein complex in reducing availability of food nitrogen or inhibiting digestive enzymes has also been reported (Wagh, 2010).

2.5 Biological Activities of Tannins

Tannins have diverse effects on biological systems because they are potential metal ion chelators, protein precipitating agents, and biological antioxidants. Because tannins can play such varied biological roles, and because of the enormous structural variation among tannins, it has been difficult to develop models which allow prediction of the effects of tannins in any system. An important goal of future work on the biological activities of tannins is the development of structure/activity relationships so that biological activities can be predicted (Tang *et al.,* 2003).

2.6 Enzymes in Leather Industry

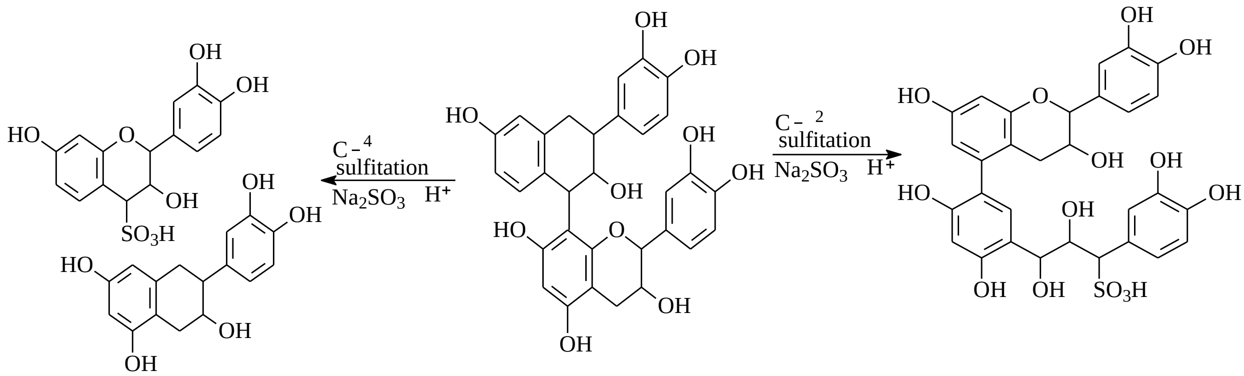
Enzymes have huge potential in the manufacturing industry in general as they can target, digest and modify very specific organic components with minimum environmental impact. Their use has been established in leather manufacture to assist the soaking, liming, and bating processes via the digestion of non-structured proteins. Other functions include breaking fat cells for fat and grease release. Other applications include the removal of non-tanned components in the grain of chrome tanned leathers for relaxation and area gain, and the breakdown of any dung from pre-slaughter (Richard *et al.,* 2013).

The hydrolysable tannins are readily hydrolysed by acids, alkalis or enzymes (tannases) into a sugar or a related polyhydric alcohol (polyol) and a phenolic carboxylic acid (Haroun *et al.,* 2013). Tannase was reported to be present in plants containing hydrolysable tannins such as myrobalan (Terminalia chebula) fruits, divi-divi (*Caesalpinia coriaria*) pods, and dhawa (*Anogeissuslatifolia)* leaves, English oak

(*Quercus robur*), Pendunculate oak (*Quercus rubra*) and in the leaves of Karee (*Rhustyphina*) tree as well as in plants containing condensed tannins like avaram (*Cassia auriculata*), babul (*Acacia arabica*), and konnam (*Cassia fistula*) (Wagh, 2010).

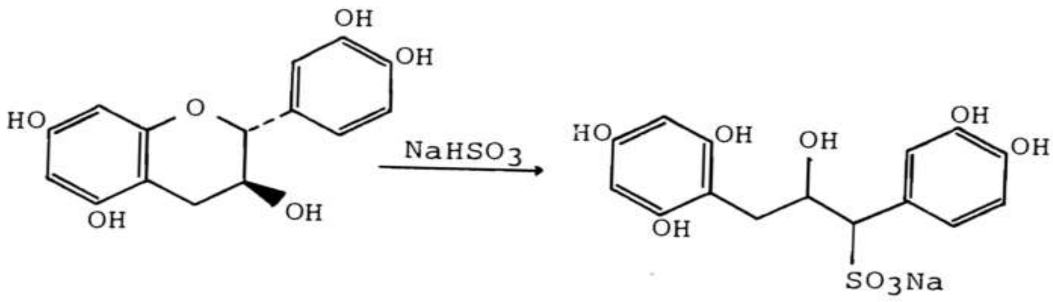
2.7 Sulphitation

This is the reaction between tannin extracts with sodium sulphite or sodium metabisulphite to produce lighter tannins or average molecular weight (500-3000 Da) that can penetrate the collagen faster. The sodium sulphite would either solubilize the large molecular weight or attach itself on C2 or C4 of the catechin backbone (2.22).



Sulphitation reaction of condensed tannin **(2.11)**

The position of sulphite can result in lowering the proanthocyanidins structures that normally penetrate faster in the skin. Sears (1972) confirms that the pyran C ring of catechin is indeed opened on sulfonation to give the compound shown in (2.11). This result perhaps convinced many that all condensed tannins reacted as in 2.11 to give C-ring opened polymers on sulfonation.Haslam (1977), carried out a research on the sulfonation of condensed tannins to improve their solubility and discovered that treating quebracho extracts with sulfites to produce a "soluble quebracho" that remained soluble in cold water or even when made ready for tanning was hailed as one of the most important for the tanning industry. The solid of this "soluble" extract was called “Crown" and contained 64-68% tannins (Bae,1989).



Sodium 1-(3, 4-dihydroxypheny1)-2-hydroxy-3- (2, 4, 6 trihydroxypheny1)-propane-l-sulfonates

Etherocyclic ring opening reaction of (+)-catechin with sodium bisulphate (2.12)

According to Hemingway (1989), all procyanidins which have a phloroglucinol A ring will similarly react with sodium hydrogen sulphite solution (2.12). However, in the case of Acacia and quebracho tannins which have a resorcinol type A ring, the interflavanoid bond does appear to be resistant to such cleavage by sulphite ion (Bae, 1989).

2.8 Structure and Function of Hide and Skin

Skin is made up of many bundles of interwoven protein fibres which are able to move in relation to one another when the skin is alive (Plate 2.6). The basic component of the skin is collagen, a fibrous protein. The name ‘‘collagen’’ is used as a generic term for proteins forming a characteristic triple helix of three polypeptide chains and all members of the collagen family form these super molecular structures in the extracellular matrix, although their size, function and tissue distribution vary considerably (Gelse *et al.*, 2003).

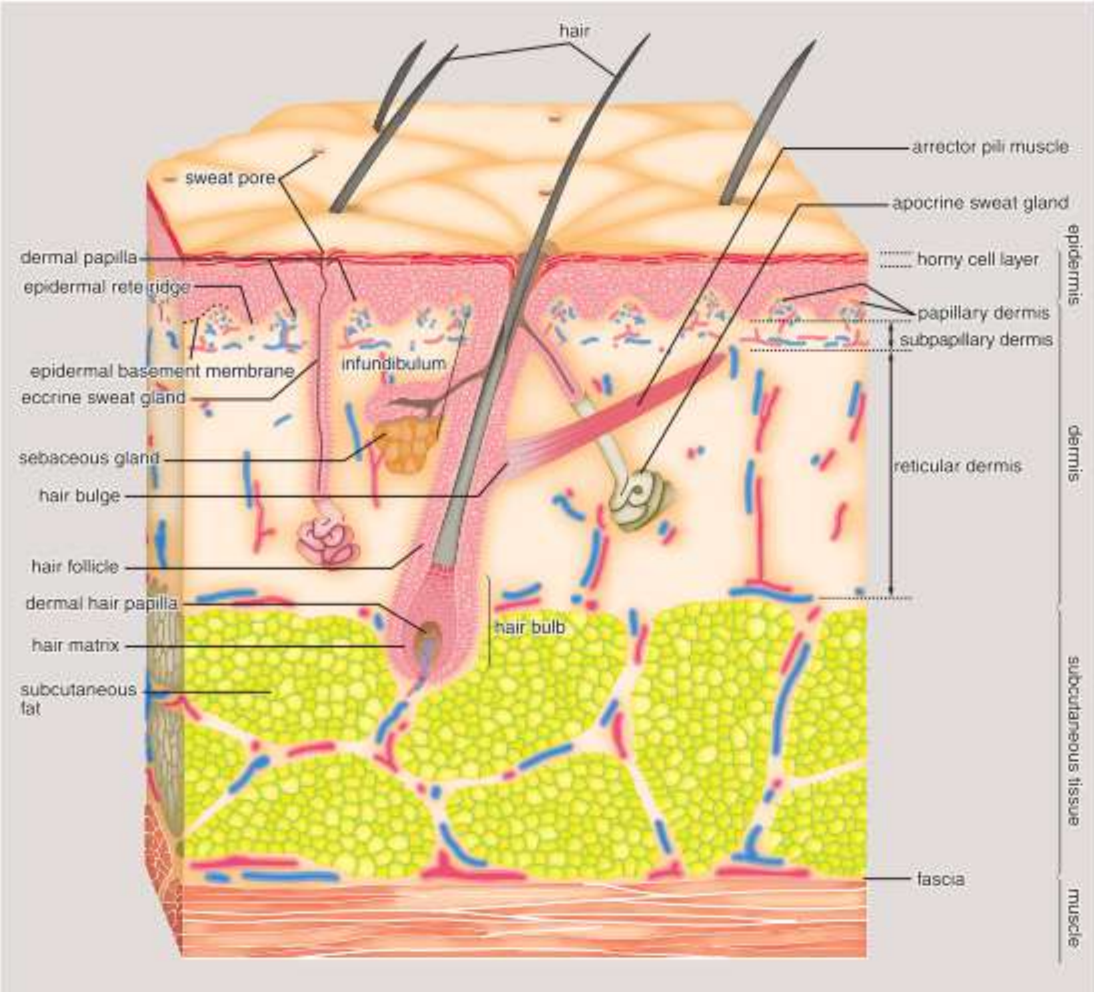
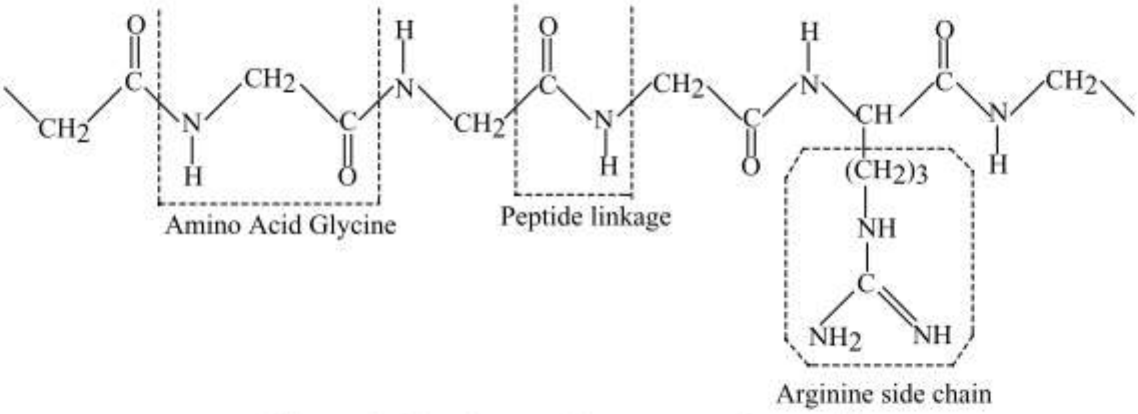


Plate 2.6: Structure of the Skin

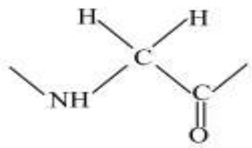
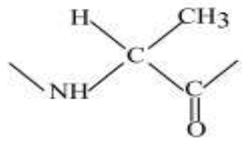
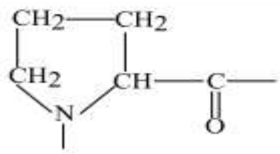
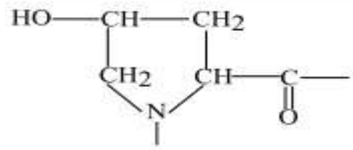
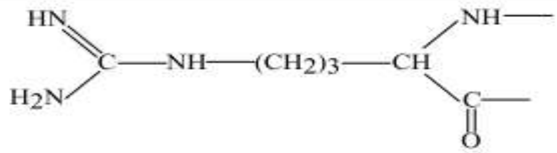
The latest research conducted by Paul and Bailey (2003) indicated that the basic collagen structure consists of twined triple units of peptide chains of differing lengths. The amino acid residues are joined together by peptide links. The peptide chains within the triple helices are held together by hydrogen bonding (2.13). When the skin dies, these fibres tend to become dry and wrinkled, shrivel and stick together. Essentially, the purpose of tanning is to permanently fix the fibres apart by chemical treatment, and to lubricate them so they can move in relation to one another. Well-tanned leather, therefore, retains the properties of flexibility, toughness and wear. It also continues to 'breathe', allowing water vapour to pass through but remaining reasonably water-proof. It is this characteristic which accounts for the comfort of genuine leather shoes and clothing.

In addition, the process of tanning imparts the advantage of resistance to heat. This is an important factor in many of the uses of leather. In conjunction with chemical processing, the tanner imparts colour, texture and finish to the leather, to enhance its appearance and suit it to the fashion requirements in vogue.

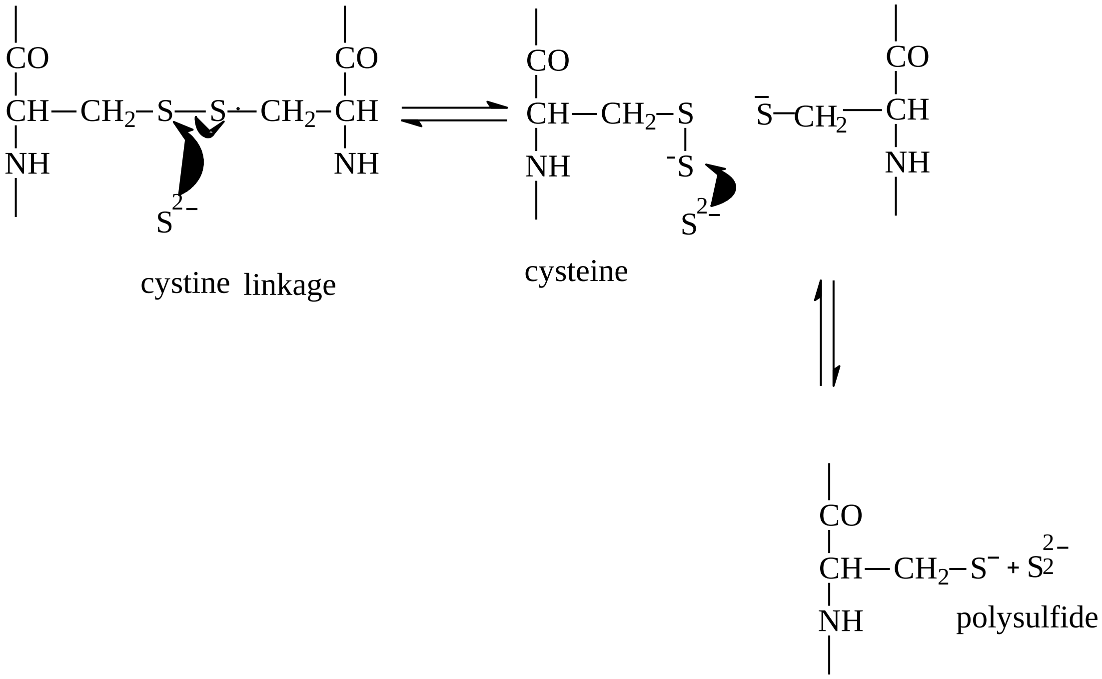


Basic structure of a polypeptide **(2.13)**

Table 2.1: Amino acids found in skin

Amino acid	Structure	Abundance
Glycine		26.8% of total nitrogen
Alanine		8.0%
Proline		9.0%
Hydroxy proline		8.0%
Arganine		15.3%

Skin collagen is usually associated with keratin (the protein in hair, wool and nails). Most mammals have an outer coat of hair, wool or fur, which acts as an insulating layer and keeps the animal warm. Keratin is a fibrous protein and different from collagen in one very important aspect: the polypeptide chains are linked together by cystine linkages. The sulphur-sulphur linkage in cysteine is susceptible to the action of alkali, and breaks down quite readily in the presence of alkali and a reducing agent. The early part of leather production is the removal of hair from the skin, and the presence of the cystine linkage makes it possible for this to be achieved (2.14).



The amino acid Cysteine **(2.14)**

Fibrous structure of true skin (collagen) are arranged in the form referred to here as fibril bundles in which the fibril bundles are a sub-structure or the constructing units of fibres (Covington, 2009). Fibre bundles consist of fibres (diameter 20-200 µm) consisting of fibres (diameter about 5 µm) consisting of fibrils (diameter 10 – 100 µm). Consisting of macromolecules (tropocollagen length about 280 µm and diameter about 1.5 µm, molecular weight 350,000) consisting of three polypeptide chains twisted together in form of a helix (triple helix) and consisting of amino acid which are linked by peptide bonds. 1 kg raw skin has 9 reactive inner surface area of 1000 – 2500 m^2.

The amino acids in collagen can be grouped/classified as alpha amino acid and beta amino acids. Collagens are characterized by a repeating triplet of amino acids: glycine–proline-hydroxyproline. One third of the amino acid residues in collagen is glycine (Table 2.1). The repeating triplet allows the determination of the helical shape of the molecule (Covington, 2009).

While tanning, the fibres are separated, the unwanted matter is removed from the structure, thereby stabilizing and preserving it. In all this, it is ensured that the useful properties are retained. The different collagen types are characterized by considerable complexity and diversity in their structure, their splice variants, and the presence of additional, non-helical domains, their assembly and their function (Gelse *et al.,*2003). The basis of measuring the collagen content in any skin and skin derivative is offered by the unique presence of hydroxyproline and proline. Meanwhile, tryptophan is absent in collagen, thereby making it deficient as a food stuff (Covington, 2009).

2.9 Goats Skins

Goats are the largest group of ruminant livestock in Nigerian totalling about 345million (FDLPCS, 1991). The West African Dwarf (WAD) goats is most commonly found in the coastal and central Nigeria (Hamagun *et al.,* 2006). The Sokoto Red goat is the predominant and most widely distributed breed in the Northern Savannah belts of Nigeria (Ngere *et al.*, 1984). They are usually deep red in colour, about 60cm long with mean male and female weights of 27 and 25 kg, respectively. The West African Dwarf and Sokoto Red goat are raised mainly for meat and are known to be well adapted to the stressful tropical environment. But in Nigeria, especially the northern part, goats are kept not only for meat; but also are used in leather manufacturing (Attah *et al.,* 2004) and for other utilities such as parchment, drumheads and sounding boards musical instruments (Yusuff *et al.,* 2013; Chemonics International Inc., 2002).

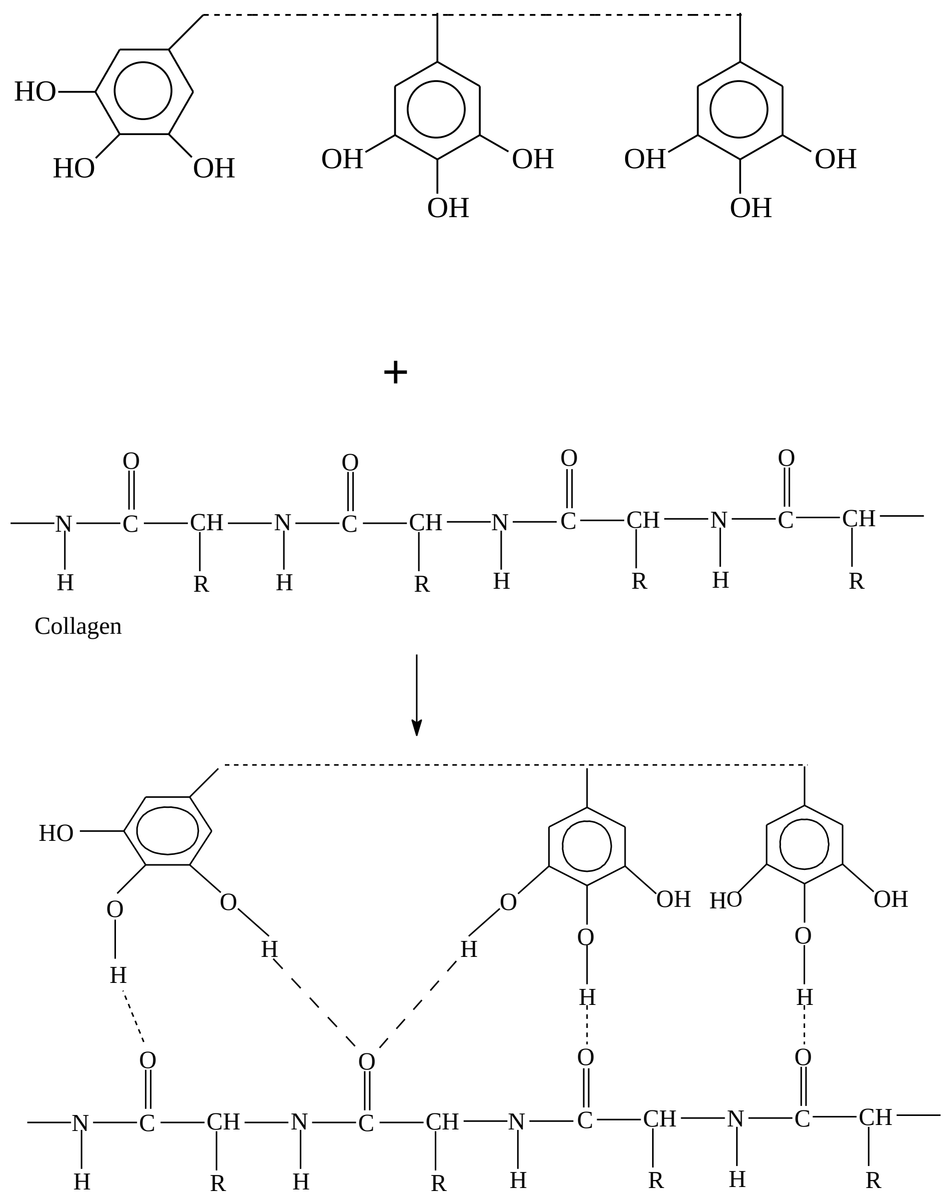
2.10 Mineral Tanning

Tanning effects of simple inorganic compounds reveal that many elements are capable of being used to make leather. However, going by the practical criteria of effectiveness, availability, toxicity and cost effectiveness, the number of useful options of these inorganic compounds is reduced (Covington *et al.,* 1998). Consequently, the benchmark for comparison is tannage with chromium (III): shrinkage temperature> 100^{o}C is by far achieved and it is readily available.

Disposal of chromium contaminated sludge produced as by-products of the waste water treatment is becoming a widely known environmental problems in leather industry (Moorthy and Mekonnen, 2013). Tannery effluent containing chrome could be toxic and consequently affects the mitotic processes and reduces seed germination (Mole, 1993). As a result of the above claimed disadvantages, leather industries are nowencouraged to source for vegetable tannins alternatives which are ecofriendly (Moorthy and Mekonnen, 2013).

2.11 Vegetable Tanning

Vegetable tannins and tanning have kept their significance for many years(Afsar and Sekeroglu, 2008). Vegetable tanning have been carried out on both single and combined use with other vegetable tannins, aldehydes, syntans and various metal salts (2.15). Although the vegetable tannins are thought as alternatives of chromium salts, the characteristics of the leather properties and tanning systems have remained irreplaceable. Attempts are being made continuously to improve the quality of the leather (Madhan, 2006). On the other hand, Madhan (2006) combined valonia and modified valonia extracts together with oxazolidine to obtain an increase in hydrothermal stability, and thus had a better tanning efficiency and produced leathers that had better properties than single valonia tanned ones (Afsar and Sekeroglu, 2008).



Reaction of vegetable tannins with polypeptide chain **(2.15)**

Vegetable tanned leathers are typical of the high-quality leathers produced within the tanning district of Santa Croce sull'Arno, Italy (Castiello *et al.*, 2008). This is the oldest and most classictype of leather, combining the values of quality and tradition. To safeguard and improve these traditional leathers, H.R. Procter, one of the pioneers in applying scientific principles to leather manufacture, said, "Science must follow before it can lead" (Reed, 2013); there is a need to study the process that local tanners use and analyse the tanning strength of the vegetable tanning material they use (Nduru, 2015). It is known that the tanning industry not only needs to pay more attention to the process, but also employ new technologies to guarantee better production constancy (Koloka and Moreki, 2011).

2.11.1 Sorptive ability and binding capacity of collagen

Sorptive ability and binding ability is the first step in determining the tanning ability of the vegetable tannins extracts. The sorptive ability is defined as an ability of a certain amount of tannin to be bound with hide powder; while the binding ability is an ability of a tannin to be irreversibly combined with collagen. Results of these tests could be used to judge the tanning ability of different Vegetable tanning materials (Teng *et al.,* 2013).

Teng in (2013), compared water and diethyl ether extract of vegetable tanning material and found that, the water fraction had the best properties in terms of binding and sorption. On the contrary, the diethyl ether fraction showed minimum values for both properties. This was the same as the Molecular weight sequence. A positive correlation between the Molecular weight and the binding ability and the sorptive ability was observed. That is to say that the higher the Molecular weight of the tannin, the stronger tanning ability it will present (Teng *et al.,* 2013).

The parameters within an established process that mainly affect the production of vegetable tanned leathers are: the temperature reached inside the drums and the mechanical action to which the hides are subjected during the processing. However, this high level of mechanical action can cause the temperature to rise and, in the early stages of tannage, the hides are very sensitive to thermal damage(Castiello *et al.,* 2008), and also to grain damage. This is due to friction within the system, and is directly related to the load size, quantity of water and chemicals, and rotational speed of the drum within the process are based on a balance between the quality of the finished product and productivity. The only aspect not connected with productivity, but crucial for the quality of the finished product, is the smoothness of the drum inner walls and any pegs or paddles that are fitted (Castiello *et al.*, 2008).

Ecological pressures on chromium have now forced the leather industry to look for possible alternatives. A vegetable- aluminium combination tannage has been studied, with special attention being given to intended final product. Aluminium is a mineral tanning agent that is widely used to stabilize collagens in the leather industry. The crosslinking of vegetable and aluminium, with collagens, has been explored(Mahdi *et al.,* 2009). This kind of chrome free tannage give us leathers with shrinkage temperature around 125^{o}C, elongation at break 65.6%, tensile strength 38 N/mm2, and tear strength 98 N/mm. The chemical properties of the combination tanned leathers are found to be quite normal. Among the combination system evaluated, a vegetable pretannage followed by retannage with basic aluminium sulphate was found to produce stronger leather with the durable characteristics.

In contrast, pre-tanning with aluminium possibly tightens the collagen fibre network, preventing high molecular weight vegetable tannins from interacting with collagen fibres. Optimal results were obtained when 10% (w/w) vegetable tannins (garad) and 2% Aluminium sulphate was used(Mahdi *et al.,* 2009). The growing interest in clean technologies and environmental requirements led tanners to increase their efforts to develop chrome-free tanning agents (Spier *et al.*, 2015). Among the various tanning options, vegetable tanning is a traditional and recognizable method and, associated with some physicochemical properties, which provides the leather with unique characteristics like retaining the natural flaws and markings employing organic biodegradable material and it is also environmentally friendly (Spier *et al.*, 2015).

2.12 Leather Processing

Tanning processes is divided into three: beam House operation, tanning operation and finishing operation.

2.12.1 Beam House Operation

The raw hides/skins contain much material unsuitable for leather manufacture and which would introduce serious complications, if not removed as early in the production process. Because of that, serious attempts are made to remove each undesirable constituent. The beam house is a stage where hides/skins are prepared for tanning. The beam house operations include soaking, trimming, fleshing, unhairing, liming, deliming, bating, degreasing and pickling. The term dates back to the time when the hair was removed from the skins by means of a hand beam, that is, on a sloping, curved table or large log using a two-handled knife. This working of the skins on the beam is of ancient origin and is still in use today. The beamhouse operations are of tremendous importance in the ultimate quality of the leather. leather is either made or marred in the beam house.

2.12.2 Soaking

The purpose of soaking is to replace the loss of water during hides/skins preservation, thereby restoring the hide to its original degree of swelling, that is, as it was on the living animal. At the same time, blood, dirt, dung, preservation salts and soluble proteins (albumins,dirts, globulins, various proteins and melanines) are removed. The soaking generally takes 4-6 hours for salted hides and several days for dried hides. The soluble proteins, if not removed from the skin prior to the tanning, will interfere by affecting the tannins that should attack the collagen in the tanning process.

Soaking is generally accomplished by placing the skins in water, which may contain additives. There are two steps involved in soaking: the preliminary or dirt soaking and main soaking. The dirt soaks serves to remove the dirt (blood, dung etc.) and the curing salt. The main soak serves to complete the rehydration and to reduce the salt and the soluble proteins of hides to a level conductive to opening-up of the hides.

2.12.3 Unhairing

Unhairing is the process of removing the hair/wool off the skin. Hair is non-collagenous; thus not necessarily needed in leather making, unless it is hair-on leather. It is known that the protein of the hair, nails, hoofs, epidermal scale is keratin. The cystine and cysteine are the predominant amino acid present in keratin. Sodium sulphide (Na_2S) gives strong nucleophilic attack, thus used as the unhairing agent (2.16 and 2.17).

$$Na_2S + H_2O \longrightarrow NaHS + NaOH$$

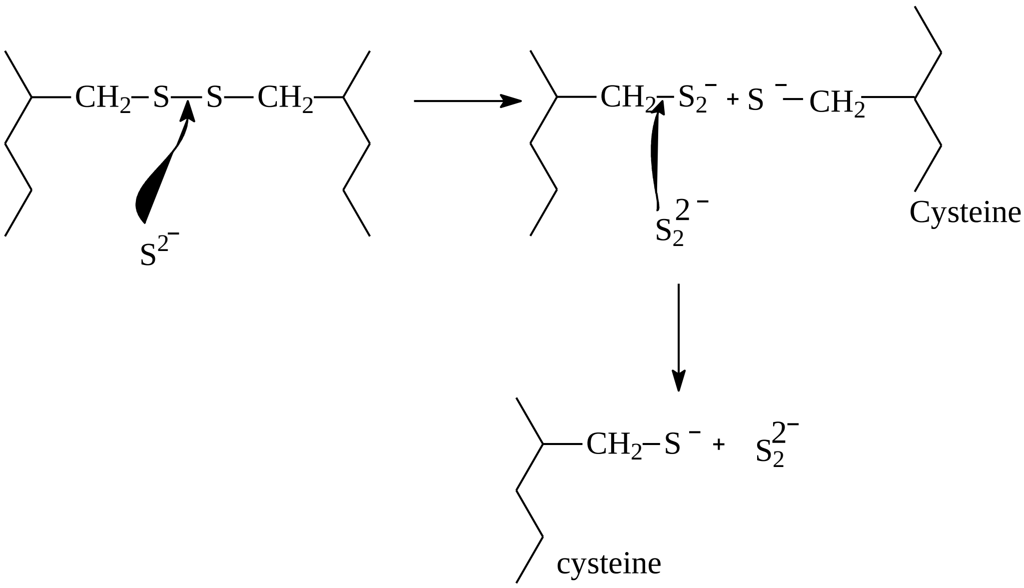
$$NaHS \longrightarrow Na^+ + HS^-$$

$$HS^- + OH^- \rightleftharpoons S^{2-} + H_2O$$

There are two major methods for unhairing: painting, and hair burning(2.16).

1. Paint Unhairing: in this method, a paste of $Na_2S/Ca(OH)_2$is painted on the flesh side of certain soaked skins (by hand or machine). After a few hours, the skins are unhaired and relimed. The advantage of this method is the preservation of the valuable wool and hair for clothing and brush carpet.
2. Hair Burning(destroying): in the hair burning system the skins are introduced into a drum and sodium sulfide is added. Very rapid destruction of the hair results and within fifteen minutes the hair loses most of its fibre structure. The hair burning

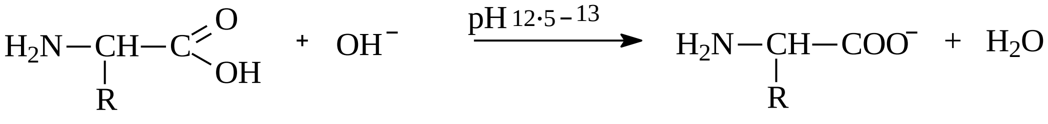
system may result in uneven swelling and in the formation of scud on the skins. The advantage of this method is that it gives a very clean surface and a tight grain and it reduces the costof production (labour, time and quantity of the lime used).



(2.17)

2.12.4 Liming

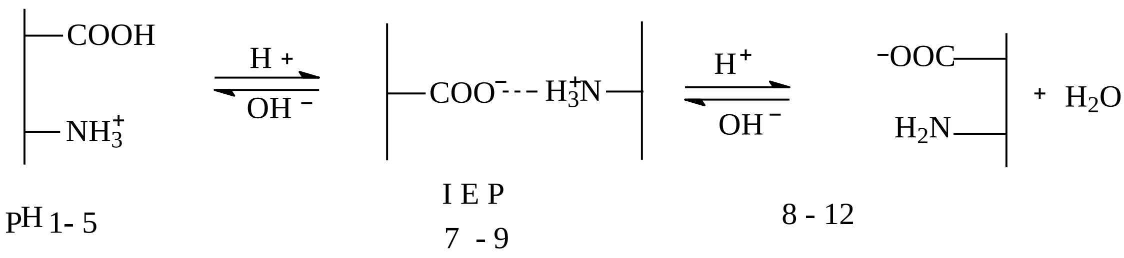
Liming is the use of calcium hydroxide (slake lime) to treat pelts with the aim of modifying the collagen (2.18). The effects of the liming process on hide and skin are as follows: the epidermis is destroyed and the hair is thereby loosened, the whole fibrous structure of the hide and skin is swollen owing to the high pH value, the cementing substance or interfibrillary tissue is attached or affected in some way which renders it more susceptible to the action of the bate.



(2.18)

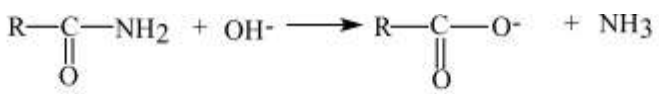
Skins, which have not been limed, cannot be efficiently bated and numerous tissues in the raw skin which are not required in the finished leather are destroyed, e.g. glands, blood vessels, muscles, nerves Also, some of the skin grease is converted into lime or alkali soaps, the adipose tissue is swollen by the liming process and is more easily removed in the fleshing operation, liming causes the following (2.20,2.21,2.22 and 2.23):

(1)amide groups hydrolysis with liberation of carboxyl group and ammonia, so the isoelectric point (IEP) of collagen shifts from 7.6 to 5 (2.19).



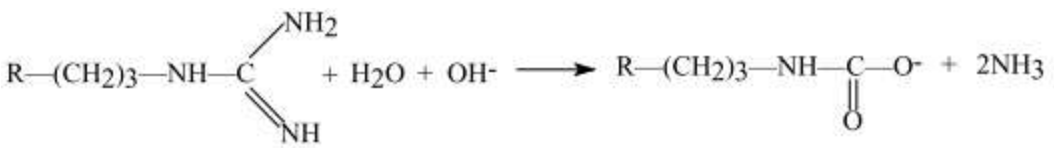
(2.19)

Hydrolysis of amide groups



(2.20)

2) Modification of guanide groups (arginine residue)



(2.21)

3) Hydrolysis of amide linkages in the backbone of the collagen chains

(2.22)

4) Asparagine, glutamic acid hydrolysis

$$R-(CH_2)-C{\overset{O}{\underset{NH_2}{\lessgtr}}} + OH^- \longrightarrow R-(CH_2)_n-CO_2^- + NH_3 \tag{2.23}$$

5) Swelling

In addition to the chemical action, in the presence of an alkali, swelling of the fibrous structure takes place. This is due to an osmotic pressure effect. The result is the separation of the fibres and the fibrils from one another and an opening up of the whole structure.

6) Removal of unwanted material

Present in the collagen structure are globular proteins and other interfibrillary substances. These are hydrolysed and removed in the subsequent washing and bating processes. Unwanted fats are saponified.The pH of the normal liming operation takes place between 12.0 and 13.0, when the hide is introduced into the lime, the grain and flesh surfaces of the skin are subject to solutions of pH approximately 12.5, while the center of the skin is still near neutral.

A differential swelling occurs between the grain, flesh, and center of the skin in accordance, with the variation in pH existing at that time. Hydrotropic substances (that is substances which make the hydrogen bonds between protein chains more flexible) have proved helpful here.

Drum liming, which has become the dominant liming method today, demands most of all, a suitable drum which means a big enough volume, wider than high and low r.p.ms, 1-3r.p.ms,which have proven to be suited best. The volume of the drum has to allow a float length of at least 300%. A too long or too frequent drumming should be avoided. Usually, we drum 5-10 minutes in the beginning of the liming cycle, and then in intervals of one hour 1-2 minutes. Another important factor is the temperature during the liming process, which first of all, should be the same time. Liming temperatures of 20-22^{o}C used to be in order. Today however, the advantages of a warmer system have been recognized, namely, a less pronounced swelling and a smoother grain of the pelts. A liming temperature of 25-28^{o}C is normal today.

2.12.5 Deliming

Deliming is the process of removing lime from inside a limed pelt and it is sometimes called deswelling of pelt. Deliming is aimed at reducing the pH to 8.5, solubilizing the calcium hydroxide and saponified calcium soaps and washing it out. One of the advantages of delimingis that it is the first stage where the loaded lime begins to be removed. It is like reversing the movement of materials from inside to outside. This is done by the use of ammonium chloride and ammonium sulphate (2.24).

$$NH_4Cl + H_2O \longrightarrow NH_4OH + HCl$$

$$(NH_4)_2SO_4 + H_2O \longrightarrow NH_4OH + H_2SO_4$$

(2.24)

Strong acid also can be used but rarely, because they ionize, completely and, hence very difficult to control the pH.

$$\underset{\displaystyle R}{H_2N-\underset{|}{CH}-COO^-} + NH_4(SO_4)_2 \xrightarrow[P^{H\,8}]{H^+} \underset{\displaystyle R}{H_2N-\underset{|}{CH}-COOH}$$

$$\left[\begin{array}{c}(H_2SO_4)\\ \text{generated insitu}\end{array}\right]$$

(2.25)

Others reagents that could be used include weak acids (HCOOH, $CH_3CH(OH)COOH$), CO_2:Although,the reagents reacts pretty fast with OH^-, penetration rate is very slow and must be subjected to strict control to prevent the reaction from coming below pH 6 because of the next process, which is bating. The extent of opening up or fibre splitting depends on the concentration of lime, the volume of water used, the mechanical action and time. For softer leather e.g. clothing, the liming time is prolonged to about 3-4 days. Prolonged liming could lead to breaking down of polypeptide backbone thereby destroying the pelts.

2.12.6 Bating

Much of ancient bating was carried out using dog dung, and was called puering (puer = boy). Up to the 1960s in United Kingdom, chicken dung was still the preferred product. The active ingredient in it is the enzyme that has photolytic effect. Nowadays, bate made from animal pancreas and from by-product of insulin production for diabetic patients is in use.The whole bating process is aimed at digesting the so-called ground substances (hair root) to impact softness and cleanliness. The reactivity of bating agent depends on the concentration, temperature (35-40 ^{o}C), the pH (8.5), and time.

2.12.7 Pickling

This is also called acidification, which is the stage where bated pelts are prepared for tanning. It involves the use of acid, salt in water in order to modify the carboxylic side chains of the amino acid. Typical Preparation: 100:10:1 of H_2O, NaCl and H_2SO_4or HCOOH respectively, depending on the type of tanning. The required pH for mineral tannage is 2.5-2.8 and for vegetable tannins is 4-5.5as shown in (2.26). The lower the pH, the faster the penetration, but the slower the fixation. Consequently, one must have to balance penetration and fixation simultaneously.

$$H_2-N-\underset{R}{\underset{|}{C}}-COOH + HCOOH + NaCl \xrightarrow[pH\ 4\cdot5-5\cdot5]{H^+} H_3\overset{+}{N}-\underset{R}{\underset{|}{C}}-COO^- \qquad \textbf{(2.26)}$$

2.12.8 Tanning

This is the process of introducing tannins into the pickled pelt, there by converting the collagen fibres into a stable material called leather (2.15). This is done at different pH depending on the type of tannin used (mineral or vegetable tannins). Tanning is a chemical process by which additional cross-links are introduced into collagen, binding the active groups of tanning agents to the functional groups of protein (Bienkiewicz, 1983). So, the tanning effect mainly depends on the extent of cross-linking between the collagen molecules and the thermodynamic stability of the cross-linking bonds. Animal skins or hides generally have a substantial thickness, thus penetration of tanning agents is also very important for characterizing the tanning process. Only complete penetration and uniform distribution of tanning materials along the hide's cross section will lead to a satisfactory tanning effect (Haroun *et al.*,2012).

2.13 Leather

Leather is the product of the conversion of hides/skins into a stable material. Thus, capable of withstanding great physical force, flexible, complex biomaterial mainly comprising of fibrous type 1 collagen. Leather is used in a wide variety of manufacturing applications where the physical properties exhibited by the material are important for both strength and aesthetic reasons. The physical attributes of leather are largely dependent on the structure of collagen fibers and the interactions among them. Since leather is processed animal skin, the collagen fiber structure of leather is derived from that of living skin and some other tissue (Sizeland, 2014).Collagen as fibre is formed as a result of the combination of layers of structure known as the 'hierarchy' of collagen structure (Covington,2009).

2.14 Uses and Applications of Vegetable Tanned Leathers

Vegetable-tanned leather is strong and resistant and is used in industry for mechanical parts such as belts that were subject to great duress. Saddlery is also a very ancient application, as are the outer soles of shoes, which remain one of the emblematic outlets for vegetable leather in the luxury sector. But footwear interiors also benefit from its capacity to absorb humidity and it is often used for linings and inlay soles thanks to its strength and its suitability for contact with human skin. It is also recommended for orthopaedic items. These are often at arnal manufactured by moulding, a technique which is unsuitable for its chrome- or synthetic- tanned competitors. Similarly, it is used for upholstery where its patina is considered to be a sign of nobility and naturalness. But it is also benefiting from the boom in leather goods production based on the progress made by drum tanning, which makes it suppler and easier to handle. Handbags in particular are currently an important outlet for vegetable- tanned leather, where it can be used as the outer or inner material (Premier Vision Leather, 2017).

2.15 Instrumentation for UV-VIS and FT-IR Spectrophotometer

UV-Vis spectroscopy is an effective technique for both qualitative and quantitative analysis of organic and inorganic compounds. This is based on the Lambert-Beer principle which states that the absorbance of a solution (A) is directly proportional to its path length (l) and its concentration (c) when the wavelength of the incidence light remains fixed (Brochure, 2013).

The double beam approach to UV-Vis spectroscopy requires two beams of light, both having the same intensity to measure the absorbance through sample and reference positions simultaneously. The sample position is used for measurement of the analyte, whereas the reference position is used for the correction against a blank solution or sample matrix. A clear advantage of the double beam optical system is the improvement in measurement stability and drift precision as a result of having a real-time feedback of both the reference and sample signals.

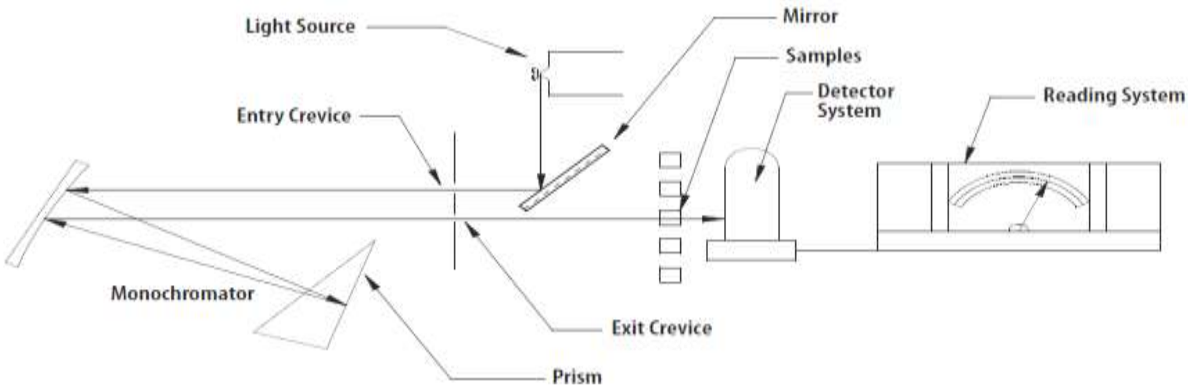


Figure 2.2: Instrumentation for UV-VIS

FT- IR Spectrophotometer

Radiation from an FT-IR source is directed through the sample cell to a beam splitter. Half of the radiation is reflected from a fixed mirror while the other half is reflected from a mirror which moved continuously over a distance of about 2.5 micrometers. When the two beams were recombined at the detector, an interference pattern is produced. A single scan of the entire distance takes about 2 seconds and is stored in the computer. In order that several scans may be added, they must coincide exactly by help of a helium-neon laser, which is simultaneously directed through the Michelson Interferometer and the interference pattern of the laser is used as a frequency reference. Generally, only a small amount of sample will produce an excellent spectrum in a fraction of the time.

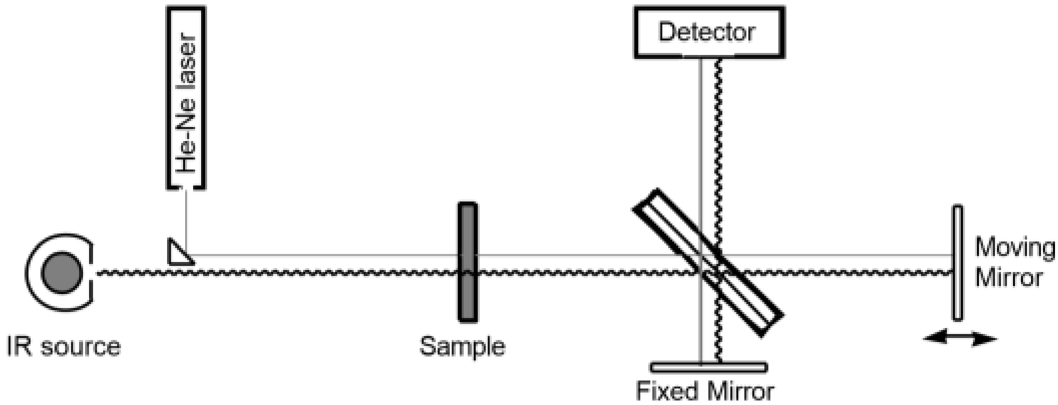


Figure 2.3: Schematic diagram of FT-IR Spectrophotometer

Recorded images contain signals that are generated by molecules or units in a sample, plotted as a function of spectral and spatial distribution. As such, a single data set consists of both spatial and spectral information. The typical dimensions of data set range from 256 to 1024 pixels, with each pixel containing a full IR spectrum. Such data will facilitate visualizing chemical distribution in a sample or chemical compositions of several samples. This type of information is of particular importance, as it is known that chemical as well as physical properties of materials are dependent on the chemical distribution within the samples (Tran, 2005).

Table 2.2:Research Gap from Prvious Related Works

S/N	Authors	Topics	Contributions
1	Adewoye *et al.*, (1986). *Journal of Leather Research, Vol* 4 (2) pp 17.	*Parkia clappertoniana* fruit husk- a promising tanning material of Nigeria- Part 1	The husk of *Parkia clappertoniana*, popularly referred to as west African locust bean was found as a new source of potential commercial vegetable tanning material in Nigeria with 45 % tanning content. It has the highest tannins among the Nigeria tanniferous plants so far screened as at 1986.
2	Adewoye *et al.*, (1987).	Phenolic constituents of	Aqueous acetone extract of the fruit husk of *Parkia clappertoniana* from Nigeria

	Journal of the Leather Technologist and Chemists, Vol.72,pp. 27	*Parkia clappertoniana* fruit husk	was successfully extracted with diethyl ether and ethyl acetate. Catechin and ellagic acid were identified from the ethereal extract, while rutin (quercetin-3-rutinoside) came from the phenolic fraction of ethyl acetate extract and both gallic and ellagic acids from its carboxylic fraction. This was accomplished by means of colour reactions, paper chromatography and co-chromagraphy.
3	Adewoye *et al.*, (1987). *Journal of the Leather Technologist and Chemists, Vol.73,pp. 20*	Anthocyanidins of *Parkia clappertoniana*	Characterization of anthocyanadins for the first time yielded: petunidin, pelargonidin, malvidin, delphinidin and cyaniding
4	Adewoye *et al.*, (1987). *Journal of Leather Research, Vol* 4 (2) pp 17.	Utilization of *Parkia clappertoniana* Fruit Husks in the Tanning Industry	A tanning process for producing vegetable tanned Leathers using the fruit husks of *Parkia clappertoniana* was proposed. In the proposed protocol, 1 % lime in the soaking stage, 10 % lime in the unhairing stage, 10 % lime in liming stage and 10 % lime in the re-liming stage was used. Making the total of 31 % lime used.
5	Mustapha *et al.*, (2012). Journal of Applied Sciences Research, Vol 8(1) pp 65-68.	Studies of tannin extract yield from *Parkia clappertoniana* Husk	The effects of particle size of the husk powder and extraction conditions such as feed (solid) to solvent (FSR), temperature and time on the extract yield were studied in this work. Using water as the solvent, a FSR of 1:20 was found to give the highest yield for the conditions investigated at 27 ^oC. The optimal extraction time and temperature were found to lie between 90 and 120 min and, 50 ^oC and 60 ^oC respectively. The highest extraction yield obtained was recorded at 70 ^oC for 0.25 mm particle size agitated for duration between 90 and 120 min to be about 78

%.

Table 2.3: The old Protocol for Tnning using *Parkia clappertoniana*

RAW STOCK:	Dried goat skin
TANNING METHOD	Weigh the skins: Skin weight
Soaking (pit)	Enough water to cover. Lime 1 % (on skin wt.) 6 hours Reverse order of the pack and leave overnight. Again reverse order of the pack and leave for 2 hours/till complete soaking. (Addition of a preservative: 0.5 % and a wetting agent: 0.25 % to the soak-bath is suggested, to create better conditions for soaking). Stretch/green-flesh on the beam. Cut into sides.
Unhairing	Paint the flesh side of the skins with a paste consisting of Sodium sulphide 2 % (%-ages on Soaked wt) Lime 10 % Water 20 % Pile (flesh-flesh) and leave overnight
Liming (pit)	Water 350 % Lime 10 % 2 days: Handling once/day. (Half of the lime and float may be substituted with old lime liquor). Scud.
Re-liming (pt)	Water 350 % Lime 10 % 3 days: Handling once/day. Scud. Re-flesh. Separate the left and right sides and note the Fleshed weight as follows: weight the left sides and mark them for mimosa tanning ("control"). Keep the right sides for the experiment ("Expt."); weigh half of the right sides and mark them for straight-Parkia tanning; separately weigh the other half and mark them for parkia-bagaruwa tanning.
De-liming (Drum)	Wash the sides in running water for 10 min. and delime with: Water 200 % (%-ages on combined fleshed wt) Ammonium sulphate 0.5 % Pancreatic bate 1 % Run for 1 hr/till deliming is complete. Scud and wash for 20 min with running water.
Pickling (Drum)	Water 70 % Sodium chloride 6 % Sulphuric acid 0.5 %. Run for 1 hr and drain the float. Take out the sides and separate them into "Control" and "Expt.".(A treatment with pre-tanning syntans is suggested at this stage for regular production).
Tanning (Drum)	Left sides: Water 150 % (%-ages on respective flesh wt)

	Mimosa extract (spray-dried) 28 %, add the extract in 4 instalments of 40 min interval and run for a total of 6 hr or till full penetration is achieved. Right side: Repeat as above using Parkia fruit hushs (powdered) 40 % in the place of mimosa extract. Take out the parkia-tanned sides and separate those meant for further processing with bagaruwa and treat the separated ones accordingly with: Water 150 % Bagaruwa pods (powdered) 15 %, add the bagaruwa powder in one instalment and run for 3 hr. (or run for 1 hr; leave overnight; and run again for 1 hr). Pile the tanned sides from the three treatments separately, overnight (well-covered against light and air). Rinse with water for 10 min and allow the water to drain. (for regular production involving parkia-bagaruwa, 30-40 % parkia on fleshed weiht may be used followed by ususal topping with bagaruwa).
Oiling (Drum)	Water 30 %, Sulphonated oil 2 % Groundnut oil 2 % (Epsom salt; 0.5 % may be added to the drum to fix the tannins). Run for 45 min. (add para-nitro-phenol : 0.2 %, after running the drum for 30 min and continue runnin for further 15 min). Horse overnight.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

Dried *Caesalpinia coriaria* (DD) pods were collected from trees within the premises of the National Research Institute for Chemical Technology (NARICT), Basawa, Zaria and *Parkia clappertoniana*Keay (PC) husks were collected from Gidan Tagwai Village, Kachia Local Government Area (LGA) of Kaduna State, Nigeria. Both were identified at the herbarium of the Department of Botany, Ahmadu Bello University, Zaria. The samples were then ground to break up the fibrous matter and a fine powder was collected after sieving with 0.4 mm mesh size. Procter extraction was carried out using water as solvent.

The Sokoto red goat skins of matured size were purchased from Samaru Market, Zaria, Nigeria. The skins were cured using sodium chloride (NaCl) just after flaying so as to prevent microbial activities.

3.2 Extraction of Tannins

Two extraction procedures were used, one involving extraction with methanol and the other involving the use of water. The Soxhlet extraction was carried out using methanol and Procter extraction which was done using water.

3.2.1 Soxhlet Extraction:

Procedure:

The ground pod sample (10 g, 0.4 mm mesh size) was packed with a filter paper and inserted into the soxhlet extractor tube. Methanol (250 cm^3) was transferred into the round bottom flask after which the extractor was coupled. The extraction was carried out at 65 ^{o}C for 2 hr. The extract was later concentrated into a thick paste using rotary evaporator and then air-dried to form a solid mass. This was then transferred to a pre-weighed vessel and the weight was determined. The % yield was calculated, as follows: yield (%) $= \frac{\text{mass of extract}}{\text{mass of dried plant material}} \times 100$

(3.1)

3.2.2 Proctor extraction (Standard, 2007)

Procedure:

The ground sample (40 g) was soaked with cold distilled water in the extractor overnight before commencing the extraction. 150 cm^3 tannin infusion was first collected after which the temperature was raised to 50 °C, and 750 cm^3 was then collected. Then the temperature was raised to boiling point and extract at this temperature was collected into a conical flask to give a total volume of 2000 cm^3. The flask containing the extract was cooled in a large vessel containing cold water until the temperature was about 27 ^{o}C was attained.

3.2 Test for the Presence of Vegetable Tannins

Reagent:

Ferric chloride (5 % w/v): 5 g of ferric chloride was dissolved in a 200 cm^3 beaker containing 50 cm^3 of water and transferred into 100 cm^3 volumetric flask and made to the mark with distilled water.

Procedure:

Powdered dry of extractPC and DD (1 g) was weighed into a beaker and 10 cm^3 of distilled water was added. The mixture was boiled for 5 min and then 2 drops of 5 % ferric chloride was added. A blue or green coloration indicated the presence of tannins (Jonathan, 2009).

3.3 Determination of tanning strength of the Extracts (SLTC, 1996)

The hide powder method, which is the official method of accurately determining the amount of tannins in vegetable tanning materials, was used. In this method, the following were determined: moisture, total solids, total soluble, non-tannins and tannins. The pH of vegetable tannins used was also determined.

3.3.1 Determination of Moisture Content

Procedure:

The finely ground sample (2.5 g) was dried to constant weight at 98.5 to 100 ^oC in an oven in a pre-weighed bottle. It was allowed to cool in a desiccator and the weight of the bottle and content were determined. The moisture content was calculated using the expression in Equation (3.2).

$$\text{moisture } (\%) = \frac{W1 - W2}{W1} \times 100 \qquad \textbf{(3.2)}$$

W1= initial weight (g)

W2 = final weight (g)

3.3.2 Determination of Total Solids

Procedure:

The extract (50 cm^3) was evaporated to dryness on a water bath. The residue was dried at 98.5 to 100 ^oC in an oven to constant weight, and the total solid evaluated when cool using Equation (3.3).

$$\text{Total Solids } (\%) = \frac{\text{Average weight of residue} \times A}{B \times C} \times 100 \qquad \textbf{(3.3)}$$

Where:

A = Weight of extract (g).

B = Volume of extract used (cm^3).

C = Weight of ground sample used for the extraction (g).

3.3.3 Determination of Total Solubles

Procedure:

The sample extracts (7 g /1000 cm^3 water) was filtered after the extraction with water. The first 250 cm^3 filtrate was discarded, and continued filtration, until the filtrate was cleared. 50 cm^3 of clear filtrate was evaporated to dryness. This represents the total solubles.

$$\text{Total soluble } (\%) = \frac{\text{Average weight of residue} \times \text{A}}{\text{B} \times \text{C}} \times 100 \qquad \textbf{(3.4)}$$

Where:

A = Volume of extract (cm^3).

B = Volume of extract used (cm^3).

C = Weight of ground sample used for extraction (g).

3.3.4 Determination of Total Insolubles

Insoluble was determined as the difference between the total dry residues and total soluble.

Insoluble (%) = Total solid (%) - Total soluble (%) **(3.5)**

3.3.5 Determination of pH of the Extracts

A solution of a sample extract was prepared by dissolving a quantity in a boiling water and adjusted with a distilled water to a specific gravity of 1 ± 0.5 at room temperature. The pH was determined using a pH meter of the model Jenway, 3505.

3.4 Chromatographic Separations of Tannins

3.4.1 TLC of Condensed and Hydrolysed Tannins

Reagents:

Solvent 1: Glacial acetic acid (2 %) 2 cm^3 was mixed with 98 cm^3 distilled water in a 100 cm^3 volumetric flask.

Solvent 2: Butan -1 -ol (60 cm^3) was mixed with 15 cm^3 glacial acetic acid and 25 cm^3 water in a 100 cm^3 volumetric flask.

Spray reagent to detect different classes of tannins:

Potassium iodate: a saturated solution of KIO_3 (potassium iodate) was prepared in 100 cm^3 distilled water in a beaker (i.e. KIO_3 crystals was added enough until some of them no longer dissolve in the water).

Procedure:

The methanol extracts (0.1 cm^3) were applied to several cellulose MN300 TLC plates (10 x 10 cm) and the spots were kept at the origin (approximately 7mm diameter) without damaging the TLC surface. The TLC plate were placed in "solvent 1" first and removed before the solvent front reached the top of the plate. The plates were air dried, then turned at 90 ^{o}C and placed into a tank containing "solvent 2". The TLC plates were subjected to the spray reagent separately.

3.4.2 Column Chromatography

The adsorbent (Silica gel) as stationary phase was slurred in 200 cm^3 of absolute methanol to equilibrate it. A small plug of glass wool was fixed at the base of a clean chromatographic column (vertical and transparent glass tube) and firmly clamped to a retort stand. The slurry was then transferred in portions to the chromatographic column; allowing the packing to settle between additions in order to ensure a homogenous column. The excess solvent (methanol) was allowed to drain out. A portion (2 g) of the tannin was dissolved in about 50 cm^3 of the solvent (methanol) to keep the sample in solution. The solution obtained was filtered through a Whatman No 1 filter paper to obtain the methanol-soluble portion and to remove any insoluble material. The solution obtained was then concentrated over water bath at 60 ^oC. The concentrated solution was then gently applied to the top of the column (Hagerman, 2002). The first solvent (pure and absolute methanol) was allowed to percolate through the column. Subsequently, the eluate was collected in the collection bottles in small portions of about 5 cm^3. Different portions of the eluates were collected while the methanol was allowed to percolate through the column.

3.4.3 Purification of Tannins

Reagents:

Ethanol (80 % v/v) - 80 cm^3 of ethanol was mixed with 20 cm^3 water in a 100 cm^3volumetric flak.

Ethanol (95 % v/v) - 95 cm^3 of ethanol was mixed with 5 cm^3 water in a 100 cm^3 volumetric flak.

Acetone (70 % v/v) - 70 cm^3 of acetone was mixed with 30 cm^3 water in a 100 cm^3 volumetric flak.

Sephadex LH 20 – 25 g of the gel was allowed to swell in 80 % ethanol and made to 100 cm^3.

Procedure:

The extract (1 g) was suspended in 100 cm^3 of 80 % ethanol, stirred and filtered using a Buchner funnel equipped with Whatman No.1 filter paper, under vacuum. 25g of Sephadex was allowed to swell in 80 % ethanol to make 100 cm^3 slurry. It was stirred gently with a stirring rod to facilitate moistening and allowed to settle. The supernatant and fine particles of the Sephadex were decanted and it was repeated three times. 100 cm^3 of the slurry was packed in a column and the filtrate of tannin extract was added. Sephadex was washed with 95 % ethanol until the eluates became almost colourless. Ethanol wash was discarded, the final eluate was colourless, and the Sephadex was brown in colour. The Sephadex was washed with 70 % aqueous acetone until the Sephadex turned white and the eluate colourless. The acetone fraction was oven dried at 40 ^oC to give a fluffy brown powder which was stored in a desiccator (Hagerman, 2002).

3.5 Preparation of the sample for FT-IR

About 2 mg of samplewas mixed with dry potassium bromide (KBr) powder and ground with an agate mortar and pestle and subjected to a pressure of about 10 ton in an evacuated die. This sinters the mixture and produced a clear transparent disc. The disc was inserted into the FT-IT sample compartment, with a wave number range of 4000 – 650 cm^{-1} at a resolution of 8 cm^{-1}, and scanned 16 times; background and sample scan were also carried out using transmittance method (Stuart, 2004).

3.6 Sorptive Ability and Binding Capacity with Hide Powder

Reagents:

Acetone/water (7:3 v/v) – this was prepared by mixing 70 cm^3 of acetone with 30 cm^3 water in a 250 cm^3 beaker.

Tannin solution: 1.5 g of the tanning material was dissolved in 30 cm^3 of acetone/water in a 100 cm^3 beaker.

Procedure:

Tannin (1.50 g) was dissolved in 30 cm^3 acetone/water mixture, 5 cm^3 of the acetone/water solution was taken into a crucible and heated at 120 ^oC to dryness. This was cooled to about 10 ^oC in a desiccator and later weighed (W_1). 2.00 g of dry hide powder was soaked with 25 cm^3 distilled water in a conical flask for 4 hr, 25 cm^3 tanning agent was added and kept in a shaker for 24 hr. The mixture was filtered with Buchner funnel and 10 cm^3 filtrate was dried at 120 ^oC and weighed (W_2). Meanwhile the tanned hide powder (A) was collected. Sorptive ability was calculated based on the Equation (3.6):

$$\text{Sorptive ability} = \frac{5(W_1 - W_2)}{2.00} \text{x } 100\ \% \qquad \textbf{(3.6)}$$

The tanned – hide powder (A) was washed with 1000 cm^3 distilled water every 8 hr, and 1 % gelatin – sodium chloride solution was used to react with the filtrate as a tannin test and after 72 hr, the washed hide powder was transferred into an oven and dried at 50 ^oC and weighed (W). Binding ability was calculated according to equation [3.19].

$$\text{Binding ability} = \frac{(W - 2.00)}{2.00} \text{x } 100\ \% \qquad \textbf{(3.7)}$$

3.7 Determination of Tannin

3.7.1 Official Shake's Method (SLTC, 1996)

Reagents:

Chrome alum (3 % w/v) – 3 g of chrome alum was dissolved in a beaker and transferred into 100 cm^3 volumetric flask and made to the mark.

Kaolin,

Hide powder

Procedure:

Hide powder (6.25 g) was taken into a 250 cm^3conical flask containing 62.5 cm^3 of water. This was swirled to ensure complete immersion of the hide powder particles in the water. The mixture was then shaken with a mechanical shaker at 15 rpm for 1 hr. 6.25 cm^3chrome alum solution was then added and the shaking was continued for 3 hr and then the mixture was left for about 16 hr. After which boiling water (800 cm^3) was added to 7 g of blended sample. The solution was stirred for five minutes and allowed to stand for 25 min. It was transferred quantitatively into a 1000 cm^3 volumetric flask and more hot water added to the mark. The extract was filtered through cotton wool and filter paper to obtain a clear solution. This was labelled "Extract A''.

"Extract A'' (50 cm^3) was evaporated in a pre-weighed crucible, dried in an oven at 98.5-100 ^oC to constant weight. It was cooled in a desiccator containing silica gel and later weighed.

The chromed hide powder was filtered using a filter cloth into a 1000 cm^3 conical flask and soaked with 93.75 cm^3 water for 15 min. The water was squeezed out. The powder was again soaked with the same amount of water three more times and transferred into a conical flask. "Extract A" (100 cm^3) was transferred into the conical flask containing the pre-chromed hide powder. 20 cm^3 of water was added and the mixture shaken vigorously by hand for about 15 s before transferring to a mechanical shaker set at 50-65 rpm for 10 min. The mixture was filtered through a filter cloth on a funnel. The filtrate was collected, and 1 g of kaolin was added and thoroughly mixed. The mixture was filtered to obtain a clear filtrate. This was labelled "Extract B''.

"Extract B'' (50 cm^3) was evaporated in a pre-weighed crucible, dried in an oven at 100 ^oC to constant weight.

Tannin % was calculated using equation:

$$\text{Percent soluble } (\%) = \frac{\text{weight of residue from Extract A} \times \text{constant}}{50 \text{ cm3} \times \text{Xg}} \times 100 \qquad \textbf{(3.8)}$$

$$\text{non} - \text{tans } (\%) = \frac{\text{weight residue from Extract B } \times \text{ Y } \times \text{constant}}{50 \text{ cm3} \times \text{Xg}} \times 100 \ \textbf{(3.9)}$$

Tannins (%) = percent soluble – percent non- tans **(3.10)**

Where:

Y = maximum volume of leached extract (cm^3).

X g = weight of sample leached (g).

Constant = 1.2 (correction for the 20 cm^3 of water of dilution by the wet hide powder into the 100 cm^3 tannin solution).

Determination was carried out in triplicate.

3.7.2 Measurement of Total Phenolics and Tannins using Folin-ciocalteu Method

Reagents:

Folin Ciocalteu reagent: 10 cm^3 of Folin-Ciocalteu reagent was mixed with an equal volume of distilled water and transferred into a brown bottle and stored in a refrigerator (4°C).

Sodium carbonate (x10 H_2O) (7.5 %): 7.5 g was weighed and dissolved in a beaker with 70 cm^3 distilled water and transferred into 100 cm^3 volumetric flask and made up to the mark with distilled water.

Gallic acid solution (1 g/dm^3): 1 g of gallic acid was dissolved in a beaker containing distilled water and transferred into 1000 cm^3 volumetric flask and made to the mark. Six working standards of 0, 10, 20, 30, 40 and 50 mg/dm^3 respectively were prepared by mixing 1000 cm^3 each with distilled water.

Procedure:

Crude extracts of the sample was diluted 50 times with distilled water prior to analysis. 1 cm^3 of the diluted extract was mixed with 1 cm^3 of Folin-Ciocalteu reagent. After incubating the mixture in the dark at room temperature for 4 min, 0.8 cm^3 of 7.5% (w/v), sodium carbonate solution was then added. The mixture was then centrifuged for 10 s and incubated in the dark at room temperature for 2 hr. The absorbance of the mixture was measured against blank at 725 nm using UV-VIS spectrophotometer. A calibration curve was prepared and the concentration of gallic acid in the sample was determined. Each crude extract was analyzed in triplicate and the results were expressed in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW)(Bouterfas *et al.*, 2014).

3.7.3 Determination of Condensed Tannins (Proanthocyanidins)

Reagents:

Butanol-HCl reagent: 950 cm^3 of n-butanol was mixed with 50 cm^3 concentrated HCl (36 %).

Ferric reagent: 2.0 g of ferric ammonium sulphate was dissolved in 100 cm^3 volumetric flask with 2 M HCl and make up to the mark and stored in an amber bottle.

Procedure:

The tannin extract (0.5 cm^3) was transferred into a test tube (100 mm x 12 mm) and diluted with 70 cm^3 acetone. To the test tube 0.3 cm^3 of the butanol-HCl reagent was added and 0.1 cm^3 of the ferric reagent. Each tube was stoppered with glass marble and the tubes were placed on a heating mantle set at a temperature of 100 ^oC for 1 hr. The tubes were allowed to cool and the absorbance was recorded at a wavelength of 550 nm. The amount of condensed tannins (X) was determined by the expression:

$$X = \frac{\text{A 550nm x 78.26 x dilution factor}}{\text{\% dry matter}} \qquad \textbf{(3.11)}$$

Note: The dilution factor is 0.5 cm^3 / (volume of extract taken) :

3.7.4 Gallotannin Determination using Rhodanine Assay

Reagents:

KOH (0.5 M): 2.8 g KOH was dissolved in a beaker containing 20 cm^3 of water and transferred into 100 cm^3 distilled water in a volumetric flask and made up to the mark.

Sulphuric acid solution (1.0 M): 1.4 cm^3 sulphuric acid (Sp gr. 1.84) % purity was measured and mixed with water in a beaker and allowed to cool at room temperature before transferring into a 250 cm^3 volumetric flask and made up to the mark.

Supernatant A (Extract): obtained from 0.50 cm^3 of the tannin extract diluted with 70 % acetone.

Rhodanine solution: about 0.79 g rhodanine was weighed and dissolved in 100 cm^3 methanol and stored in a refrigerator.

3.7.5 Determination of free Gallic acid

About 0.2 cm^3 supernatant A (Extract) was transferred into a test-tube (160 mm x12 mm) in quadruplicate. Acetone was removed from the sample using a vacuum drying oven adjusted at 40°C for 2 hr. And 0.2 cm^3 of 1.0 M sulphuric acid was transferred into the three tubes containing dried supernatant A. To the fourth test tube, about 0.3 cm^3 of the rhodanine solution and 0.3 cm^3 methanol was added. This fourth tube acted as a blank. After 5 min 0.2 cm^3 of 0.5 M KOH solution was added to all the tubes followed by 4.0 cm^3 of distilled water and the absorbance was taken at wavelength 520 nm.

3.7.6 Determination of Gallic acid present in free and in gallotannin Forms

About 3.34 cm^3 of supernatant A was transferred in duplicate, in the above mentioned test- tubes and 0.1 cm^3 of 3.5 M Sulphuric acid was added. The tubes were kept at 100 ^{o}C for 26 hr to hydrolyse gallotannins to gallic acid. After the hydrolysis, the volume was made up to 11 cm^3 with distilled water. Sulphuric acid concentration in this solution was 0.1 M. This solution was labelled as hydrolysed supernatant A. About 0.2 cm^3 of the hydrolysed supernatant A was transferred (4 tubes per sample: one blank and the rest 'test' samples) and 0.3 cm^3 of the rhodanine solution was added to the test-tubes and 0.3 cm^3 methanol to the blank tube. After 5 min, 0.2 cm^3 of 0.5 M KOH solution and 4.3 cm^3 distilled water were added respectively. Then absorbance was measured at wavelength 520 nm after 10 min.

3.7.7 Preparation of Gallic acid Solution

Gallic acid (0.11 g) was weighed and dissolved in 100 cm^3 of 0.1 M sulphuric acid, which was kept for two weeks. Working gallic acid solution (0.001 g/cm^3) gallic acid – stock solution was diluted 1:10 with 0.1 M sulphuric acid (1 cm^3 of the stock solution with 9 cm^3 distilled water) which was also stored in a refrigerator for at least 2 weeks.

3.7.8 Determination of Stabilization Time between ferric chloride and the sample solution.

The time needed for the complex to stabilize was determined, using 0.4 cm^3 of 0.05 mol dm^{-3} Iron (III) chloride solution was added to about 20 cm^3 of the sample solution. The absorbance of the complex formed was measured at 570 nm and was recorded at 2 min intervals to constant values and each observation was repeated.

3.8 Tanning Procedure

The stages and procedures for tanning as follows (Covington, 1997):

Soaking: - The skin was pre-soaked in 1:5 water at room temperature with 1: 0.04 detergent for 2 hr to remove dirts.

Main skin soaking was carried out in 1:10 water at room temperature with 1:0.03 detergent for 24 hr and drained on beam.

Unhairing: - Water (1:1) was added to the skin at room temperature, followed by sodium sulphide (1:0.03) and pulped for 60 min.

Liming: - Calcium hydroxide (0.03:1) was added to the pelt for 2 hr, followed by 1:10 water to cover and left for 24 hr.

Deliming: - The limed pelt was delimed in water and ammonium sulphate salt 1: 0.7: 0.03 for 60 min and was checked for deliming using phenolphthalein (pH 8 to 9).

Bating: -This was carried out with bate powder 1: 0.008 for 25 min, then drained and washed twice.

Pickling: -The pelt was acidified in water, Sodium chloride and formic acid 1:1:0.1: 0.01 respectively at room temperature and it was run for 20 min and then run for 1 hr after it was left stationary overnight. pH was checked to be between 4.0 and 5.5.

Tanning: -This was carried out using the same pickle liquor in short float tannage. For Divi-Divi tannage, 1: 0.3 crushed Divi-Divi was used in 3 lots (0.1 each) at intervals of 30 min. The same was carried out for *Parkia clappertoniana* tannage.

Fatliquoring: -This was carried out for both tannages in 1: 0.6 water at 50 ^{o}C with 1: 0.04 fatliquor for DD and 1: 0.05 for PC for 45 min, then 1: 0.01 formic acid was added and run for another 15 min.

Finally, the tanned leather was drained, washed, tested for shrinkage temperature and hung to dry; furthermore, it was conditioned, staked, toggled, trimmed, buffed and de-dusted and then taken for physical and chemical tests.

Table 3.1: The quality of leather produced by combination of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertonia* (PC) and *Acacia nilotica* (AN) used in tanning goat skin samples

Group	**Ratios**			
	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Key: DD/PC – Ratios, M-Modifier (Sodium bisulphite)

***Acacia nilotica* (AN)- control**

3.9 Physical Analysis of the Tanned Leather

The quality of the leathers was determined according to the International Union of Leather Technologists and Chemist Society (IULTCS) official methods 1996.

3.9.1 Shrinkage Temperature Determination

The shrinkage temperature of the tanned skin was measured using SATRA TM 17:1997 Shrinkage Temperature apparatus (Allen, 2015). A small strip (10 cm X 5 cm) of leather fibre was cut and immersed in a closed convection circuit comprising the transparent sample chamber (sight glass) connected to a pipe with a small water reservoir which was heated with a Bunsen burner. The temperature of the water in contact with the leather strip was monitored with the thermometer immersed alongside the leather strip in the transparent sample chamber. A safety valve was fitted to the apparatus which limits the pressure corresponding to a temperature of 120 ^{o}C. The temperature at which the leather shrinks to one–third of its length was taken as the shrinkage temperature.

3.9.2 Measurement of Thickness

Six (6) samples of leather were cut in a circular shape. A standard dial micrometer gauge was loaded with a mass of 500 gcm^{-2} at the presser foot. The gauge was set to zero and then used to measure the thickness of the leather in four (4) positions at 1 cm from the edge. The central position was marked and then the other three formed an equilateral triangle. Each reading was taken 5 s of dwell and on the grain side.

3.9.3 Measurement of the Apparent Density of Leather

The procedure in 3.8.2 was repeated. Here, the diameter of the leather was measured at two positions at right angles using Vernier Calipers. The diameter readings were taken both on the flesh and the grain sides. The weight of the leather sample was taken three (3) times using a weighing balance. The mean diameter was determined and the thickness was used as the height to calculate the volume. The apparent density was calculated as in (3.12).

$$\text{Apparent density} = \frac{\text{mass}}{\text{Volume}} \qquad \textbf{(3.12)}$$

Where: mass :- mass of leather (g), volume :-volume of the leather (cm^3)

3.9.4 Measurement of the Resistance to Compression

The thickness of the leather was measured in four positions under a pressure of approximately 20 g cm^{-2} (no added weight). The mean thickness (d_o) was determined and then thickness was measured again in the same positions using a 200 g load. The mean thickness (d_1) was determined as in 3.8.2.

The measurement was repeated for both the flesh and grain sides.

Resistance to compression 'R'

$$R = \frac{\text{Increase of load per unit area}}{\text{Decrease in thickness per unit thickness}} \quad \textbf{(3.13)}$$

$$R = \frac{(200 \text{ x } 4/\pi) \text{ x do}}{1000 \text{ (do} - \text{d1)}} \text{ kg cm}^{-2} \quad \textbf{(3.14)}$$

A low ‘R’ figure indicates a softer surface characteristic.

3.9.5 Measurement of Indentation Index

The thickness of the leather was measured in four positions with a load of approximately 20 g. The mean thickness (d_o) was determined and then the thickness was measured again in the same positions with a load increase of 1000 g. The mean thickness (d_1) was also determined. The measurement was repeated for both the flesh and grain sides. Indentation index is the difference between the two means ($d_o - d_1$) for either surface (grain or flesh) expressed in 1/100 mm.

3.9.6 Measurement of Tensile Strength

The samples were cut parallel and perpendicular to the back bone in a dumbbell shape – the thickness and width of the specimen were measured in the same position using a standard micrometre screw gauge and Vernier Caliper, one at the midpoint and the other two midway. The width was measured on the flesh and grain side, and then the mean thickness (mm) and width (cm) was determined. The area of cross section of each specimen was calculated by multiplying its width by its thickness (SLTC, 1996). The jaws of the tensile machine (Instron 1026) were set 50 mm apart, and then the sample was clamped between the jaws, so that the edges of the jaws lay along the mid line. The machine was run until the specimen was broken and the highest load reached was taken as the breaking load. Tensile strength load was expressed in Newton’s (SLTC, 1996) as calculated using equation 3.15.

$$\text{Tensile strength} = \frac{\text{maximum breaking load}}{\text{initial free length}} \times 100 \quad \textbf{(3.15)}$$

3.9.7 Percentage Elongation at Break

The initial free length between the clamps before and after final free length was set at 5 cm and the elongation calculated from a graphical read out (SLTC, 1996) as calculated using equation 3.15, while the percentage elongation at break was evaluated thus:

$$\text{Elongation (\%)} = \frac{\text{final free length} - \text{initial free length}}{\text{initial free length}} \times 100 \qquad \textbf{(3.16)}$$

3.9.8 Lastometer Determination of Grain Crack Properties of Leather(Kuria et al., 2016a)

Discs of upper leathers were cut and the location, leather type, and tannage of the leather were noted. Thickness gauge was used to measure the thickness of the grain side in 3 positions, under a load of 500 gcm^{-2}. Then, the Lastometer was set to zero. Thereafter, the circular leather specimens were placed (flesh side down) on the Lastometer and securely damped. The distension increased at a rate of 1 mm per 5 s. while the grain surface was observed for appearance of grain crack/burst. The grain crack, the load (N) and the distension (mm) at 10 N intervals were determined to a maximum of 80 kg using the official standard samples position (butt, shoulder and belly).

3.9.9 Measurement of Water Vapour Absorption

The mass of circular leather sample was measured with a weighing balance. The leather sample (test piece) with an impermeable material were clamped over the opening of a metal container containing 50 cm^3 of water for 1 hr at room temperature. The test piece was measured to determine the amount of water vapour absorbed (3.17).

Water vapour absorbed = Final weight of test piece - Initial weight of test piece **(3.17).**

3.9.10 Measurement of the Absorption of Water

A disc leather sample was cut using a cutter and hammer and the location, leather type and tannage type were noted. The thickness of the leather sample was measured under a load of 500 g cm^{-2} using a standard thickness gauge. The average thickness (mm) was calculated. The diameter of the leather was measured using a Vernier caliper and was weighed. The Kubelka apparatus was washed and filled to the mark. The sample was immersed and left for about 15 min, after which the water was drained and the volume was re-measured after 1 min. The leather sample was re-immersed and the above procedure was repeated at 15 min intervals for 24 hr. The changes in volume of liquor (Q and P) in the Kubelka apparatus were noted:

$$Q = 100\ v/m; \quad P = 100\ v/V \tag{3.18}$$

Where v = total volume of water absorbed (cm^3) at time't'

m = mass of sample (g)

V = initial volume of leather (cm^3)

P and Q = were plotted against time

3.9.11 Measurement of Water Vapour Permeability

A bottle (100 cm^3) was half-filled with dry desiccant (silica gel) and a pre-weighed leather sample (grain side) was placed at the mouth of the bottle sealed and made air-tight with adhesive. The water vapour permeability machine was allowed to equilibrate for 1 hr. The leather samples were re-weighed. The internal diameter (free area) of the samples in the bottle was measured and the water vapour permeability, P was determine by taking the weight difference of the leather sample and plotted against time (1 hr interval).

$$P = \frac{7639.m}{d^2.t}\ (g\ cm^{-2}h^{-1}) \tag{3.19}$$

Where d = 'free area' diameter (mm)

t = hr

m = final – initial weight (g)

3.10 Chemical Analysis on Leather

3.10.1 Preparation of Leather Sample

The leather was cut into small pieces to pass through a screen with circular perforations of 4 mm. The pieces were thoroughly mixed and brought to a state of homogeneity by keeping them in a closed container for one night. After determining the moisture content, they were stored in a jar.

3.10.2 Determination of Ash Content

Leather sample (5 g) was placed in a pre-weighed porcelain crucible and carbonized at 400 ^{o}C on a hot plate in a fume cupboard and then transferred to a furnace set to 800 ^{0}C (SLTC, 1996).

The ash content was calculated according to equation. **(3.20)**

$$\text{Ash (\%)} = \frac{W2}{W1} \times 100 \quad \textbf{(3.20)}$$

Where:

W_1 = Weight of the sample (g).

W_2 = Weight of the ash (g).

3.10.3 Determination of Fat Content

Leather sample (20 g) was weighed and wrapped in a filter paper and placed in a Soxhlet apparatus, equipped with 250 cm^3 of round bottom flask containing 200 cm^3 petroleum- ether (boiling point 40-60 ^{o}C). Extraction was carried out for 5 hr using a heating mantle set at 50 cm^3. After extraction the extract was concentrated in a rotary evaporator at 40 ^oC to thick syrup and then heated in an oven at 103 ±3 ^oC (SLTC, 1996). The fat content was evaluated.

Thus:

Fat content (%) $= \frac{W1}{W2} \times 100$ **(3.21)**

Where:

W_1 = fat weight (g)

W_2 = Weight of leather sample (g)

3.10.4 Determination of Total Matter Soluble in Water

The defatted leather samples from Section 3.9.3 were placed in a 250 cm^3 conical flask with distilled water and shakes for 4 hr then left at room temperature overnight. The extract was then collected in 1 dm^3 volumetric flask and made up to volume with water. 50 cm^3 of the extract was transferred into a porcelain dish and evaporated on a water bath. It was later dried in an oven at 100 ^oC to constant weight (SLTC, 1996). The total matter soluble in water was determined (3.22).

Total matter soluble in water, (%) = $\frac{20 \times W2}{W1} \times 100$ **(3.22)**

Where:

W_1 = Weight of final residue (in g).

W_2 = Weight of initial residue after water extraction (in g).

3.10.5 Determination of Hide Substance

Tanned leather (0.6 g) was taken and placed into a dry 250 cm^3 Kjeldahl flask containing 15 cm^3 of concentrated sulphuric acid with the specific gravity of 1.84 g/cm^3 as well as some glass beds. The flask was equipped with a thistle funnel to prevent loss of acid during hide destruction. Potassium sulphate (5 g) and of copper sulphate (5 g) were added to the flask, and heated to boiling. Heat was withdrawn when the solution became clear. After cooling for 2 hr, the solution was quantitatively transferred into distillation flask and sodium hydroxide solution (0.05 M) was added until the solutions became black. The solution was then distilled into a 250 cm^3 conical flask containing 100 cm^3 of sulphuric acid (0.05 M) in the presence of methyl orange as indicator. After obtaining 150 $cm^3$of distillate, the excess acid was back titrated with 0.05 M NaOH (SLTC, 1996). The percentage hide substance was calculated using the Equation (3.23)

$$\text{Hide substance (\%)} = \frac{W1}{W2} \times 100 \qquad \textbf{(3.23)}$$

Where:

W_1 = Hide substance weight (g).

W_2 = Leather sample weight (g).

3.11 Hide Powder Preparation

Wet salted hide was soaked in water 1:1 for 12 hr and washed to remove blood, dung and salt from the hide. The hide was unhaired and limed using depilating agent (Na_2S) 1: 0.04 and $Ca(OH)_2$ 1: 0.025, respectively in hide 1:1 water for 2 hr, to effect plumping and swelling of the hide. This was allowed to stand overnight. The pelt was fleshed using a fleshing machine. Deliming was carried out using 1: 0.03 $(NH_4)_2SO_4$ in 1: 0.7 H_2O for 4 hr to achieve complete deliming. The delimed pelt was thoroughly washed with water, drained and soaked in acetone/water solution (10:1) for 2 weeks to remove bound water from the pelt. The pH of the pelt was adjusted using formic acid to pH 5 to 5.5. The pelt was cut into pieces and dried prior to crushing.

Table 3.2:Real Variables studied in the Box-Behnken design

Factor	Name	Units	Type	Low Actua	High Actual
A	$Ca(OH)_2$	%	Numeric	1.50	3.50
B	Time	h	Numeric	20.00	30.00
C	H_2O	cm^3	Numeric	250.00	350.00

3.12 Statistical Treatment

Box-Behnken Design, an allied of Response Surface Methodology, was performed using Design Expert software (Version 6.1.0) to investigate the effects of three (3) independent variables (liming concentration, temperature and volume of water) in order to optimize the liming condition. A quadratic polynomial model was used to characterize the liming process.

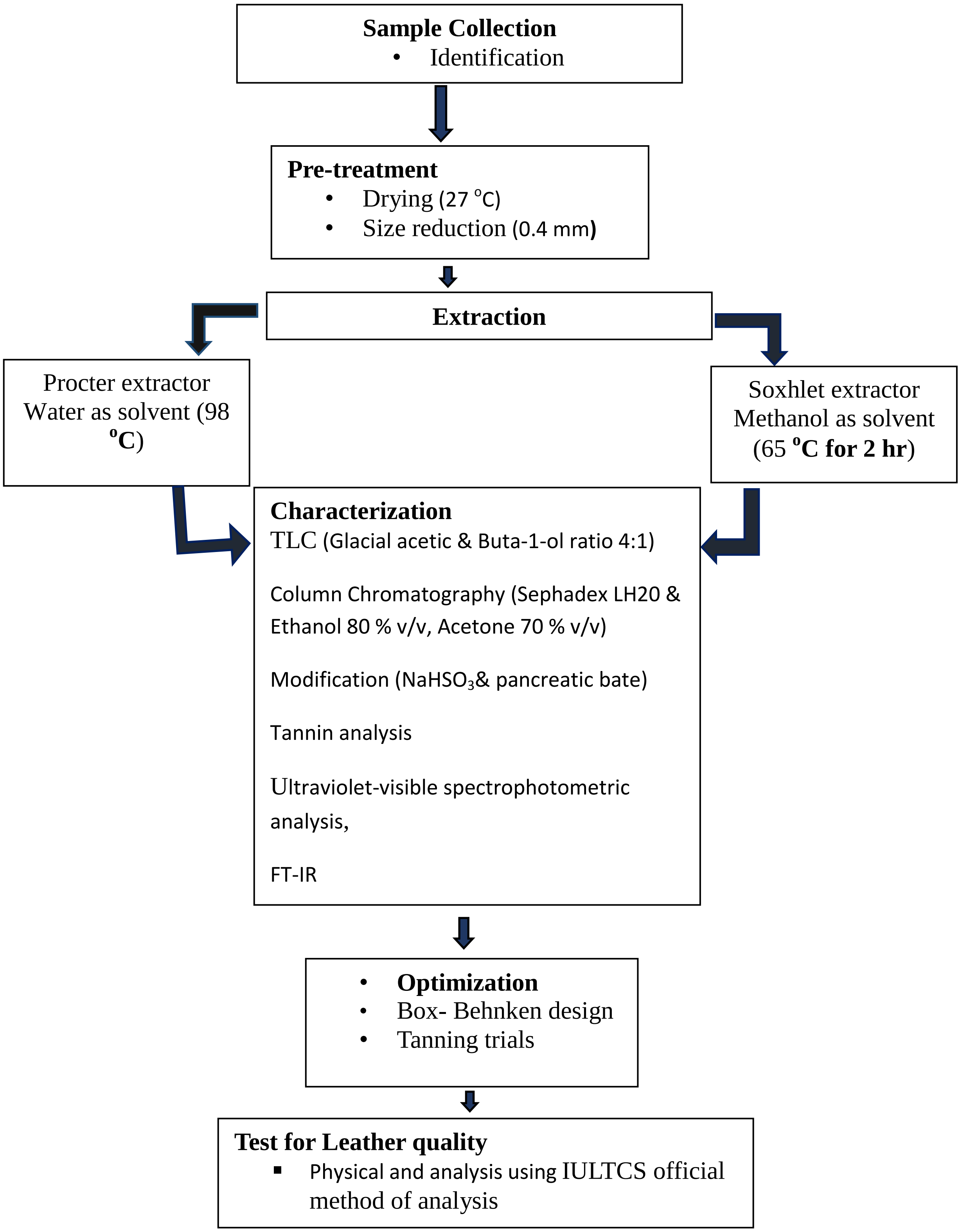


Figure 3.1: Summary of the Experimental

CHAPTER FOUR

RESULTS

4.1 ANALYSIS OF TANNIN EXTRACT

The results of the qualitative test for the type of tannin carried out on *Parkia clappertoniana* (PC) and *Caesalpinia Coriaria*(DD)are displayedin Table 4.1. Table 4.2 shows the tannin strength of the extracts, which is 42.33% for PC and 47% tannins for DD. Results for thin layer chromatography of methanolic extracts of DD and PC, are given in Table 4.3. Table 4.4 shows the total gallic acid, free gallic acid and gallotannins in methanolic and ethyl acetate extract (25.54 %, 20.48 %, 5.11 % and 24.86 %, 19.38 %, 5.48 % respectively). Figure 4.1 shows ultraviolet visible spectra of modified and unmodified PC and DD using $NaHSO_3$ and Pancreatic trypsin.The spectra exhibit differences in the intensities of modified and unmodified samples. The ultraviolet-visible spectrum of the modified tannin with pancreatic bath is given in Figure 4.2. This shows a higher absorption by PC than DD in the pancreatic bath.

Figure 4.3 shows the sorptive ability of modified PC and DD. This shows an increase in sorptive ability as the concentration for the modifier increases. The binding capacity of PC and DD is shown in Figure 4.4. The maximum binding capacity for PC (45 %)was found to be greater than that of DD(15 %). Figures 4.5 and 4.6 show sorptive ability of PC and DD modified with pancreatic trypsin. PC has a higher sorptive ability of 35%.and binding capacity of 25%,while DD was found to be 25% and 10% respectively. Figures 4.7 and 4.8 show FT-IR spectra of modified and unmodified PC and DD respectively.

Table 4.1: Qualitative Test on Tannins

TEST	OBSERVATION	INFERENCE
PC Extract +$FeCl_3$	Greenish-black colouration	Condensed tannin present
Extract + formaldehyde	precipitate formed and persist on heating	Condensed tannin present
Extract + Conc HCl	Red insoluble precipitate formed	Condensed tannin confirmed
DD Extract +$FeCl_3$	Bluish-black precipitate	Hydrolysable present
3 drops of extract+ a drop of formaldehyde + 10%HCl	It forms a pinkish precipitate after 1minute of adding 10%HCl	Hydrolysable tannins Confirm

Table 4.2: Analysis of the Tanning Strength of the Extracts

Parameters	DD	PC
Moisture (%)	4.62	6.70
pH	4.03	5.56
Total soluble (ts %)	65.00	78.33
Non-tans (nt %)	18.00	36.00
Tannins (t %)	47.00	42.33
Total solid (%)	95.00	93.30
Total insoluble (%)	35.00	21.67
Tannin strength(t/nt)	2.61	1.20
Purity ratio(t/ts)	0.72	0.54

Note: DD=*Casalpinia coriaria*, PC=*Parkia clappertoniana*

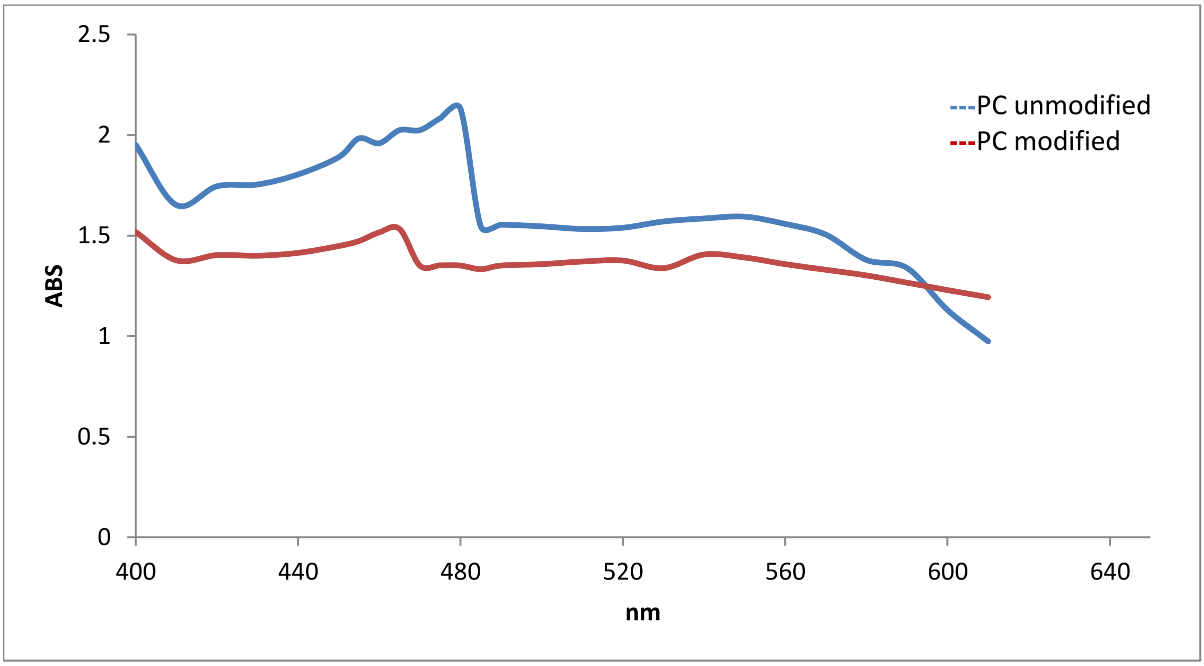
Table 4.3:TLC of Tannins in Methanol Extract of DD and PC

DD Components in 6 % Acetic acid	**Rf Values**	**Saturated** KIO_3
3,4,6-tri-O- Galloyl-D-glucose	0.37	Orange pink to brown
Tannic acid	0.43	Orange-brown
Pyrogallol	0.81	Orange pink to brown
Gallic acid	0.84	Orange pink to brown
PC Components in 6 % Acetic acid	**Rf Values**	**Saturated** $\mathbf{KIO_3}$
Gallic acid	0.84	Orange pink to brown
Catechol	0.88	Orange-brown

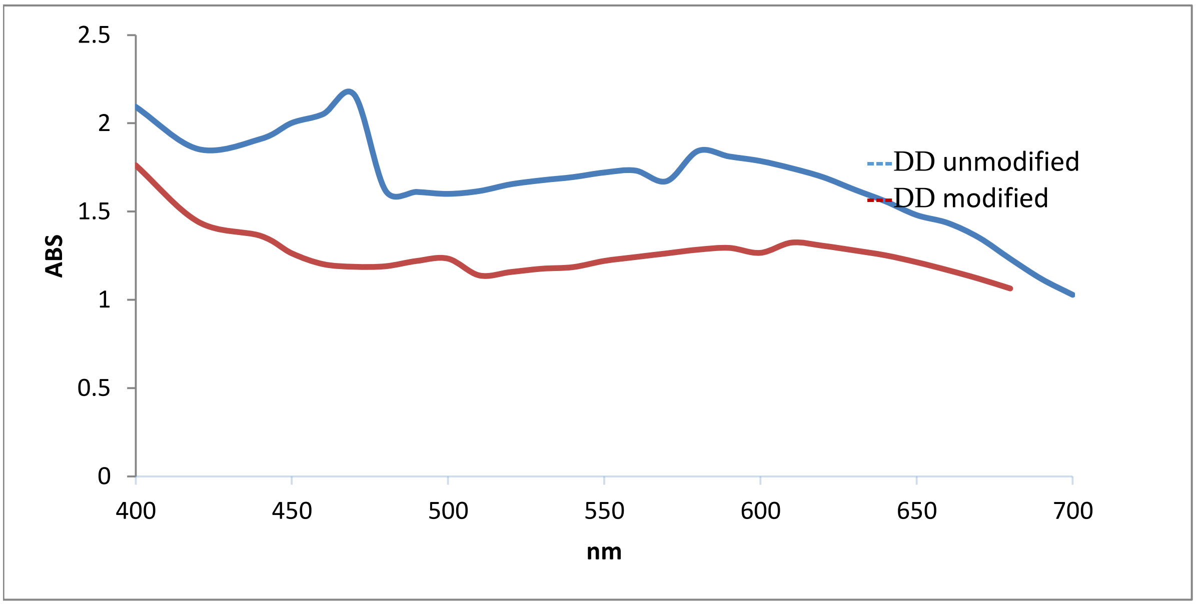
Table 4.4: Total Gallic Acid, Free Gallic Acid and Gallotannins of PC Extract

EXTRACTS	TOTAL GALLIC ACID (%)	FREE GALLIC ACID (%)	GALLOTANNINS (%)
Methanol	25.54	20.48	5.11
Ethylacetate	24.86	19.38	5.48

Key: The gallotannins was obtained by the difference between total gallic acid and free gallic acid.



PC



DD

Figure 4.1: UV-VIS Spectrum of modified and unmodified PCandDD

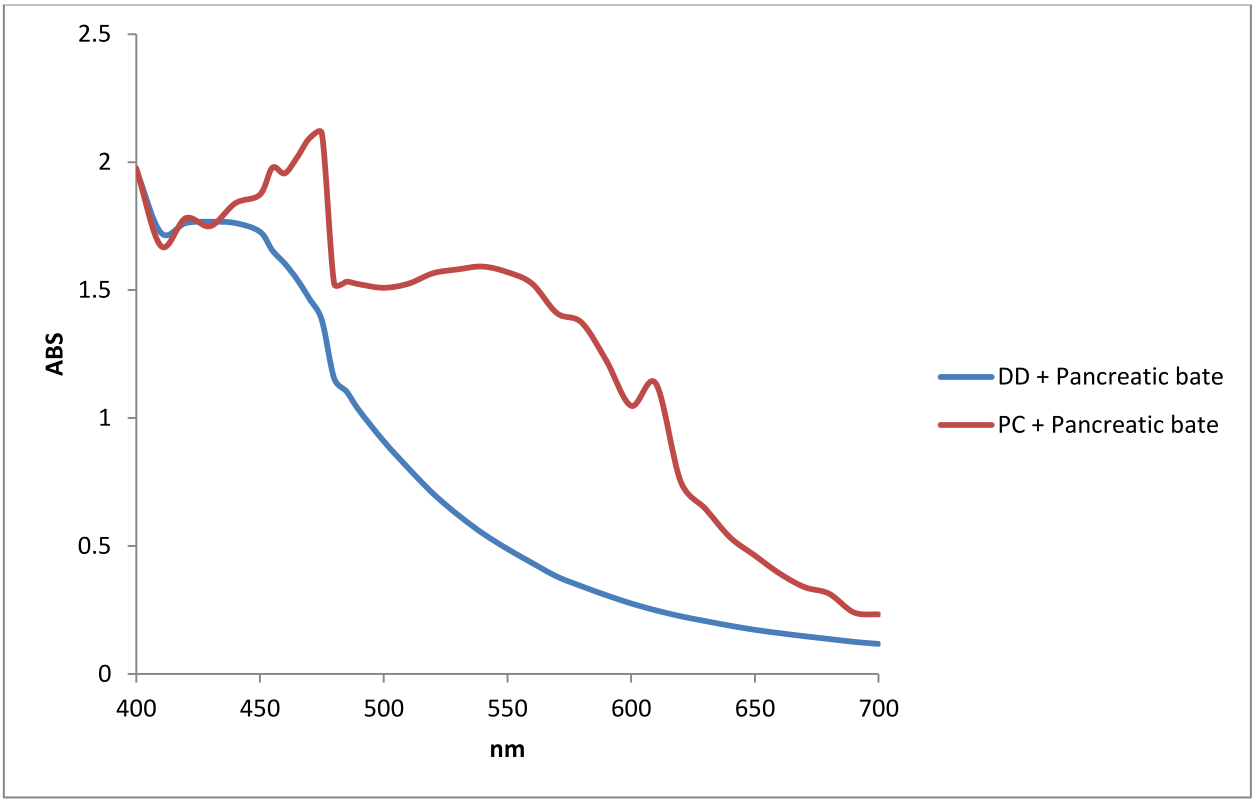


Figure 4.2: UV-VIS Spectrum of Tannin modified with BT (pancreatic trypsin)

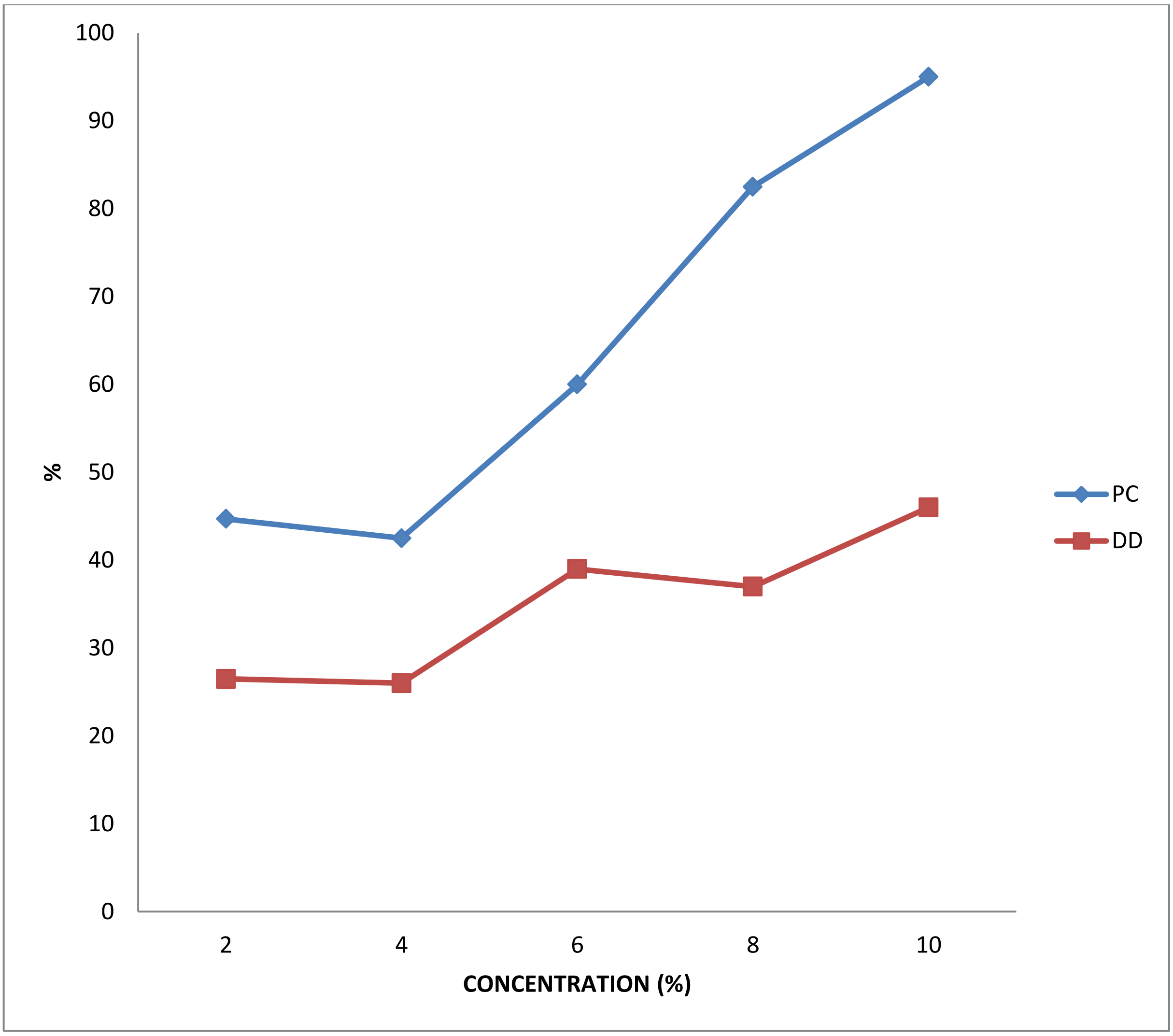


Figure 4.3:Sorptive Ability of Modified PC and DD

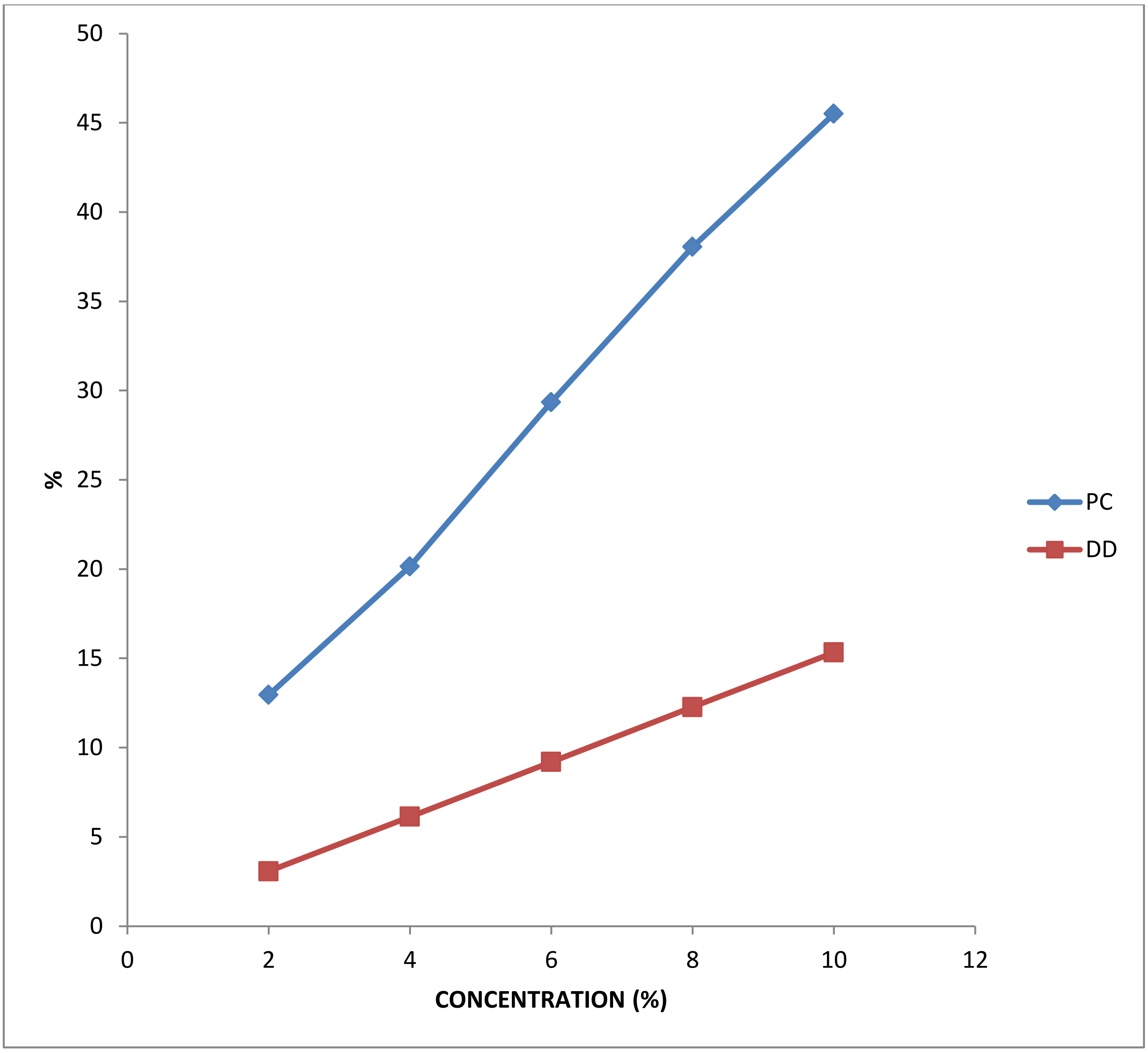


Figure 4.4: Binding Capacity of Modified PC and DD

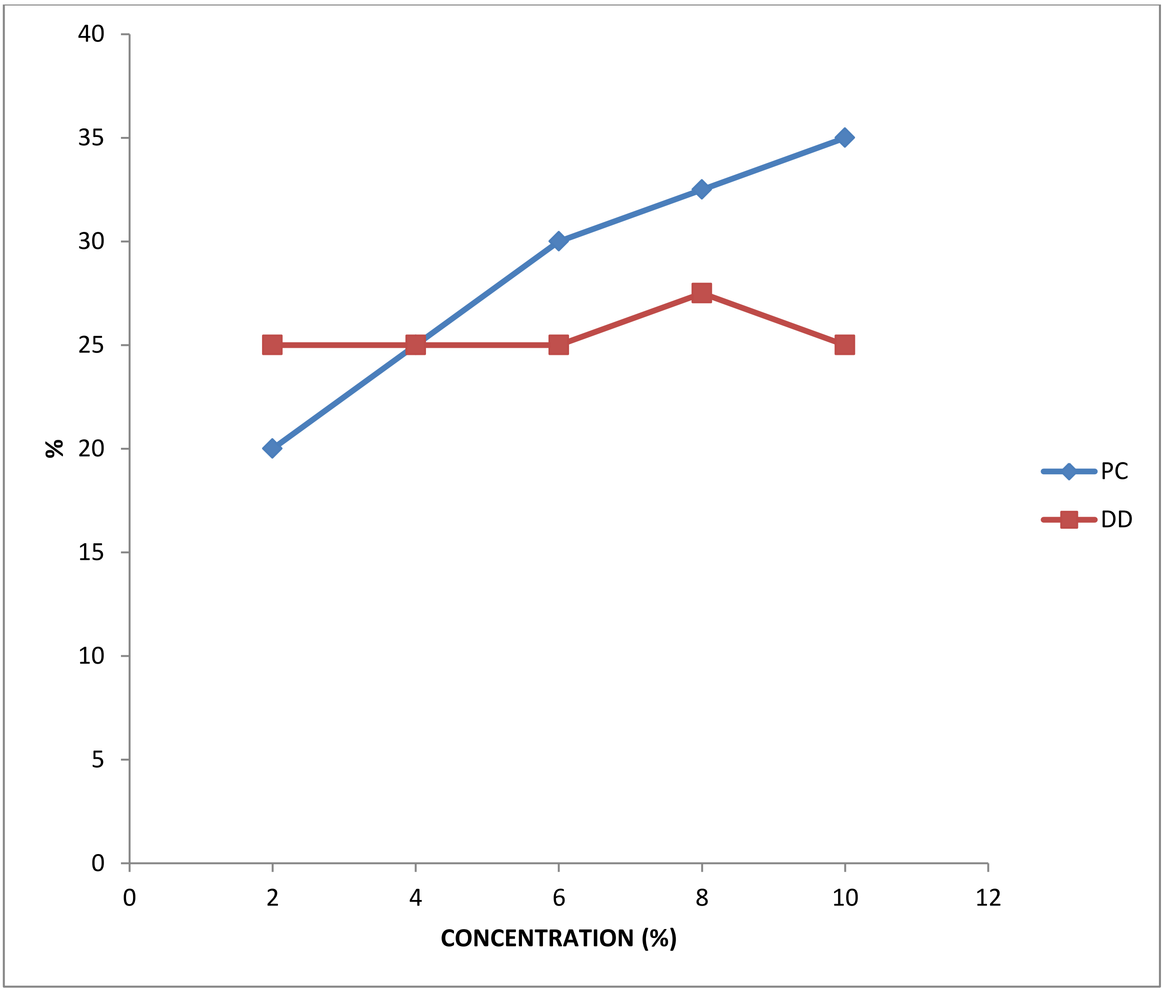


Figure 4.5: Sorptive Ability of PC and DD Modified with BT

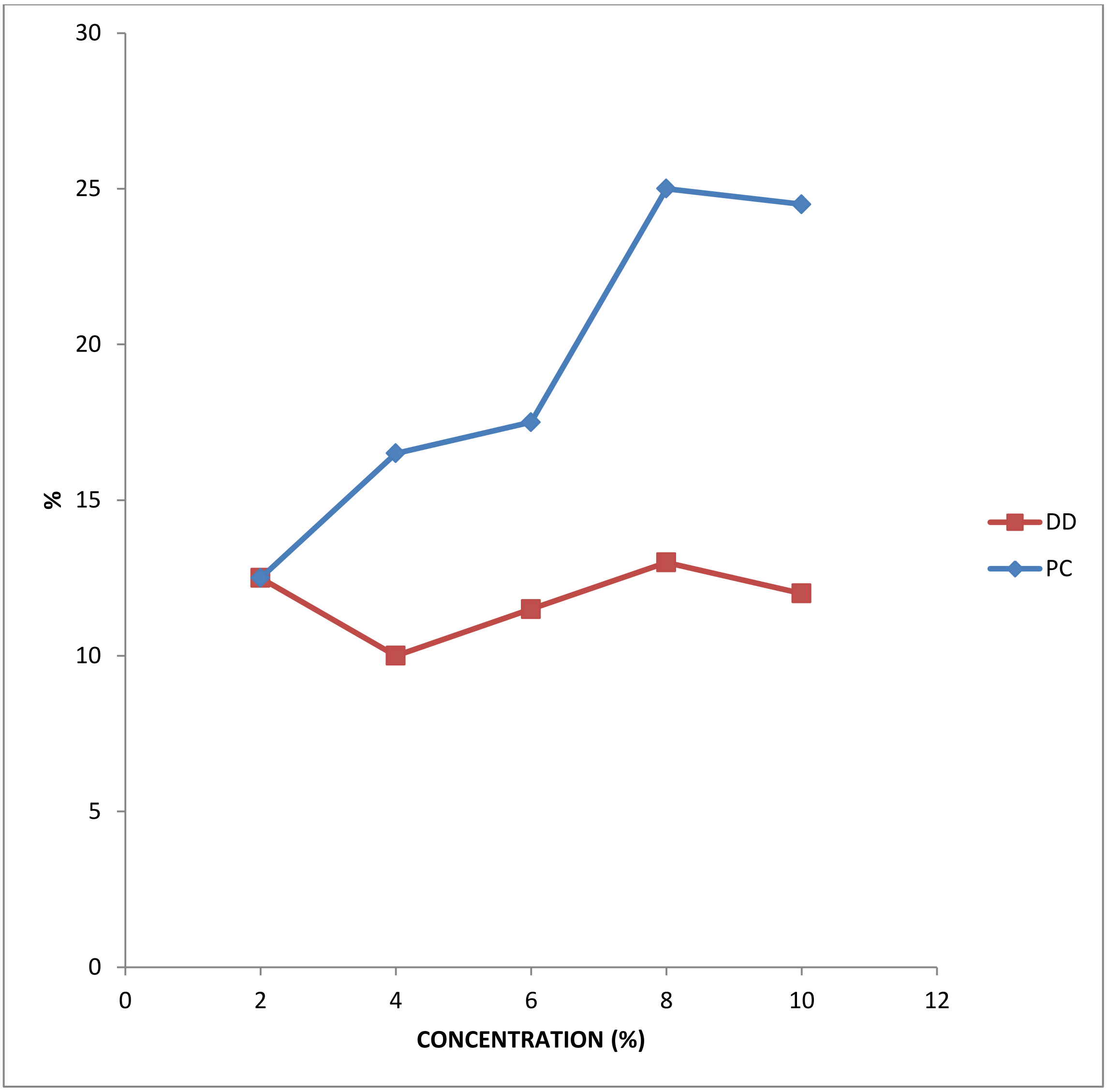
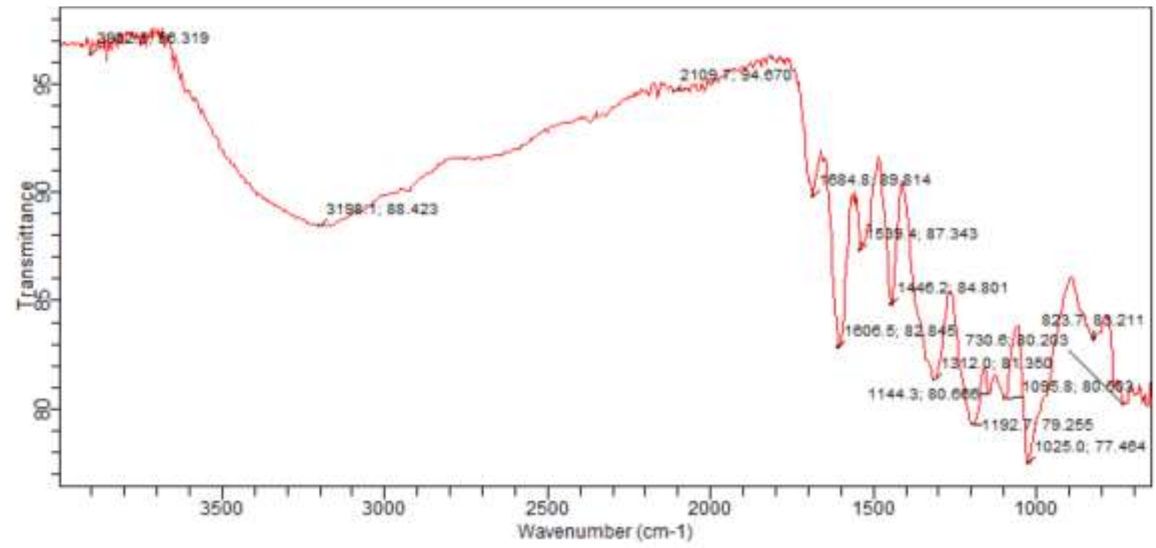
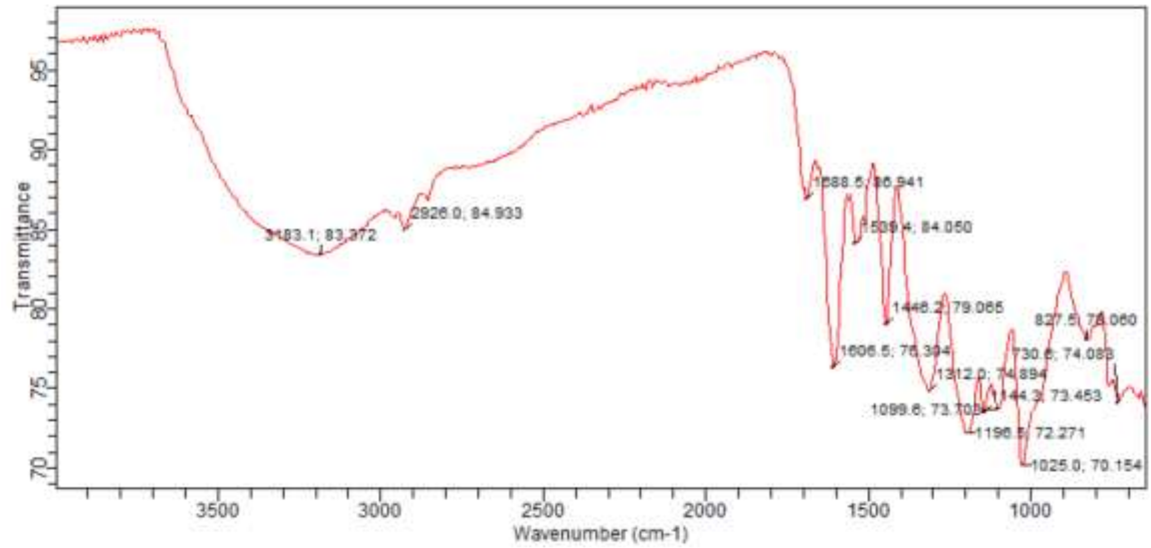


Figure 4.6:Binding Capacity of PC and DD Modified with BT

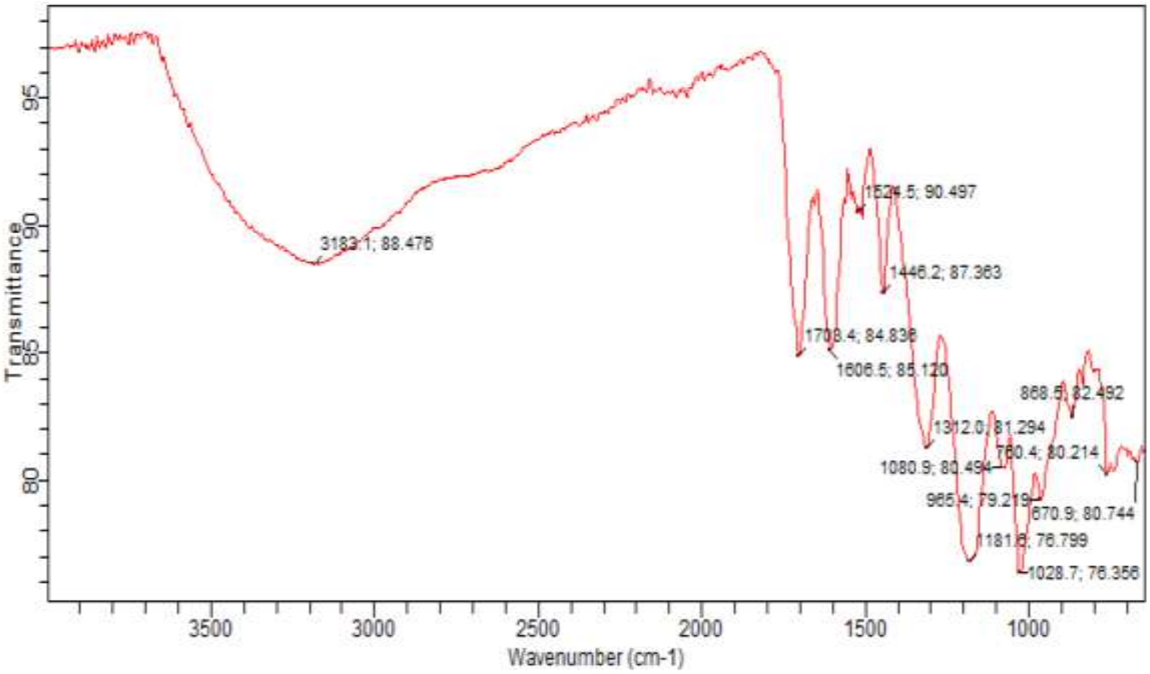


PC



PC Modified

Figure 4.7 FT-IR Spectrum of PC Modified and unmodified



DD

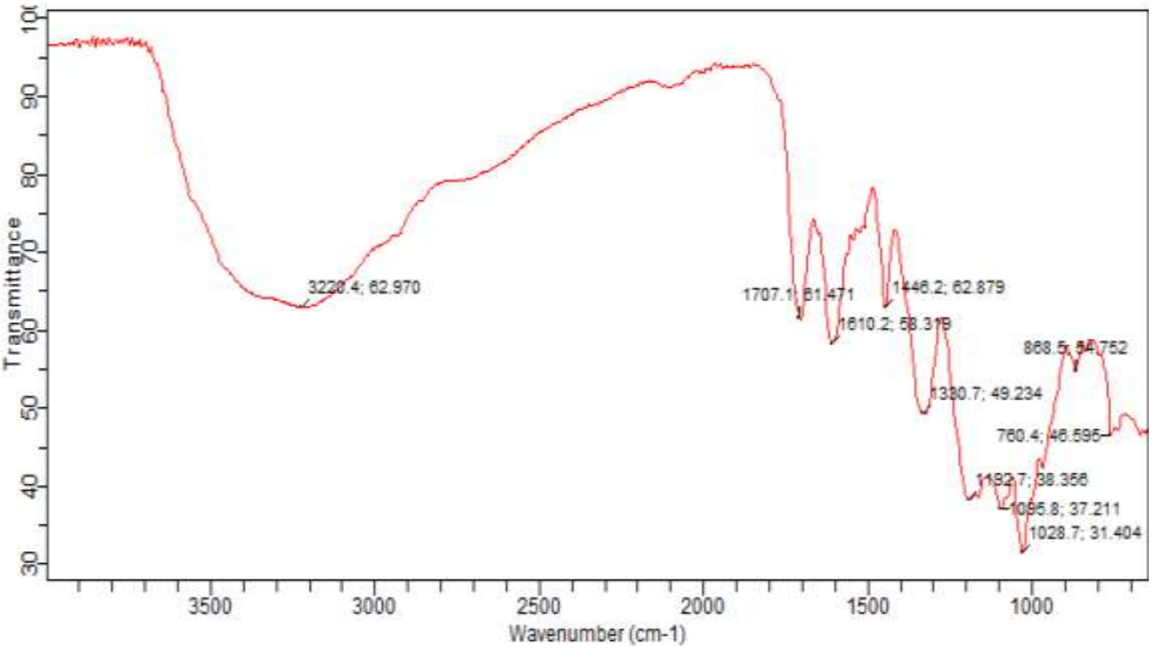


Figure 4.8: FT-IR Spectrum of DD Modified and unmodified

4.2 PREPARATIONOF DIFFERENT VEGETABLE TANNINS RATIOS

Table 4.5 presents the shrinkage temperatures of the different ratios of vegetable tannins (PC:DD) with and without modification. The results show increase in shrinkage temperature on addition of the modifier. Table 4.6 shows the effect of fat liquor on the shrinkage temperature (Ts) of the resultant leathers using different lime concentrations. The shrinkage temperature was increased on addition of fat liquor.

Table 4.5: Results of Shrinkage Temperature (T_S) obtained for Raw Skin and after Tanning with Different Ratios of PC, DD and AN Extracts

Group	Ratio of tannins/Modifier	Ts of Raw skin (°C)	Tsof Leather (°C)
A1	DD	52	70
A1M	MDD	48	76
A2	DD: AN (1:1)	52	70
A2M	M(DD: AN) (1:1)	52	71
B1	PC	54	80
B1M	MPC	54	84
B2	PC:DD (1:1)	56	76
B2M	M(PC:DD) (1:1)	50	76

Key:DD =*Caesalpinia coriaria,*

PC = *Parkia clappertoniana,*

AN= *Acacia nilotica as control*

M= Modified tannin (using Sodium bisulphite)

Table 4.6: Effect of Different Liming Concentrationsand Fatliquoring on the Shrinkage Temperature (Ts) of Leathers Tanned with PC and DD

Lime Concentration %	PC		DD	
	(Ts ^oC) Before fatliquor	(Ts ^oC) After fatliquor	(Ts ^oC) Before fatliquor	(Ts ^oC) After fatliquor
1.50	77	89	72	81
3.50	78	84	71	79
3.50	77	90	71	80
1.50	75	86	73	81
2.50	78	83	70	80
2.50	70	86	68	82
1.50	74	88	70	80
2.50	79	86	71	79
1.50	73	82	68	78
3.50	74	87	72	80
3.50	80	88	74	82

Key: DD =*Caesalpinia coriaria*, PC = *Parkia clappertoniana*

(Ts ^oC) Before = Shrinkage Temperature of the Tanned leather before fatliquor,

(Ts ^oC) After = Shrinkage Temperature of the Tanned leather after fatliquor

4.3 ASSESSMENT OF LEATHER QUALITY

The standard parameters used to assess the quality of leather after tanning are presented in Table 4.7and 4.8 and Figure 4.9 and 4.19. Theresults of light fastness of the tanned leathers are given in Table 4.7. Figure 4.9 shows shrinkage temperature of the leathers tanned with different tannin ratios. Indicating tannin ratios of B4 to have the highest shrinkage temperatures value of 86 ^{o}C among other tannin ratio. Figure 4.10 shows tensile strength of the leather tanned with different tanninratios. This result reveals that the tensile strength of the leather to be highest in the ratio C3 and D1 respectively.Figure 4.11 shows the % elongation of the leather to be highest (80%) when tanned with A1 tannin preparation. Figure 4.12 shows the young modulus of the leather which is highest when tanned with D1, C1 and C3,respectively. Figure 4.13 presents resistance to compression of the leather tanned with different tannin ratios. The leather tanned with tannin ratios A3 had the highest Resistance to compression (9 kgcm^{-2}). Figure 4.14 shows thickness of leather tanned with different tannin ratios. The tannin ratio A4 was found to give 3 mm thickness of leather which is the highest value. Figure 4.15 shows the indentation index of the leather tanned with different tannin ratios. The tannin ratio D2, A2followed by D3 gave the highest indentation values of 65 mm, 55 mm and 52 mm respectively. Figure 4.16 shows apparent density of the leather tanned with different tannin ratio. The apparent density of the tanned leather is highest (7 gdm^{-3}) with the tannin ratio C1. Figure 4.17 presents water vapour permeability of the leather tanned with different tannin ratios. The result show higher vapour permeability with tannin ratio D1 (0.7 %). Figure 4.18 show water absorption of the leather tanned with different tannin ratio. The result is highest for the tannin ratio C2 and C3. Figure 4.19 present distention of leathers tanned with different tannin ratios. The distention of leather was found to be highest with the tannin ratio D2. Table 4.8 shows the chemical analysis of tanned leathers from tannin ratios B, B4gave the highest % hide substance followed by B2.

Table 4.7: Light Fastness of the Tanned leathers

Group	Ratios			
	1	**2**	**3**	**4**
A	3-4	2	1	1
B	2-3	2-3	1-2	1-2
C	3	2	1-2	3
D	3-4	1-2	3	3

Key: 4= excellent, 3 = very good. 2-3 = good, 1 = fair

Fastness Rating Standards	Colour Index (C.I) Designation
1	C.I Acid Blue 104
2	C.I Acid Blue 109
3	C.I Acid Blue 83
4	C.I Acid Blue 121
5	C.I Acid Blue 47
6	C.I Acid Blue 23
7	C.I Solubilized vat Blue 5
8	C.I Solubilized vat Blue 8

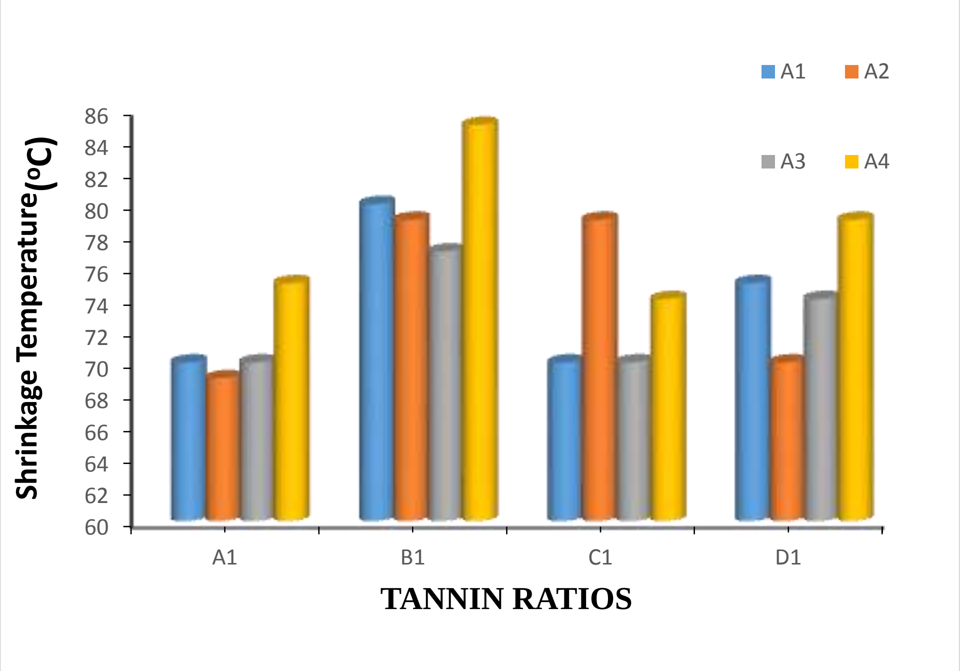


Figure 4.9: Shrinkage Temperature of the Leathers Tanned with Different Tannin Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD),*Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as a control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

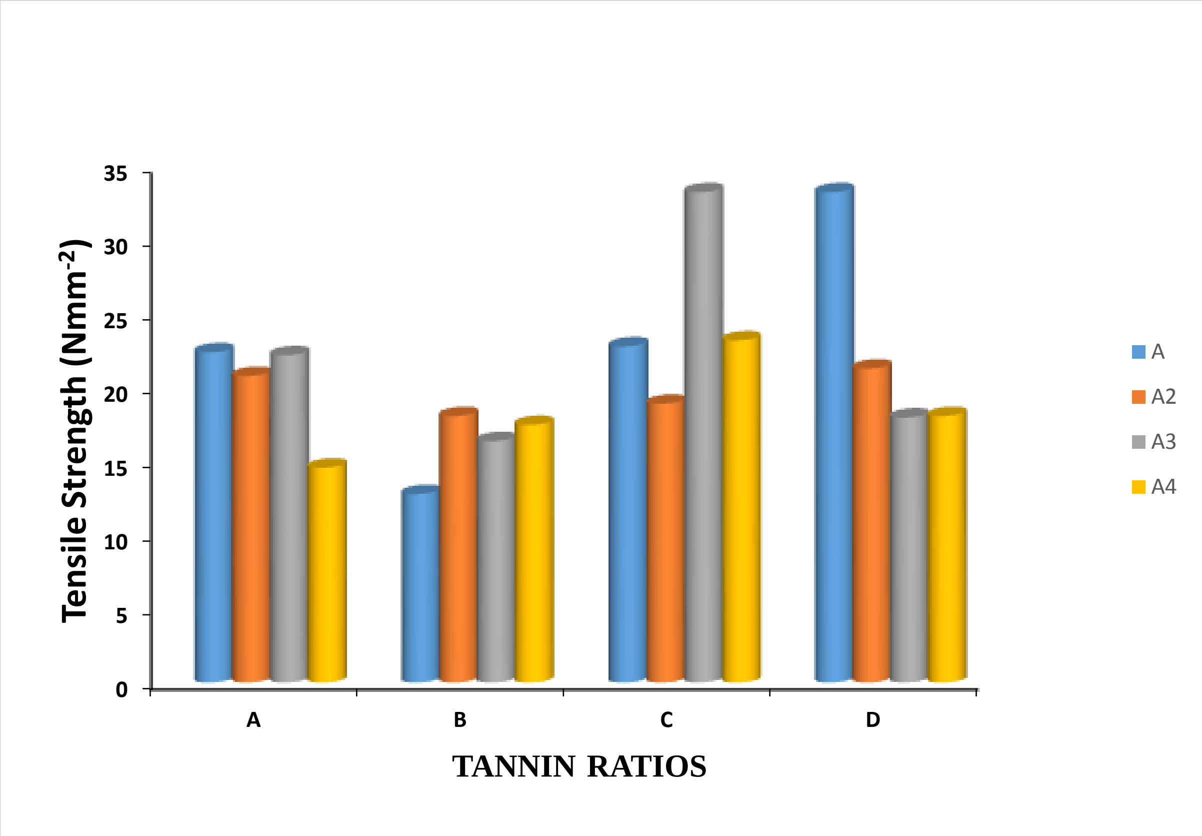


Figure 4.10: Tensile Strength of the Leathers Tanned with Different Tannin Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and*Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note:M = Sodium bisulphite

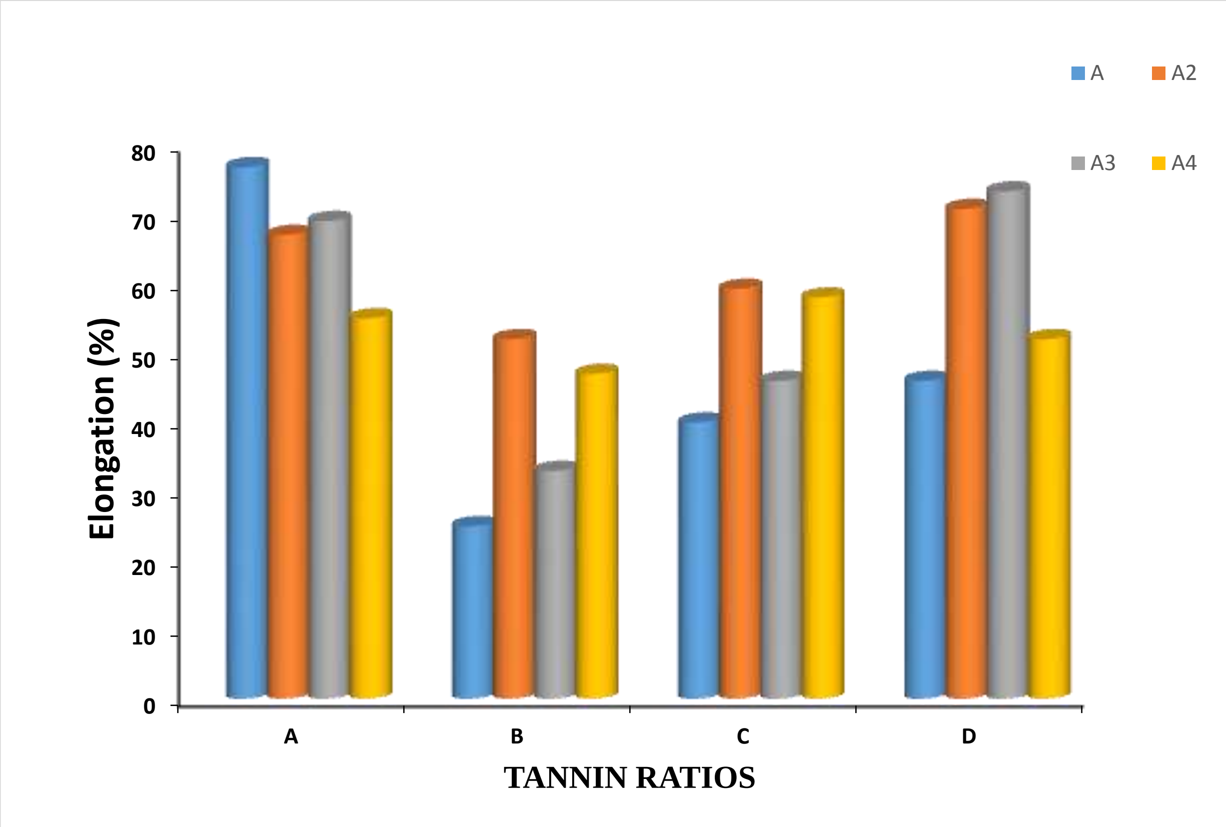


Figure 4.11 Elongation of the leathers Tanned with different Tannin preparations

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

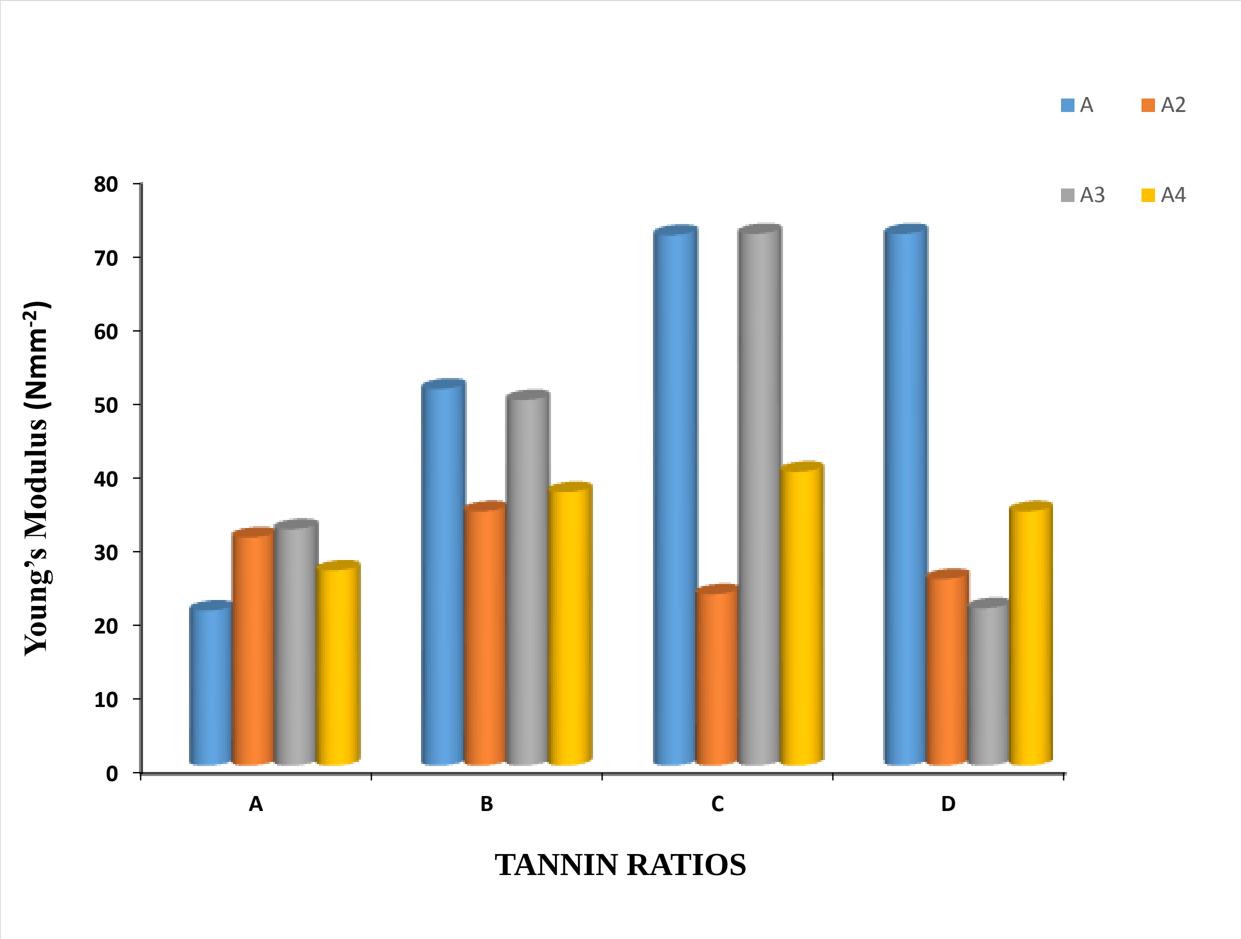


Figure 4.12: Young's Modulus of the Leathers Tanned with Different Tannin Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

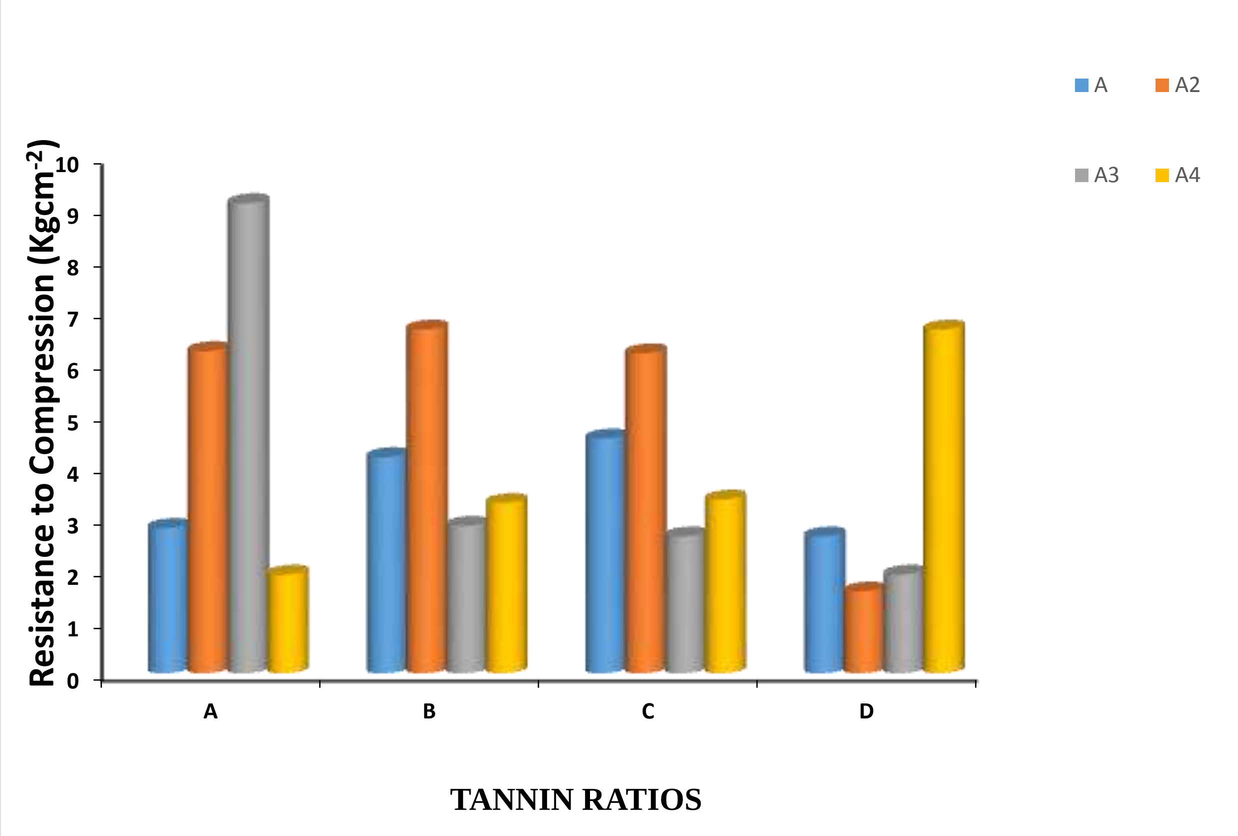


Figure 4.13: Resistance to Compression of the Leathers Tanned with Different Tannin Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

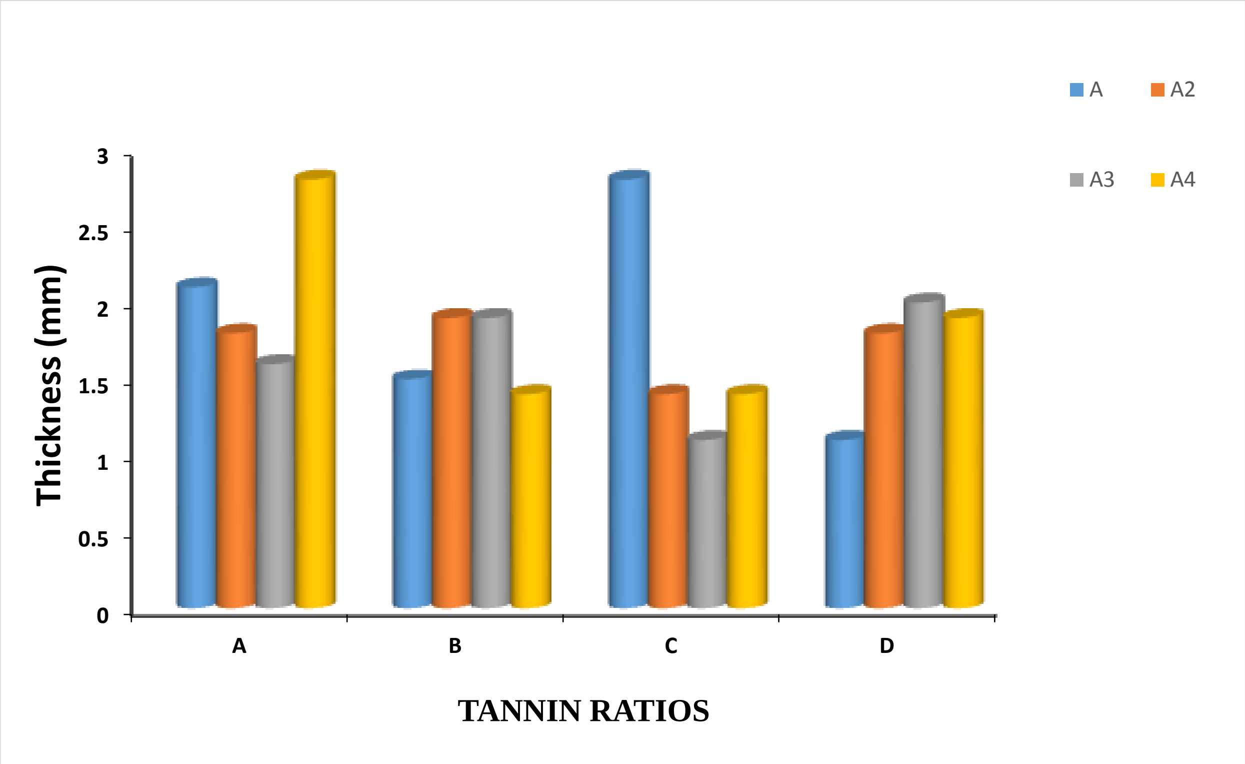


Figure 4.14: Thickness of Leathers Tanned with Different Tannin Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

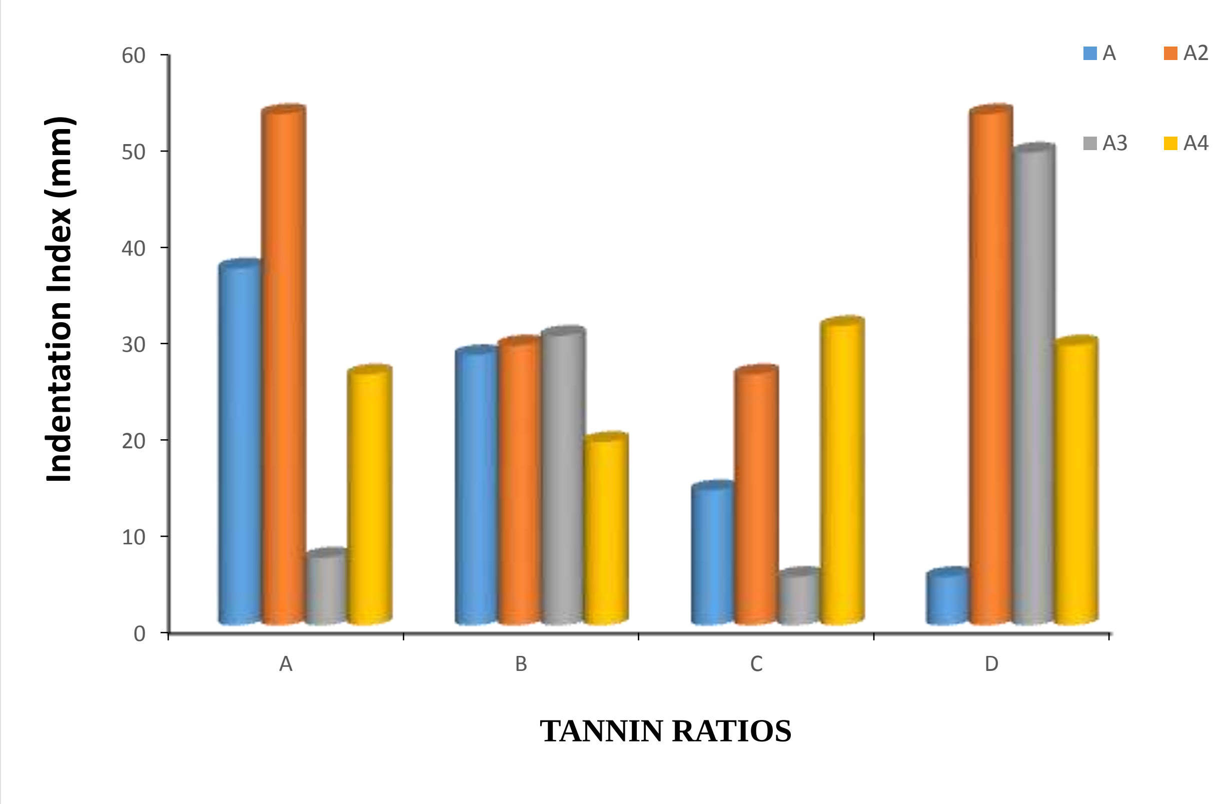


Figure 4.15: Indentation Index of the Leathers Tanned with Different Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

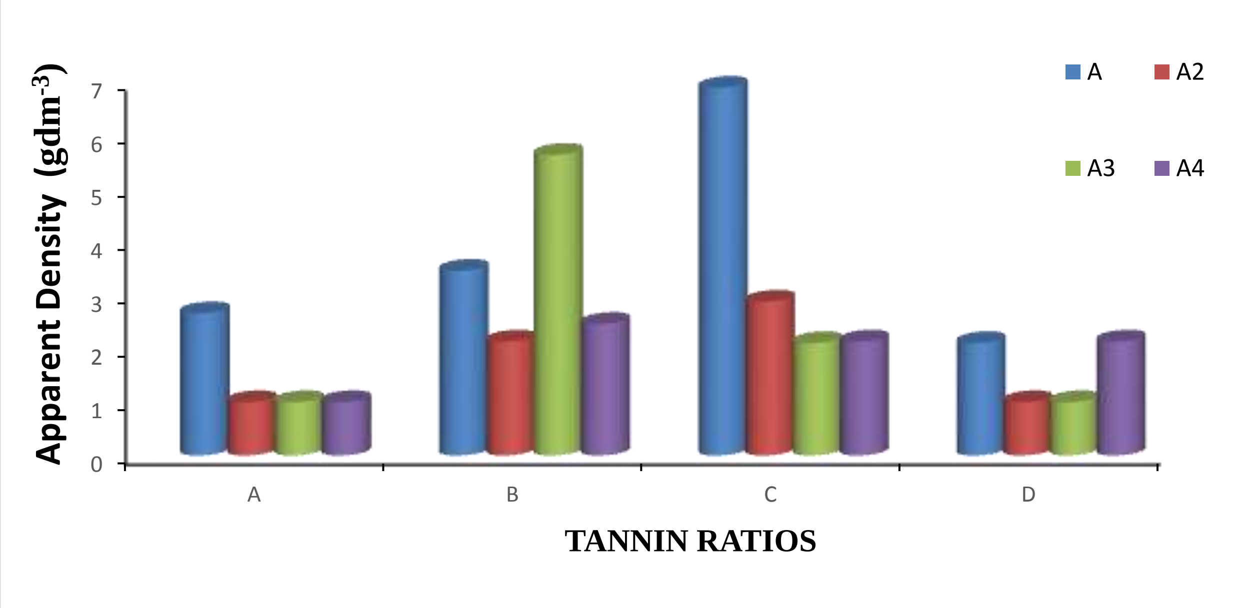


Figure 4.16: Apparent Density of the leathers Tanned with Different Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

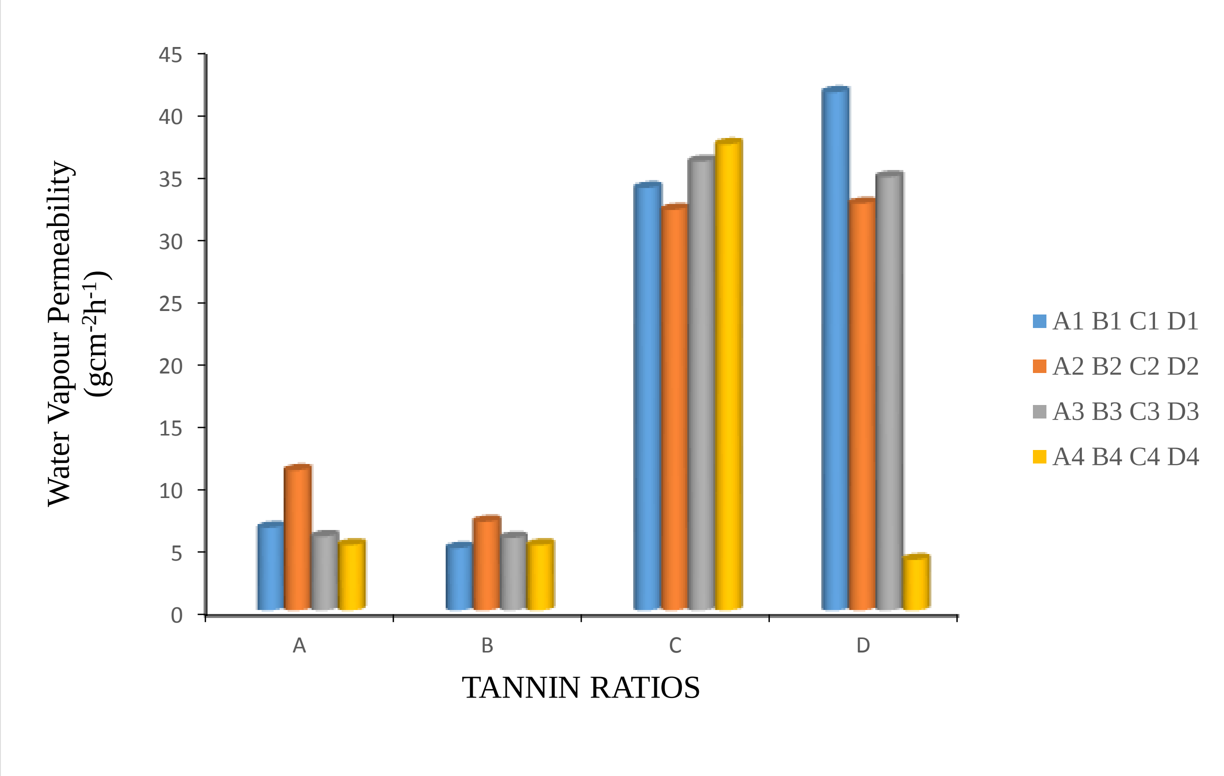


Figure 4.17: Water Vapour Permeability of the leathers Tanned with Different Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

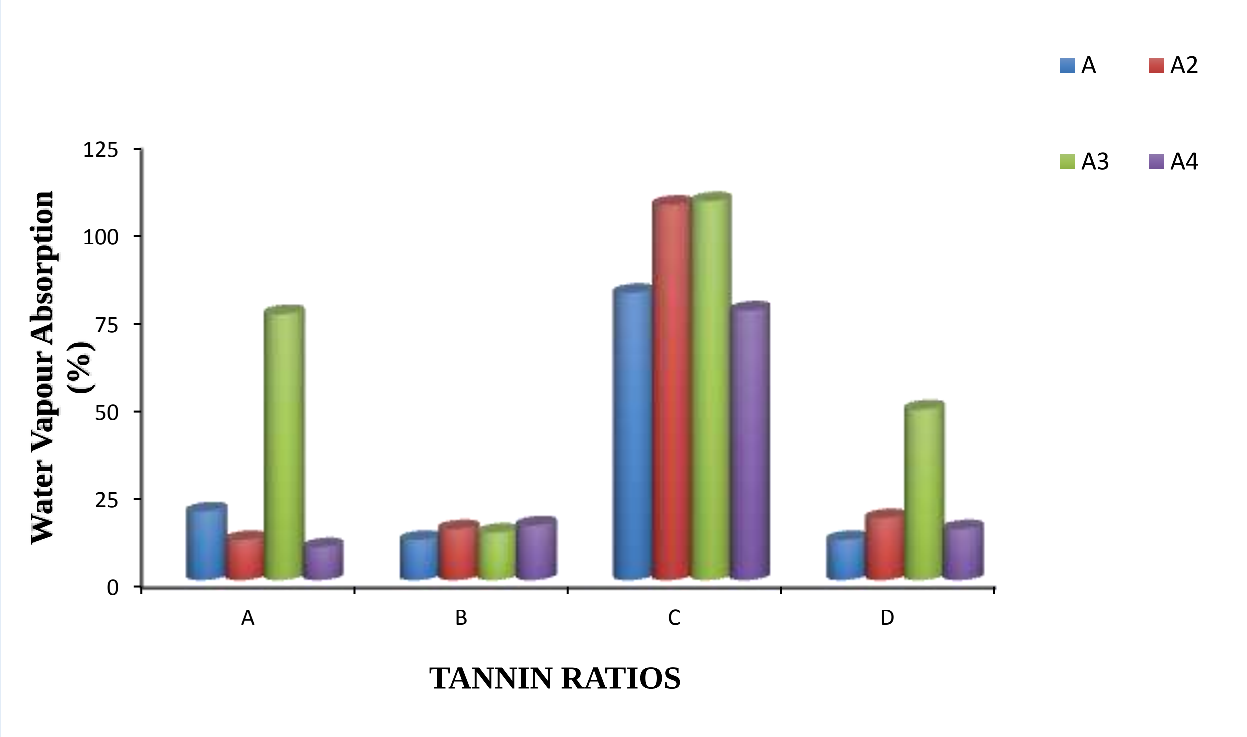


Figure 4.18: Water Vapour Absorption of the leathers Tanned with Different Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	MDD:AN(1:1)	PC:DD(1:1)

Note: M = Sodium bisulphite

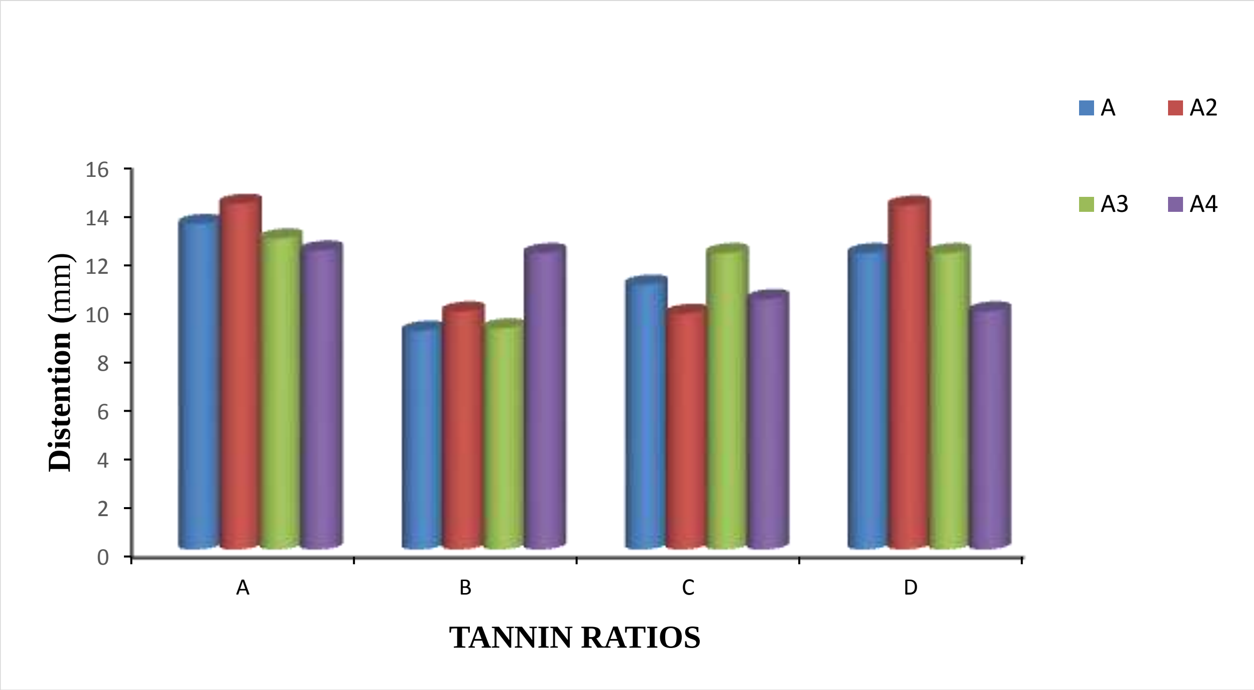


Figure 4.19: Distention of Leathers Tanned with Different Ratios

Key: Preparations of different ratios of *Caesalpinia coriaria* (DD), *Parkia clappertoniana* (PC)and *Acacia nilotica* (AN) as control

	1	**2**	**3**	**4**
A	DD	DD:AN(3:1)	DD:AN(1:1)	DD:AN(1:3)
B	PC	PC:DD(1:1)	PC:DD(1:3)	PC:DD(3:1)
C	PC	PC:DD(1:1)	DD:AN(1:1)	DD:AN(1:3)
D	DD	DD:AN(1:1)	DD:AN(1:1)+T	PC:DD(1:1)

Note: M = Sodium bisulphite

Table 4.8:Chemical Analysis of the Tanned Leathers fromGroup B

Leathersamples	Hide substance (%)	Moisture (%)	Fat soluble (%)	Ash content (%)
B1	33.83	13.96	1.88	0.679
B2	39.34	13.24	1.66	0.713
B3	31.47	13.87	1.02	0.679
B4	40.00	9.68	2.42	0.061

4.4 OPTIMIZATION OF THE LIMING PROCESS

Table 4.9 presents the experimental matrix for the Box-behnken design with three factors (lime concentration, time and water) and shrinkage temperature as response for PC and DD. From the table 3.50 % lime, 25 hr and 250 % (w/w) water gave the highest shrinkage temperature for PC and DD to be 80 °C and 74 °C respectively.

Figure 4.20 presents 3 dimensional(3D) plots between %lime and time for PC shrinkage temperature with actual factors of water (a) 250 cm^3 (b) 300cm^3 (c) 350 cm^3 from the plots, as the % of lime increase with increase in time, the shrinkage temperature increases. Figure 4.21 shows the 3D plots between % lime and water for PC shrinkage temperature with actual factor of time: (a) 20 hr, (b) 25 hr and (c) 30 hr. As the % lime concentration increases with an increase in volume of water, the shrinkage temperature increased.

Figure 4.22 shows 3D plots between water and time for PC shrinkage temperature with actual factor of lime (a) 3.50 %, (b) 3.01 %, (c) 1.50 % and (d) 2.50 % from the plots, the shrinkage temperature increases with the increase in time and volume of water and later declined with increase in the volume of water.

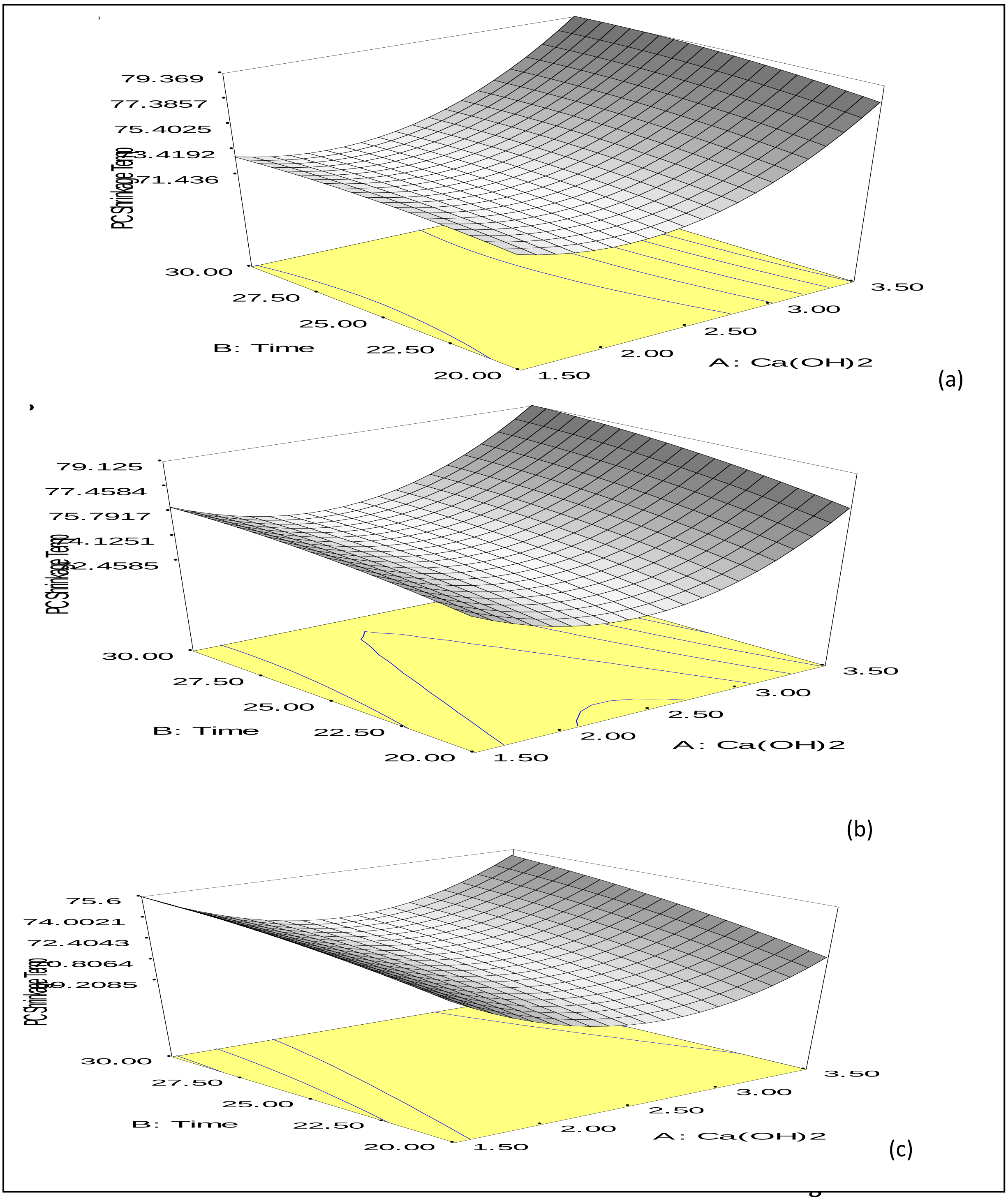
Figure 4.23 shows 3D plots between % lime and time for PC shrinkage temperature with actual factor of (a) 250 % (b) 300 % water. From the plots, the shrinkage temperature decreased with the increase in % lime concentration. Figure 4.24 shows the 3D plots between % lime and water for DD shrinkage temperature with actual factor of time: (a) 20 hr (b) 25 hr and (c) 30 hr. From the plots, the shrinkage temperature decreases with the increase in lime concentration and later increased with increase in lime concentration. Figure 4.25 presents the 3D plots between water and time for PC shrinkage temperature with actual factors of lime (a)

1.50 %, (b) 2.50 % and (c) 3.50 %. From the plots, at 250 % water at 20 hr the shrinkage temperature was high.

Table 4.9: Experimental matrix for the Box-Behnken Design and Shrinkage Temperature

Run	Factor 1 A:$Ca(OH)_2$ %	Factor 2 B:Time hrs	Factor 3 C:H_2O %	Ts PC (^oC)	Ts DD (^oC)
1	3.50	25.00	250.00	80	74
2	2.50	25.00	300.00	79	71
3	2.50	25.00	300.00	70	68
4	1.50	30.00	300.00	75	73
5	3.50	25.00	350.00	74	72
6	2.50	25.00	300.00	78	70
7	3.50	20.00	300.00	78	71
8	2.50	25.00	300.00	70	64
9	2.50	25.00	300.00	72	66
10	1.50	20.00	300.00	77	72
11	2.50	20.00	250.00	70	68
12	1.50	25.00	350.00	74	70
13	3.50	30.00	300.00	77	71
14	2.50	30.00	250.00	72	64
15	2.50	30.00	350.00	74	67
16	1.50	25.00	250.00	73	68
17	2.50	20.00	350.00	68	66

Note: Ts – Shrinkage Temperature



4.20: 3D plots between $Ca(OH)_2$ and Time for PC shrinkage Temperature with actual factors of Wates:(a) 250 cm^3, (b) 300 cm^3 and (c) 350 cm^3

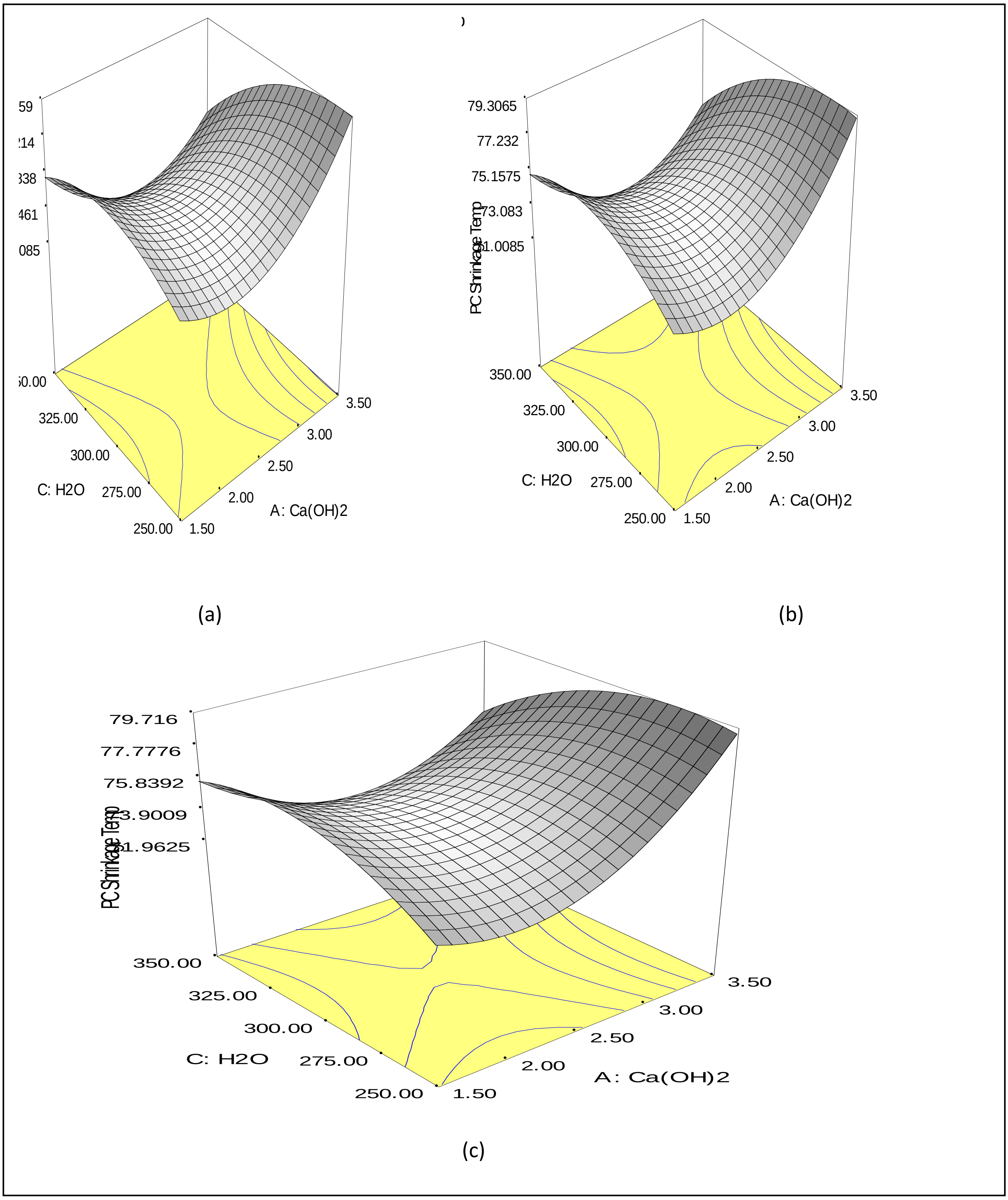


Figure 4.21:3 D Plots Between $Ca(OH)_2$ and water for PC Shrinkage Temperature with actual factor of Time: (a) 20 hr (b) 25 hr and (c) 30 hr

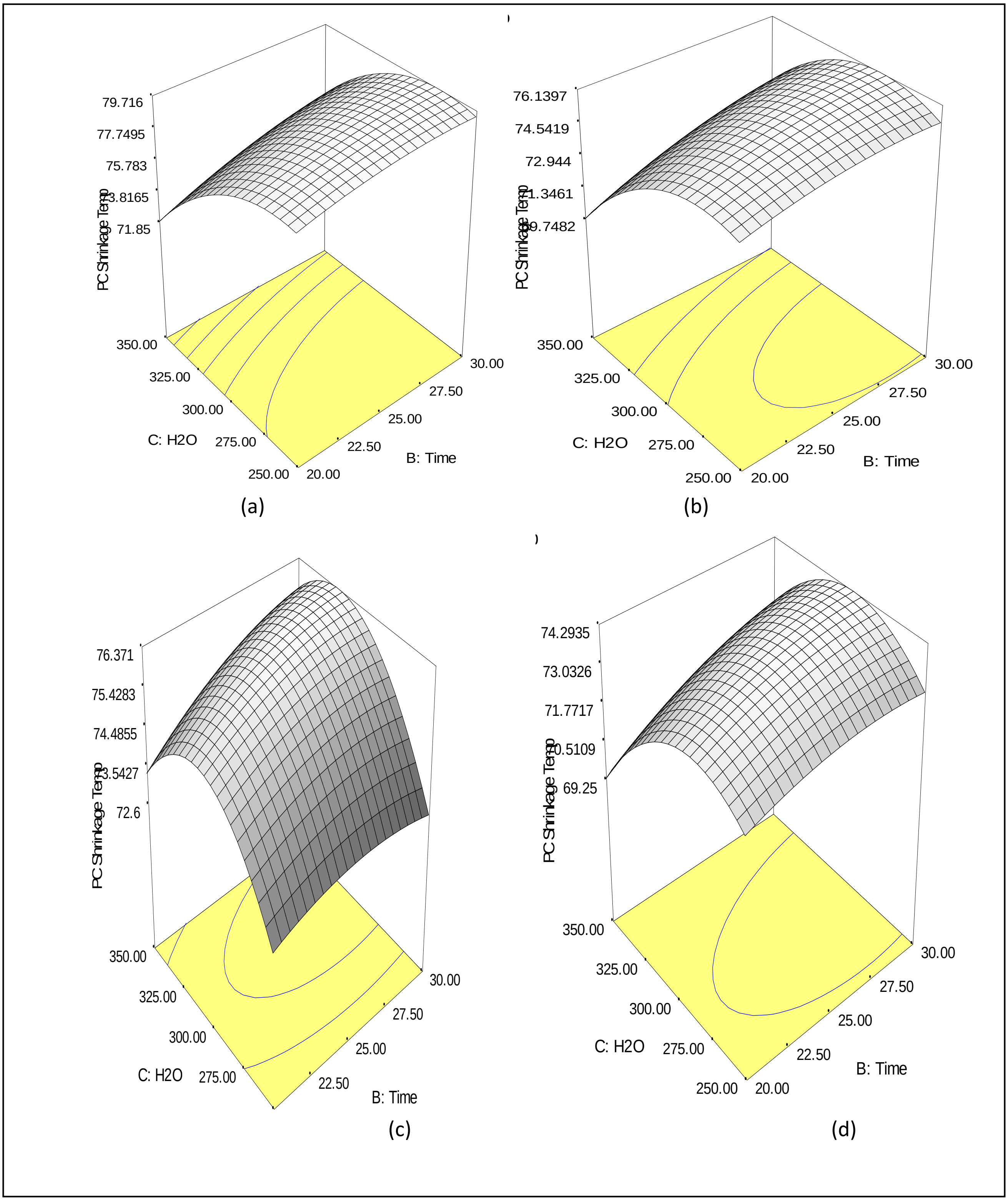


Figure 4.22:3D plots between Water and Time for PC shrinkageTemperature with actual factors of $Ca(OH)_2$:(a) 3.50 %, (b) 3.01 %,(c) 1.50 % and (d) 2.50 %

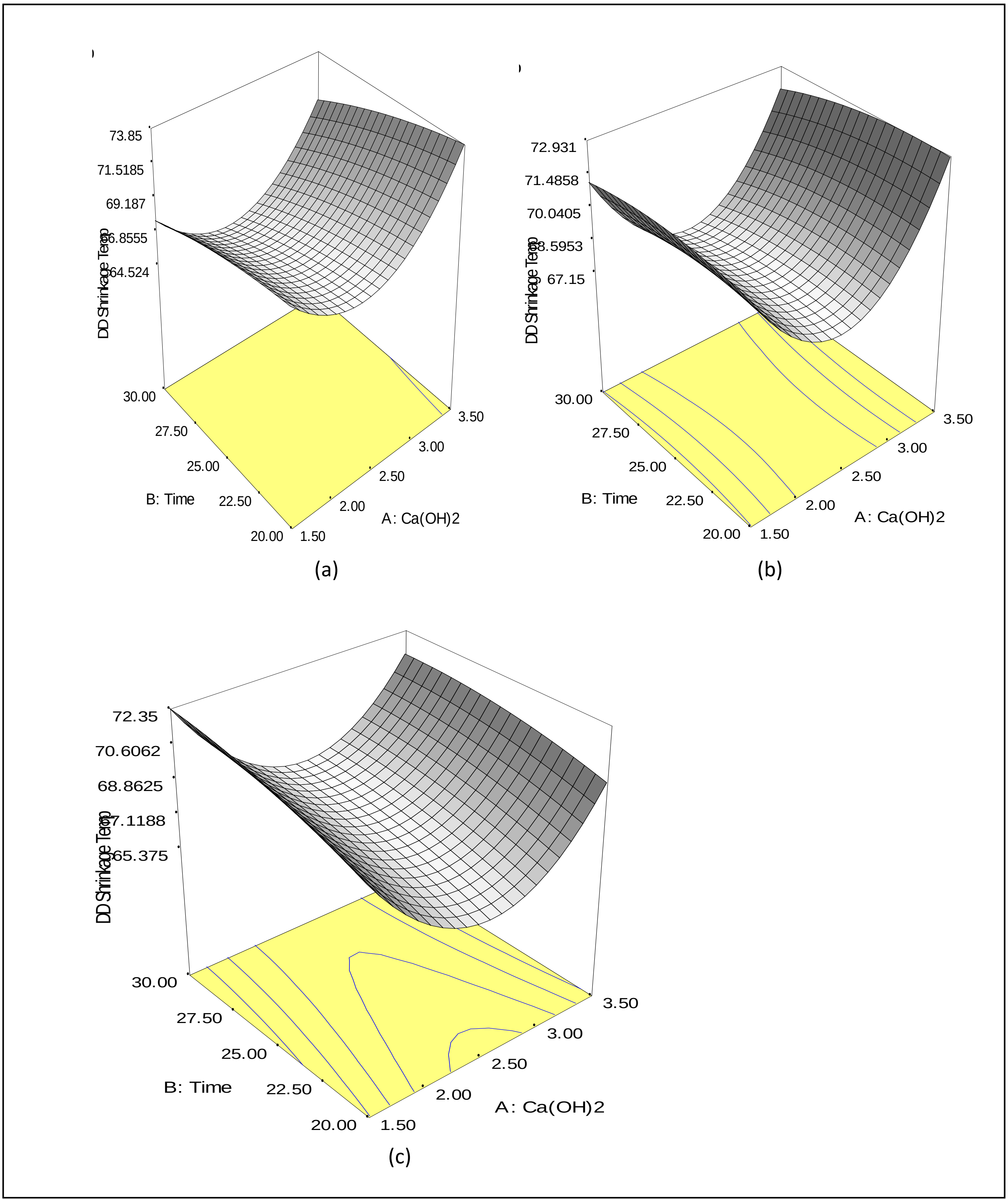


Figure 4.23:3D plots between $Ca(OH)_2$ and Time for PC shrinkage Temperature with actual factor of :(a) 250 cm^3 water, (b) 300cm^3water

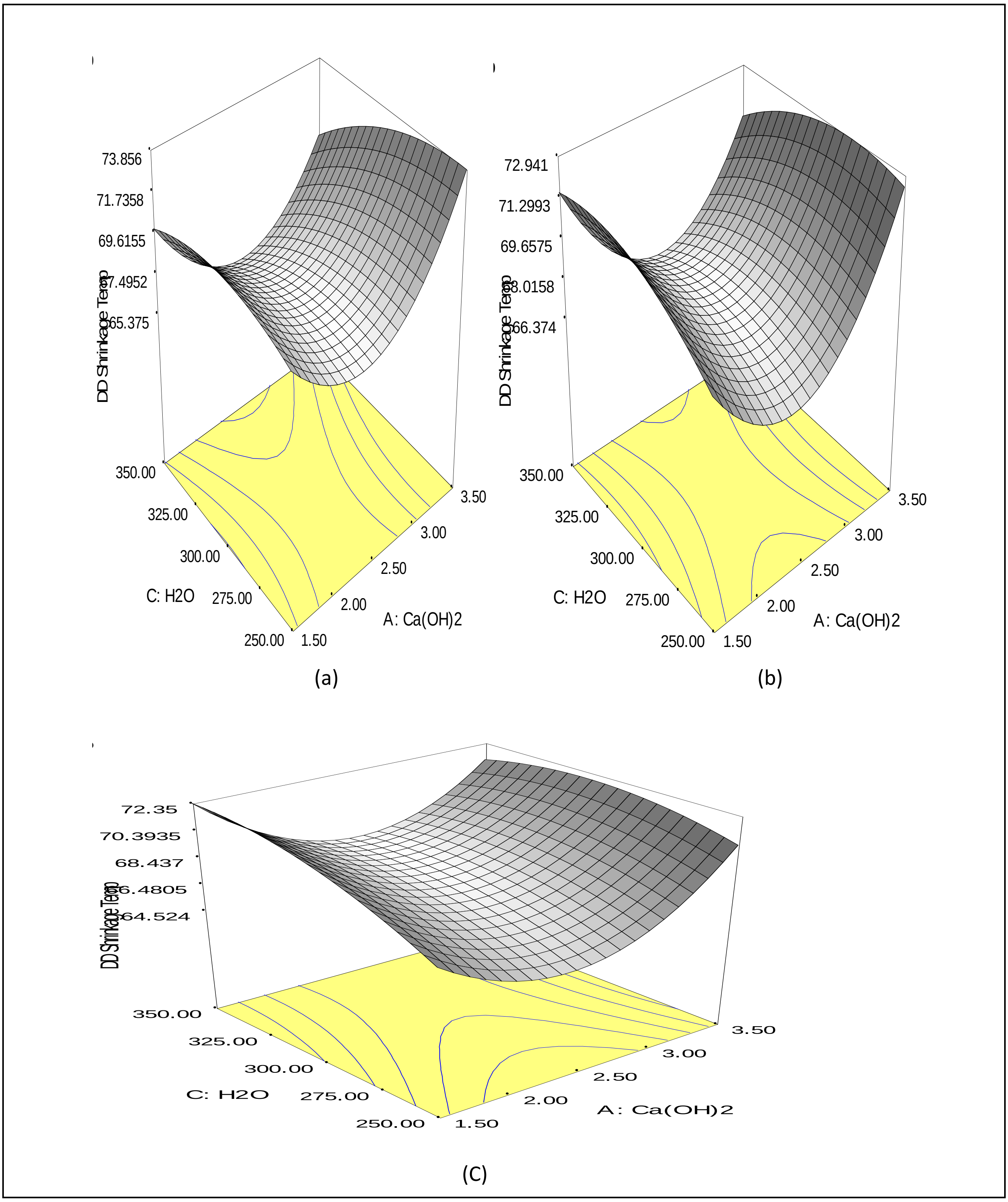


Figure 4.24:3D plots between $Ca(OH)_2$ and Water for DD shrinkage Temperature with actual factors of Time :(a) 20 h, (b) 25 h and (c) 30 h

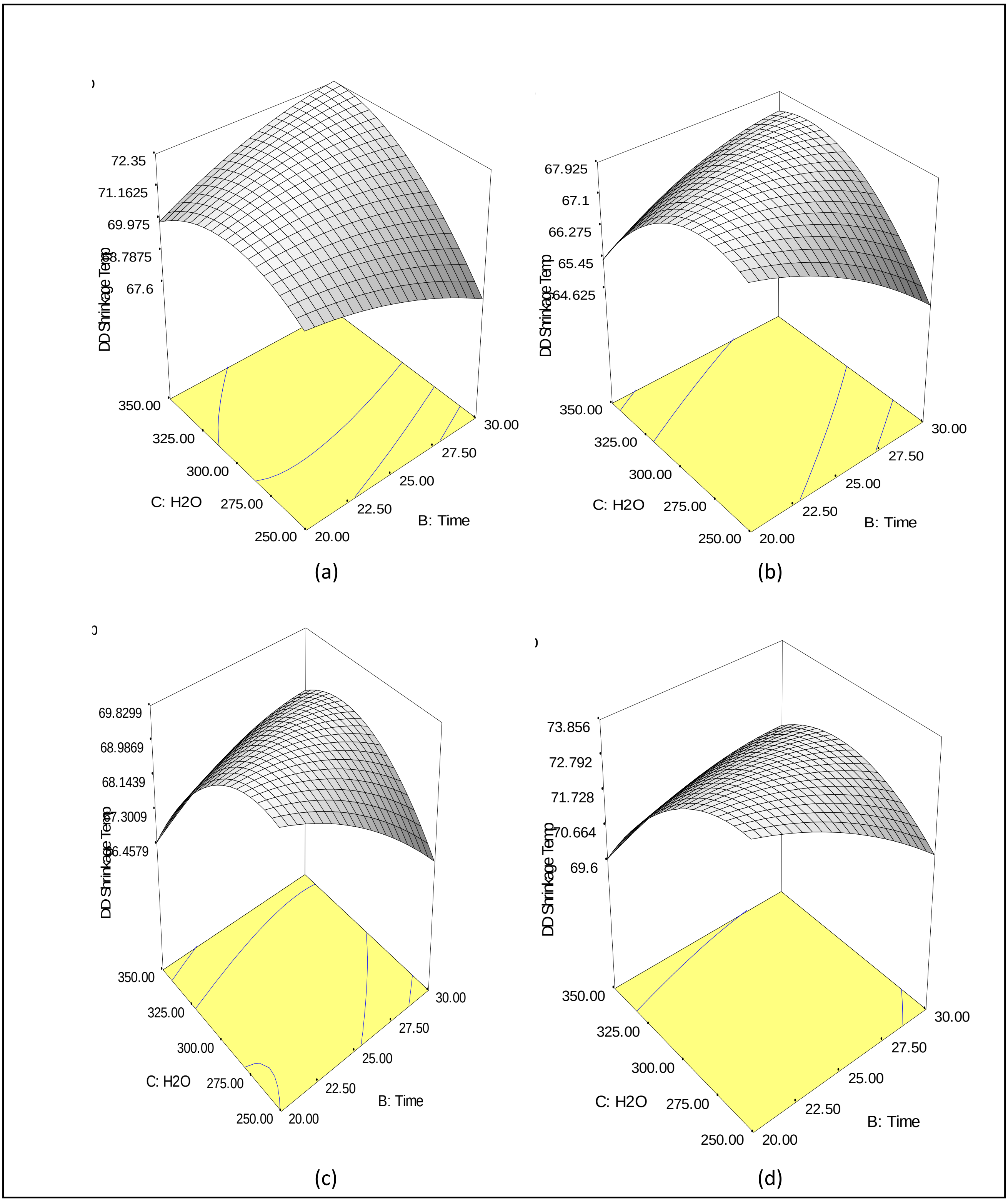


Figure 4.25:3D plots between Water and Time for PC shrinkage Temperature with actual factors of $Ca(OH)_2$:(a) 1.50 %, (b) 2.50%,(c) 3.5 % and 3.01 %

4.5 OPTIMUM CONDITIONS FOR LIMING

This section contains tables and figures that show the results of experiment for two (2) optimum conditions of liming (3.50 % and 1.50 %) chosen from the recommended conditions for Box-Behnken design in Table 4.9. Table 4.10 shows the electronic lastometer of three (3) leather samples cut symmetrically. The values of distention shows no significance difference. Figure 4.26 shows the hydrothermal stability of three (3) leather samples, giving sample 2A and 2B to be the highest (86 ^{o}C) among others. Figure 4.27 shows the tensile strength results,indicating sample 2 to have 25 N/mm^2 as the highest. Figure 4.28 shows the elongation results,revealing sample 1A to be the highest among others. Figure 4.29 present water vapour permeability which shows sample 2 to be the highest. Figure 4.30 shows resistance to compression with sample 3A and 3B to be the highest.

Table 4.10: Electronic Lastometer Test on the Leather Samples using 1.5 % and 3.5 % Liming Concentration

SAMPLE	LIME CONCENTRATION(%)	FORCE (Kg)	DISTENSION (mm)
A1	1.5	29.3±1.24	9.592±0.29
B1	3.5	29.444±3.01	9.296±0.39
A2	1.5	37.746±5.21	9.556±0.58
B2	3.5	41.806±7.42	9.340±0.29
A3	1.5	40.184±5.67	9.454±0.43
B3	3.5	44.942±2.42	9.580±0.39

Key: 1 to 3 represent the different animal skins while A and B symmetrical sets of the skins were cut

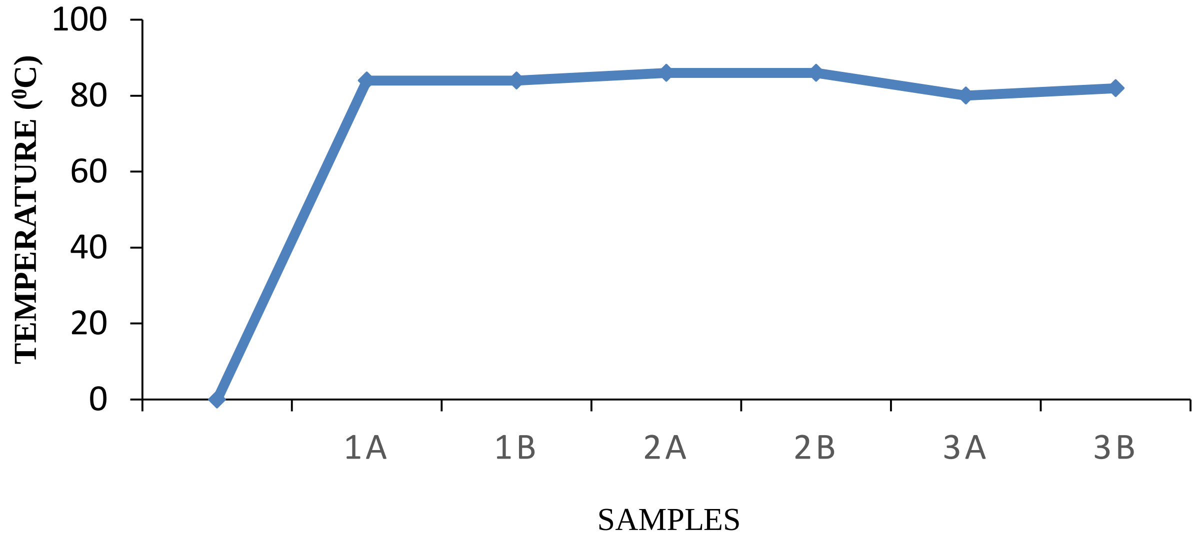


Figure 4.26: Hydrothermal Stability Test on the Leather Samples using 1.5 % and 3.5 % Liming Concentration

Key: 1 to 3 represent the different animal skins while A and B symmetrical sets of the skins were cut

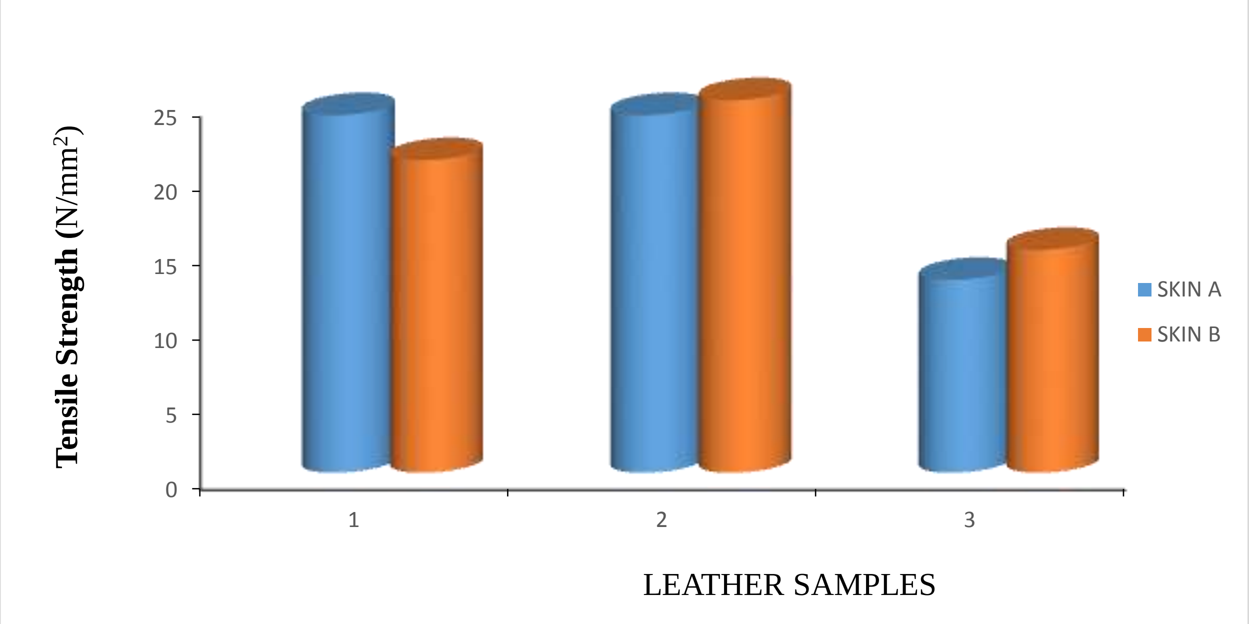


Figure 4.27:Tensile Strength Test on the Leather Samples using 1.5 % and 3.5 % Liming Concentration

Key: 1 to 3 represent the different animal skins while A and B symmetrical sets of the skins were cut

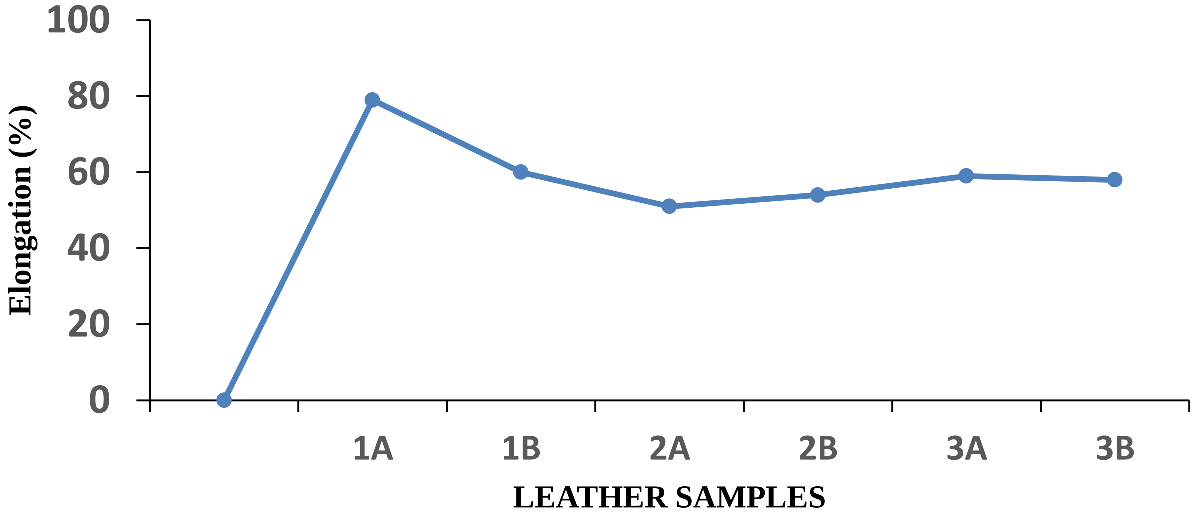


Figure 4.28:Elongation Test on the Leather Samples using 1.5 % and 3.5 % Liming Concentration

Key: 1 to 3 represent the different animal skins while A and B symmetrical sets of the skins were cut

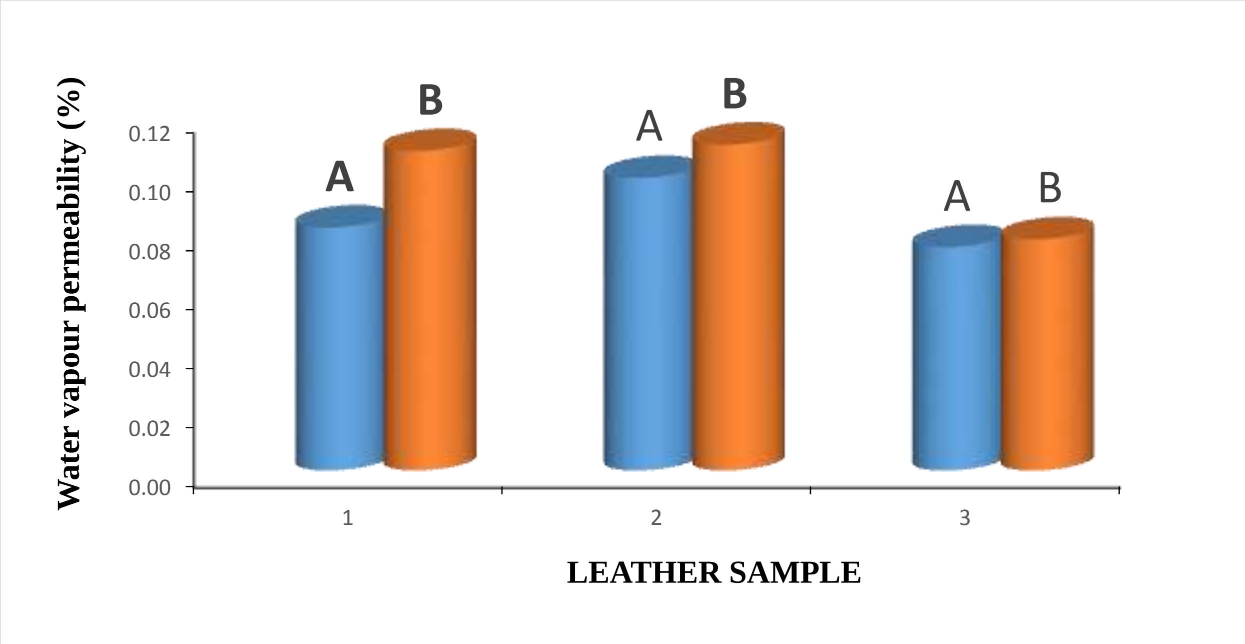


Figure 4.29: Water vapour permeability Test on the Leather Samples using 1.5 % and 3.5 % Liming Concentration

Key: 1 to 3 represent the different animal skins while A and B symmetrical sets of the skins were cut

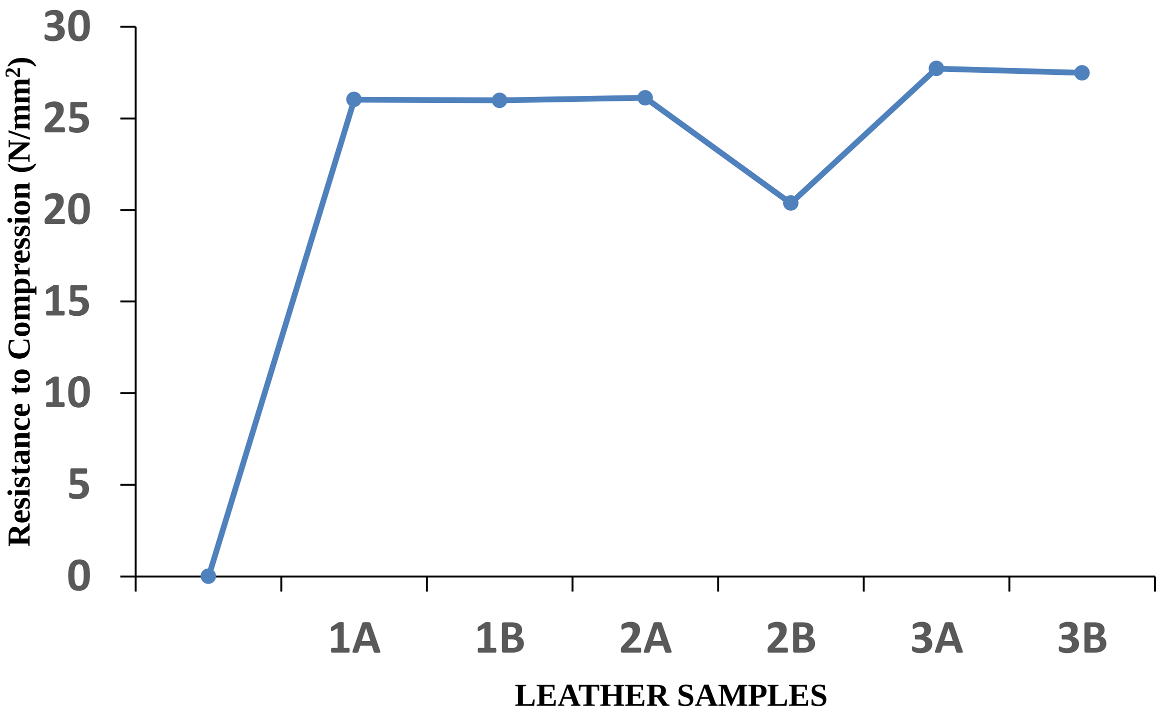


Figure 4.30: Resistance to CompressionTest on the Leather Samples using 1.5 % and 3.5 % Liming Concentration

Key: 1 to 3 represent the different animal skins while A and B symmetrical sets of the skins were cut

4.6 PREPARATION OF HIDE POWDER

The Tables 4.11 and 4.12 show results of hide powder preparation, the analysis of the hide powder and comparison with the standard and its application on the vegetable tanning materials. Results of ash content, pH and water soluble matter are presented in Table 4.11 present better than the standard hide powder, while the tanning potential of PC using the two hide powders is given in Table 4.12.

Table 4.11: Powdered Hide Model for Vegetable Tanning

Parameter	Hide Powder (Standard)	Hide powder (Formulates)
Moisture content(%)	6.5000	6.8000
Ash content(%)	**0.5800**	**0.4100**
Alkalinity (CaO)(%)	0.0098	0.0099
pH	5.9	5.0
Water soluble matter(%)	0.2710	0.0390

Table 4.12: Analysis of Tanning Potential of PC Using Powdered Hide

Parameter	Hide Powder (%) (Standard)	Hide Powder (%) (Formulates)
Non-tans	15.0000	15.0000
Total soluble	57.0000	56.0000
Tannins	42.0000	41.0000

CHAPTER FIVE

5.0 DISCUSSION

The qualitative and quantitative assessment (Tables 4.1 and 4.2) show that *Caesalpinia coriaria* (DD) pods have the highest amount of tannins, 47 % compared to *Parkia clappertoniana* (PC) husks whose tannin content is 42.33 %. This shows that the two vegetable tanning materials have more than the 10 % threshold tannins content required for commercial extraction (Kuria *et al.,* 2016). Characterization of the tannins by thin layer chromatography (Table 4.3), using glacial acetic acid gave retardation factors (Rf): 0.37, 0.43, 0.81, 0.84 indicating that the components of the tannins as 3,4,6-tri-O-Galloyl-D-glucose, tannic acid, pyrogallol and gallic acid for DD respectively; and 0.88, 0.84 indicated catechol, gallic acid for PC respectively (Eldin *et al.*, 2016). Tannins from *Caesalpinia coriaria* are hydrolysable tannins while that of *Parkia clappertoniana* contain both condensed and hydrolysable tannins in the ratio 95:5 respectively (Table 4.4). This is similar to the results reported by Kuria *et al.* (2016), which stated that condensed and hydrolysable tannins can occur in the same plant. However, condensed tannins are preferred to hydrolysable tannins during tanning process. This is because they have a higher affinity for skin collagen due to their high molecular weight and the fact that bonding may occur with carbonyl groups of the polypeptide.

The pH of DD pods extract was found to be 4.03 while PC husks extract gave a pH value of 5.56. The pH of both tanning liquors are within the recommended pH range of 4-6 (SLTC, 1996). This shows that PC and DD are unlikely to cause plumping of the pelt during tanning. Therefore the spaces between the fibres will not decrease and the rate of diffusion of the tanning liquor will not be affected. Furthermore, the tendency of the tannins to combine with the collagen on the surface of the pelt increases when the pH is low and thus leads to leather with poor tannage. At pH 4 to 6, obtained for these tannin liquors, the leather is at its isoelectric point; therefore, the tannins are expected to penetrate well into the pelt and fixation should take place at later stages.

The purity ratio (ratio of tannins/total soluble) was found to be 0.72 for *Caesalpinia coriaria* and 0.54 for *Parkia clappertoniana* (Table 4.2). This is good for the two vegetable tanning materials because it is above the minimum recommended value of 0.5 (Kuria *et al.,* 2016). Thus on the basis of pH and the purity ratio, tannins obtained from these plants are adjudged to be suitable for tanning, however, DD is better.

Generally, the ultraviolet visible absorption spectra for the modified PC and DD using sodium bisulphite was found to be lower than that of the unmodified. A prominent peak (λmax) for modified PC was found to be at 460 nm while the unmodified was at 480 nm (Figure 4.1). A hypsochromic shift was observed as a result of the modification .This is a shift towards shorter wavelength .It could be caused by a change in the medium or the disappearance of conjugation as a result of break of bond in the condensed tannin structure.

However, shift from 470 nm to 500 nm, 570 nm to 620 nm was observed for unmodified and modified DD respectively. A bathochromic shift was observed. This is a shift of the absorption maximum towards the longer wavelength .It could be caused by a change in the medium or the presence of an auxochrome, that is the addition of SO_3Na to the carbonyl group in hydrolysable tannin structure. The chemical catalysis using $NaHSO_3$ to modified tannin of PC and DD shows a positive effect by reducing the large molecular weight of PC (condensed tannin) and increased reactivity of PC and DD.

In the same vein, biological catalysis was used in the modification using pancreatic trypsin commonly known as bate powder (BT) (Figure 4.2). The ultraviolet visible absorption spectra shows a higher absorption at 470 nm and 620 nm for PC tannin material. Whereas there was no observable absorption for DD tannin. This lack of absorption could be attributed to the pH of DD which was found to be 4.03 (Table 4.2) .Enzymes work effectively at pH range of 5 to 11 maximally. Therefore the use of pancreatic trypsin would only be suitable for PC (condensed tannin).

Surface reactions as well as large molecular weights limit the use of vegetable tannins in tanning of hides and skin, reducing penetration into the collagen and lowering the thermal stability or tanning effect (Leticia *et al.,* 2015). Survey into the utilization of small phenolic compounds (ranging from 500-3000Da) such as Catechin to improve penetration by using biological and chemical catalysis, and synergetic effect may provide a novel and alternative tanning agent. Tannin strength of DD and PC, the sorptive ability and binding capacity are the first steps in determining the tanning ability of modified DD and PC using water fraction. The sorptive ability is defined as an ability of a certain amount of tannin to be bound with hide powder, while binding capacity is the ability of tannin to be irreversibly combine with collagen.

The different concentrations of the sodium bisulphite are shown in Figures 4.3 and 4.4. As the concentration of the sodium bisulphite was increased, the sorptive ability and binding capacity also increased. Results of these tests are used to judge the tanning ability of the vegetable tanning materials (Teng, 2014). Figures 4.3 and 4.5 show that PC extracts have the best properties in terms of binding and sorption by 45.50 % and 95.00 % respectively at 10 % concentration of sodium bisulphite. This is followed by DD extracts which have 15.33 % and 46.00 % binding and sorption properties respectively at 10 % concentration of sodium bisulphite. The results shown by *Parkia clappertoniana* (condensed tannins)was the best and can be attributed to its class of tannins which have high molecular weight (Teng, 2014).

Sulphitation is considered to be a very important step of the commercial tannin making process, which enhanced the water solubility, while joining the sulphonyl group to the tannins. The values of sorptive ability and binding capacity increases as the modifier (SO_3Na) increased for PC more than that of DD (Figures 4.3 and 4.4). The hydrophobic – hydrophilic co-effect theory is generally accepted for illustrating the binding interaction between tannins and proteins (collagen). Manchado (1999), reported that binding ability and reliability are positively related to the molecular weight of the tannins. In addition, if the tannins are excessively provided, protein combine preferentially with higher molecular weight tannins. Also, positive relation between – OH groups content and binding energy was reported by Bo (2016), it revealed that the more the – OH groups were presented, the easier the binding occurred.

Tannins extracts of PC and DD were oxidized by enzymatic catalysis using pancreatic trypsin (BT) (Figures 4.5 and 4.6). The sorptive ability of PC increased as the concentration of BT increased from 2 to10 %. On the contrary, DD showed no significant change as the concentration of BT increased. Also, the binding capacity of PC increased with the increase in the concentration of the BT, whereas DD showed no significant change. The resistance to change of DD could be attributed to its class of tannins that is hydrolysable (ellagitannins) which hydrolysis easily. The pH of DD was found to be 4.03 and this could inhibit enzyme activity due to its antioxidant properties (Table 4.2,).

Enzymes are applied at various stages of leather processing, from beamhouse operations to the final stages (DeSouza and Gutterres, 2012). Enzyme activity depends on the pH, temperature and the amount and type of enzyme offered. Most Pancreatic trypsin (BT) are shown to work at pH 5 to 11, but work optimally at pH 8 to 9. In bating, BT is still considered the best enzyme in terms of performance and safe use. It serves to further remove the scud which has been loosened and partially broken down in the liming. The enzymes come into contact with the protein in the tanning material by splitting it at the chain ends where the structure is not ordered. The gap zone of the collagen molecule, measuring approximately 1.4 nm in diameter and 40 nm in length consists in the structure. The empty spaces facilitate the infiltration of polyphenol molecules, suggesting a possible site for binding vegetable tannins molecules. The amino acid sequence in the gap zone is strongly hydrophobic, rich in alanine, proline, hydroxyproline and phenylalanine amino acids.

Covington (1997) reported that vegetable tannins react with collagen mainly by means of hydrogen bonding and thus is inferred from studies of reaction with polyamides. Furthermore, polyphenols fix to amino side chains by electrostatic salt links with carboxylate or hydrogen bonding with carboxylic acid groups depending on the pH. In addition to the above mentioned interactions, condensed tannins have an additional mechanism for reaction that is, covalent reaction between the protein and aromatic carbon in the tannin molecules by means of quinoid structures.

Quinine also can tan protein effectively, raising the shrinkage temperature to 90 to 95^{o}C (Covington, 1997). There is a positive correlation between the molecular weight, the binding capacity and the sorptive ability of tanning. The higher the molecular weight of the tannin, the stronger the tanning ability it will present and that the water fraction had a molecular weight of 2808 Da (Teng, 2015).

Basic structural information of samples were obtained through FT – IR analysis and shown in Figures 4.7. The bands and peaks are from OH stretching and bending vibration (3183.1cm^{-1}), C – H stretching and bending in aromatic methoxyl groups (2926.0cm^{-1}), aromatic skeleton vibrations (1688.5cm^{-1}), C – H deformation (1446.2cm^{-1}), C – O stretching in aromatic ring (1312.0 and 1144.3cm^{-1}), and C – O stretching of methoxyl groups (1099.6cm^{-1}) confirm that the purified modified and unmodified samples were tannins. The only difference was the presence of C – H stretching in aromatic methoxyl groups (2926.0; 84.933cm^{-1}) in modified PC which was not found in the unmodified. This could be attributed to the break in of bond of the high molecular weight of the tanning by the reagent (sodium sulphite) (Ping, 2012; Teng, 2016).

In Figure 4.8, the bands and peaks are as a result of OH stretching vibration (3183.1cm^{-1}) C = O stretching in carboxylic acid (1708.4cm^{-1}), aromatic skeleton vibration (1606.5 and 1524.5cm^{-1}), C – O stretching in aromatic ring (13120 and 1181.7cm^{-1}), C – O stretching in methoxyl groups (1028.7cm^{-1}), C = O stretch in carboxylic acid (1624.5cm^{-1}), C = CH, Ar H bending out of plane (670.9 and 965.4cm^{-1}) confirm that the purified (modified and unmodified) sample of DD were tannins. The modified sample of DD gave similar absorption bands except with the removal of three bands (1624.5, 965.4 and 670.9cm^{-1}) which arose from C = O, C = CH and Ar-H bending out of plane. This is in agreement with what Teng (2016) reported.

Generally, there was a significant increase in shrinkage temperature (Ts) when all the different ratios of tannins obtained from DD and PC and their modified forms (A2M and B2M) were used. The shrinkage temperature of the leathers produced from the unmodified tannin ratios varied from 70 to 80 ^{o}C and the difference between shrinkage temperature of raw skin and the leather produced are generally greater than

18 ^{o}C. PC (B1) gave Ts of 80 ^{o}C. This is consistent with reports that condensed tannins always give high shrinkage temperature (Covington, 2009). When modified (B2M) a leather having a higher Ts values (84 ^{o}C) was obtained. A combination of PC and DD (1:1) (A2 and B2) which represents equal combinations of condensed and hydrolysable tannins, gave high Ts value (76 ^{o}C). This suggests that the combination tannins from DD and PCshowed a synergetic behaviour which increased Ts values of leather. Modification of the various tannins and their combinations (A1M, A2M, B1M, and B2M) with sodium hydrogen sulphite enhanced the Ts of all the leathers produced. Since the character of leather is determined by the first tannage, this combination of condensed and hydrolysable tannins when modified could serve as effective, eco-friendly tanning materials which gave Ts of leather that is comparable to that obtained from mineral tanning using chrome. These differences in shrinkage temperature observed in the different skin samples used may be attributed to the differences in the amino acid content of different animal collagen. The report by Covington (2009) showed that the concentration of amino acid determines the reactivity of the protein towards reagents such as tanning compounds. Also, the sequence influences the formation of electrostatic links, which are not only important for protein stability but are also exploited in leather making. The amino acid content determines the isoelectric point which, with pH, controls the charge on the protein. Therefore, these features of the protein influence its affinity for different tanning reagents, but there is little difference between animal species in terms of the outcomes of stabilizing reactions. Shrinkage temperature is the effect of wet heat on the integrity of the material usually at the denaturation transition. The discerned initiation of the transition is referred to as the shrinkage temperature reflecting the observation that collagenic materials respond to wet heat by shrinkage (SLTC, 1999, Covington,

2009). The shrinkage temperature which indicates additional cross linking between the tannin and the collagen (Table 4.5) was determined before and after tanning. The shrinkage temperature was found to increase after tanning because of the increasing cross linkages between hydroxyproline and bound water of the collagen and the tannins. These cross linkages are as a result of hydrogen bonds between the groups of the hydroxyproline, the bond water molecules and the tannins; additional bridges are formed with a growing amount of hydroxyproline(Mahdi *et al*., 2009). Extreme variations in skin source is the only factor in which the discernible difference in hydrothermal stabilities of collagen is observed.

In a separate experiment, the effect of different liming concentrations and fat liquoring on Ts of leathers tanned with PC and DD, and result given in Table 4.6. This showed that these two parameters increased Ts to different extents. Generally, these effects on Ts was comparable for leathers tanned with PC and DD. The largest Ts of 90 ^{o}C was obtained for PC after fatliquoring when liming was carried out using 3 % lime and Ts 82 ^{o}C was obtained for leathers tanned with DD when 2.5 % and 3,5 % lime were used. This suggests that fatliquoring and liming plays an important role in enhancing the shrinkage temperature, Ts and by extension the quality of leather. A careful selection of the percentage liming used in the liming process and fatliquoring of the tanned leather with vegetable tannins such as DD and PC could be used in place of chrome tanning or complementary to it. Since it is possible to get Ts of up to 90 ^{o}C(Nasr, *et al*., 2013).

The amount of light acting upon vegetable tanned leathers was assessed with the aid of the fading of a set of dyes of differing light fastness on a special kind of textile, known as “blue wool references”. The results are given as classes on the blue scale, with Class 1 corresponding to the greatest degree of change (= very low light fastness) and Class 8 to the lowest (e.g. showing no change after irradiation = excellent light fastness). The demands to be met by a footwear material clearly depend upon its intended use. As a matter of principle, all materials used for the exterior of shoe uppers or which are exposed to light in the interior of footwear by virtue of design should attain Class 3 light fastness (Nasr, *et al*., 2013). The degree of fading with relation to the gray scale for assessing change in colour was the underlying condition for estimating the rate of oxidation of the tannins. The leather sample tanned with DD ratio A1 and D1 alonegave better light fastness (3-4) than PC ratio B1(2-3,), but PC ratio C1 made the required standard (3)(Table 4.7). Consequently, when combining DD with PC and AN in different ratios a decreased in the light fastness was observed. However, this decreased in the light fastness could be compensated when the leathers are dyed. The behaviour of DD tanned leather suggests better background colour for leathers to be dyed with a broad range of light colours.

Tannin ratio A4(DD:AN)1:3 gave the highest shrinkage temperature of 75 ^{o}C. Tannin ratio (A1) DD, ratio (A2) (DD:AN)3:1 gave the shrinkage temperature of 70 ^{o}C and 69 ^{o}C respectively. While tannin ratio A3(DD:AN) 1:1 gave the Ts of 70 ^{o}C and the addition of 10 % of sodium bisulphite as seen in the Table 4.5, improves the Ts to 74 ^{o}C of the mirror image of the leather. This improvement was as a result of the nucleophilic reaction between bisulphite and the carbonyl group in the tannin structure. Similarly, the etherocyclic ring opening reaction of (+)-catechin with sodium bisulphite result in lowering the proanthocyanidins structures that normally penetrate faster in the collagen. Sears in 1972 as cited by Bae (1989) confirmed that the pyran C ring of catechin is indeed opened on sulphonation to give the compound shown in (2.11 and 2.12). The shrinkage temperature result in the ratio 1 implies that tannin ratio A4 (DD: AN) 1:3 gave good hydrothermal stability. The values obtained concurred with the range already shown for the vegetable tanning materials (Brown *et al.,* 2015).

Tannin ratio B4 PC: DD (3:1) gave the highest shrinkage temperature of 85 ^{o}C. This shows that synergetic effect of PC and DD in the ratio of 3:1 was capable of improving the shrinkage temperature of B1 (PC) from 80 ^{o}C to 85 ^{o}C. While tannin ratio B2 (PC:DD) 1:1, B3 (PC:DD)1:3 decreased the shrinkage temperature by 1 ^{o}C and 2 ^{o}C respectively. This shows that the more DD in the mixture, the less the shrinkage temperature (Salminen *et al.,* 2011,IFC, 2007). The result in the tannin ratio B4suggests that tannin ratio (PC:DD) 3:1 could be used whenever the hydrothermal stability is of utmost importance to the users (Figure 4.9). The range of shrinkage temperature values found agrees with what has been reported by (Mahdi, 2012). The results in all the four ratios suggests an improvement in the quality of the resultant leather.

In Figure 4.10, the combination of different ratio of PC and DD show a noticeable increase in the tensile strength. Tensile strength of leathers produced from these different ratios ranged from 15 N/mm^2 to 35 N/mm^2. A combination of DD and AN (1:1) ratio C3 and DD ratio D1 gave a high tensile strength value of 35 N/mm^2.On the other hand, the tensile strength of modified tannins mixture M(DD:AN(1:1)) decreased to 19 N/mm^2. This implies that, the addition of sodium bisulphite had a negative effect on the tensile strength of the leather. According to the standards by UNIDO, the suggested tensile strength value of the leathers tanned with Chromium salts is 10 N/mm^2 minimum. Sharphouse (1983), reported that the value of 10 N/mm^2 tensile strength was also a good value for the vegetable tanned leathers. All the tensile strength values of leather samples tanned with PC, DD and the modification, were above the recommended standard for shoe upper leather. A higher value of tensile strength is a desirable property for all vegetable tanned leathers and is an indicator regarding the quality of leather. Tensile strength is one of the most important physical quantities of characterizing the mechanical properties of materials. It is a routine quality control test in the leather industry where maximum stress and breaking elongation of leathers are determined.

The elongation of the leathers tanned with PC alone (B1 and C1 as seen in Figure 4.11) were found to be 23 % and 30 % respectively. These values are below the recommended standards of 40 % to 70 %. However, the mixture of PC: DD and PC: AN in different tannin ratios gave the % elongation values within the recommended standard(Yusuff *et al.*, 2013). DD alone (A1 and D1 as seen in Figure 4.11) gave 78 % and 42 % elongation value respectively. The different values obtained for PC and DD may be attributed to the different class of tannins present in the two plants. By modifying the tannins both DD and PC an increase in the % elongation value was observed.

Young's Modulus of the leathers tanned with different tannin ratios (Figure 4.12) gave the value to be highest when tanned with DD (D1) 70 which was slightly above PC (C1) 69 and (DD:AN (1:1) (C3)). The results indicated that the tensile strength, % elongation and Young's modulus increased as the tannin ratio of DD increased in all the mixtures. This suggests that, the combination of two tannins PC and DD could enhance the quality of the tanned leather(Yusuff *et al.*, 2013).

The result in Figure 4.13 shows that as the combination of DD:PC varies, the resistance to compression also changed. A3 (DD:AN 1:1) gave the highest resistance to compression of 9.093. In B2 (PC:DD 1:1) gave the highest resistance compression of 6.657. C2 (PC/DD 1:1) gave the highest value of 6.190. In tannin ratio, D4 (PC:DD 1:1) gave the highest resistance compression of 6.651. From the result above, it can be suggested that ratio 1:1 of DD:PC and DD:AN gave the highest resistance to compression. This implies that all the leathers tanned with PC and DD will have shape retentions properties and solidity. These results also suggests that the shape retentions properties and solidity is better than leathers tanned with chrome(Nandy*et al.*, 1999). Vegetable tanning is generally adopted where certain desirable physical properties like solidity, feel, colour etc. are desired, viz., heavy leather or in-sole leathers. This is because the large molecular weight tannins serves as fillers thereby giving the leathers its shape retention properties and solidity. While leathers meant for shoe upper, glove, garment, luggage, etc., are invariably processed by chrome tanning(Nandy*et al.*, 1999).

Generally, all the leathers produced with different tannin ratios gave the thickness within the required standard (0.8 mm to 2.6 mm) (Figure 4.14), except tannin ratio A4 (DD:AN) 1:1 and C1 (PC) gave thickness of 3 mm which was above the recommended standard. This may be attributed to the nature of the animal. There was no significant increase in thickness when all the different tannin ratios obtained from DD, PC and their modified form M(DD:AN) 1:1 (C3) were used. From the results, this implies that, these leathers are suitable for the production of shoes and leather goods of any kind. The leathers are supple enough to bend freely or repeatedly without breaking and exhibit shape retention property(Premier Vision Leather, 2017).

The indentation index results of the leathers tanned with different ratios A3 (DD:AN)(1:1) and C3(DD:AN)(1:1) and D1(DD) gave values of 8 mm, 7 mm and 6 mm respectively(Figure 4.15) . These values could be traced to their corresponding thickness in Figure 4.14. It is not unexpected that this values were low because, these same tannin ratio gave low thickness in Figure 4.14. This property measures the compressibility of a leather under severe conditions. Therefore all the leathers are expected to give good compressibility except ratio A3, C3, and D1 respectively.

One of the useful signs for thermal insulation and durability of leather is the apparent density (Figure 4.16). To obtain a greater comfort, lower density is translated to lower weight. From the values obtained, tannin ratio B1(PC),B3(PC:DD)(1:3) and C1(PC) gave results of higher weight of 4 g/dm^3, 6 4 g/dm^3 and 7 4 g/dm^3, respectively. All the leathers from other tannin ratio gave lower weight 3 and below. However, the apparent density depends on the use that it would be subjected into eventually(Bitlisli, *et al.,* 2004).

The permeability of water vapour through surface materials and its behaviour on contact with water, is the major factors influencing comfortable use of footwear. Figures 4.17 and 4.18 show the behaviour of the permeability of water vapour and water vapour absorption for different tannin ratios. These results imply that leather tanned with ratios A and B displays minimum capacity for water vapour and the minimum permeability of vapour, the so-called “breathing”. Leather tanned using ratios C and D, on the other hand, showed the maximum capacity for removing foot sweat of 37.35 and 41.53 $mg/cm^3/hr$(Nasr, *et al*., 2013).These differences in water vapour in tannin ratios A and B, C and D could be attributed to the different tannin ratios used and the nature of the raw hides and skins. The results suggest a distinct effect on the sense of hygiene and comfort in wear.

Figure 4.19 shows distention of leathers tanned with different tannins ratios. Distention (burst strength) reveal the deformation of leathers under the impact of force. The standard requirement for the burst strength test is generally given as 7.0 mm distention at grain crack at a load of 20 kg. The burst strength values suggest that all the samples displays good results. This implies good response of the leather in toe-lasting conditions(Kuria *et al.*, 2016b).

In Table 4.8, moisture content varied between 9.68 % and 13.96 % in all the tannin ratios. It was observed that tannin ratios A, B and C gave similar values, but ratio D gave values lower than the standard (12 % to 14 %). The relative humidity of an area is a determinant factor in the moisture content of all leathers. Low moisture content affects the tensile strength, flexing endurance and ball burst test of the leathers. But the moisture content of all leathers falls within the range for shoe upper leathers(Haron *et al.*, 2012;Oladunmoye, 2007).

The amount of natural fats in various ratios also varied between 1.02 % and 1.88 % (Table 4.). Tannin ratios A, B and C gave similar values, while tannin ratio D gave values (2.42 %) higher than the three. The higher the fat content the lower the moisture content. This property has direct implication on the softness of the leather. Softness is treated as a key viscoelastic property of footwear materials.Hide substance values varied from 31.47 % and 40.00 % for the four tannin ratios (Table 4.8). The minimum standard value for hide substance is 12.5 %. The hide substance content for all the ratios satisfies the requirement for shoe upper leather. And ash content values ranges 0.061 % to 0.679 %. It was shown that ash content varies in proportion to the amount of salt used in the tanning process. Ash content were found to be lower than the conventional tanned leathers which is good. This is attributed to the reduction in lime concentration, during the tanning process. All the chemical characteristics of the combination tanned leathers were found to be quite normal. Haron *et al.* (2012) reports that, animal hides are not uniform, which means there are big differences in the thickness and type of fibril weaving existing in the different areas of a hide or skin. Results within experiments are comparable (due to samples being adjacent), but results between experiments are not easily comparable, due to inhomogeneity between samplings.

In Table 4.9, the three factors studied in the box-behnken design were Ca (OH) $_2$, Time (hr) and water (%) which is aimed at minimizing the use of lime (Ca (OH) $_2$) and at the same time not compromising the quality of the resultant leather. This Table shows the seventeen (17) experimental run for the box-behnken design with their responses (shrinkage temperatures for PC and DD) which enable the execution of this experimental design. The results were analysed for each variable to get the optimal response for shrinkage temperature (Ts).

From the table, 3.5 % lime, 25 hr and 250 % water gave the highest shrinkage temperature for PC and DD to be 80 ^{o}C and 74 ^{o}C respectively. While 2.5 % lime, 20 hr and 350 % w/w water gave the lowest shrinkage temperature of 68 ^{o}C for PC and 2.5 %, 25 hr and 300 % w/w water gave the lowest shrinkage temperature of 64 ^{o}C for DD. Considering the overall results, 1.5 % lime, 20 hr and 300 % w/w water gave the shrinkage temperature of 77 ^{o}C and 72 ^{o}C for PP and DD respectively. Comparing the three (3) percentages of lime 3.50 %. 2.50 % and 1.50 % and their corresponding time 25 hr, 20 hr and 20 hr with the volume of water 250 %, 350 % and 250 % respectively. This indicates the use of 1.50 % lime, 20 hr and 250 % water to be the best and efficient. This is because the differences in shrinkage temperature when using 3.50 % and 1.50 % was not significant. That is, 3.5 % lime gave 80 ^{o}C and 1.50 % gave 77 ^{o}C. Also, the reduction in % lime concentration and time would reduce the cost of leather production and the total suspended solid in the waste.

Figure 4.20 (a, b and c) shows the three-dimensional plots for shrinkage temperature values between lime and time at a fixed volume of water. In these plots, shrinkage temperature value initially increased with increasing lime concentration and then decreased when lime concentration increased further at a fixed volume of water and finally increased at the maximum lime concentration of 3.5 %. In other words, with increase in the length of time, the effect of volume of water is less significant.

The interaction in Figure 4.21 shows an increase in the shrinkage temperature as the lime concentration and volume of water increased at a fixed time (25 hr). However, at the maximum concentration of lime (3.5 %) and maximum volume of water (350 cm^3), shrinkage temperature decreased. This implies that the shrinkage temperature (Ts ^oC) is not significantly different. Liming can be comfortably carried out at 1.5 % lime while maintaining the hydrothermal stability. Figure 4.22 shows the interaction between water and time with actual factor of lime. In this plot, a linear increase in the shrinkage temperature value with increasing the liming time at a fixed concentration of lime was observed. For water, there was a linear increase in the shrinkage temperature value initially which later declined at the maximum volume of water (350 cm^3) Figure 4.22c). This means that the effect of water is less significant in a shorter time. Therefore, the liming process can be carried out in a shorter time by maintaining the shrinkage temperature.

Figure 4.23 shows a similar increase in the shrinkage temperature value with increasing liming concentration and time at a fixed volume of water. The shrinkage temperature value declined at a certain lime concentration (2.5 %) and later rose at 3.5 % lime. This implies that, liming can be comfortably carried out at 1.5% lime while upholding the hydrothermal stability. Figure 4.24 shows the interaction between lime and water with the actual time (Figure 4.24 a, b and c). A similar effect as mentioned in Figure 4.23 was observed between lime and water for DD shrinkage value. Figure 4.25 shows the interaction between water and time with the actual factor of lime 1.5 %, 2.5 %, 3.01 % and 3.5 %. In these plots, a linear decrease in shrinkage temperature value with increasing time at fixed lime and also decrease in shrinkage temperature value as the water increases. Based on results in Table 4.12, six (6) optimum conditions were observed after analysing the responses (Ts, ^{o}C) using PC and DD as a tanning agent. The highest shrinkage temperature was found to be 79.22 ^{o}C at 3.5% lime, 76.46^{o}Cat 1.5% lime for PC and 73.54 ^{o}Cat 3.5%, while at 1.5 % lime the Ts was found to be 72.16 ^{o}Cfor DD. The differences shown in these results are not significant. As a way of minimizing wastage (economy) and environmental pollution, the liming process can continue with less concentration of lime (1.5 %).

In addition, two conditions out of sixteenexperimental runs (high lime concentration of 3.5 and low lime concentration of 1.5 %) were selected on the basis that they gave the highest responses (Ts ^{o}C) using PC as tanning agent at 3.5 and 1.5 respectively to validate the experimental design carried out under these conditions.

Three (3) goat skins were used after they were divided into two equal half through the backbone at the unhairing stage. 3.5 % lime was used for one part while 1.5 % lime was also used for the other part to avoid inter-skin variations. The tannages for both PC and DD were done using the best ratio (PC/DD 3:1) obtained.

The leathers were subjected to some physical tests such as Lastometer test, shrinkage temperature, tensile strength, % elongation, water vapour permeability and resistance to compression to determine its quality. Table 4.10 shows the Lastometer test, also known as distension and strength of grains by the ball-burst test. This test revealed the positive response of the leathers tanned with 3.5 % and 1.5 % lime in toe lasting conditions. The standard performance needs are generally quoted as 7.0 mm distension at grain crack and load of 20 kg (1UPa, 200l). Comparing the results obtained in the two leathers, no difference was observed; all the values were above 9 mm. Figure 4.26 show the hydrothermal stability of the leathers tanned with 3.5 %

and 1.5 % lime. There was no significant difference in the results. This test is one of the major properties that are routinely used to characterize the tanning effect of tannins and reflect the chemical status of the collagen.

The tensile strength of the tanned leathers were realized when various concentrations of lime (1.5 and 3.5 %) where used (Figure 4.27). However, almost all the samples were above the standard (15 N/mm^2). These results ranged from 13 N/mm^{2}to 25 N/mm^2 from different leathers, which further confirmed what had been shown in Table 4.5 that different skins would display different hydrothermal stability even after tanning with the same percentages. This is attributed to the phenomenon of inter hides/skin variations. Figure 4.28, % elongation test reveals the extension of the leather fibre after being stretched. The minimum standard requirement is 40 mm and the three samples gave the elongation above the standard. One of the factors that influence comfortable use of footwear is water vapour permeability also called breathability of leathers. The results in Figure 4.29 show that leathers tanned using 1.5 % lime have the maximum permeability of 0.10 % while leathers tanned with 3.5 % lime display the maximum permeability of 0.11 %. The results imply that these leathers can be comfortably used thereby giving a distinct effect on the sense of hygiene and comfort to wear. The results obtained for the resistance to compression (Figure 4.50) shows the “fullness” of the leather. The lower the value, the softer the surface characteristic.

A powdered hide was produced for the purpose of exploring possible vegetable tanning mechanism and quantification to compare it with the powdered hide (A) imported from Germany. The specification for hide powder in the literature as seen in Table 4.11 shows that ash content was 0.3 to 0.5 %, moisture < 14 %, pH 5 to 6.5. The powdered hide (B) which was produced in this work show ash content to be

0.41 %, moisture content 6.8 % and pH 5.01 compared to powdered hide (A) which gave ash content 0.58 %, moisture content 6.5 %, pH 5.9. According to the standard specification(Teng *et al*., 2013), the powdered hide produced and the standard were within the range. Different methods have been used for the determination of tanning concentration. These include lowenthal permanganate, folin-ciocalteau colorimetric tannic acid and the official Shake's method, among others. The principle of these methods depend on either the oxidation of the phenolics on permanganate in the presence of indigo carmine or by folin-Ciocalteau reagent – a solution of complex polymeric ions formed from phosphomolybdic and phosphotungsticheteropoly acid. When the phenolics are oxidized, the heteropoly acids are reduced to a blue colouration. Colour formation and oxidation determine the above mentioned methods due to the ability to affect the measurement easily (SLTC, 1996). This method requires hide powder as the major reagent for the determination of tanning concentration (Gimba *et al.*, 2013). However, this hide powder is not produced here in Nigeria. The official standard method uses hide powder produced from bovine and by the specification, bovine (calf) should be between 0-3 years of age. A calf from 0-12 months of age was used to produce powdered hide and to apply on the named tannin, PC (Table 4.12). The result is as follows: total soluble for standard powdered hide (A) was found to be 57 %, whereas powdered hide produced (B) gave 56 %; non-tannins of powdered hide A and that of B gave the same value of 15 %. Tannins concentration of powdered hide A was 42 %, whereas that of B gave 42.33 %. From the result so far, it can be seen that there is little or no difference between the two powdered hides.

The protocol (Table 5.1) for processing the skins using vegetable tanning materials were designed based on the available information on problems associated with vegetable tanned leathers, particularly the low hydrothermal stability.The modification made to the conventional method for tanning procedure (liming, combination of different tannin ratios and fat liquoring) is as shown in Table 4.5 and Table 4.6 above. Table 4.5 shows the effect of modification of tannins using sodium bisulphite, and combination of different tannin ratios which raised the initial Ts for PC and DD from 80 ^{o}C and 76 ^{o}C to 84 ^{o}C and 76 ^{o}C respectively. This modified tannins could be utilized in the production of shoe upper providing high shrinkage temperature and strength properties. In Table 4.6, the application of the fat liquor volume from 4 % to 5 % is capable of changing the D-spacing of a fibril structure of the collagen, thereby increasing the hydrothermal stability of the resulting leather from 70 ^{o}C to 82 ^{o}C for DD and 80 ^{o}C to 90 ^{o}C for PC. This change in D-spacing serves to increase the length (and therefore perhaps the volume) of the collagen fibrils in leather. Fat liquor is considered in the industry to help in the mechanical properties of leather by preventing the fibril structure from restickening and enable the fibres to slide over one another (Sizeland *et al.*, 2016).

The results obtained showed that optimization of the liming stage, modification of PC and DD and the combination of different ratios of PC and DD improved the penetration/fixation of tannins into the collagen fibre leading to a better quality leathers. This modified tanning protocol using 1.5 % lime is expected to give tanners a sustainable product and further lower the use of lime by 1.5 %. Besides, a clear improvement in environmental control was obtained by decreasing the suspended matter in the waste, thereby reducing the cost of procurement of the lime.

Table 5.1: Protocol for the production of shoe upper leather using PC/DD

OPERATION	REAGENTS	%(w/w) /TEMP(^{o}C)	TIME	pH	
Washing (dirtwash)	water	400/RT			
Unhairing	water	100/RT			
	Na_2S	3	45’		
Liming	$Ca(OH)_2$	1.5 %		11-12	
	+water	300-400%			Beam house
Deliming	water	80/RT	43’		
	$+NH_4SO_4$				
Bating	reduce float		40’		
	+ Bate wash check porosity properly				
Drenching	water	100/RT			
	+ salt	7	10’		
	+HCOOH	0.8	45’		
	in 3 slots				
Tanning	PC/DD(3:1)	30	3hrs.		
	2 slots				
	Drain wash pile leave overnight				
Setting out					Tanning yard
Stripping	water	100/RT			
	$+NaHCO_3$	1	30’	5.5	
	Drain wash				
Fatliquoring	water	100/50-60			
	4 % for DD				
	5 % for PC		1hr		
Clearing	oxalic Acid	1	30’		
	Drain wash pile				

Note: RT-room temperature

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Tannins from the pods of *Caesalpinia coriaria* (DD) and the husks of *Parkia clappertoniana* (PC) were Procter and Soxhlet extracted using water and methanol as a solvent and modified usingSodium bisulphite and Pancreatic trypsin. The sorptive capacity, binding ability and purification (column chromatography, gel permeation column chromatography) were determined and the modified extract was characterized by UV-VIS, FT-IR, sorptive capacity and binding ability. The sorptive capacity of the two extracts using Pancreatic trypsin was found to be 41, 25 % as control, while, 35, 25 % as the capacity for PC and DD respectively. Binding ability: 12, 15 % as control, while 25 and 12 % as binding ability for PC and DD respectively. Sulphitation: 41, 25 % as control, while 95, 46 % as the capacity for PC and DD respectively. Binding ability: 11.5, 15 % as control, while, 45.5 and 15.3 % as the binding ability for PC and DD respectively. The results obtained show that Pancreatic trypsin and Sodium bisulphite increase the sorptive capacity and binding ability of the PC extracts to the hide powder much more than that of DD, and this could be attributed to the pH of DD. FT-IR results confirmed that the purified modified and unmodified samples of PC and DD were tannins. The FT-IR spectra of modified sample of DD gave similar absorption bands with the unmodified except with the removal of three bands (1624.5, 965.4 and 670.9 cm^{-1}) which arose from C = O, C = CH and Ar-H bending out of plane. Also, PC spectra gave similar bands except the presence of C – H stretching in aromatic methoxyl groups (2926.0; 84.933 cm^{-1}) in modified which were not found in the unmodified. This could be attributed to the breaking of bond or hydrolysis of the high molecular weight of the tanning by the reagent (sodium bisulphite).

In the same vein, 16 different tannin ratios of DD and PC were blended to examine its synergetic improvement on the resultant Leather. The tanning trials were carried out using 30 % offer for shoes upper leather. The quality of the resultant leather were determined according to the International Union of Leather Technologists and Chemist Society (IULTCS) official methods. The results showed that tannin ratio B4 gave the highest shrinkage temperature of 85 ^{o}C, while tannin ratio B2 gave the highest resistance to compression of 6.65 N/mm^2, water vapour permeability of 0.12 % and elongation of 52 %. Tannin ratio B3 gave water vapour adsorption of 13.5 % and apparent density of 5.63 kg/m^3 and tannin ratio A1 gave the indentation index of 37, young's modulus of 72.17 N/mm^2 and tensile strength of 33.2 N/mm^2. The Lastometer test results were above the standard value of 7 mm distension. Chemical parameters of hide substance, moisture content, fat-soluble and ash content met the standard need for the shoe upper leather (Table 4.9).

Box-behnken design was used to investigate the effects of three independent variables (the volume of water, liming temperature and time) for the optimization of liming conditions. The quadratic polynomial model was used to characterize the liming process. The plots showed that these independent variables significantly influenced the liming conditions. The optimal condition to gain the highest shrinkage temperature of 79 ^{o}C were concentration ($Ca(OH)_2$) of 3.5 %, Time 26.65 hr and solvent (water) 288.44 % . In all the eighteen (16) different trials, tannin ratio B2, gave the highest resistance to compression, while, tannin ratio A1 gave the highest indentation index. At tannin ratio 1:1 of PC and DD, the shrinkage temperature was found to be 79 ^{o}C but at 1:3 of PC and DD, the shrinkage temperature dropped to 77

^{o}C. However, as the ratio of PC increases, the shrinkage temperature increased to 85 ^{o}C. This reveals that, the more the PC, the higher the shrinkage temperature and vice versa. And these results were above the minimum standard. The smaller values in Figure 4.13 indicate higher compressibility and better wear index component. Water vapour permeability and adsorption gave good results, and this is the most important properties inherent on the mammalian skin that enables it act as an osmo-regulator and if lost during tanning, the leather would behave like plastic. The standard tensile strength value for the goat leather is 15 N/mm^{2}, and all the tannin ratios gave the leathers tensile strength that were above the standard.

6.2 Conclusion

Caesalpinia coriaria (DD) pods and*Parkia clappertoniana* (PC) have been successfully extracted using water and methanol. This places DD to be hydrolysable tannin and PC to be condensed tannins. The tannin strength in DD and PC was found to be 47% and 42.33% respectively. This was above the 10 % concentration required for commercial extraction of tannins.The tannin extract was modified using sodium bisulphite and pancreatic trypsin. An increase in the sorptive ability of the concentration of the modifier was observed for both PC and DD. Maximum binding capacity for PC (45%) was found to be greater than that of DD (15%) when modified with sodium bisulphite. While modified with pancreatic trypsin, PC has a higher sorptive ability of 35% and binding capacity of 25% .Also, DD was found to be 25 % and 10% respectively. The result of modification shows that PC has the best tanning properties in terms of binding and sorption.

The characterization of the extracts before and after the modification shows a shift in the ultra –violet visible absorption to the shorter wavelength (nm) for PC extract and to the longer wavelength for DD. The result shows the break of bond in catechin structure in the condensed tanning leading to the absorption in the shorter wavelength and addition of SO_3Na leading to the absorption in the longer wavelength, thereby increasing the penetration and fixation of modified tannins into the collagen fibres. The FT-IR spectra of modified DD tannin gave similar absorption bands with the unmodified tannin except with the removal of three bands (1624.5, 965.4 and 670.9cm^{-1}) which arose from C=0, C=CH and Ar -H bending. This removal is as a result of the addition of SO_3Na to the carbon to carbon double bond. However, PC spectra gave similar bands except with addition of C-H stretching in aromatic methoxyl group in the modified. These additional bands is a result of break of bond from large molecular weight tannins to lower molecular weight ranging from 500-3000 Da.

The thin layer chromatography indicated the component of the tannins as 3,4,6-tri-O-Galloyl-D-glucose, tannic acid, pyrogallo and gallic acid for DD respectively and 0.88, 0.84 indicated catechol, gallic acid for PC respectively. There was asignificant increase in shrinkage temperature, tensile strength, percentage elongation, thickness and indentation index when all the different tannin ratios from DD and PC and their modified forms were used.Optimization of the liming process of the collagen indicates the use of 1.5 % lime, 20 hr and 300 % water to be the best and efficient. This is because, the differences in shrinkage temperature was not significant when using 3.5 % lime (Ts 80 ^oC) and 1.5 % lime (Ts 70 ^oC). The reduction of percentage lime concentration and time will reduce the cost of leather production. Fat liquoring and liming places an important role in enhancing the shrinkage temperature and by extension the quality of leather. Modified protocol for PC and DD tanning of leather using the optimum condition obtained are lime (1.5 %), time (20 hr), water

(300 %) tannin, (PC:DD 3:1) and fatliquor (5 %). Hide powder was produced, analyzed on PC tannins and found out to be better compared to the imported one.

This study concludes that a careful selection of the percentage liming used in the liming process and Fatliquoring of the tanned leather with vegetable tannins such as DD and PC, and the modification using sodium bisulphite and pancreatic trypsin could be used as a substitute for chrome tanning or as a complementary process, since it is possible to get Ts of 90 ^oC.

6.3 Recommendations

1. The high tannins content in the husks and pods of *Parkia clappertoniana* and *Caesalpinia coriaria* plants growing in Nigeria should be exploited for commercial use in the leather Industry.

2. Despite the availability and wide spread of the Plants (*Parkia clappertoniana*), Government should embark on deliberate planting programmes to increase the populations of both *Parkia clappertoniana* and *Caesalpinia coriaria* plants.

3. Methods of species multiplication through seeds, cuttings, layering and genetic improvements which are extremely important for the future improvement of the species, need to be developed and adapted for application at village level. The need to promote the current social status will renew planting interest of *Parkia clappertoniana* and *Caesalpinia coriaria* as a viable resource through improved market outlets and research effort in the bi- products utilization of both plants in leather production.

4. The best Liming condition (1.5 % $Ca(OH)_{2,}$ 20 hrand 300 % H_2O) should be put into use. This would reduce the cost of procurement of the lime and also a preferable means of minimizing the tannery effluent.

5. Sheep's skin/hides should also be used to compare with the goat's skin.

6. The use of Cr as the sole tanning agent should be minimized.

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APPENDIX I

Plate I: ***Caesalpinia coriaria*** **Plant and Pods**

Plate II: ***Parkia clappertoniana*** **Plant and Pods**

Plate III: ***Acacia nilotica*** **Plant and Pods**


(A)


(B)


(C)

(D)

(E)

Plate IV (a, b, c, d, and e): Samples of resultant leather from tannin preparations

Plate V: Samples for physical testing

Plate VI: Traditional tanners from Kano

Plate VII: Samples of Traditional tanned Leathers from Kano

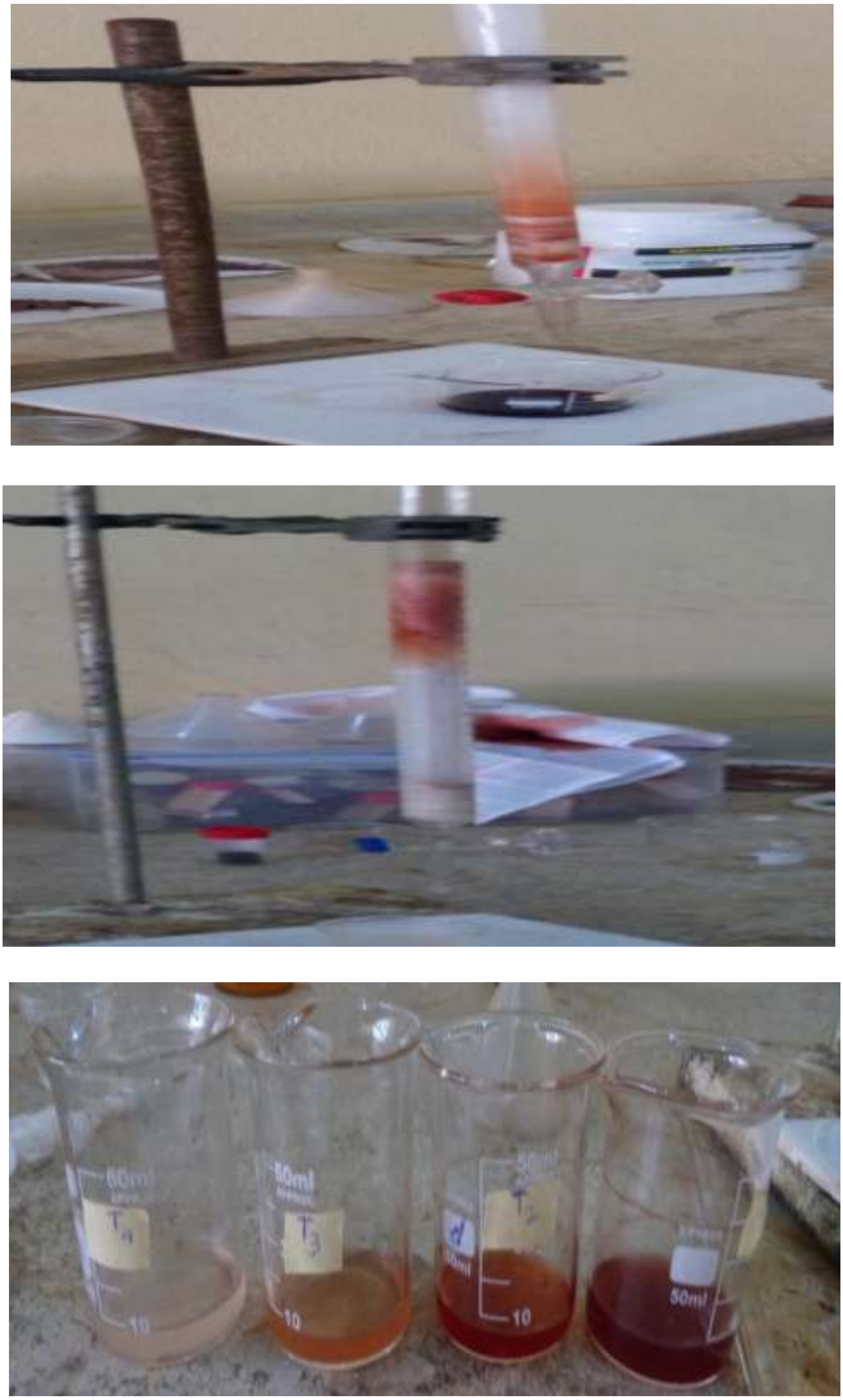

Plate VIII: Gel permeation chromatography

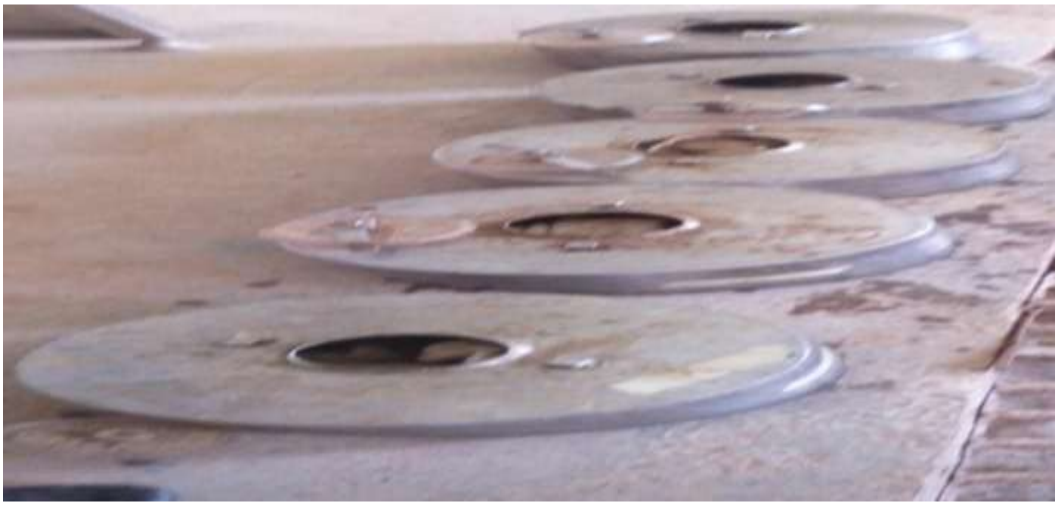

Plate IX: Experimental Tanning Drums

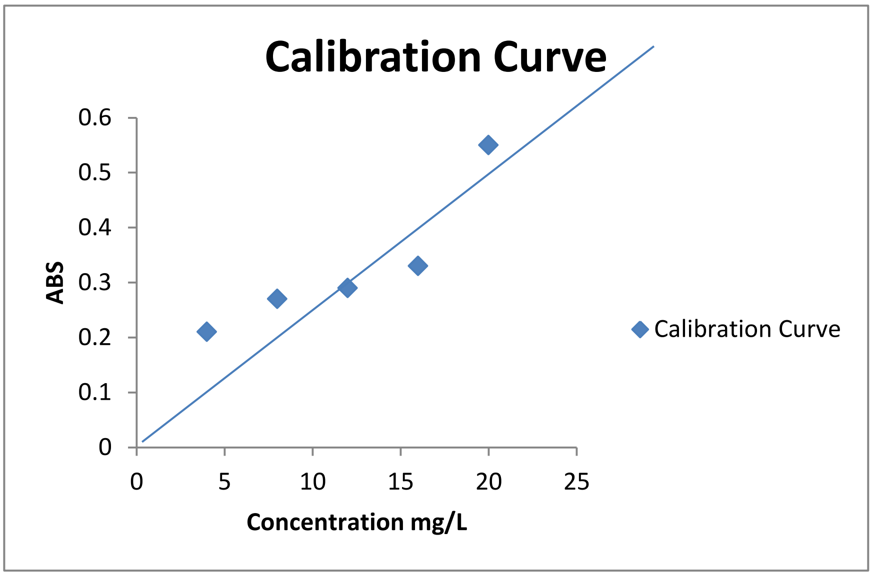

Figure A: Calibration Curvse for Gallic Acid Standard

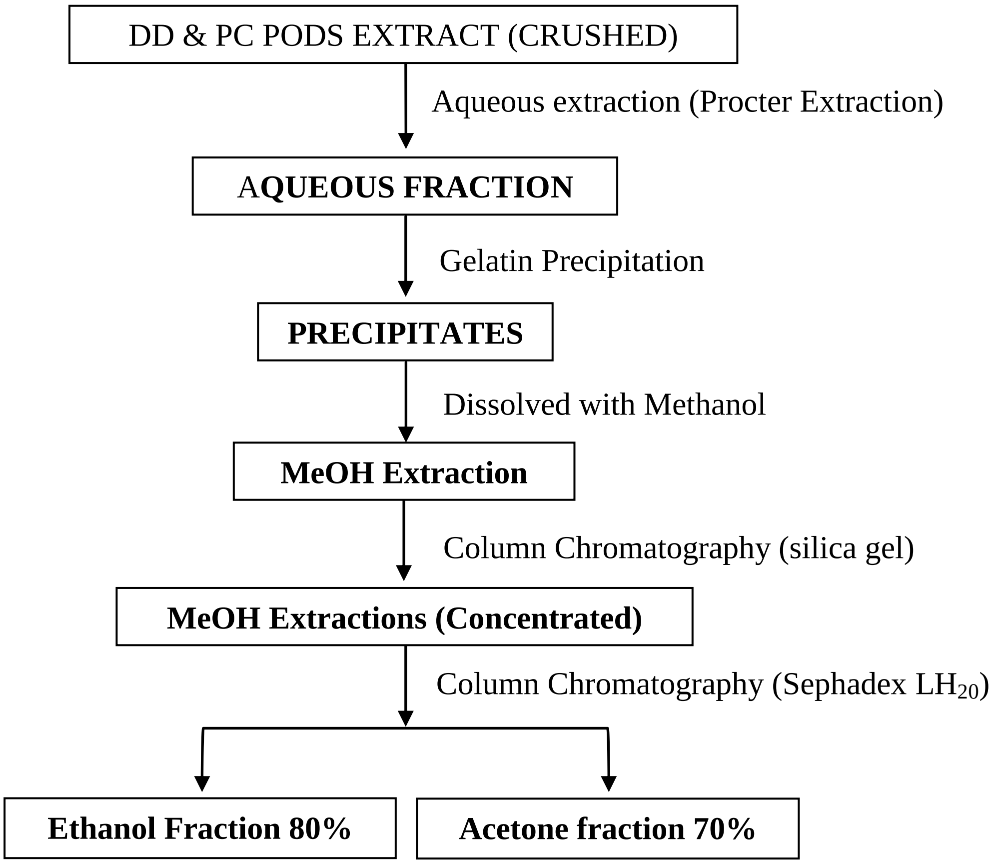

Figure B: Tannins Purification