EVALUATION OF *PLECTRANTHUS BARBATUS* AS A POTENTIAL VEGETABLE TANNING AGENT IN NYAMIRA COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other University.

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DEDICATION

This work is dedicated to my late grandfather Enos Ombui and my parents George Obiero and Teresa Obiero.

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ABBREVIATIONS AND ACRONYMS

KIRDI: Kenya Industrial Research and Development Institute.

LDC: Leather Development Centre.

IPPC: Integrated Pollution Prevention and Control.

Ts: Shrinkage temperature.

N: Newton.

mm: Millimeter.

ISO: International Organization for Standardization.

IULTCS: International Union of Leather Technologists and Chemists Societies.

ISB: International Standardizing Body.

DVS: Director of Veterinary Services.

EPA: Environmental Protection Agency.

BIS: Bureau of Indian Standards.

KEBS: Kenya Bureau of Standards.

SLTC: Society of Leather Technologists and Chemists.

UNIDO: United Nations Industrial Development Organization.

ABSTRACT

Chrome tanning is popular in industrial production of leather but the residual chrome in tannery waste pollutes the environment with concerns of oxidation of chrome(iii) to carcinogenic chrome (vi) in leather articles. Eco-friendly vegetable tanning is an alternative to chrome tanning but the technology is not widely used in Kenya due to lack of cheap sources of these tannins in the market.

This study was designed to evaluate *Plectranthus barbatus* for its potential use as a vegetable tanning agent. The tannin content in *Plectranthus barbatus* was determined using the hide powder method while the tanning potential was assessed by tanning wet salted goat skins using *Plectranthus barbatus* leaves and stem extracts and thereafter determining the quality of the leather produced. Anova statistical test was used to compare the mean tannin content in leaves, stems and a combined leaves and stems extracts and also the physical properties of tanned leather.

Plectranthus barbatus crude extracts were found to contain hydrolysable tannins ranging between 8-20 % depending on the part of the plant. The tannin contents in leaves and stems were significantly different ($p<0.05$) with values of 20% and 8% and respectively.

The physical properties of *Plectranthus barbatus* extracts tanned leathers significantly improved after retanning to attain the following mean values: Shrinkage temperature: 67.5°C ; Grain crack range: 8.7 mm; Grain burst range: 9.1 mm; Tear strength: 78.2 N with % elongation: 75.9; Tensile strength: 40.8N/mm^2 with % elongation of 42.2 and thickness of 1.2 mm. They also endured 100,000 dry flexes without damage and all these figures were above the recommended values.

The study concluded that *Plectranthus barbatus* leaves have adequate tannin content required for tanning and the plant can produce leather with quality comparable with conventional *Mimosa* tanned leathers. It was recommended that leaf extract from *Plectranthus barbatus* can be used for tanning and retanning light leathers and commercial production of the plant should be encouraged through intercropping with other crops especially in small holder farming communities.

CHAPTER ONE: INTRODUCTION

1.1. Background

Vegetable tannins or natural organic tannins are astringent bitter plant polyphenolic compounds that are known to precipitate proteins, amino acids and alkaloids (Aerts *et al.*, 1999). The term tannin is used to mean any large polyphenolic compound containing enough hydroxyl groups and other suitable groups capable of forming complexes with several macromolecules. There are two main classes of tannins, condensed tannins (catechols) and hydrolysable tannins (pyrogallols) (Covington, 2011).

Tannins can be used for tanning hides and skins into leather, making them non putrescible while giving them a soft and flexible feel especially after being subjected to wet and dry cycles thus making them suitable for various uses (Thorstensen, 1993). Vegetable tanning is used in the manufacture of all types of leathers especially those used for sole, belting and harness. In addition, retanning of chrome tanned leather with vegetable tanning or vice versa is common in producing most upper leather and garment leather (Tuck, 1981). This technology has been used in Sudanese rural *garad* tanned crust leathers for production of semi-chrome shoe upper leathers (Musa & Gasmelseed, 2014). Tannins are also used in the manufacture of ink, iron-tannic pigments, alkaloid antidotes, clarification of beer and wine and in the manufacture of wood adhesives (Jingge *et al*, 1998).

Many plants have been subjected to extraction and respective extracts studied as possible tanning agents but only a small number of these materials have shown true value as commercial products in the leather industry (Mole, 1993). Tannins in plants may be concentrated in wood, leaves, nuts, twigs and barks of various plant species (Mole, 1993). Their primary role in plants is protection from decomposition, predation and wild fire (Katie *et al.,* 2006).

In early 1800, tannins used for leather tanning were extracted from oak bark and chestnut trees. Commercial tannic acid is extracted from *Tara* pods (*Caesalpinia spinosa*), Black wattle (*Acacia mearnsii*) and gallnuts from *Rhus semialata* or *Quercus* (Romer *et al.,* 2011). Globally barks of black wattle tree commonly known as *Mimosa* are used in commercial vegetable tanning (Gujrathi and Babu, 2007). In Kenya it grows in the tropical highlands of western part, Rift Valley and central regions. Other plant species used for tanning in Kenya include *Acacia nilotica* locally known as *Mucemeri* in Mbeere (Infonet-biovision, 2004) and *Boswelia neglecta* known as *Halale* in Rendile. In Ethiopia boiled fruits of *Solanum incanum* are also used in leather tanning and in soap manufacture (Abebe *et al.,* 2014).

Plectranthus barbatus also commonly known as *Coleus forskorhlii* or Indian coleus and locally known as *Omoroka* (Kisii), *Mwaraka* (Embu) and *Mumbu (Digo)* is a tropical perennial plant. One of the most widely studied compounds derived from this plant is the labdane forskolin which has a range of diverse medicinal uses (Lukhoba *et al.,* 2006). *Plectranthus barbatus* grows perennially over the tropical and subtropical regions of the Indian subcontinent and is cultivated commercially for its use in pickles (Schultz *et al.,* 2007). Pickles are salt solutions used to preserve perishable foods such as meat and vegetables.

Plectranthus barbatus is distributed in Egypt, Ethiopia, Brazil, India, Sri Lanka and tropical East Africa including Kenya, Uganda and Tanzania (Lukhoba *et al.*, 2006). In India the plant is found on dry barren hills and at an altitude of about 2400 m with moderate rainfall of 400-500 mm and a mean annual temperature of 18-27°C (Lukhoba *et al.*, 2006, Alasbahi and Melzigh, 2010).

The crop is also being commercially grown in South Africa, Zimbabwe, Rajasthan, Maharashtra, Karnataka and Tamil Nadu in an area of about 2500 ha (Lukhoba *et al.*, 2006). In Kenya, *Plectranthus barbatus* grows in Kisii, South Coast, Central province, Western Kenya, Eastern province and South Turkana.

The plant is known to contain tannins (Saksena *et al.*, 1985) however, its potential for use as a vegetable tanning agent is not known. Its resistance to fire is a salient characteristic of plants that are rich in tannins (Katie *et al.*, 2006).Tannins can be concentrated in stem, bark and/or leaves (Ruedi, 1986) and vegetable tanning in Kenya has only been done using barks of *Acacia mearnsii* and *Acacia nilotica* (Infonet-biovision, 2004). Therefore, most indigenous plant species in Kenya have not been studied for their suitability as tanning agents.

The aim of this study was therefore to determine the tannin content in *Plectranthus barbatus* and assess its potential use as a vegetable tanning agent.

1.2. Objectives

1.2.1 General objective

To determine the tannin contents in *Plectranthus barbatus* and its suitability for use as a vegetable tanning agent in the manufacture of leather.

1.2.2 Specific objectives

- To identify the type of tannins present in the *Plectranthus barbatus* plant species growing in Nyamira County.
- To determine the tannin content and tanning strength of *Plectranthus barbatus* leaves and stems.
- To tan goat skins with *Plectranthus barbatus* leaves and stem extracts and compare the physical properties of *Plectranthus barbatus* tanned leathers with the conventional *Mimosa* tanned leather.

1.3. Research hypothesis

Ho: *Plectranthus barbatus* does not contain adequate tannin content and strength for effective tanning of leather.

1.4. The problem statement and justification

Challenges in environmental pollution caused by mineral tannages especially chrome discharge are a threat to the environment in terms of potential exposure and accumulation of toxic heavy metals (IPPC, 2003). Chrome is the most common commercial mineral used in tanning hides and skins into leather. This tannage releases chrome to the environment in tannery effluent after its utilization in tanning process. Chrome is toxic to plants, fish and animals including humans due to bioaccumulation and biomagnification in food chains. Chrome (iii) migration occurs in chrome tanned leather and it sometimes undergoes oxidation to carcinogenic chrome (vi) where both forms cause allergic contact dermatitis. Traces of Cr^{6+} have been found in leather articles that include watch straps and shoes (Graf, 2001).

Oxidation can be caused by fatliquours that contain unsaturated fatty acids especially sulphited fish oils and wetting auxiliaries with reducing abilities prior to dyeing in temperatures of about 80ºC (Hauber and Germann, 2000). Furthermore, contamination of ground and surface water systems with severely high levels of chromium that pose risks to human health and the environment has been noted around tanneries in many low-income countries (Hossain and Bhuiyan, 2010). Chromium is a metallic element which is listed by the Environmental Protection Agency (EPA) as one of 129 priority pollutants (Mohan and Pittman, 2006). Chromium (vi) is outlined by EPA as a class A human carcinogen and is ranked as one of the 14 most toxic heavy metals (Mohan and Pittman, 2006., Das and Mishra, 2008).

Vegetable tanning has been identified as an alternative to chrome (Covington, 2011) as we move towards green chemistry and eco-labeling. However, vegetable tanning is not as widely used as

chrome due to the high cost of *Mimosa,* the only available vegetable tanning agent for commercial tanning. There is also limited knowledge and information of other cheaper indigenous plants that have a potential for use as vegetable tanning agents in Kenya. *Mimosa* is derived from barks of *Acacia mearnsii* which have tannin content of 38.6% which when concentrated yield a commercial product with a tannin content of between 63-70% (Gujrathi and Babu, 2007).

Research on new vegetable tanning materials is necessary in order to find plants with high tannin content that can be exploited for commercial tanning. *Plectranthus barbatus* an indigenous plant growing in many parts of the country was identified as a potential candidate for study due to its resistance to fire, a salient characteristic of plants that are rich in tannins (Katie *et al.*, 2006).

The findings of the study will provide cheap locally available tanning materials for the leather industry in Kenya. This will lower the cost of production of leather products through reduced importation and use of chrome and will contribute to the development of an eco-friendly leather industry.

CHAPTER TWO: LITERATURE REVIEW

2.1. Structure and classes of tannins

Tannins are large polyphenolic compounds with sufficient hydroxyls and other suitable groups such as carboxyls which contribute to their high molecular weight that allows them to form strong complexes with proteins and other macromolecules (Ashok and Upadhyaya, 2012). Tannin molecules must have at least 12 hydroxyl groups and a minimum of five phenyl groups in order for them to precipitate proteins (Haslam *et al.*, 1992).

Oligostibenoids (oligo- or polystilbenes) are oligomeric forms of stilbenoids and constitute a class of tannins (Boralle *et al.*, 1993) however, there are three major classes of tannins considering the basic units or monomers in each category (Covington 2009). The three classes are as follows:

a) Hydrolysable tannins (pyrogallols)

Hydrolysable tannins will tend to disperse when boiled in an acid solution and are less likely to develop red colours upon addition of alkali. They are easily purified to relatively simple light coloured tannic acids. In addition they develop blue colours on addition of ferric chloride (Yisa, 2009). The tannins of *myobalans*, *chestnut*, *sumac* and *divi-divi* are of hydrolysable type (Afsar and Sekeroglu, 2008). Hydrolysable tannins are further divided into two groups i.e. gallotannins and ellagitannins (Covington, 2009):

i) Gallotannins

The glucose core in gallotannins is esterified only by gallic acid and bound gallate groups can undergo depside esterification through their phenolic hydroxyls. Variation in structures arises from the degree of esterification of glucose centre and the magnitude of depside esterification. The astringency polyphenols depends on the concentration of hydroxyl groups(Covington, 2009). A typical gallotannin is pentagalloyl glucose (1,2,3,4,6-pentagalloyl-O-D-Glucopyranose) (Hagerman, 2002). Its structure is shown in figure 2.1 below.

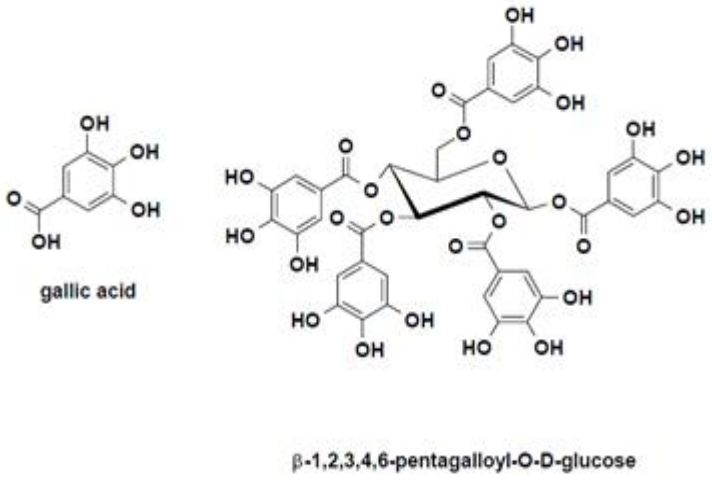

Figure 2.1: Structure of pentagalloyl glucose (Hagerman, 2002)

ii) Ellagitannins

In ellagitannins the esterification moieties include gallic acid, ellagic acid and chebulic acid however, gallotannins are more astringent than ellagitannins. Pyrogallols are highly astringent because of the large number of closely associated phenolic hydroxyl groups and their acidity is due to the presence of carboxylic acid groups (Covington, 2009). Gallotannins are converted to

ellagitannins through oxidative coupling of galloyl groups and simple ellagitannins are esters of hexahydroxydiphenic acid (HHDP) (Hagerman, 2002) as shown in figure 2.2. A colloid of hydrolysable tannins left to stand overnight has a tendency to decompose into ellagic and chebulinic acids (SLC 112, 2001).

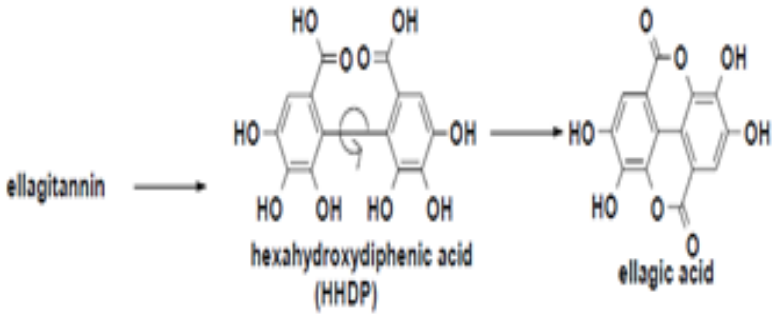

Figure 2.2: Structures of hexahydroxydiphenic acid and ellagic acid (Hagerman, 2002).

b) Condensed tannins (catechols)

Catechols have a flavanoid ring structure and will show an increase in weight (polymerize) when boiled in an acid solution (Thorstensen, 1993). Condensed tannins will disperse upon addition of an alkali and are oxidized to yield red colours (Thorstensen, 1993). They will develop green colours on addition of ferric chloride (Yisa, 2009). Tannins of black wattle, *quebracho*, *hemlock*, *cutch*, *gambier*, *mangrove*, spruce, *harch* and tea are catechols (Afsar and Sekeroglu, 2008). The structures of the most common condensed tannins based on flavan-3-ols -(-) epicatechin and (+)-catechin are shown in figure 2.3.

(Flavan-3-ols)

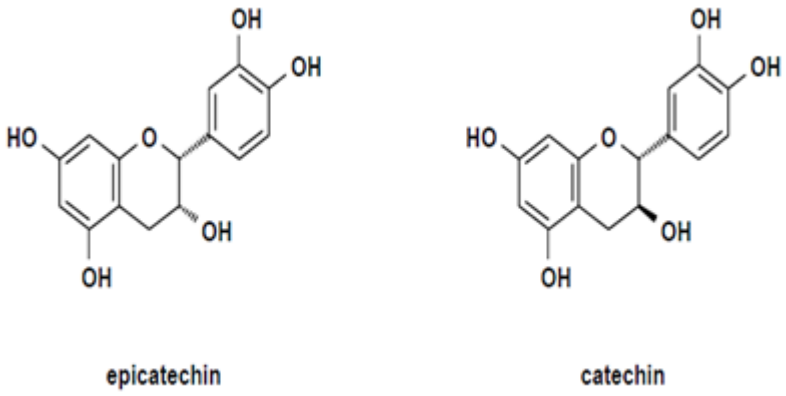

Figure 2.3: Structures of epicatechin and catechin (Hagerman, 2002).

c) Complex tannins

Complex tannins are mixtures of tannin types where hydrolysable gallotannin or ellagitannin moiety is bound glycosidically to a condensed tannin moiety. This category exhibits properties of both types of polyphenols (Covington, 2011).

2.2. Nature of tannins

Tannins are an amorphous yellowish or light brown powder, flakes or sponge. Tannins are soluble in water and alcohol but insoluble in organic solvents. Extracts usually have three fractions (Covington, 2011): non tannins characterized by low molecular weight <500, tannins that have medium molecular weight i.e. 500 – 3000 and gums of high molecular weight >3000.

2.3. Occurrence of Tannins

Vegetable tannins are known to occur throughout the plant kingdom and common in both gymnosperms and angiosperms (Mole, 1993). However, tannins do not occur in the kingdom Fungi but phlorotannins are found in brown algae. Mole (1993) studied the distribution of tannins in 180 families of dicots and 44 families of monocots. Most families of dicots studied contain tannin free species. Monocots families of Najadaceae and Typhaceae have all their member species containing tannins as is seventy three percent (73%) of the oak family (Mole, 1993), Fagaceae were found to contain tannins in the species tested but in the family Mimosaceae, only 39 per cent of the species tested were found to contain tannins (Mole, 1993). Only six per cent of Solanaceae and four per cent of Asteraceae tested positive for tannins (Mole, 1993). Condensed tannins are the most abundant and are found in almost all families of plants consisting up to 50 per cent of the dry weight of leaves (Doat, 1978).

2.4. Classification and characteristics of *Plectranthus barbatus*

Plectranthus barbatus belongs to the family Lamiaceae also known as Labiatae which is the largest family of the order Lamiales that has herbs and shrubs with distinct four sided stems and blue raceme inflorescence (Mariya *et al.,* 2013). The entire plant is aromatic with leaves and roots having different oduors (Lukhoba et al., 2006). A photograph of *Plectranthus barbatus* is shown in plate 2.1.

Plate 2.1: *Plectranthus barbatus* growing along a natural fence

2.5. Phytochemistry of *Plectranthus barbatus*

Plectranthus barbatus contains a compound known as forskolin that was discovered in 1974 and was initially referred to as coleonol (Saksena *et al.,* 1985). After identification of other coleonols and diterpenoids the name was later changed into forskolin (Saksena *et al.,* 1985). Forskolin is in great demand in Japan and European countries for its medicinal use and other research purposes (Kavitha *et al.,* 2010). The plant also contains phenolics, monoterpenoids and sesquiterpenoids (Misra *et al.,* 1994).

Although the majority of abietane diterpenoids were isolated from the leaves and stems of *Plectranthus barbatus* growing in Brazil (Lukhoba *et al.,* 2006) and from the leaves of

Plectranthus barbatus distributed in East Africa (Lukhoba *et al.,* 2006) some of them were also obtained from the leaves, roots and whole plant as well as from the roots of *Plectranthus barbatus* growing in China (Lukhoba *et al.,* 2006) and India respectively (Ruedi, 1986).

Plectranthus barbatus leaves are resistant to decomposition perhaps because of significantly high tannin content in them. This aspect has been studied through observation since leaves soaked in water do not evolve putrid smell but will leach giving a brown solution predominant with tannin rich plant materials (Sims and Morris, 1986).

2.6. History of tanning

Man's interaction with polyphenols is ancient perhaps, because of his diet and this must have been the origin of vegetable tanning but where and how it started is not clear. Plant polyphenols´ ability to stabilize collagen in the skin against putrefaction has been applied since ancient times and must have begun through observation. Prehistoric man must have realized suitable alterations in hide or skin after it had accidentally lain in a pool with plant material (Covington, 2011).

The first evidence of leather tanning was found in the remains of ancient settlements in northern Germany dating back 10,000 BC. The earliest known practice of vegetable tanning was by Egyptians who used *Acacia nilotica* 7000 years ago. The Hebrews used oak bark while Romans used a variety of barks, woods and berries. The most prolific relevant tree of the desert wadis is *acacia seyal* and other types of acacia whose barks and pods yielded tannin extracts. These plants grew in wadis in Palestine, Sinai, Egypt and other parts of Asia and Africa. *Acacia nilotica, Acacia Arabica, Acacia tortilis* and *Acacia adamsonia* are collectively called *Babul* in India,

Babar in Sind, *Babla* in Arabia, *gabarua* in Nigeria and *garad* or *sunt* in Sudan (Hingham, 1996). The Arabs used barks and roots whereas in Spain, leathers were tanned with *sumac* and then retanned with alum.

The use of *quebracho* for vegetable tanning in South America started in 1870 whereas black wattle was discovered in 1814 and has its origin in Australia but its plantations were established in South Africa in 1880. Up to the end of the nineteenth century nearly all leathers were vegetable tanned and the onset of chrome tanning can be traced in Knapps monograph on tanning of 1858 in which he described the use of chrome alum (Covington, 2011). Commercial chrome tanning began in 1884 and the new two bath process in which chromic acid was the chemical infused through hides and skins in one bath followed by reduction and fixation in the second bath was patented by Schultz (Covington,2011).

A clear change of this technology was observed at the beginning of the twentieth century with a new reaction which was faster and reliable than the then current vegetable tanning thus bending the global leather industry towards chrome tanning. The chemistry of chrome tanning involves several simultaneous competing reactions influenced largely by the pH and temperature during the process (Thorstensen, 1993). The skill of the tanner has continuously improved in dealing with these prevailing factors in order to make consistent high quality leather in line with the current market demand and customer satisfaction.

2.7. Current tanning practices

Radical changes for future tanning chemistries will take place through research with improvements on combination tanning which clearly suggest that many activities will surround vegetable/ organic tanning. The interactions between tanning agents, skin collagen and solvent are to be considered but options on organic tannages based on organic plant polyphenols plus their derivatives together with synthetic polymers or a synergistic effect of both seems to be more likely (Covington, 2000). More and new tanning methods will be linked to the latest development in the theory of tanning which include the thermodynamics of tanning reactions that create a matrix around the collagen triple helix (Covington, 2000).

The present trends of tanning chemistries are constantly changing because they are required to respond to the shifting consumer demands and stricter environmental regulations and therefore it is possible to contemplate of tannages with inbuilt instability to microbes with the aim of ecologically recycling leather back to the earth (cradle to grave). This means that the many years work by tanners to develop tanning mechanisms in order to impart high stability to leather especially resistance to microbial attack will be reversed and relatively unstable leathers will be more desirable by 2050 (UNIDO, 2000).

2.8. Methods of tanning

Tanning of hides and skins is broadly classified as mineral (inorganic) and vegetable (organic) based tanning but when the two forms are employed in the same process then they result into combination tanning. Emerging environmental impact issues are looking at a possibility of

substituting metal tanning by reviewing the chemistries of various elements and their compounds together with their reactivity towards collagen with an objective of conferring to leather the same versatility as chrome. (Covington, 2011).

2.8.1. Mineral tanning

Apart from chrome tanning, experiments with other metals have been conducted and based on feasibility of the reactions Kuntzel and Droscher concluded that satisfactory mineral tannage must involve aggregation and polymerization in order to allow multiple interactions with collagen and complexation reactions (Covington, 2011). Although through studies on thermodynamics of tanning by Weir and Carter and reviews made by Borasky in 1957 followed by another comprehensive study by Chakravorty and Nursten in 1958 (Covington, 2011), there seems to be no option that can rival chromium (iii). The only practical options as alternatives of chrome which have been in use in leather industry are: Aluminium (iii), titanium (iv), Zirconium (iv) and to a lesser extent iron (ii) (Covington, 2011).

2.8.2. Combination tanning

Combination tannages use more than one tanning agent which is either a metal or a non-metal for instance oxazolidine. Retanning of chrome tanned leather with vegetable tannage is a typical combination tannage (Musa and Galsmelseed, 2013). Several useful combination tannages are applied in which various components especially, aluminum interacts synergistically with plant polyphenols to confer high hydrothermal stability to leather (Covington, 1997).

Generally condensed tannins do not participate in semi-metal reactions except for the presence of pyrogallols B-ring however, they readily engage in reactions with aldehydic reagents. The exception, with regard to tannin type is *Mimosa*, which is effective because it contains a major component which has a pyrogallol group with a B ring. It is possible that the ability of *Mimosa* to react with collagen partially covalently (Covington, 2011) contributes to its powerful semi metal tanning reaction. Sykes and Cater (1980) proposed that the critical requirements of the semi-metal reaction are the presence of a polyhydroxy aryl ring with more than one such moiety in a molecule although Orszulik *et al*., (1980) demonstrated that more specifically the reaction requires pyrogallols moieties in the polyphenols compounds. The commonly used combination tannages include: Semi-metal tanning, Semi-chrome tanning and Condensed tannins with aldehydic crosslink.

2.8.3. Organic (vegetable) tanning

Vegetable tannins are found in a wide variety of plants and may be concentrated in wood, leaves, nuts, twigs, bark and roots. The extract of a particular plant has numerous substances hence there is no such thing as single tannin from a particular plant source (Thorstensen, 1993). The extracts usually contain different tannins, gums, starches, sugars, lignins and other materials. Sources of vegetable tannins include the following: *Oak* bark, *Mimosa* bark, *Mangrove* bark, *Valonia*, *Myrobalans*, *Divi divi*, *Sumac*, *Quebracho*, Pine bark, *Chestnut*, *Hemlock* and *Gambier* (Thorstensen, 1993).

Organic tanning is the reaction between tannin molecules and the pelt collagen to form leather during which the polyhydroxyl groups in tannin molecules react with the functional groups on the

polypeptide chain especially –COOH groups (Covington , 2011). The particles upon deposition on collagen fibers, they become chemically bound either through hydrogen bonds, ionic bonds or covalent bonds (Covington , 2011). The latest thinking invokes the notion of preferential binding in gap region of the quarter stagger structure (Haslam, 2007). Figure 2.4 illustrates cross-linking of vegetable tannins with polypeptide chain.

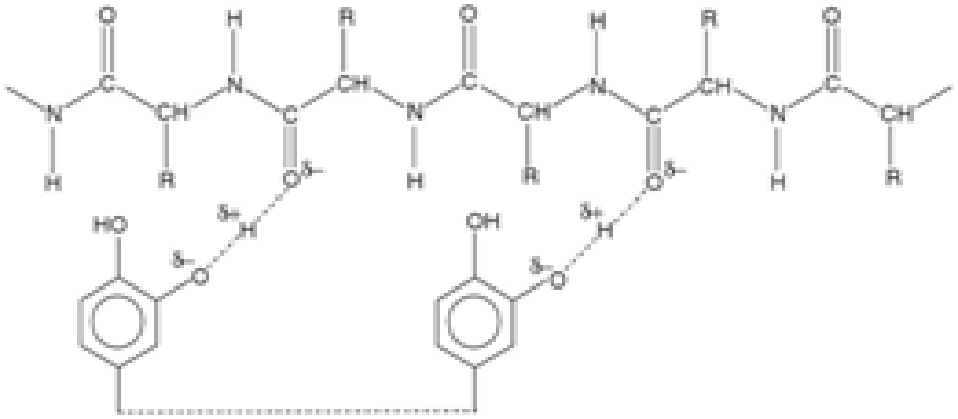

Figure 2.4: Bonding in organic tanning (Covington 2011)

In vegetable tannage of light leather, the intention is to fill up the collagen structure and to confer weight and firmness (Covington, 2000). In heavy leather, the filling action and weight increases are important (Covington, 2000). Hydrolysable tannins are used in combination tannages where specifically less toxic metals like titanium other than chrome are used to produce superior leathers that are eco-friendly (Covington, 2000). This practice has been viewed as one of the best available techniques that will lead to increased production of leather products, environmental sustainability and proper use of available natural resources (IPPC, 2003).The oxidation of chrome (iii) to carcinogenic chrome (vi) in leather products tanned with chrome further threatens the

future of chrome tannages and the application of green chemistry in cleaner technologies calls for increased use of vegetable tanning technology.

2.9. Tanning processes

2.9.1. Beamhouse operations

Beamhouse refers to processes in a tannery between removal of hides and skins from storage or their arrival and their preparation for tanning (Thorstensen, 1993). These processes entail modification and partial purification of collagen fibres in hides and skins at the wet end section of the tannery with an aim of exposing collagen fibres to tanning chemicals, a process normally called opening up. Beamhouse operations are critical and closely attended controlled process steps since it is known by most practical tanners that leather is made in the beamhouse.

Reasonably any deviation from the required regulations and details of these preparative process steps will compromise the quality of the final product which will not be compensated for by subsequent operations or even refined corrective measures (Covington, 2011).There is emphasis in ‘get it right the first time’ because the fundamental properties and ultimate performance parameters of leather are conferred to the pelt in the beamhouse (Covington, 2011).The beamhouse operations include :Shaking off excess salt and loosely attached contaminants, dirt soak, main soak, unhairing, liming, fleshing, deliming, bating, scudding and pickling.

a) Soaking

The soaking process has various objectives which include: rehydration of the skins and hides, removal of salt, cleaning the pelt, removal of non-structural proteins, removal of manure and removal of hyaluronic acid. Several parameters have to be considered according to Stockman *et al.*,(2008) and they include: history of the pelt, type and degree of curing, water content of the raw stock, nature of the protein solubilised in the soak, condition of the soak water, concentration of the salt in the soak liquor and the rate of diffusion from the pelt into the solution, pH and temperature of the soak liquor, float to pelt ratio, time in soak, effect of changing the soak float, and impact of biocide in the soak and the chemistry of the reagent.

b) Unhairing

Unhairing involves the process of removal of hair from the pelt and this process is underlined by the chemistry of hair and soft keratin proteins but traditional methods of hair dissolving and alkaline hydrolysis combines liming and unhairing (Covington, 2011). This process is considered to be the dirtiest based on the oduor emanating from sulphide, decomposed protein and tannery effluent load generated (Money, 1996).

Unhairing can be achieved through various means: hair burning (El Baba *et al.*,1999, Onyuka, 2009) hair saving (Frendrup and Buljan, 1998), immunisation (Cantera, 2001), Heidemanns Darmstadt process (Heidemann, 1993), oxidative unhairing, reductive unhairing, acid unhairing, enzyme-assisted chemical unhairing, chemical - assisted enzyme unhairing, enzyme hair saving (Paul *et al.,* 2001), keratinase (Covington *et al.,* 2005) and painting (Covington, 2011).

Sweating system of hair removal is a traditional method that possibly gave birth to enzyme based dehairing where skins were dipped into water and then hanged in their moist condition in a room for a period until hair slip occurred by natural wild bacterial proliferation. However, the approach more often than not resulted into uncontrolled excessive damage to hide collagen (Thorstensen, 1993). Recent approaches in dehairing are inclined towards hair saving method where hair is removed intact (Frendrup and Buljan, 1998) because it is a cleaner technology that reduces tannery effluent as compared to hair burning process. The best known method in modern industry is the Sirolime process developed at CSIRO in Australia (Covington, 2011).

c) Liming

After dehairing is complete the liming operation is conducted for a period of up to 18 hours. This is a pH controlled step dependent on solubility of lime where swelling of the pelt that usually occurs due to high pH values (pH>13) should be monitored to avoid incidences of irreversible structural damage of the pelt collagen fibres (Covington, 2011). The conditions to consider include: weight of raw stock, float length (200%), pH (12-13), temperature (25-30˚C) and time (18hrs).

Purposes of liming constitute controlled destabilization of collagen in the pelt that occurs due to amide hydrolysis leading to lowering of shrinkage onset temperature which affects conventional shrinkage temperature (Covington and Alexander, 1993). Other liming objectives consist of: removal of non-collagenous components of the hides and skins and splitting the fibre structure at the level of fibril bundles. In addition liming also contributes to swelling of the pelt, hydrolysis

of peptide bonds, hydrolysis of amide side chains, hydrolysis of guanidine side chains (Covington, 2011), removal of dermatan sulfate (Alexander, 1988) and hydrolysis of fat.

d) Deliming, bating and pickling

The main reason for deliming, bating and pickling is preparation of the pelts chemically and physically for tanning. Deliming is the partial removal of alkali and adjustment of pH to 8.2-8.3 in readiness for bating. Bating is an enzymatic action that degrades unwanted non-structural proteins including hair roots that can be easily scudded away using a slicker followed by washing with plenty of cold running water. Pickling is the adjustment of pH of the pelt using mostly sulphuric acid to the level desired for either chrome or vegetable tanning or hide preservation (Thorstensen, 1993).

2.10. Analysis of tannins

There are three groups of methods for the analysis of tannins: precipitation of proteins or alkaloids, reaction with phenolic rings and depolymerization (Scalbert, 1992). However, modern quantitative and qualitative analysis of natural compounds is by use of chromatographic methods.

2.10.1.Ferric chloride test

The presence of tannins in plant extracts can be done using ferric chloride test (Yisa, 2009). On addition of few drops of ferric chloride solution hydrolysable tannins (pyrogallols) have a tendency of producing blue colours while green colour is observed in the same reaction with condensed tannins (catechols) (Yisa, 2009). The two types of tannins are distinguished since a colloid containing condensed tannins readily forms a red colour when some drops of aqueous

potassium hydroxide are added but no colour change is observed in the case of hydrolysable tannins (Thorstensen, 1993).

2.10.2. Hide powder method

The hide powder method is used globally for analysis of vegetable tannin content of materials used for leather tanning (Zheng *et al.,* 1991). This method is based on shaking and filtering where materials from the extract are absorbed by the hide protein. It is not based on chemical analysis of the true tannin molecule. Another closely related method that has been used to quantify tannins for the manufacture of particle boards is known as Stiansny′s method (Xiangming *et al.,* 2007).

2.10.3. Reaction with phenolic rings

Precipitation reactions and quantitative determination by the methods of Lowenthal- Procter and Deijis have been used based on formalin and hydrochloric acid (Eskin and Shahidi, 2012). Calorimetric methods such as Neubauer- Lowenthal method which uses potassium manganate (vii) as an oxidizing agent and indigo sulphate (Kim *et al.,* 2012) have also been used. This method lacks convenience since it is extremely difficult to obtain pure tannin hence determining the amount of tannins becomes difficult. However, this method has since been modified and used in the quantification of tannins in wine (Feldmans method) where calcium hypochlorite is used instead of potassium manganate (vii) and indigo sulphate (Romero-Gonzales and Aymard, 2015).

2.10.4 Tannin analysis using polymers

Resio (1996) studied the use of polymers derived from polyvinyl pyrrolidine that are able to fix polyphenols in an acid environment. Solid phase extraction was adapted followed by elution of

solutions containing *chestnut* and *quebracho* tannins as well as syntan based on dihydroxy diphenylsulphone. The non-tannins were found to have eluted through the polymer and the methods seemed to be quick and repeatable.

2.10.5. Chromatographic methods of tannin analysis

Chromatography is an important tool in separation and identification of natural chemical compounds including hydrolysable tannins and other plant polyphenols (Tuominen and Sundmen, 2013) . This method is based on the adsorptivities of compounds of interest on the stationary phase and their degree of polarity which will subsequently determine elution in the mobile phase and resolution on the readout (Levens, 2015). The analyte peaks are usually compared with a known standard and the concentration of the analyte is calculated using either its peak height or peak area in relation to the standard peaks and concentration.

Chromatographic methods for tannin qualitative and quantitative analysis include high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC) and gas chromatography (GC) (Zhang and Bao, 2014).

2.11. Leather testing and analysis

Leather by nature is an inconsistent material (Thorstensen, 1993) hence independent leather testing and analysis becomes important before shipping to manufacturers or cutting (Sterlacci, 2010). However, for instance in the United States there are no consensus industry specifications for garment leather and product criteria are generally established between buyers and sellers through evaluation and acceptance of initial production samples (Sterlacci, 2010).

According to Thorstensen, (1993), leather tests can be divided into the following main classes: physical/ mechanical tests of strength which include: stitch and tear resistance, tensile strength, shrinkage temperature, leather thickness and distension tests. The second class consists of moisture related tests which include: water absorption, water vapour transmission and resistance to water penetration and lastly chemical analysis that features the following tests: oil content, tannin content, hide substance, ash content, moisture content and pH among others.

Organisations that participate in leather testing and analysis are: American Society for Testing Materials (ASTM), American Leather Chemists Association (ALCA), International Standards Organization(ISO), Society of Leather Technologists and Chemists and International Union of Leather Technologists and Chemists Societies (IULTCS) (Thorstensen, 1993).

Leather that is subjected to testing must undergo the standard sampling procedure such that the samples are representative of the test lot. Samples should be cut from an agreed location and direction on the hide and the number of samples be sufficient to give statistically meaningful data (Thorstensen,1993).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study Area

The study was done in Gesima Ward of Kitutu Masaba constituency Nyamira County. Gesima ward is located about 300 km west of Nairobi city and about 150 km east of Kisumu city. The Ward lies to the North east of Keroka town and to the south of Kebirigo town. Gesima ward is sandwiched by Rigoma and Mekenene wards from west and east respectively and has five locations: Gesima, Riamoni, Esani, Nyamakoroto and Mochenwa. The Ward covers an area of approximately 65.69 km^2 and has a population of 33,189 people. The population density is 505 people per square km (IEBC, 2012). The major economic activity in Gesima ward is subsistence farming characterized by diminishing demarcated plots and small scale tea farming dominates in the area with tea being the only cash crop.

The study was done in Sungututa, Nyatieno and Rioga sub-locations of Gesima, Riamoni and Esani locations respectively. The vegetation communities of *Plectranthus barbatus* form releve's mainly on the hills and slopes where the vegetation is less disturbed. However, *Plectranthus barbatus* is conspicuously part of the relic vegetation along roads and land demarcations. The Ward has one existing tannery near Gesima trading centre which uses *Mimosa* in its tanning operations.

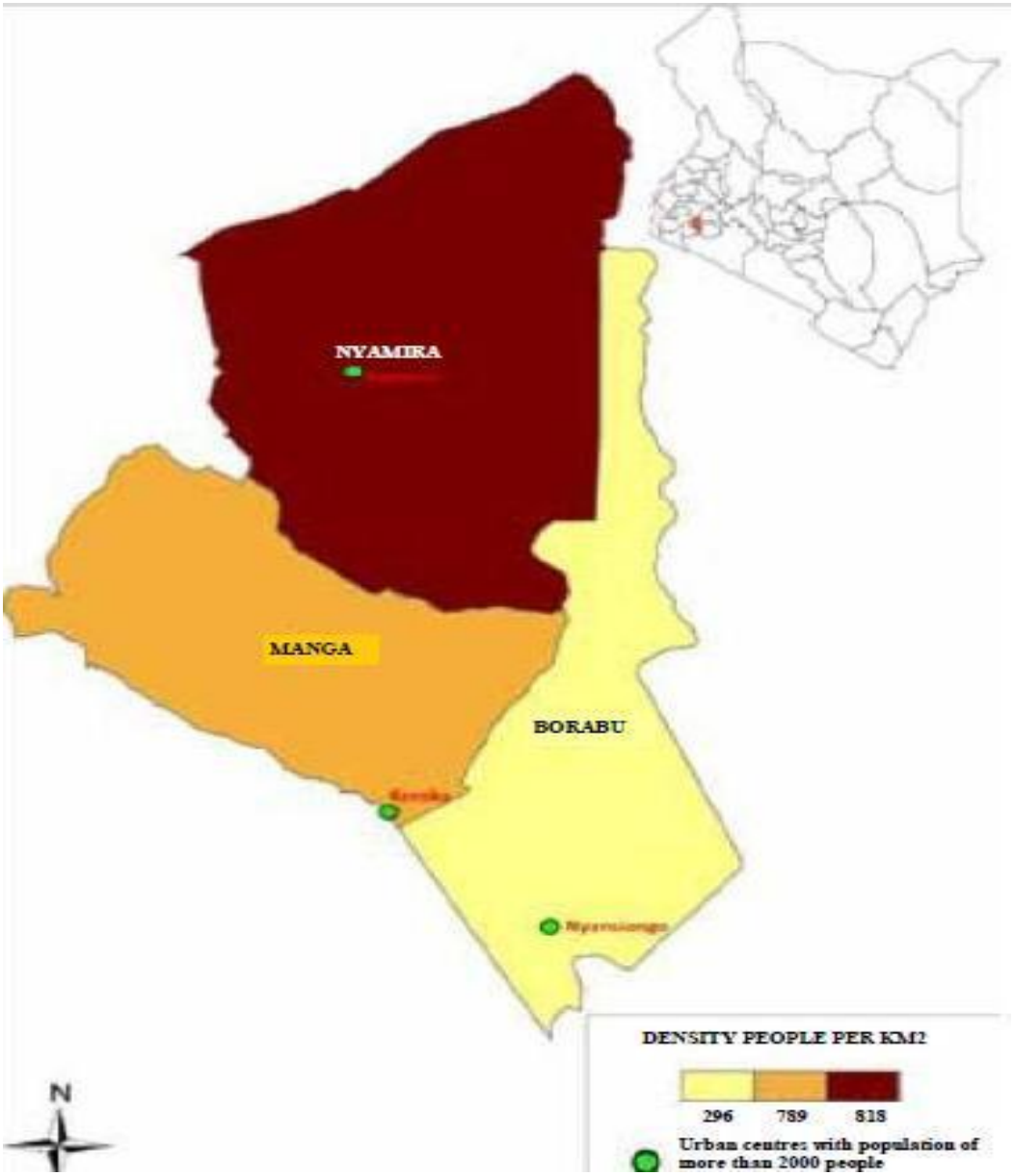

Fig 3.1: Map of Nyamira County (IEBC, 2012)

3.2. Study Design

This experimental study adopted a randomized block design and each village formed a block and sampling was done in three villages representing three locations. The study employed multistage sampling method where three locations were selected randomly from the five locations.

In each of the selected locations there are two sub-locations where one was chosen randomly. Each of the selected sub-locations has 11 villages where one village was randomly selected in each case from which the samples were collected. Randomization was done by picking pieces of mixed folded papers containing names of the areas under consideration.

3.3. Plant identification

The plant was positively identified by the Kenya National Museum as *Plectranthus barbatus Andrews* and successfully compared with various herbaria available at the institute.

3.4. Sample collection

The samples collected comprised of apical leaves and stems of *Plectranthus barbatus* shrub. These were picked from several shrubs to form a representative sample. Care was taken such that there was no disturbance of plant communities at the collection site or danger of spread of weeds. In addition accurate records for all plant collections were kept. The samples were then cut into small pieces and separately kept in sealed clear plastic bags thereafter transported to the leather testing laboratory at Kenya Industrial Research and Development Institute (KIRDI) Nairobi for processing and analysis. The samples were labeled 1A, 2A and 3A for leaves and 1B, 2B and 3B for stems indicating the three sampling blocks respectively.

3.5. Laboratory Procedures

3.5.1. Phytochemical screening for active ingredients

A few leaves and stems that had been chopped earlier from each sample were selected and boiled in 500 ml of distilled water for 30 min at 100°C. The resultant extract solutions were allowed to cool and then transferred to six separate universal bottles and labeled 1A, 2A, 3A, 1B, 2B and 3B respectively. Each sample was tested for: tannins and flavonoids, using ferric chloride test, ferric chloride and lead acetate tests respectively according to (Yisa, 2009, Payne *et al.*, 2013, Sermakkani and Thangapandian, 2010).

3.5.1.1. Tests for tannins

i) Ferric chloride test: each sample extract was mixed with 1% ferric chloride solution and development of blue black and/or green colours were recorded as positive for tannins (Yisa, 2009).

3.5.1.2. Tests for flavonoids

i) Ferric chloride test: Alcoholic extracts of each sample were mixed with few drops of neutral ferric chloride solution and development of green colour was recorded as positive for flavonoids (Yisa, 2009).

ii) Lead acetate tests: Alcoholic solutions of the sample extracts were mixed with few drops of 10% lead acetate and development of a yellow precipitate indicated presence of flavonoids (Yisa, 2009).

3.5.1.3. Tests for condensed and hydrolysable tannins

Each sample extract was mixed with few drops of aqueous potassium hydroxide and rapid development of red colour confirmed the presence of condensed tannins and where there was no observable change the presence of hydrolysable tannins was concluded (Thorstensen, 1993).

3.5.2.Determination of tannin content

Determination of tannin content was done using hide powder method as outlined in the SLC 116 official method of analysis (SLTC, 2001). Subtraction of non-tannins from soluble solids gives total tannin content. The tannin content is expressed as a percentage of the soluble solids.

3.5.2.1. Sample drying and grinding

Preparation of the samples for analysis was done according to the official method SLC 112 (SLTC, 2001) where each sample was shredded further and dried separately at room temperature (22-25°C) for a period of up to one month until there was insignificant change in weight. Each sample was then ground separately using a suitable mill until it passed through a BS sieve, Mesh 12 (BS No 410/1943).The ground material was further separated by passing through a BS sieve, Mesh 25 (BS 210/1943). The weight of each sample was determined using an electronic balance (Kern & John ABJ 320-4) and recorded and the samples were put in plastic bags which were sealed and labeled respectively. Plates 3.1a and 3.1b show dry ground leaves and stems of *Plectranthus barbatus* respectively.

Plate 3.1a: *Plectranthus barbatus* dry ground leaves

Plate 3.1b: *Plectranthus barbatus* dry ground stems

3.5.2.2. Preparation of extracts

This process was done using a proctor extractor as outlined in official method SLC 112 (SLTC, 2001). The ground sample material was not soaked overnight because it was suspected to be of hydrolysable type and hence could have yielded deposits of ellagic and chebulinic acid. Twenty grams of the ground samples were weighed accurately using an electronic balance (Kern & John ABJ 320-4) and extraction was began at 50°C and after one litre was extracted at this temperature the extraction was completed at near boiling point. The infusion was then cooled by immersing the conical flask in a sink containing water at 25°C and the temperature maintained throughout the cooling process by agitating the flask from time to time. The cooling process was continued until the contents in the flask had reached 25°C then made up to the mark at the same temperature.

3.5.2.3. Determination of total solids

This procedure was carried out using official method SLC 114 (SLTC, 2001).The weight of labeled empty beakers was determined and recorded before they were used to separately evaporate to dryness on a steam bath 50 ml of uniformly turbid tannin infusions prepared in duplicates from 20 g of dry ground leaves and stems of *Plectranthus barbatus*. The residues were then dried in an oven (Gallen kamp Oven BS –Size Two) at 100°C to constant weight. The weight was considered constant when two weightings at an interval of 1 hr did not differ by more than 2 mg after the residues were cooled in a dessicator (Kartell-Italy) for 20 min.

Calculation:

$$\text{Total solid, percent by weight} = \frac{W2}{W1} * 100$$

Where:

W1= Weight of material taken for test,

W2= Weight in g of material left after drying.

3.5.2.4. Determination of total soluble

150ml separate tannin infusions of leaves and stems of *Plectranthus barbatus* were put in 250 ml glass beakers and 1 g of kaolin added to each then mixed and left to stand for 15 min as indicated in SLC 115 (SLTC, 2001). The mixture was then stirred and then poured through a fluted filter paper (Whatman No 11) in a filter funnel supported by a conical flask. The filtrate was returned to the filter paper when approximately 25 ml had been collected and the process was repeated for 1 hr and all kaolin was transferred to the filter paper. The filter paper with kaolin was refilled with the filtrate which was then collected in a fresh conical flask while care was taken not to disturb the kaolin. The filter papers were kept full and the funnels together with collecting flasks covered throughout the period of filtration. Fifty millitres (50 ml) of the separate filtrates was then pipetted into labeled beakers in duplicates for evaporation. The dried residues were then weighed and the process of drying and weighing was repeated until constant weight was obtained.

Calculation:

Total Solubles, Percent by weight = $\frac{W_2}{W_1} * \frac{V_1}{V_2} * 100$

Where:

W_2 = Weight in g of the residue left after drying,

W_1= Weight in g of tanning material taken,

V_1= Volume in ml made up originally,

V_2= Volume in ml of test solution taken.

3.5.2.5. Preparation of chrome alum solution

Thirty grams (30 g) of chrome potassium sulphate (analytical grade) was dissolved in distilled water at room temperature and the solution made up to one litre according to SLC 110.8 (SLTC, 2001).

3.5.2.6. Preparation of chromed hide powder

For every analysis 6.25 g of dry hide powder was digested with 62.5 ml of distilled water for 1 hr as described in SLC 116 (SLTC, 2001). One ml of chrome alum solution was then added for each gram of dry hide powder taken and the mixture then shaken frequently for 2 hrs using a Stuart Flask Shaker (Stuart Scientific CO LTD- GB) before it was left to stand overnight.

The following morning the chromed hide powder was transferred to a clean linen cloth then it was drained and squeezed thoroughly. The cloth containing the hide powder was then placed into a 250 ml glass beaker and the cloth bag opened and 95 ml of distilled water poured onto the

chromed powder. The contents were then mixed thoroughly and digested for 15min, after which the cloth with the powder was lifted and the powder was immediately drained and squeezed such that it contained approximately 75% moisture.

The powder was further digested three times the same way with distilled water then the caked chromed powder was broken and mixed uniformly until lumps were eliminated before it was weighed with an electronic balance (Kern & John ABJ 320-4).

3.5.2.7. Preparation of gelatin salt reagent

One gram (1g) of photographic grade gelatin and 10 g of pure sodium chloride were dissolved in 100ml distilled water at 27°C and pH adjusted to 4.7 according to SLC 110.10 (SLTC, 2001).

3.5.2.8. Determination of non-tannins

The quantity of wet hide powder that contained 6.25 g dry hide powder was weighed and immediately added to100 ml of unfiltered tannin infusion plus 20 ml of distilled water present in a 300 ml wide mouth flask and this was according to the test method SLC 116 (SLTC, 2001).

The wide mouth flask was then stoppered tightly and shaken vigorously first by hand for 15sec before it was transferred to a mechanical rotary shaker (Shaking Machine for Leather Analysis-Satra Test Equipment, STM 145) and shaken for exactly 10 min at 60 rev/min. The powder and the solution were then poured on a dry linen filter cloth supported by a funnel then drained and squeezed by hand. One gram of kaolin was then added to the filtrate and the contents then poured into a single 15 cm pleated filter paper (Whatman No.11) by returning the filtrate repeatedly until it was clear.

The funnel and the collecting conical flask were kept covered during filtration. The filtrate was tested with gelatin salt and 10ml did not show turbidity with 2 drops of the reagent. Fifty (50 ml) of the filtrate was then evaporated in a tarred 250 ml glass beaker and the residue dried in an air oven (Gallen Kamp Oven BS – Size Two) at 100°C overnight. The residue was then cooled in a dessicator for 20 min and then weighed until constant weight was obtained. The tests were done in duplicates for every sample. The weight of the residue was multiplied by 1.2 to correct for 20 ml of water introduced to wet hide powder.

Calculation:

$$\text{Non-tannins, Percent by weight} = \frac{W_2}{W_1} * \frac{V_1}{V_2} * 100 * 1.2$$

Where:

W_2= Weight in g of the residue left after drying,

V_1= Volume in ml made up originally,

W_1= Weight in g of the material taken,

V_2 = Volume in ml of the test solution taken.

3.5.2.9. Determination of tannins

The tannins were determined in duplicates by the difference between the percentages of the total soluble and the non-tannins according to SLC 117 (SLTC, 2001). Calculations were done as follows:

Tannins percent by weight= X-Y

Where:

X= Total solubles, percent by weight,

Y= Non-tannins, percent by weight.

3.5.2.10. Determination of insolubles

The insolubles were determined by the difference between 100% and the sum of the percentages of moisture and total solubles according to SLC 118 (SLTC, 2001).

Calculation:

Insolubles percent by weight= 100 - (X+Y)

Where:

X= Moisture content,

Y= Total soluble percent by weight.

3.5.3. Determination of moisture

Three grams (3g) of finely ground sample was accurately weighed in a tarred 250 ml glass beaker and dried at 100°C in an oven (Gallen Kamp Oven BS –Size Two) for 3 hrs. The sample was then cooled in a dessicator (Kartell- Italy) for 20 min and then weighed accurately. The process of drying was repeated until two weighings at an interval of 1 hr did not differ by more than 2 mg as outlined in official test method SLC 113 (SLTC, 2001).

Calculation:

Moisture, percent by weight= $\frac{W_1 - W_2}{W_1} * 100$

Where:

W_2= Weight in g of the material taken for the test,

W_1= Weight in g of residue left after drying.

3.5.4. Determination of pH

The pH of prepared sample solutions was determined first by adjusting their relative densities to 1.05g/ml at room temperature according to official test method SLC 120 (SLTC, 2001) and then the pH measured using a calibrated pH meter (Metler Toledo 86505). The conductivity of the solutions was also measured using a conductivity meter (JENWAY 4071).

3.6. Evaluation of *Plectranthus barbatus* for suitability as a tanning agent

This was done by tanning nine goat skins using *Plectranthus barbatus* extracts which were prepared by soaking the ground plant material to yield the tanning liquor. Tanning was also done with *Mimosa* for the purpose of comparison.

3.6.1. Pre-tanning and tanning

Leather tanning was done using water extract of *Plectranthus barbatus.* Bated pelts from wet salted goat skins were tanned under controlled conditions of temperature, pH and concentration of the tan liquor (Appendix 3).

Four pairs of bated pelts: S1, S2, S3 and S4 were subjected to the same tanning procedure and the level of penetration of *Plectranthus barbatus* was checked by cutting a small piece of the skin from the thickest part i.e. the neck region and assessing the uniformity of the extract colour

through the pelt cross-section. The following process steps were followed in the beamhouse and tan yard operations: Dirt soak, main soak, liming, unhairing, scudding, fleshing, deliming, bating, tanning, fatliquoring and dyeing. This procedure was repeated twice.

a) Dirt soak and main soak.

The wet salted goat skins weighing 15 kg sourced from Dagoretti slaughter house in Nairobi which were contaminated with soil, sand, blood and manure and therefore were rid of this dirt by washing them in 200% float of water at 25˚C followed by draining. The float was then changed and another 200% added followed by eventual draining. Washing was done in stationary water in a trough to avoid any mechanical injury on the grain due to cracking.

Washing of the skins was followed by pre-soak that involved addition of 1% detergent, 1% wetting agent in 200% float. This operation was done in the drum (plate 3.2) at 6 rev/min for 30 min. The float was then drained and main soak carried out in fresh 200% float. This process was aided by addition of 1% soaking enzyme, 0.5% sodium sulphide, 0.5% lime, 2% bactericide, 2% liquid detergent. The drum was run for 1hr at 6rev/min and the skins were left to stand overnight (16 hrs) at 20˚C.

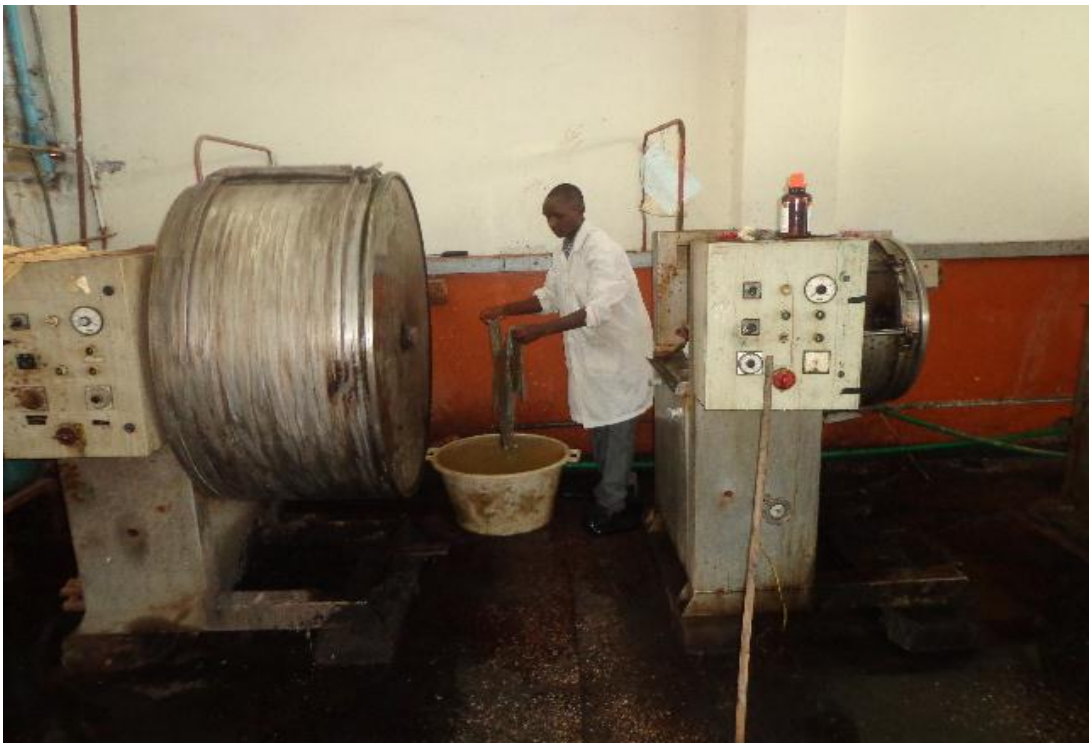

Plate 3.2: Experimental tanning drums at KIRDI

b) Liming and unhairing

This process step was done by normal liming and the following additions were done in 150% float in respect to the weight of the skins: 2% sodium sulphide and 1.5% lime at 20˚C. The drum was run for 6 hrs at 6 rev/min and the skins were left to stand overnight for 16 hrs.

c) Fleshing and scudding

Scudding was done with a slicker to remove hair roots and the epidermis together with melanin as shown in plate 3.3. The pelts were then washed with plenty of clean running water at 20˚C before they were fleshed with a fleshing knife on a beam as shown in plate 3.4. The weight of the

fleshed pelts was determined to be 13.5 kg and this was used for calculations in subsequent processes.

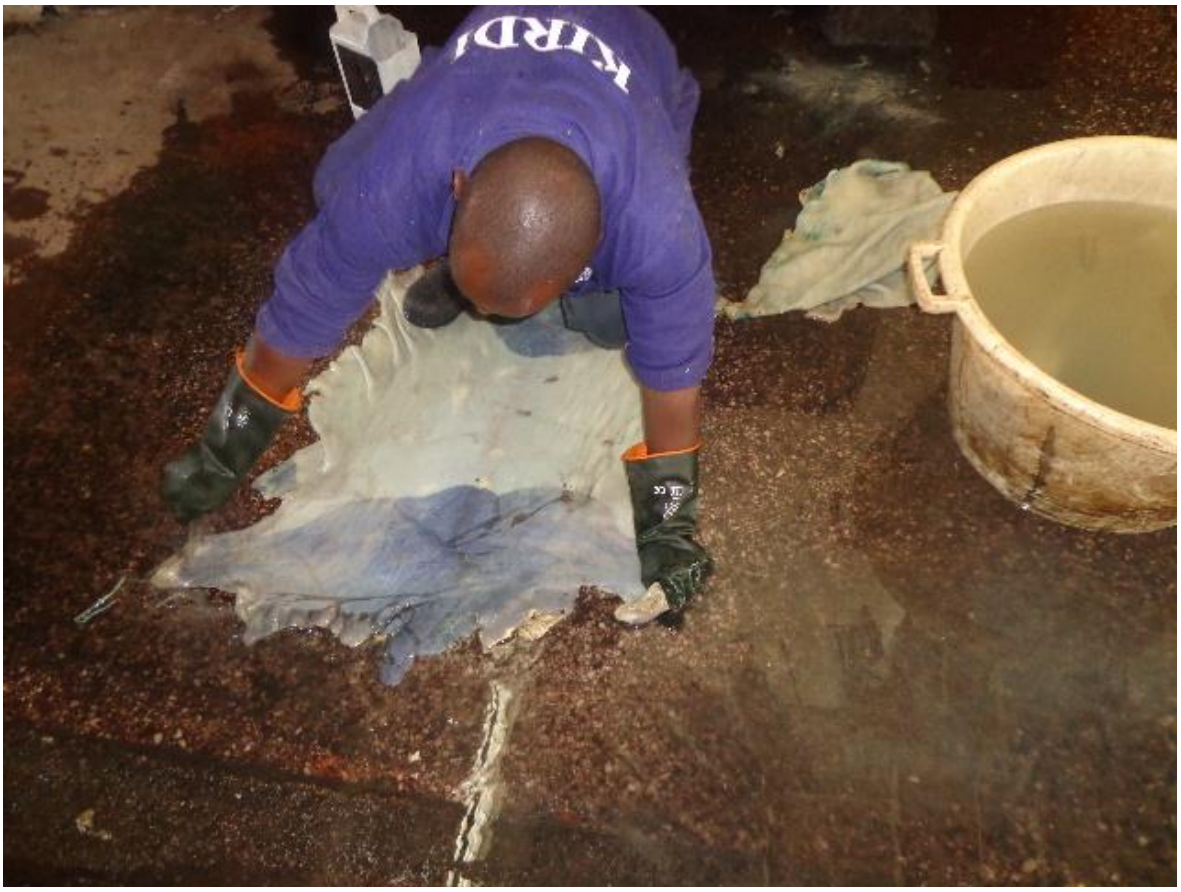

Plate 3.3: Scudding operation to remove hair roots and non- structural proteins.

Plate 3.4: Fleshing operation to remove excess fat.

d) Deliming

Deliming was carried out to increase the pH of the pelt from 12 to 8.3 to make it suitable for the subsequent bating operation. This was done by adding 2% ammonium sulphate, 1% sodium metabisulphite in 200% float. The drum was run for 30 min at 6 rev/min followed by washing and draining. The pH was checked by cutting the pelt cross-section at its thickest region i.e. the neck area and putting three drops of phenolphthalein indicator. A clear colour was observed which confirmed that the pH of 8.3 had been attained hence deliming was complete. This process step was also followed by net outflow of dissolved components from the pelt that led to further opening up of collagen fibres in preparation for tanning mechanism.

e) Bating

Bating was done in 100% float with 1% microbate (Microzyme P) at a temperature of between 37ºC- 38ºC. The drum was run for 1 hr at 6 rev/min before the pelts were drained and washed with cold running water.

f) Vegetable tanning

To each vegetable liquor float of 150%, 5% ground vegetable tanning materials that had been premixed with warm water at a ratio of 2:1 were added to respective drums for *Mimosa, Plectranthus barbatus* leaves extract, *Plectranthus barbatus* leaves mixed with stems extract and *Plectranthus barbatus* stems extract. 0.5 % bactericide (Mirecide) was added and drums were run for six hours at 16 rev/min after two weighed bated pelts had been put into each of the tanning liquors. The level of penetration was then checked including the pH which was at 6 and the pelts were left to stand in the liquors overnight for 14 hours. This was followed by respective further additions of 3% tanning materials and the drums were run for 8 hours at 16 rev/min.

Penetration of the tannins was again checked by cutting a piece from the neck region and then the pelts were left to stand in the liquor for two days. Uniform penetration through the pelt cross section was checked and for the pelts in liquors containing *Mimosa, Plectranthus barbatus* leaves and *Plectranthus barbatus* leaves mixed with stems, uniform penetration had been achieved hence they were fixed with 1% formic acid for 20 min then washed before they were horsed up to age for one day as shown in plate 3.5.

Plate 3.5: Horsing up of *Plectranthus barbatus* tanned leather to allow aging.

For the pelts in liquor with *Plectranthus barbatus* stems further additions of the tanning material were made until uniform penetration was achieved before the tannins were fixed with 1% formic acid for 20 minutes thereafter the tanned leathers were washed and horsed up for one day to age.

g) Hang drying

The tanned leathers were hang dried as shown in plate 3.6 on an overhead drier for one day.

Plate 3.6: Hang drying of *Mimosa* tanned leathers

h) Fatliquoring and toggling

The dried leathers were fatliquored in separate drums with 4% vegetable oil fatliquor in100% float at 50˚C. The drums were run for 1 hour at 16 rev/min. Before exhaustion of the fatliquor, the float was checked. The fatliquor was then fixed with 1% formic acid for 20 min and when pH indicator paper turned yellow then a pH=3.5 had been attained hence fixation was complete. The leathers were later washed and then toggled for two days as shown below in plate 3.7

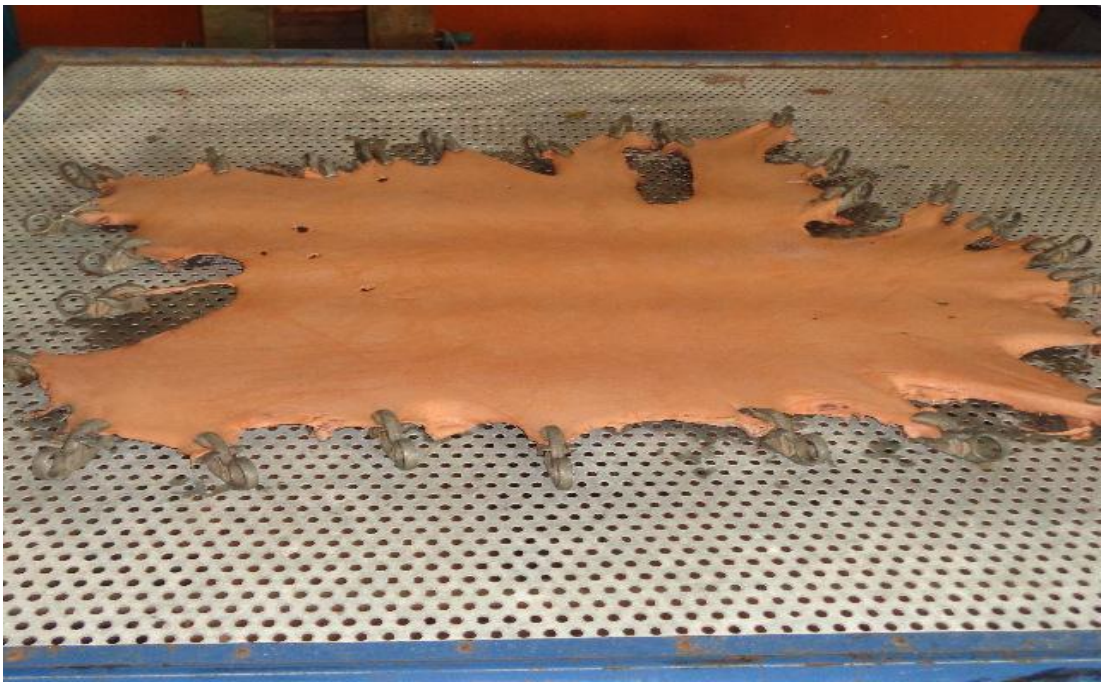

Plate 3.7: Toggled *Mimosa* tanned leather to increase area yield.

3.6.2. Physical testing of leather

The following properties were analyzed in the physical testing Laboratory at KIRDI Nairobi:-

a) Shrinkage temperature determination/ hydrothermal stability

The shrinkage temperature of the tanned skins was measured according to IUP/16 physical test method (IULTCS, 2001) using SATRA STD 114 instrument. Strips of leather 50 mm * 2 mm were cut from the *Plectranthus barbatus* and *Mimosa* tanned leather . The samples were cut along and across the backbone. Holes were punched at the ends of the leather to allow the samples to be held vertically in the test chamber filled with water and a small weight was attached to the lower end. The position of the lower end was indicated by an adjustable marker outside the tube to help note when the shrinkage occurred. The instrument was then closed and

water heated at approximately 4^{o}C/min by applying the external heat source to the boiler components. The temperature at which the leather started to shrink was taken as the shrinkage temperature.

a) Flexing endurance

The measurement of flex resistance was carried out by flexometer method as described by IUP/20 physical test method (IULTCS, 2001). On a Bally Flexometer model 2184. Leather samples of dimension 70 mm by 60 mm were folded and fixed to the jaws of the instrument in such a manner that the grain side remained outside with fold on the sample. The leather samples were thus flexed in the folded state up to 100,000 cycles and they were observed periodically for any signs of crack on the grain surface of the leather.

Sampling and preparation for measurement of tensile strength and tear strength.

Six pieces of the tanned hide were cut by applying the cut knife to the grain surface. Three were cut with the longer edge parallel to the backbone and three with the longer edge perpendicular to the backbone. The pieces were then conditioned and thickness of each piece was measured. The upper end of the metal test piece holder was gripped in the upper jaws of the tensile testing machine. The perforated end of the test piece was placed between the arms of the test piece holder and the mandrel passed through the holes in the test piece holder and the slit in the test piece. The free end of the test piece was then clamped in the lower jaws of the tensile testing machine.

The tensile test machine was then run until the piece was torn apart and the force to initiate tearing was recorded in Newton.

c) Measurement of tear strength

The tearing strength of tanned leather samples was measured by Instron 1011 according to the official test method IUP/8 (IULTCS, 2001). Leather samples were cut as a rectangle 50 mm long and 25 mm wide using a press knife which cut out the samples and slotted them in one operation (Template machine) parallel and perpendicular to each position.

The instrument was ran at uniform speed of separation of the jaws of 100 mm per minute and the readings of load fall in that part of the scale which had been shown by calibration had to be correct within 1%. The machine was run until the specimen was torn apart and the highest load reached during tearing was recorded as the tearing load in Newton (N).

d) Tensile strength

The measurements of tensile strength, elongation at break and maximum force are necessary in determining the fitness of the material for end-use applications and were carried out according to the physical test method IUP/6 (IULTCS, 2001) using test instrument, Instron 1011. The samples were cut parallel and perpendicular to the backbone using a dumbbell shaped press knife. Each sample was measured in triplicate. The jaw of the tensile instrument was set 50 mm apart, and then the samples were clamped in the jaws, so that the edges of the jaws were along the mid line. The machine was run until the samples broke and the highest load reached was taken as the breaking load and recorded.

Tensile strength =maximum breaking load (Newton/cross sectional area(mm^2))

e) Ball burst test (lastometer)

Measurement of strength of grain by the ball burst test (Lastometer) is important in determining the suitability of leather for a particular use such as shoe manufacture and was done according to the physical test method IUP/9 (IULTCS, 2001) using Lastometer STD 104 (Satra Test Equipment). A disc shaped specimen of the leather was firmly held with the grain side up between the clamping rings, with the spherical tip of the steel rod just touching the flesh surface. The specimen was moved downward against the rod, distending the grain of the leather immediately above the rod, while the surface was watched for incipient cracking and bursting. The force and three distention values at the points which the grain of samples cracked and burst were read off gauges on the instrument and recorded.

3.7. Data Analysis and Statistics

Data was entered in excel and then imported to INSTAT+ V3.36 and STATA 12 for statistical analysis. It was arranged and summarized using descriptive statistical method for categorical and numerical data and charts and tables were used to present data. Two-way and One-way ANOVA statistical tests were used to analyze the difference between the tannin content in leaves and stems and location effect on the content of tannins in *Plectranthus barbatus* together with physical properties of tanned leathers (Significance level; $\alpha < 0.05$).

CHAPTER FOUR: RESULTS

4.1. Phytochemical screening of *Plectranthus barbatus*

The phytochemistry conducted on ground *Plectranthus barbatus* leaves and stems showed that both leaves and stems contained hydrolysable tannins by giving blue- black precipitate when reacted with ferric chloride and the samples did not develop red colour on addition of a few drops of aqueous Potassium hydroxide. The results are summarized in table 4.1.

Table 4.1: Phytochemical screening of *Plectranthus barbatus* leaves and stems.

Phytochemical	**Test method**	**Phytochemical level**		
		Leaves of *Plectranthus barbatus*	Stems of *Plectranthus barbatus*	*Mimosa* (+ve Control)
Tannins	Ferric chloride test	+++	++	+++
Flavonoids	a)Ferric chloride test	++	+	-
	b)Lead acetate test	++	+	-
Saponins	Froth test	++	++	++
Hydrolysable tannins	Potassium hydroxide (aq)	+++	++	-
Condensed tannins	Potassium hydroxide (aq)	-	-	++

Key

+++ Present in high amounts.

++ Present in moderate amounts.

+ Present in small amounts.

- Absent

4.2. Analysis of the level of tannins in *Plectrantus barbatus* using the hide powder method

The average concentration of tannins in *Plectranthus barbatus* was 20% in leaves, 8 % in stems and 10% in the mixture of leaves and stems and calculated tanning strengths of 1.7, 0.7 and 1.3 respectively. The *Plectranthus barbatus* stems collected from Esani location had the highest concentration of tannins at 9% while those from Riamoni and Gesima locations had similar concentration of 8% and their tanning strengths were 0.8 and 0.7 respectively. Comparatively, the percentage of tannins tested in *Mimosa* which was used as a positive control was 66% with a tanning strength of 2.8.

Table 4.2 shows the concentration of tannins and non-tannins of *Plectranthus barbatus* crude extracts from Gesima, Riamoni and Esani locations and their physicochemical characteristics in comparison with conventional *Mimosa*.

Table 4.2: Concentration and characteristics of tannins and non-tannins of *Plectranthus barbatus* crude extracts from Gesima, Riamoni and Esani locations.

	Concentration level/Characteristics												
Test Samples	*Plectranthus barbatus* Leaves				*Plectranthus barbatus* Stems				*Plectranthus barbatus* Leaves + Stems				*Mimosa* (+ve Control)
Location	G	E	R	A	G	E	R	A	G	E	R	A	
% Tannins	20	21	19	20	8	9	8	8	10	11	9	10	66
%Non- tannins	12	13	11	12	12	12	12	12	8	9	8	8	24
% Moisture	11	10	7	11	7	10	10	7	9	8	9	9	9
%Total solids	89	90	93	89	93	90	90	93	91	92	91	91	91
%Tannins in total solubles	63	62	63	63	53	43	40	53	56	55	53	56	73
Tanning strength	1.7	1.6	1.7	1.7	0.7	0.8	0.7	0.7	1.3	1.2	1.1	1.3	2.8
Av pH	6.0	6.0	6.0	6.0	5.8	5.8	5.8	5.8	6.2	6.2	6.2	6.2	4.6
Av conductivity (Mv)	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	1.0	1.0	1.0	1.0	0.5

Key:

G= Gesima location,

E= Esani location,

R= Riamoni location,

A= Average.

4.3: Tanning of goat skins

4.3.1: The colour of tanned leathers

Tanned leathers showed different physical characteristics and most notably the colour of wet leathers which changed upon drying. *Plectranthus barbatus* leaves extract and *plectranthus barbatus* leaves mixed with stems extract tanned leathers were yellow-brown when wet but turned brown after drying. *Plectranthus barbatus* stems extract tanned leathers showed a dark brown colour when wet but then became black when dry while those tanned with *Mimosa* were reddish when wet but a shift of colour to gray-brown was observed on drying.

The yellow-brown colour of wet *Plectranthus barbatus* leaves extract tanned leathers was probably influenced by the yellow colour of non-tannins (plate 4.1b) but when they lost water, the observed brown colour was similar to that of tannins as shown in plate 4.1a. The observed colour behavior of tanned leathers is shown respectively in plates 4.2a, 4.2b, 4.3a, 4.3b, 4.4a, 4.4b, 4.5a and 4.5b.

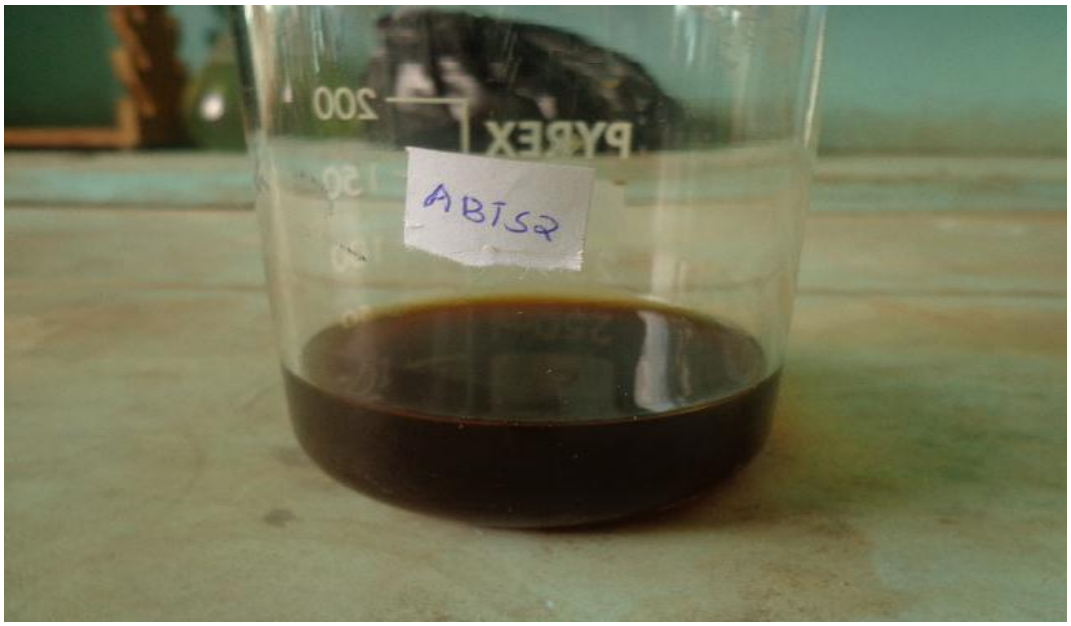

Plate 4.1a: Brown colour of tannins in *Plectranthus barbatus* leaves infusion

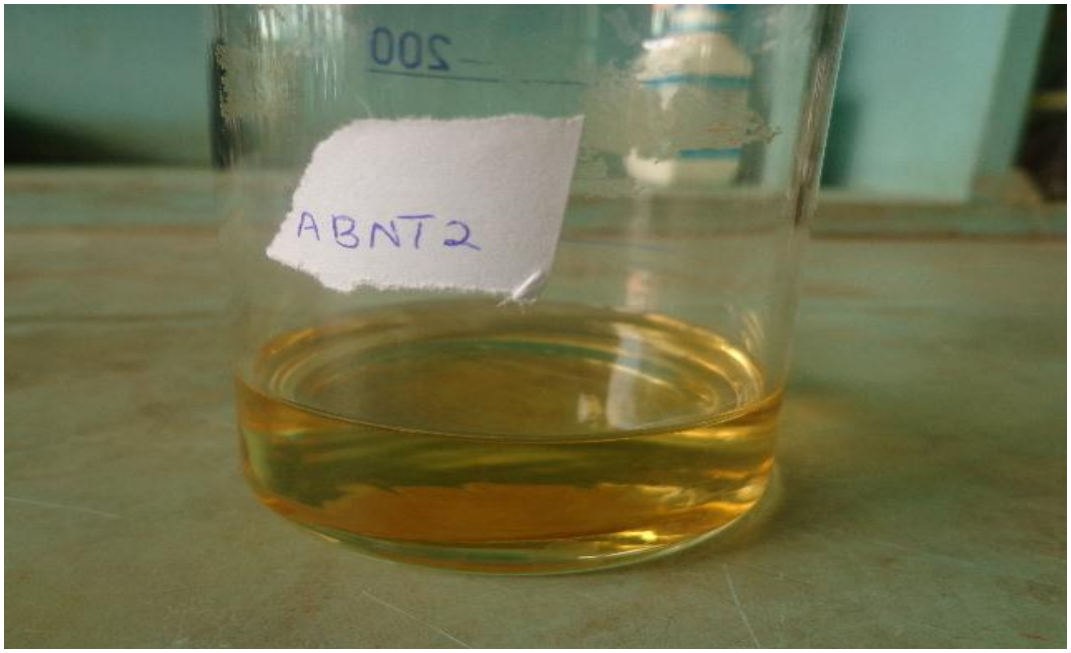

Plate 4.1b:Yellow colour of non-tannins in *Plectranthus barbatus* leaves infusion

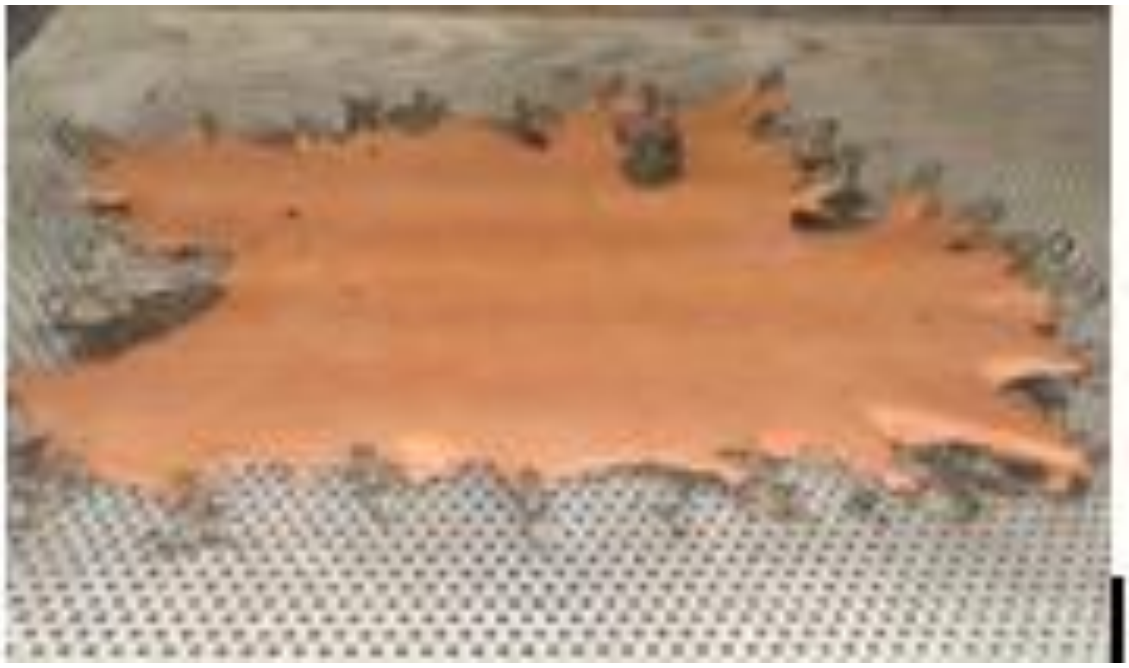

Plate 4.2a:Reddish wet *Mimosa* tanned leather

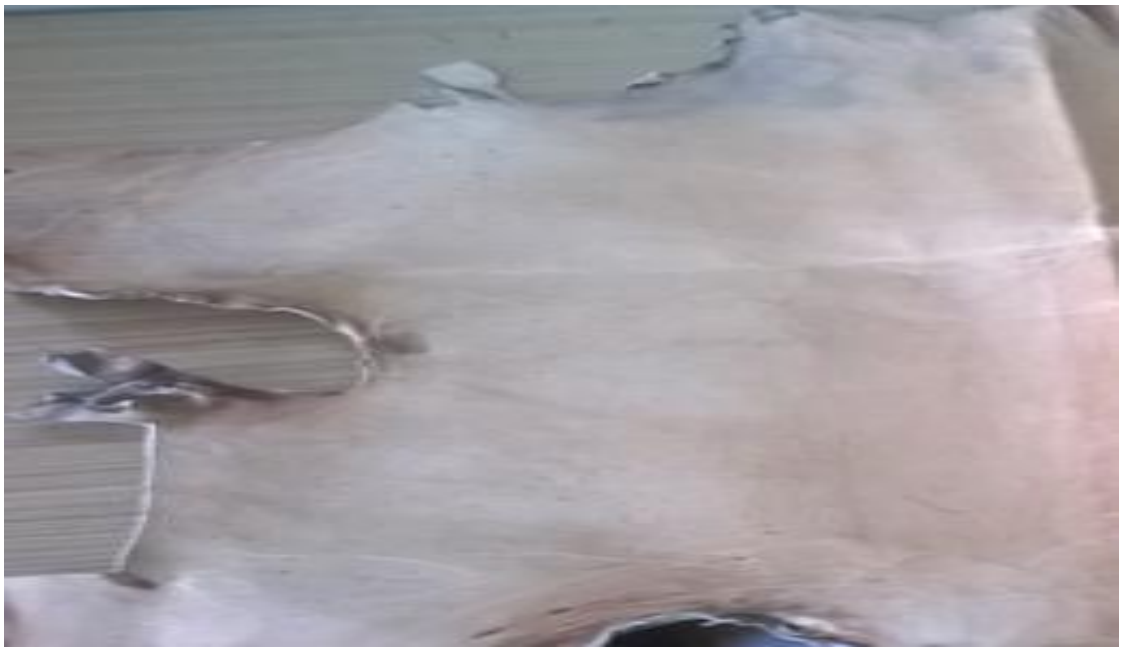

Plate 4.2b: Gray-brown dried *Mimosa* tanned leather.

Plate 4.3a: Yellow-brown wet *Plectranthus barbatus* leaves extract tanned leather

Plate 4.3b: Brown dried *Plectranthus barbatus* leaves extract tanned leather

Plate 4.4a: Yellow-brown wet *Plectranthus barbatus* Leaves+stems extract tanned leather

Plate 4.4b: Brown dried *Plectranthus barbatus* leaves+stems extract tanned leather

Plate 4.5a: Dark brown wet *Plectranthus barbatus* stems extract tanned leather

Plate 4.5b: Black dried *Plectranthus barbatus* stems extract tanned leather

4.3.2. A comparison of shrinkage temperature of tanned and retanned leathers

The shrinkage temperature of tanned leathers was measured during the first sampling (I) and second sampling (II) and in each case two samples were used. Independent sampling averages were then divided by two to give rise to the mean shrinkage temperatures of leather samples. The mean shrinkage temperatures of *Plectranthus barbatus* leaves extract tanned leather (SA), leaves mixed with stems extract tanned leather (SAB), stems extract tanned leather (SB) and *Mimosa* tanned leather (+ve control) were 64.6ºC, 61ºC, 64.7ºC and 74**ºC** respectively.

The mean temperature rise of individual leathers was calculated as the difference between the shrinkage temperature of raw goat skins (55^{o}**C)** and the respective mean shrinkage temperatures. Results are recorded in the table 4.3.

Table 4.3: Comparison of shrinkage temperatures of *Plectranthus barbatus* extracts and *Mimosa* tanned and retanned leathers.

Leather Samples	Temp (˚C)						
	I		II		Avr.	Mean Avr.	▲
	T	R	T	R			
SA1	60.0	67	63.0	67	64.3	64.6	9.6
SA2	64.0	65	65.0	65	64.8		
SAB1	61.0	62	62.0	62	61.8	61.0	6.0
SAB2	60.0	61	59.0	61	60.1		
SB1	61.0	67	63.0	67	64.5	64.7	9.7
SB2	61.0	68	62.0	68	64.8		
SM1	72.0	-	73.0	-	72.5	73.5~74	19.0
SM2	74.0	-	75.0	-	74.5		

Key:

SA- *Plectranthus barbatus* Leaves tanned leather sample,

SAB- *Plectranthus barbatus* Leaves+stems tanned leather sample,

SB- *Plectranthus barbatus* stems tanned leather sample,

SM- *Mimosa* tanned leather sample,

T- Tanned, R- Retanned,

▲- Temperature change,

I- First sampling, II- Second sampling,

Avr.- Average,

Mean Avr.= Average in 1^{st} sampling + Average in 2^{nd} sampling /2.

4.3.3. Ball burst (lastometer test)

Lastometer test conducted on *Plectranthus barbatus* extracts and *Mimosa* tanned and retanned leathers recorded ball burst and grain crack values that were above minimum recommended values of 7.0 mm and 6.5 mm respectively except *Plectranthus barbatus* stems extract tanned leather. These values are actually measured distension of leather fibres in all directions subject to specified stress. After retannage the distension values improved and even those leathers retanned in *Plectranthus barbatus* stems extract showed values above minimum recommended figures. This test is mainly used for leathers destined to manufacture sole leather and results are presented in table 4.4.

Table 4.4: Distension values of grain crack and grain burst of *Plectranthus barbatus* and *Mimosa* tanned and retanned leathers.

Sample	Grain crack (G.C) (mm)		Grain burst (G.B) (mm)	
	Tanned	Retanned	Tanned	Retanned
M (+ve control)	7.7	-	8.2	-
A	7.7	8.7	7.8	9.1
AB	7.0	8.1	7.1	8.5
B	6.4	7.5	6.7	7.9

Key:

M=*Mimosa* tanned leather.

A= *Plectranthus barbatus* leaves extract tanned leather.

AB= *Plectranthus barbatus* leaves+ stems extract tanned leather.

B= *Plectranthus barbatus* stems extract tanned leather.

4.3.4. Tear strength of tanned leathers

The Tear strength of tanned leather is the force required to propagate a cut in a specified direction of a test specimen due to applied force in Newton. Leathers tanned with *Plectranthus barbatus* stems showed the highest tear strength of 85.2 N for samples cut perpendicular (→) to the backbone and this value was slightly higher by 0.6 N when compared with samples cut parallel (↓) to the backbone in regard to the direction of collagen fibre run. All samples of *Plectranthus barbatus* extracts tanned leathers showed increase of tear strength when retanned regardless of the orientation of fibres however, this aspect did not apply to *Plectranthus barbatus* stems extract tanned leathers which depicted a negative trend.

All samples of *Plectranthus barbatus* extracts retanned leathers showed improved % elongation but the leaf extract gave the highest value of 82.6 for samples cut perpendicular to the fibre orientation whereas those of stems extract had a relatively lower value of 74 with a similar fibre orientation. The *Mimosa* tanned leathers that were used as a positive control recorded tear strength values of 70.6 N (→) that was lower than 77.7 N (↓) while the highest % elongation value for this sample was 78.8 (↓). All other results are recorded in table 4.5.

Table 4.5: Tear strength values of *Plectranthus barbatus* extracts and *Mimosa* tanned and retanned leathers.

Sample	Fibre run	Tear strength (N)		% Elongation	
		Tanned	Retanned	Tanned	Retanned
M (+ve control)	→	70.6	-	67.7	-
	↓	77.7	-	78.0	-
A	→	32.2	70.8	74.2	82.6
	↓	31.1	58.7	74.8	76.2
AB	→	34.5	41.8	74.2	80.4
	↓	32.0	51.3	74.2	82.2
B	→	85.2	75.6	69.0	75.2
	↓	84.6	78.2	56.6	74

Key: →- perpendicular to the backbone, ↓- parallel to the backbone,

M=*Mimosa* tanned leather, A= *Plectranthus barbatus* leaves extract tanned leather,

AB= *Plectranthus barbatus* leaves+ stems extract tanned leather,

B= *Plectranthus barbatus* stems extract tanned leather.

4.3.5. Tensile strength of tanned leathers

Tensile strength or fracture stress of tanned leather is the stress required to fracture a test specimen of specified thickness, fibre orientation and location on the skin. Percentage elongation also measured is related to strain and is the ability of leather to stretch when subjected to stress without breaking. *Mimosa* tanned leather (+ve control) posted an average % elongation value of 34.6 with respective tensile strength of 23.9 N and average thickness of 1 mm for samples cut perpendicular to the backbone **(→).** All *Plectranthus barbatus* extracts tanned leathers had high tensile strength which decreased on retanning presumably because of reduced stiffness and increased lubrication that was desirable.

Average thickness for all *Plectranthus barbatus* tanned leathers improved after they were retanned with stems extract tanned leather having the highest value of 1.2 mm (→) as compared to the minimum value of 0.7 mm (→) measured in leaves extract tanned leather however, all were above the minimum recommended value of 0.5 mm. All related results are presented in table 4.6.

Table 4.6: Tensile strength, thickness and % elongation values of *Plectranthus barbatus* and *Mimosa* tanned and retanned leathers.

Sample	Fibre run	Aver Thickness (mm)		Aver Tensile strength (N/mm^2)		Aver % Elongation	
		Tanned	Retanned	Tanned	Retanned	Tanned	Retanned
M (+ve Control)	→	1.0	-	23.9	-	34.6	-
	↓	1.0	-	26.1	-	21.4	-
A	→	0.3	0.7	67.2	31.2	12.2	46.4
	↓	0.4	0.9	41.2	27.9	12.6	40.4
AB	→	0.3	0.85	73.5	17.6	10.0	35.4
	↓	0.3	0.8	56.4	21.6	24.4	26.2
B	→	0.8	1.2	54.3	40.8	20.4	21.4
	↓	0.7	1.2	72.2	26.5	17.2	16.6

Key:

→- perpendicular to the backbone.

↓- parallel to the backbone.

M- *Mimosa* tanned leather.

A- *Plectranthus barbatus* leaves extract tanned leather.

AB- *Plectranthus barbatus* leaves+stems extract tanned leather.

B- *Plectranthus barbatus* stems extract tanned leather.

4.3.6. Flexing endurance of tanned leather

Good flexibility of leather prevents emergence of cracks and deterioration of surface finish after repetitive flexing during a leather items life cycle. *Mimosa* tanned leathers which were used as positive controls endured 100,000 flexes without damage for samples cut perpendicular to the fibre run (→) as well as those cut parallel (↑**)** to the fibre orientation. Leathers tanned and retanned separately with *Plectranthus barbatus* leaves extract and leaves mixed with stems extract also endured 100, 000 flexes without damage for samples cut in both fibre orientations.

This trend however, was different for leathers tanned with *Plectranthus barbatus* stems extract where samples cut perpendicular (→) to the backbone failed at 20,000 flexes with observable considerable change in colour coupled with powdering. Comparatively, similar samples cut parallel to the skin collagen fibre run failed at 25,000 flexes with similar physical manifestations.

Plectranthus barbatus stem extract tanned leathers showed improved flexibility after they were retanned with the same extract since samples cut in regard to both fibre orientations completed 100,000 flexes without observable damage. The results are summarized and compared with other measured leather physical parameters in table 4.7 while other relevant results are recorded in appendix1.

Table 4.7: Summary table for physical properties of *Plectranthus barbatus* retanned leathers and *Mimosa* tanned leathers.

Physical properties		*Plectranthus barbatus* Leaves	*Plectranthus barbatus* Stems	*Plectranthus barbatus* Leaves+stems	*Mimosa*	Minimum recommended value
Ball burst extension (mm)	Grain crack	8.7	7.5	8.1	7.7	6.5
	Grain burst	9.1	7.9	8.5	8.2	7.0
Shrinkage temperature (^oC)		63	62	61	74	60
Flexing endurance		→No damage @ 100000	→Creasing @ 100000	→No damage @ 100000	→No damage @ 100000	No damage @ 100000 flexes
		↑ No damage @ 100000	↑ creasing @ 100000	↑ No damage @ 100000	↑ No damage @ 100000	
Thickness (mm)		→ 0.7	→ 1.2	→ 0.9	→ 1.0	>0.5
		↑ 0.9	↑ 1.2	↑ 0.8	↑ 1.0	
Tensile strength (N/mm^2)		→ 31.2	→ 40.8	→ 17.6	→ 23.9	>12
		↑ 27.9	↑ 26.5	↑ 21.6	↑ 26.1	
Elongation at break (%)		→ 46.3	→ 21.3	→ 35.4	→ 34.3	>40
		↑ 40.3	↑ 17.6	↑ 26.2	↑ 21.0	
Tearing strength (N)		→ 70.8	→ 75.6	→ 41.8	→ 70.6	>20
		↑ 58.7	↑ 78.7	↑ 51.3	↑ 77.7	

Key:

↑ parallel to the backbone

→ perpendicular to the backbone

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussion

5.1.1. Phytochemical screening of *Plectranthus barbatus*

From the phytochemistry conducted on *Plectranthus barbatus*, its contents in the leaves and stems differ in terms of the concentration of tannins and flavonoids (monomeric or dimeric species) in that the former tend to be concentrated in leaves and the later in stems. The tannins were found to be predominantly of the hydrolysable type since they did not readily form red colour when reacted with potassium hydroxide. Hydrolysable tannins structure is predominantly based on glucose but shows variations in which its derivatives can be the central moiety such as sucrose (Khanbabaee and Ree, 2001) and it is possible that *Plectranthus barbatus* constitution is dominated by such structure. Similar studies conducted on *Garad* pods which are used for rural tanning in Sudan contain gallic acid esterified with flavanoid, chebulinic acid and high sugar content an aspect common in hydrolysable tannins (Musa and Gasmelseed, 2013).

The widely used *Mimosa* contains only small amounts of hydrolysable tannins that are masked by condensed tannins and are only noticed during its pyrogallol B ring participation in combination tannages (Covington, 2011). Other condensed tannins used include the prodelphinidins (*Myrica esculenta*, *pecan* and green tea) because they have the required B ring (Covington *et al.,* 2005). *Plectranthus barbatus* is therefore the first indigenous plant in Kenya found to contain hydrolysable tannins that has been used successfully in tanning. Experiments to investigate its

participation in semi-metal tanning and other combination tannages will expose the efficacy of the plant′s water extract in green leather production.

5.1.2. Level of tannins and non-tannins of crude extracts

The level of tannins in *Plectranthus barbatus* from the three locations in Nyamira County did not differ significantly ($p>0.05$) and this means that the soils in this regions from which the samples were collected had relatively uniform properties. The uniformity of the prevailing climatic factors further explains the lack of significant variation in the levels of tannins found in the plant growing in different locations. The plant thrives well in porous and well drained soils having a pH range of 5.5-7. It is grown in many parts of India at an altitude of 2400 metres in relatively poor soils preferably red and sandy loam soil (Mariya *et al.*, 2013).

Esani recorded the highest levels of tannins in leaves at 21% while leaves from Riamoni location contained the lowest levels at 19% hence these results suggest that all the *Plectranthus barbatus* plants growing in Nyamira County are not significantly different ($p>0.05$) in the level of tannins they contain and therefore they can be used for tanning if sourced from any part within the County. Elsewhere in Sudan several indigenous tanning materials such as *garad* pods (*Acacia nilotica sub. Sp. Nilotica*) and Talh bark (*Acacia seyal*) are used by rural tanners and *garad* pods have a tannin content of 30% and 20% soluble non-tans (Musa and Gasmelseed, 2013).

The stems of *Plectranthus barbatus* which do not undergo considerable secondary growth contained the least amount of tannins at an average of 8% with stems from Esani location having the highest amount at 9%. Tests carried out on *chestnut* wood record 5-15% tannins while *oak*

wood has 4-10% tans (Covington, 2011) hence this shows that tannins are not generally concentrated in stems as also noticed in *Plectranthus barbatus*. The smell of the water extracts from the stems was different from that evolved from the leaves. This indicated that the two parts of the plant differed not only in the level of tannins and flavonoids they contain but also in other components that were not tested that are actually part of non-tans and there is a possibility that they too participated in tanning because the physical characteristics of tanned leathers showed variation.

When the leaves and the stems of *Plectranthus barbatus* were mixed at a ratio of 1:1 the level of tannins recorded was slightly higher than that of stems with an average content of 10% and the combination from Esani Location recorded the highest level at 11%. There is a significant difference ($p<0.05$) between the tannin levels measured when the leaves and stems were combined in respect to that measured in leaves or stems alone. However, considering the effect of the locations and the effect of the parts of *Plectanthus barbatus* on the level of tannins tested, there occurs some significant level of interaction ($p< 0.05$) between blocks and treatments hence it is not of much importance to look at variation between the plant parts.

Plants collected from Esani location contained the highest percentage of tannins and non-tannins in leaves, stems and leaves combined with stems while those from Riamoni location were found to contain the lowest levels of the tested polyphenols. The location from which the plants were collected slightly influenced the level of tannins and non-tannins in the plant.

Other studies have reported the following plant materials with hydrolysable type of tannins with the following concentrations: Sumac leaves; 22-35% tannins and 14-17% non-tannins, *chestnut* wood; 5-15% tannins and 1-2% non-tannins, *oak* wood; 4-10% tannins and 1-2% non-tannins and *myrobalan* nuts; 25-48% tannins and14-17% non-tannins (Covington, 2011). From this data it is evident that the level of tannins seems to concentrate in leaves and nuts with stems having a lower concentration and also the level of non-tannins depend on the level of tannins and this trend was noted in *Plectranthus barbatus*.

5.1.3.Vegetable tanning using *Plectranthus barbatus*

Leather tanned with *Plectranthus barbatus* extracts: Leaves extract liquor, leaves and stems extract liquor and stems extract liquor showed different characteristics but most conspicuously in their colours which also changed when wet leather dried. Leathers tanned with liquor made from *Plectranthus barbatus* leaves looked yellow-brown when wet but after drying they became brown. The same characteristics were evident in those tanned with liquor made from a mixture of leaves and stems meaning that the stems were suppressed from showing their colour.

Undyed vegetable tanned leather will show varying shades of brown, yellowish brown or reddish brown depending on source and type of tannins (Mongkholrattanasit *et al.,* 2011). Catechols produce red, pink and dark brown leathers while pyrogallols give rise to paler tans but hard wearing leather for instance those tanned by *sumac* and *chestnut* extracts (Covington, 2011).

This feature can be attributed to the fact that the molecules of the respective tannins deposited on the surface of leather were unstable and changed their structure by either combining with air

(oxidation) or breaking away from each other to gain stability. For instance catechols are oxidized in an oxygen environment to *ortho*-benzoquinone resulting in dark brown-black leather (Covington, 2011). Recombination of molecules after bond breaking could have occurred as the leather lost water thus yielding structures that absorbed light at regions of white light giving corresponding colours. There usually occurs intermolecular hydrogen bond between catechin hydroxyl and water and intermolecular hydrogen bond of ellagic acid (Zheng and Shi, 2005).

Discolouration of phenols in vegetable tannins i.e. darkening and reddening depends on the formation of phenyl radicals by loss of hydrogen to atmospheric oxygen. Free radical formation allows bond shifting and oxidative coupling leading to polymerization that results in linking of chromophores to develop colour (Covington, 2011). In hydrolysable tannins, the chromophores (benzene rings) of ester moieties are far apart and not linkable and in this way they are lightfast and resistant to reddening. *Tara*, a gallotannin is known to be the most lightfast vegetable tannin and produces very pale coloured leather (Covington, 2011). For this reason it is expected that the final colour developed by *Plectranthus barbatus* tanned leathers will also have good light fastness and maintain the brown colour as long as possible until proved otherwise by time.

The proximity of aromatic nuclei in flavonoid structure in condensed tannins favours free radical oxidative bond rearrangements hence tannins redden allowing rapid colour change on the surface of leather. *Mimosa* tanned leather suffers this effect by darkening visibly shortly after exposure to sunlight in air (Covington, 2011) however, this phenomenon is less likely to occur in *Plectranthus barbatus* tanned leather because hydolysable tannins are not easily susceptible to this effect as seen in *tara* tanned leather (Covington, 2011).

5.1.4. *Plecranthus barbatus* Leaves tannage

The quantification of tannins in *Plectranthus barbatus* leaves showed an average concentration of 20% and tanning strength of 1.7 and since the nature of the tannins present is hydrolysable with comparatively low molecular weight, the uniform penetration of the tannins was achieved within two days. Similar research has indicated that *Sumac* leaves have 22-35% tannin content and 14-17% non-tans (Covington, 2011). This study has also shown the efficacy of the low molecular weight fraction of tannins called non-tannins (Covington and Shi, 1998) and the use of organic materials containing either monomeric or dimeric flavanoid species has two advantages for the leather industry: Problems of penetration of the primary component of the tannage are minimized and the components of the tannage may be used as precise reagents, not used in excess, which is a typical feature of vegetable tanning (Covington and Shi, 1998).

Six hundred grams of crude ground *Plectranthus barbatus* leaves was used to achieve full tannage of 4 kg bated pelts made from goat skins. This result therefore shows that 150 g (13%) of the crude tanning material will be needed to tan 1kg of unhaired bated pelts prepared from goat skins and 150 kg of the same tanning material will be required to tan one tonne of the same pelts. Comparatively a recipe extracted from *Bagaruwa (Acacia nilotica)* tannage in Nigeria indicated usage of 30% of this crude material followed by occasional inspection for uniform penetration (Yusuff *et al.,* 2013).

5.1.5. Tannage of the combination of *Plectranthus barbatus* leaves and stems

Leaves and stems of *Plectranthus barbatus* when mixed at a ratio of 1:1 were found to contain an average of 10% tannins and 9% non-tannins with a tanning strength of 1.3. 260 g of tanning material was used in converting 2 kg bated pelts into leather. Five percent (100 g) tanning mixture was later used to impart more weight and improve the opacity, colour and handle of the tanned leather during retanning. In total therefore, 360 g (18%) of the crude tanning mixture was used to tan 2 kg of unhaired bated goat pelts meaning that 180 g of the mixture of ground leaves and stems of *Plectranthus barbatus* could be required to sufficiently tan 1 kg of the same pelts.

5.1.6. Tannage of *Plectranthus barbatus* stems

Plectranthus barbatus stems were found to have the lowest amount of tannin concentration at 8% and 12% non-tannins content. The level of non-tannins exceeded that of tannins by 4% giving a tanning strength value of 0.7. A total of 1000 g was used in completing the stems extract tannage but the leather produced was hard and inflexible hence further retannage was done followed by fatliquoring giving rise to dark brown leather that turned black on drying.

In total 1.5 kg of ground stems was used to tan 2.5 kg of goat bated pelts hence, 1 kg of this tanning material will be needed to tan 1.7 kg of dehaired bated pelts and one tonne of the same material will be enough to tan 1.7 tonnes of these pelts. The use of *Plectranthus barbatus* stems for tanning will therefore be uneconomical because of the quantity of the crude tanning material and the time required since the whole process will take 18 days. Comparatively 1 kg of bated pelts were completely tanned in 11% *Mimosa* which means that this control tannage used 110 g

in two days whereas the *Plectranthus barbatus* leaves extract tannage required 150 g of ground tanning material per 1 kg of bated pelts in 2 days.

5.1.7. Physical properties of *Plectranthus barbatus* and *Mimosa* tanned leathers

The most outstanding difference among the leathers tanned with various plant parts of *Plectranthus barbatus* and *Mimosa* is based on their respective colours when wet and when dry. This is an indication that besides the variation of the levels of hydrolysable tannins among the different plant parts, there was undoubtedly other variations in the components that were not tested that formed the bulk of non- tannins.

Wet fatliquored *Plectranthus barbatus* leaves tanned leathers and those tanned with a combination of leaves and stems showed yellow-brown colour but they both turned brown upon drying. Pyrogallols (hydrolysable tannins) are known to deposit a pale-colored sediment called 'bloom' (ellagic acid) which when deposited in the leather contributes to its colour and improves its solidarity, wearing properties and resistance to water (Covington, 2011). The resultant leather is of pale color varying from creamy or yellowish to light brown (Covington, 2011) and this previous research is not inconsistent with the results of *Plectranthus barbatus* tannages where almost similar observations were made. Such kinds of leathers are preferable for bookbinding, upholstery and other purposes where longevity is essential (Covington, 2011).

The colour observed in *Plectranthus barbatus* tanned leathers must have also been contributed by some other sediments in the non-tannins since laboratory tests show that after absorption of tannins by the hide protein from a previously brown *Plectranthus barbatus* ground leaves

infusion the resultant solution containing non-tannins was yellow in colour. It is therefore clear that the colour of the dry leathers was determined by the colour of tannins present in *Plectranthus barbatus*.

5. 1.8. Ball burst test (lastometer)

This test which is also known as distension test measures the leather bursting strength and height (mm) after grain crack. The busting height is a measure of the elongation of leather fibre in all directions at a specified stress and which has some direct relationship with leather softness (Jianzhong *et al.*, 2003). The higher the busting height, the greater the softness achieved in the leather (Jianzhong *et al.*, 2003) and results of tested leathers showed good softness with no significant difference ($p>0.05$) among the three forms of *Plectranthus barbatus* tanned leathers and *Mimosa* tanned leather.

The mean values were above 7.0 mm which is the minimum recommended value according to Bureau of Indian Standards (BIS). De- Britol *et al.*, (2002) also recorded the minimum recommended bursting height of good leather to be 7.0 mm. Sudan *garad* tanned leathers had a measured grain crack height of 10 mm with a standard deviation of 0.52 mm and semi-chrome showing improved softness by 2 mm (Musa and Gasmelseed, 2013).

When the three *Plectranthus barbatus* retanned leathers were compared, the leaves tanned leathers showed more satisfactory softness with the highest mean bursting height of 8.3 mm but still there was no significant difference ($p>0.05$) among them in regard to this parameter.

Retanning wet blue leather with between 1-3% vegetable tannin extract has been shown to afford lasting antioxidant protection that prevents chromium (vi) formation (Rius *et al.,* 2002).

5.1.9. Tensile strength of tanned leathers

This test method covers the determination of the load required to rupture a leather test specimen having a 1⁄2-in. (12.7 mm) width. The load to rupture divided by the original unstretched cross-sectional area gives the tensile strength. It may be used for all types of leather that are smooth and firm enough to permit accurate thickness measurements. The tensile strength test gives a reliable indication of the quality of the leather. Improperly lubricated and partially degraded leathers give low values of tensile strength. The orientation of the specimen in relation to the backbone and the location of the specimen on the hide influence the results significantly (Yusuff *et al.,* 2013). This test method is excellent for development, control, specification acceptance, and service evaluation of leather (Sterlacci, 2010).

Elongation refers to leather′s ability to lengthen/stretch, when stress is applied to it and represents the maximum extent leather can stretch without breaking (Kuria, 2015). Elongation is usually considered when choosing garment leathers because a low elongation value results in easy tear while a high elongation value causes leather goods to become deformed very quickly or even lose usability (Ork *et al.,* 2014).

There was no significant difference ($p>0.05$) among the *Plectranthus barbatus* tannages i.e. leaves, leaves combined with stems and stems alone however, generally retanned leathers showed more satisfactory physical properties. Stem tannage showed the highest tensile strength of 48.5

N/mm^2 while leaves tannage recorded the lowest value of 43.2 N/mm^2. When compared with *Mimosa* which had a mean tensile strength of 25.0 N/mm^2 there was no significant difference ($p>0.05$) among the four tannages.

Garad tanned goat skins recorded a tensile strength of 23.5 N/mm^2 and Bureau of Indian Standards (BIS) sets the value at 19.6 N/mm^2 (Musa and Gasmelseed, 2013). Kenya Bureau of Standards (KEBS) has also documented a minimum standard of 15 N/mm^2 and 6 N/mm^2 for leather uppers and linings respectively (KEBS, 2012).

Looking at percentage elongation at break values among the *Plectranthus barbatus* tanned leathers there was no significant difference ($p>0.05$) with a mean of 22% and a maximum value of 50% posted by both *Plectranthus barbatus* leaves and leaves mixed with stems tannage. When this property was compared with *Mimosa* which had a highest value of 53% and a mean % elongation value of 28% which was the highest among the four types of leathers, statistical analysis showed no significant difference ($p>0.05$) among them. Comparatively, leathers produced by tanning goat skins with *garad* pods by Sudan rural tanners had a percentage elongation value of 41% and semi-chrome giving a value of 58% with BIS range between 40-65% (Musa and Gasmelseed, 2013). KEBS has also put its minimum standard value at 30% for leather intended for manufacture of linings and highest at 80% (KEBS, 2012).

In terms of thickness of the leathers there was a significant difference ($p<0.05$) among the four types of leathers with *Mimosa* tanned leathers having the highest mean value of 1.1 mm followed by *Plectranthus barbatus* stem retanned leathers at 1.0 mm and leaves retanned leather being the

thinnest at 0.6 mm with an overall coefficient of variation of 38.6%. Leather thickness of *Acacia nilotica* tanned leathers of West African dwarf goats and Sahelian goats recorded thickness of above 0.98 mm and 0.87 mm respectively which was also reported for Lori goat breed of Iran (Salehi *et al.,* 2013). KEBS has maintained a minimum value for upper garment leathers at 1.0 mm while that for upper linings is put at 0.6 mm (KEBS, 2012). These results give evidence that the type of tanning material and also breed of the animal greatly influence the thickness of tanned leather and which conventionally will influence its tensile strength (Kuria, 2015).

5.1.10. Comparison of tear strength of *Plectranthus barbatus* tanned and *Mimosa* tanned leathers

The Tear strength is the median force required to propagate a cut in a specified test specimen due to applied force in Newton (ISO, 2012). Good quality leather should have high flexibility to prevent appearance of cracks and tears for instance in the ball area of the footwear upper (SATRA, 2011). Leathers tanned with *Plectranthus barbatus* stems had the highest tear strength of 80.0 N that was 0.5 N higher than the strength tested in *Mimosa* tanned leather. Leaves_stem tanned leather showed the lowest tear strength of 39.0 N whereas leather tanned with *Plectranthus barbatus* leaves was second last with a tear strength value of 47.3 N.

There was a significant difference ($p<0.05$) between the tear strength means of the four tannages. This difference was between *Plectranthus barbatus* Leaves_stems and *Mimosa,* combined leaves and stems and stems ($p<0.05$), stems and *Mimosa* ($p<0.05$), leaves and *Mimosa* ($p<0.05$) and leaves compared with stems ($p<0.05$) tannages. There was no significant difference ($p>0.05$) between *Plectranthus barbatus* leaves and combined leaves and stem tanned leathers.

Other studies show that *garad* tanned leathers have a tear strength of 40 N, semi-chrome 47 N and BIS value of 30 N (Musa and Gasmeseed, 2013). All the leathers tanned with *Plectranthus barbatus* and including those tanned with *Mimosa* had tear strength values well above the minimum BIS value of 30N. KEBS tear strength value for leather uppers meant for children's sole leather is 50 N while leather used for upper linings is set at 20 N (KEBS, 2012). This means that all the leathers made from the three *Plectranthus barbatus* liquors can be used for light garment leather, ladies shoes, children's shoes and other leathers that may require high tear resistance (Tuck, 1981).

5.1.11. Comparison of flexing endurance of *Plectranthus barbatus* and *Mimosa* tanned leathers

The Vamp and Linear Flexes are used to determine the flexing endurance of leather for instance uppers and their surface finish after repetitive use in the same and opposite flexing cycle.

The tendency for cracks to form in the crease caused by walking can be used to determine suitability of the leather for various purposes especially in applications that encounter continuous flexing (SATRA, 2011). High flexibility therefore, in leather and its finish prevents the appearance of cracks and tears in the ball area of the footwear upper and other leathers that require flexing endurance (UNIDO, 1994).

Plectranthus barbatus leaves tanned leathers and those tanned with a combination of leaves and stems endured 100,000 dry flex cycles with no apparent physical change and this performance was also recorded from *Mimosa* tanned leathers. However, the *Plectranthus barbatus* leathers tanned by liquor made from the stems failed the test at 25,000 dry flexes where powdering and

surface cracks appeared for the sample cut parallel to the fibre run and the same failure was noticed for the sample cut perpendicular to the fibre run at 30,000 dry flexes. The leather tanned with *Plectranthus barbatus* stems lacked flexibility but when it was retanned with additional 20% offer, it showed improvement in the flexing endurance since it completed the 100,000 dry flexes with minimum creasing and change in colour. KEBS value for flex resistance is set at 50,000 cycles for appearance of initial crack and 150,000 cycles for crack propagation for bottom or sole leather (KEBS,2012). Therefore, with these observations, then leather tanned with *Plectranthus barbatus* stems can only be used in applications that need tough rigid leather for instance in belting and harness.

For leathers tanned with leaves extract or a combination of leaves and stems extract, because of their high endurance to flexing they can be suitable in the manufacture of ladies shoes, children shoes and light garment but looking at the relative opacity and handle of the two tannages then, leaves extract yields more superior leather with satisfactory physical properties. It is important also to remember that several factors such as breed, age at slaughter, nutrition and environment also influence the physical properties of leather (Yusuff *et al.,* 2013). For this reason, it is therefore expected that these properties will vary looking at studies done in different parts of the world. Results of flexing endurance of *Acacia nilotica* tanned goats skins in Nigeria from three breeds: West African dwarf, Sahelian and Sokoto red goats showed that 5.83% of the samples subjected to flexometer test showed emergence of cracks however there was no significant difference ($p>0.05$) among them (Yusuff *et al.,* 2013).

With regard to contributing to mitigation of eminent climate change arising from reduced carbon sinks by deforestation due to overdependence on *Mimosa* for tanning, then *Plectranthus barbatus* leaves can be used in the manufacture of high quality leathers comparable to those tanned with *Mimosa* and *Acacia nilotica*. Furthermore, the regeneration of leaves harvested from *Plectranthus barbatus* is about two weeks whereas harvesting of *Acacia nilotica* and *Mimosa* requires felling of entire trees and even when afforestation is considered, it takes many years to restore the ecological balance.

It now remains to be confirmed whether combination or semi-metal tannages with *Plectranthus barbatus* leaves hydrolysable tannins will produce more superior leathers that are cheaper, environmentally friendlier and more competitive in the world market as compared to chrome and *Mimosa* tanned leathers. There is a possibility that high hydrothermal stability will be achieved with combination tanning preferably through crosslinking of polyphenols by oxazoladine or any other suitable crosslinker in situ in collagen (Holmes, 1996).

5.1.12. Comparison of Shrinkage temperature of *Plectranthus barbatus* and *Mimosa* tanned leathers

Shrinkage temperature is a basic test that isolates good quality leather from poor quality leather in terms of its resistance to wet heat and it is defined by the temperature at which tanned leather or raw leather starts to shrink when subjected to wet heat (Covington, 2011). In garment leather shrinkage is exclusively a problem with commercial dry cleaning where leathers have been noticed to shrink during the process. This happens when manufacturers use poor quality hide and skins (Sterlacci, 2010).

All leathers have a shrinkage temperature of above 60ºC but for smaller molecular weight tanning materials such as *Plectranthus barbatus*, Phloroglucinol and green tea, high hydrothermal stability can still be achieved in combination tannage (Covington and Shi, 1998). Simple Phenols such as phloroglucinol have little tanning ability and the stability of collagen molecules may even be lowered because phenols can break hydrogen bonds (Shi and Di, 2000). Green tea tannin, which is a mixture of flavanol monomers in which epigallocatechin gallate is prevalent shows tanning ability that results in shrinkage temperature of 70ºC (Dalluge and Nelson, 2000). Modified vegetable tannages for high stability will require precision in the way reagents are applied where pyrogallol groups and a metal salt with a high affinity to phenolic hydroxyl groups are used.

All hydrolysable tannins including *Plectranthus barbatus* are suitable, but their astringency can be extremely high and any reduction of the offer leads to inadequate penetration but the problem can be solved by applying either a syntan or aldehyde pretannage (Covington, 2011). *Plectranthus barbatus* leaves extract tanned leathers had a mean shrinkage temperature of 64.2ºC that showed a mean increase of 9.3ºC and this change in temperature was slightly lower by 0.1ºC in respect to the shrinkage temperature of *Plectranthus barbatus* stem extract tanned leather.

There was no significant difference ($p>0.05$) in shrinkage temperature between these two tannages, however, there was a significant difference ($p<0.05$) in the shrinkage temperatures of leaves extract and leaves_stem extract tanned leathers and this was also the case with the comparison of *Mimosa* and *Plectranthus barbatus* leaves extract tanned leathers ($p<0.05$). Combined leaves and stem tannage recorded the lowest mean shrinkage temperature of 60.7 ºC

i.e. a temperature change of 5.7 ºC and *Mimosa* showed the highest mean shrinkage temperature of 74.9ºC i.e. a temperature change of 19.9ºC thus there was a significant difference ($p<0.05$) between these two tannages in regard to this physical test.

Comparatively, Sudanese *garad* tanned goat leathers showed a shrinkage temperature of 84ºC (Musa and Gasmelseed, 2013) probably because *garad* pods contain a mixture of hydrolysable and condensed tannins that seem to synergistically improve the heat stability of the tannage. Semi-chrome *garad* tanned leathers showed further increase of shrinkage temperature to 104ºC (Musa and Gasmelseed, 2013) and it is expected that semi-chrome and other combination tannages with *Plectranthus barbatus* will yield more heat stable leather. Research has witnessed positive tanning effects and more specifically shrinkage temperature of aluminium, titanium and Zirconium together with vegetable tannins and other organic molecules (Sundarrajan *et al.,* 2003, Fathima et al., 2005).

The huge deposits of titanium in Kwale district (Kenya) further offer a commercially viable future of this proposed tannage that will utilize two sets of locally available natural resources. Studies of combining vegetable tannins with oxazolidine and zinc have also yielded elevated shrinkage temperatures (Covington and Shi, 1998., Morera *et al.,* 1996).

Generally there was a significant difference ($p<0.05$) in shrinkage temperature among the four tannages that were tested but since all values were above the minimum value of 60ºC, then all of them can be used for purposes that do not need endurance of very high temperatures. Studies have shown that there is no direct relationship between shrinkage temperature and leather

stability (Covington, 2011). All the leathers tanned with *Plectranthus barbatus* including that of *Mimosa* failed the qualitative boil test therefore, this shows that shrinkage temperature is not the acid test for good quality leather but rather fitness for purpose. *Mimosa* though expensive with relatively poor colour fastness is a conventional tannage in Kenya that continues to produce excellent eco-friendly leather articles for various uses including the popular sole leather hence this perspective seems to support Covington's thinking that the future of tanning probably lies with creating unstable leather for environmental sustainability (Covington, 2011).

However, it is also assumed that the shrinking reaction is independent of the stabilizing effect because the enthalpy of denaturation is independent of stabilizing chemistry (Covington *et al.,* 1989). Hydrothermal stability depends on the chemistry of bonding with hydrogen bonding conferring only moderate rise in shrinkage temperature and this was the case with *Plectranthus barbatus* tannage. Previous studies show that shrinkage temperature of leather tanned with condensed tannins (*Mimosa*) is between 80-85ºC and for hydrolysable tannins (*sumac*) the shrinkage temperature of is < 80ºC (Covington, 2011). *Divi-divi* and *myrobalan* tannages (hydrolysable) both have a shrinkage temperature of 68 ºC and *quebracho* and wattle tannages (condensed) produce leather with a shrinkage temperatures of 76°C and 78°C respectively (Covington, 2011).

In vegetable tanned leathers, shrinkage temperature is not of commercial importance (Thorstensen, 1993) and there is a possibility that the high shrinkage temperature of chrome, semi-chrome and semi- metal tannages is due to the metallic character of the participating species and therefore it is unjustifiable to compare them with vegetable tannages. It seems that the

metallic character influences this physical property than the proposed crosslinking which might be independent, at least for this case.

5.2. Conclusions

- *Plectranthus barbatus* was found to contain hydrolysable tannins ranging between 8-20 % depending on the part of the plant.
- *Plectranthus barbatus* was found to contain 20% tannin content in leaves 8% in stems, and 10% in a combination of leaves and stem extracts. The tannin content in leaves and stems was significantly different ($p<0.05$)
- The physical properties of the tanned leathers were above the minimum set values for good quality leathers. However, retanning significantly improved the physical properties of these leathers.
- Approximately 150 g of dry ground leaves of *Plectranthus barbatus* will be adequate to tan 1.0 kg of goat skin.

5.3.Recommendations

- *Plectranthus barbatus* should be considered as one of the vegetable tanning agents to tan goat skins for production of light cloth garments, children shoes and ladies shoes that require hard wearing light flexible leather.
- Retanning of *Plectranthus barbatus* tanned leathers be encouraged to improve their quality.

- The growing of the *Plectranthus barbatus* as a cash crop be promoted in rural areas to improve income of rural farmers. The plant can be grown along fences or even intercropped with other crops without much effect.
- There is need to sensitize the local community on the value of *Plectranthus barbatus* and the need to add value to hides and skins using locally available materials and appropriate technologies.

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APPENDICES

Appendix 1: Raw data tables

Raw data for the levels of tannins in *Plectranthus barbatus* crude extracts

	Leaves(A)			**Leaves + Stems(A+B)**			**Stems (B)**		
	% Tannins			% Tannins			% Tannins		
Location	Gesima	Esani	Riamoni	Gesima	Esani	Riamoni	Gesima	Esani	Riamoni
I	22	22	18	11	12	10	9	9	8
II	18	21	20	9	10	9	7	8	7
III	20	20	19	11	11	8	8	10	9
Aver.	20	21	19	10	11	9	8	9	8

Flexing endurance values of tanned and retanned leathers.

Sample	**→M**	**↓M**	**→ A**		**↓ A**		**→B**		**↓ B**		**→ AB**		**↓AB**		**→ B**		**↓ B**	
Flexes	T	T	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R
10,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
15,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
20,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	3X	✓	✓	✓
25,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	2,3X	✓	3X	✓
30,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	3,2X	✓
35,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
40,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
50,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
55,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
60,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
65,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
70,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
75,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
80,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
85,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
90,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
95,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓
100,000	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓

Key:

M= *Mimosa* tanned leather.

A= *Plectranthus barbatus* leaves extract tanned leather.

B= *Plectranthus barbatus* stems extract tanned leather.

AB= *Plectranthus barbatus* leaves + stems extract tanned leather.

↓- parallel to the backbone.

→- perpendicular to the backbone.

✓- Pass.

X- Fail.

T- Tanned.

R- Retanned.

1. Change in colour or finish film.

2. Creasing with smaller or greater surface cracks.

3. Loss of adhesion of finish with slight or considerable change in colour or powdering.

4. Flaking of the finish.

Appendix 2: Statistical tables

Analysis of variance for crude extract tannin concentration

```
. anova Percentagetanins treatments

                          Number of obs =      27     R-squared     =  0.9482
                          Root MSE      = 1.27294     Adj R-squared =  0.9438

                 Source | Partial SS    df       MS           F     Prob > F
------------------------+----------------------------------------------------
                  Model |  711.185185     2  355.592593     219.45     0.0000
                        |
             treatments |  711.185185     2  355.592593     219.45     0.0000
                        |
               Residual |  38.8888889    24  1.62037037
------------------------+----------------------------------------------------
                  Total |  750.074074    26  28.8490028

. anova Percentagetanins location

                          Number of obs =      27     R-squared     =  0.0167
                          Root MSE      =  5.5436     Adj R-squared = -0.0653

                 Source | Partial SS    df       MS           F     Prob > F
------------------------+----------------------------------------------------
                  Model |  12.5185185     2  6.25925926       0.20     0.8171
                        |
               location |  12.5185185     2  6.25925926       0.20     0.8171
                        |
               Residual |  737.555556    24  30.7314815
------------------------+----------------------------------------------------
                  Total |  750.074074    26  28.8490028

. anova Percentagetanins treatments location

                          Number of obs =      27     R-squared     =  0.9648
                          Root MSE      = 1.09483     Adj R-squared =  0.9585

                 Source | Partial SS    df       MS           F     Prob > F
------------------------+----------------------------------------------------
                  Model |  723.703704     4  180.925926     150.94     0.0000
                        |
             treatments |  711.185185     2  355.592593     296.66     0.0000
               location |  12.5185185     2  6.25925926       5.22     0.0139
                        |
               Residual |  26.3703704    22   1.1986532
------------------------+----------------------------------------------------
                  Total |  750.074074    26  28.8490028
```

Analysis of variance for distension (lastometer test)

General Analysis of Variance

YVA 'Distension_mm'
: SWE 'treatmen';BLO 'tannage'

GENVAR

No contrasts confounded

ANALYSIS OF VARIANCE FOR Distension_mm

Source	d.f.	s.s.	m.s.	F	Prob >F
Treatments	2	4.1206	2.0603	2.60	0.0897
Residual	32	25.329	0.79153		
Stratum total	34	29.449			

TREATMENT MEANS

Level	Mean
Leave-st	7.6833
Leaves	8.325
Stem	7.55

General Analysis of Variance

YVA 'Distension_mm'
: SWE 'treatmen';BLO 'Test'

GENVAR

No contrasts confounded

ANALYSIS OF VARIANCE FOR Distension_mm

Source	d.f.	s.s.	m.s.	F	Prob >F
Treatments	2	4.1206	2.0603	2.04	0.1460
Residual	32	32.242	1.0076		
Stratum total	34	36.363			

TREATMENT MEANS

Level	Mean
Leave-st	7.6833
Leaves	8.325
Stem	7.55

Analysis of variance for tensile strength

OneWay Analysis of Variance

YVAr 'Tensile_strength_Nsqmm'
: ONEway 'treatmen'

ANOVA TABLE for Tensile_strength_Nsqmm

Source	DF	SS	MS	F	Prob.
treatmen	3	6477.1	2159	6.9	0.002
Residual	20	6234.2	311.71		
Total	23	12711			

Overall Mean = 52.87 s (Residual) = 17.66
Coefficient of Variation = 33.4 %

The following observations have large residuals

Observation	Value	Residual	se	Ratio	%RSS
22	94.9	31.67	16.1	1.96	16.1

MAIN EFFECTS

treatment

Level	Mean	Count	S.E.
Leave-st	66.33	6	7.208
Leaves	56.87	6	7.208
Mimosa	25.03	6	7.208
Stem	63.23	6	7.208

Analysis of variance for % elongation

OneWay Analysis of Variance

YVAr 'Elongation_mm'
: ONEway 'treatmen'

ANOVA TABLE for Elongation_mm

Source	DF	SS	MS	F	Prob.
treatmen	3	153.66	51.221	2.2	0.118
Residual	20	462.16	23.108		
Total	23	615.82			

Overall Mean = 9.692 s (Residual) = 4.807
Coefficient of Variation = 49.6 %

The following observations have large residuals

Observation	Value	Residual	se	Ratio	%RSS
1	26.5	12.7	4.39	2.89	34.9
17	20.7	12.07	4.39	2.75	31.5

MAIN EFFECTS

treatment

Level	Mean	Count	S.E.
Leave-st	8.633	6	1.962
Leaves	6.95	6	1.962
Mimosa	13.8	6	1.962
Stem	9.383	6	1.962

Analysis of variance for leather thickness

OneWay Analysis of Variance

YVAr 'Avr_Thickness'
: ONEway 'treatmen'

ANOVA TABLE for Avr_Thickness

Source	DF	SS	MS	F	Prob.
treatmen	3	2.43	0.81	30.6	0.000
Residual	20	0.53	0.0265		
Total	23	2.96			

Overall Mean = 0.6 s (Residual) = 0.1628
Coefficient of Variation = 27.1 %

The following observations have large residuals

Observation	Value	Residual	se	Ratio	%RSS
1	0.7	-0.35	0.149	-2.36	23.1
2	1.5	0.45	0.149	3.03	38.2

MAIN EFFECTS

treatment

Level	Mean	Count	S.E.
Leave-st	0.3	6	0.0665
Leaves	0.3	6	0.0665
Mimosa	1.05	6	0.0665
Stem	0.75	6	0.0665

Analysis of variance for tear strength

OneWay Analysis of Variance

YVAr 'Tear_strength_N'
: ONEway 'treatmen'

ANOVA TABLE for Tear_strength_N

Source	DF	SS	MS	F	Prob.
treatmen	3	18044	6014.7	31.7	0.000
Residual	28	5316.1	189.86		
Total	31	23360			

Overall Mean = 56.11 s (Residual) = 13.78
Coefficient of Variation = 24.6 %

The following observations have large residuals

Observation	Value	Residual	se	Ratio	%RSS
6	110	35.85	12.9	2.78	24.2
25	55.9	-29.04	12.9	-2.25	15.9
27	129.8	44.86	12.9	3.48	37.9

MAIN EFFECTS

treatment

Level	Mean	Count	S.E.
Leave-st	33.25	8	4.872
Leaves	32.1	8	4.872
Mimosa	74.15	8	4.872
Stem	84.94	8	4.872

General Analysis of Variance

YVA 'Ts_RC'
: SWE 'tretment';BLO 'tannage';CON X5, X6, X7, X8, X9, X10, X11

GENVAR
No contrasts confounded

ANALYSIS OF VARIANCE FOR Ts_RC

Source	d.f.	s.s.	m.s.	F	Prob >F
Treatments	3	240.97	80.323	31.43	0.0000
Residual	9	23	2.5556		
Stratum total	12	263.97			

TREATMENT MEANS

Level	Mean
Leave-st	60.768
Leaves	64.268
Mimosa	74.893
Stem	64.393

The above means have been adjusted for non-orthogonality

Contrast	Value	se	ssq	F	Prob >F	Theta	Efficiency
l_m	-10.625	1.4593	135.47	53.01	0.0000	0.833	0.900
ls_l	-3.5	1.1304	24.5	9.59	0.0128	0.500	1.000
X7	10.5	1.4593	132.3	51.77	0.0001	0.833	0.900
l_s	-0.125	1.1304	0.03125	0.01	0.9144	0.500	1.000
m_s	10.5	1.4593	132.3	51.77	0.0001	0.833	0.900
ls_m	-14.125	1.4593	239.42	93.69	0.0000	0.833	0.900
ls_s	-3.625	1.1304	26.281	10.28	0.0107	0.500	1.000

Analysis of variance for shrinkage temperature(Ts)

OneWay Analysis of Variance

YVAr 'Ts_RC'
: ONEway 'treatmen'

ANOVA TABLE for Ts_RC

Source	DF	SS	MS	F	Prob.
treatmen	3	209.62	69.872	12.8	0.001
Residual	10	54.688	5.4688		
Total	13	264.3			

Overall Mean = 64.82 s (Residual) = 2.339
Coefficient of Variation = 3.6 %

The following observations have large residuals

Observation	Value	Residual	se	Ratio	%RSS
3	61.5	-3	1.98	-1.52	16.5
12	61.5	-3.125	1.98	-1.58	17.9
14	68	3.375	1.98	1.71	20.8

MAIN EFFECTS

treatment

Level	Mean	Count	S.E.
Leave-st	61	4	1.169
Leaves	64.5	4	1.169
Mimosa	73.5	2	1.654
Stem	64.63	4	1.169

General Analysis of Variance

YVA 'Ts_RC'
: SWE 'treatmen';BLO 'tannage';CON X5, X6, X7, X8, X9, X10

GENVAR
No contrasts confounded

ANALYSIS OF VARIANCE FOR Ts_RC

Source	d.f.	s.s.	m.s.	F	Prob >F
Treatments	3	240.97	80.323	31.43	0.0000
Residual	9	23	2.5556		
Stratum total	12	263.97			

TREATMENT MEANS

Level	Mean
Leave-st	60.768
Leaves	64.268
Mimosa	74.893
Stem	64.393

The above means have been adjusted for non-orthogonality

Contrast	Value	se	ssq	F	Prob >F	Theta	Efficiency
l_ls	-3.5	1.1304	24.5	9.59	0.0128	0.500	1.000
l_s	-0.125	1.1304	0.03125	0.01	0.9144	0.500	1.000
s_m	10.5	1.4593	132.3	51.77	0.0001	0.833	0.900
l_m	-10.625	1.4593	135.47	53.01	0.0000	0.833	0.900
ls_m	-14.125	1.4593	239.42	93.69	0.0000	0.833	0.900
ls_s	-3.625	1.1304	26.281	10.28	0.0107	0.500	1.000

Appendix 3: Tannery process sheet

PROCESS	%	PRODUCT	RUN TIME	pH	COMMENTS
Main SOAK	0.5 2.0	H_2O @ 20ºc in a steel drum Biosoak SLT (Soaking enzyme) General clean (Liquid Detergent)			
	1 0.5 0.5 0.5	Bactericide Sulphide Na_2CO_3 lime	60’	7.5 -8.0	LEAVE FOR 18 HOURS Remove the dirt, blood and dung Break over the beam on the flesh side of the hide
LIMING	200 3 2	H_2O Sulphide Hydrated lime	60’	11.5-12	LEAVE FOR 18 HOURS Do the fleshing on the flesh side of the skins Weigh fleshed pelts
DELMING	200 2	H_2O $(NH_4)_2SO_4$	30’	8.2-8.3	Scud the pelt
BATING	100 1	H_2O@ 38º C Microbate	1hr	8.5	Scud the pelt Wash and rinse with cold running water
PICKLING	100 8 0.3 0.5 0.1	Water Salt Sodium formate Formic acid Sulphuric acid	20’ 20’ 20’ 60’ run drum	5 4.5-5	Run drum Run drum Run drum Leave overnight

TANNING	150 5 3 2 0.5 1	water Vegetable tannins Vegetable tannins Vegetable tannins Biocide Formic acid	6hrs run drum 6 hrs run drum 6hrs 20’ run drum	 6-6.3 3.5	Leave overnight Leave overnight Leave overnight Wash and rinse with cold water and Horse for 2days for aging
FATLIQUORING	100 4 1	H_2O @50ºC Veg oil fatliquor Formic acid	1hr run drum 20’ run drum	 3.5	Horse overnight, hang dry for 5hrs and toggle for 1 day