EVALUATION OF FEED VALUE OF LEAVES OF TANNIN RICH TREES WITH OR WITHOUT TANNIN BINDING AGENTS

MSc Thesis

By

Afework Bedewi

December, 2012

Jimma University

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MSc Thesis

Submitted to the School of Graduate Studies,

College of Agriculture and Veterinary Medicine,

Jimma University

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Animal Production

By

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December, 2012

Jimma, Ethiopia

APPROVAL SHEET
School of Graduate Studies

As thesis research advisor, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Afework Bedewi Hasen entitled: Evaluation of Feed Value of Leaves of Tannin Rich Trees with or without Tannin Binding Agents. We recommend that it be submitted as fulfilling thesis requirement.

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As member of Board of Examiners of the M.Sc. Thesis Open Defense Examination, We certify that we have read, evaluated the thesis prepared by Afework Bedewi Hasen and examined the candidate. We recommended that the thesis could be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Animal Production.

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DEDICATION

This thesis is dedicated for my family who thought me everything.

“Nothing is more than a family”

STATEMENT OF AUTHOR

I declare that the thesis hereby submitted for the M.Sc. degree at the Jimma University, College of Agriculture and Veterinary Medicine is my own work and has not been previously submitted by me or others at another University or institution for any degree. I grant copyright of the thesis in favor of the Jimma University, Collage of Agriculture and Veterinary Medicine.

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ACKNOWLEDGEMENTS

In the completion of this thesis, and to a greater level than at any other stage of my academic career, I received help, advice and friendship from an outstanding group of professionals. I like to acknowledge that help, without which, I would not be able to complete this task.

I would like to express my sincere appreciation to my Major Advisor, Yisehak Kechero, for all the extra hours he put in on my behalf. I appreciate your patience and support while I struggled through scientific writing. Your interest for nutrition and teaching, and your willingness to go the extra distance to help your students is an encouragement. Also I should have to thanks my co-advisor Dr. Getnet Assefa for his invaluable support. I want to send my special thanks and appreciation to Dr. Martha Yami, Mr. Alemayehu Mengistu, Professor Tegenea, Holeta Agriculture Research center for their technical and material assistance. My gratitude and appreciation also goes to Proffesor Solomon Demeke, Dr. Berhanu Belay, Dr. Abegaz Beyene, Dr. Taye Tolemariam and all staffs of JUCAVM department of animal science for being with me all the time.

I would like to extend extra special thanks to my best friends at JUCAVM: Mekdelawit Yeshitela, Esubalew Tadele, Terhas Meberatu, Mulegeta Gebeyehu, Yemisrach Abate, Yebirzaf Yeshiwas, Meseret Shiferaw, Tinsae Demese, Serawdink Fikreyesus, Tsega Lema and Elsabet Tekabe. You have unquestionably made my time at JUCAVM interesting and entertaining. I appreciate your moral support and willingness to sacrifice your dignity for a laugh. I couldn't have made it through this program without your material, technical and moral supports.

I could not have done any of this without my family. My parents, brother and sisters: your Prayers, financial support, and encouragement have been much appreciated. Believe it or not, I feel very lucky to have you all. They have supported and encouraged me through all of my ventures, and listened to my whine when things did not go right. You are the best parents anyone could ask for.

My gratitude and appreciation also goes to Mr. Jan and his family (SolAgro) for their moral and financial support. I would also like to send special thanks to my special friends whom I consider them as brother and sister: Teshale Wolka, Selamawit Alemu, Addisalem Egedo, Gelila Tesfaye, Anteneh Admasu, Metinaf Dereje and Seleshi Abiy who showed me the height and length of friendship. They have been and will continue to be a big influence on my life.

Finally, everything is done because of his willing and blessing. Thanks to God for making everything happen with love and blessing.

LIST OF ABBREVIATIONS

CA	Crude Ash
CHO	Carbohydrate
Cm	Centimeter
CP	Crude Protein
CT	Condensed Tannin
DCHO	Digestible Carbohydrates
DCP	Digestible Crude Protein
DEE	Digestible Ether Extract
DM	Dry Matter
H	Hour
HT	Hydrolysable Tannin
IAEA	International Atomic Energy Agency
IVDMD	*In vitro* Dry Matter Digestibility
IVOMD	*In vitro* Organic Matter Digestibility
M	meter
Max.	Maximum
MCT	Mono Carboxylate Transporter
Mm	Millimeter
MPTS	Multi Purpose Tree Species
OMD	Organic Matter Digestibility
PA	Proanthocyanidins
PEG	Poly Ethylene Glycol
PSM	Plant Secondary Metabolites
PVP	Polyvinyl Pyrolidone
PVPP	Polyvinyl Poly Pyrolidone
RUSITEC	Rumen Simulation Technique
SCFA	Short Chain Fatty Acids
TBA	Tannin-Binding Agents
TP	Total Phenols
TT	Total Tannins

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ABSTRACT

The study was undertaken to evaluate nutrient value of tannin rich tropical browse species and the interaction effects of these plant species and different level of tannin binding agents on in vitro fermentation parameters and to examine the relationship between some chemical composition parameters and the in vitro fermentation values of the plants. The studied plant species were Albizia gummifera, Carissa edulis, Draceana steudneri, Ficus sycomorus, Grewia ferruginea, Millettia ferruginea, Prunus africana, Rhus glutinosa, Syzygium guineense and Ekebergia capensis. The species were subjected to proximate, detergent, polyphenolic and in vitro analysis. Rumen liquor was collected from three Boran- Holestein Friesian cross bred bulls. Plant species was incubated for two run in three replications in each run. An in vitro gas production measurement was performed after 2, 4, 6, 8, 12, 24, 32, 48, 58, 72 and 96 h after incubation and their fermentation kinetics were described using the equation $y = a + b(1-e^{-ct})$*. Organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acids (SCFA) were estimated for 24h gas production. The measurement of pH was performed before and after incubation in vitro. A wide variability in chemical composition including polyphenolics, in vitro digestibility and fermentation and estimated parameters recorded for plants as well as interaction of plant-tannin binding agents. The total phenols (TP) content (g/kg DM) ranged from 101 in D. steudneri to 288 in C. edulis, which also had the higher total tannins (TT) (204g/kg DM)(P<0.001). The highest soluble gas fraction (a) were recorded for C. edulis (20.73 ml) and R. glutinosa (20.71 ml), where as the lowest value was estimated for M. ferruginea (5.85ml (P<0.001)). Fermentation of insoluble fraction (b) was highest in G. ferruginea (190.77ml), P. africana (192.47ml) and S. guineense (188.17 ml) as compared to other species (P<0.001). C. edulis and R. glutinosa had the highest rate of gas production (c; 8.58, and 8.57 ml/h, respectively) as compared to rest of the species (P<0.001). Addition of* PEG_{4000}*,* PEG_{6000}*, PVP and PVPP to incubation medium increased the extent and rate of gas production all plant species as compared to species incubated without tannin binding agent (P<0.001). There were negative correlations between CT, NDF and ADF content of the plant species and the gas production at all incubation times. Negative correlation were also observed between CT and CP (r=-0.565), IVOMD (r=-0.722), IVDMD (r=-0.669), TDN (r=-0.668), DCP (r=-0.662) and DCHO (r=-0.427) (P<0.01). Addition of PEG, PVP and PVPP inactivate effects of tannins and increased gas production, SCFA, ME, and OMD in all the plants (P<0.001). Generally, treatments received* PEG_{6000} *and* PEG_{4000} *were found to be superior over other treatments in terms of rate and extent of gas production (p<0.001). Addition of* PEG_{6000}*,* PEG_{4000}*, PVP and PVPP could help to overcome adverse effects of tannins on nutrient availability as indicated by gas production parameters. This increase was noticeable in all plant species. Based on their nutrient content and fermentation kinetics as when its tannin content is biologically inactivated by using tannin binding agents the browse species may play an important feed supplements in providing sustainable fodder for herbivores.*

Key words: Browse species, chemical composition, *in vitro* gas production, Polyphenolics, Tannin binding agents, and Short chain fatty acids

1. INTRODUCTION

The major constraint to increasing livestock productivity in Sub-Saharan African countries and many other developing nations is the scarcity and fluctuating quantity and quality of the year-round feed supply (Hassan *et al*., 2007; Besharati and Taghizadeh, 2009; Arigbede *et al*., 2011). The native pastures and crop residues are the major feed sources available in these areas for the ruminants (Salem *et al*., 2006); however, tropical graminaceous fodder and crop by-products have a low nutritive value due to their low protein and fermentable energy content (El hassan *et al*., 2000). Moreover, with the increase in human population, more land is being dedicated to crop production and hence unavailable for pasture production. This has resulted in animals increasingly being fed on crop residues. Natural pastures, especially during the dry season, and crop residues are low in crude protein and digestible nutrients and high in fiber (Leng, 1990).

The low intake and digestibility of such feeds is mainly due to their low content of essential nutrients such as proteins, minerals and vitamins and coupled with high lignifications of their structural carbohydrates (Nair *et al*., 2002), which slows rumen fermentation. It has been suggested that supplementing these feed resources with nitrogen-rich feeds such as multipurpose trees species (MPTS) is one way of alleviating the feed intake and rumen fermentation (McSweeny *et al*., 1999; Abdulrazak *et al*., 2000; Yisehak *et al*., 2010, 2012). Addition of foliages of MPTS are of importance in animal production because they compete less with human food and can provide significant protein supplements, especially in the dry season and can improve the utilization of low quality roughages mainly through the supply of protein to rumen microbes (FAO/IAEA, 2002; Makkar, 2003; Ozturk *et al*., 2006).

Although MPTS have important nutritional merits (McNabb *et al.,* 1993; Wang *et al.,* 1994; Silanikove *et al.,* 1996; Kaitho *et al.,* 1998; du Plessis *et al.,* 1999; Norton, 2000; Yisehak *et al*., 2012), there are also reports which indicate that anti-nutritional factors found in many fodder trees such as condensed tannins (CTs) (Makker and Bakker, 1998; Frutos *et al*., 2002) can affect feed intake, digestibility, growth, onset of puberty and reproductive functions via interference in the metabolic process or reduction of nutrient availability or a combination of these pathways.

Studies on evaluation of browse species have indicated their potential as feed supplements and a need to identify other species to be included in the list of potential fodder with tannin deactivating agents. *In vitro* gas production has been used to assess the nutritive value of browse species. *In vitro* gas production basically results from fermentation of carbohydrates and other organic compounds to short chain fatty acids (SCFA)-mainly acetate, propionate and butyrate (Blümmel and Ørskov, 1993) and from the buffering of the SCFA (CO_2 released from the bicarbonate buffer). Hence, the extent of gas production reflects the efficiency and/or extent of degradability of the feed organic matter (OM). Besides SCFA and gas (mainly CO_2 and CH_4) the feed OM is also fermented to microbial biomass (microbial protein) (Getachew *et al*., 1998). *In vitro* fermentation of protein produces ammonium carbonate and the ammonia. The ammonia produced from protein breakdown may be directly used by bacteria for protein synthesis. Hence, low amount of gas is recorded from protein synthesis (Gonzalez *et al*., 1998).

There are various species of fodder trees and shrubs grown in the Omo-Ghibe basin of Ethiopia (Yisehak and Belay, 2011; Yisehak *et al*., 2011). Variations in species of MPTS have an effect on the tannin contents of shrub and tree leaves (Ozturk *et al*., 2006).

As the demand for food rises, tanniferous plants must play an increasingly important part in the diet of animals, in particular for ruminants in smallholder subsistence farming in developing countries. It is therefore critical that techniques be developed to measure and manage the anti-nutritional components that they contain. Addition of CT deactivating agents (Hagerman, 2011), polyethylene glycol (PEG), poly vinyl pyrrolidine (PVP), poly vinyl polypyrrolidine (PVPP) would be efficient in decreasing effects of CT, neutralizes their negative effects and improves nutrient utilization. Recently, it has been suggested that occurrence of tannins and their effect on the kinetics of *in vitro* fermentation can be assessed using the gas production techniques coupled with the use of various tannin-binding agents. Very limited work has been reported on CT deactivation using tannin-deactivating agents, PEG_{4000}, PEG_{6000}, PVP and PVPP, there *in vitro* fermentation levels in sub-Saharan Africa particularly to Ethiopia. All of the compounds are commercially available in a range of molecular weights.

The knowledge of digestion kinetics and rate of passage not only provide us an opportunity to understand the factors limiting digestive process but also to develop feeding strategies for optimizing system output. Understanding the relationship among changes in rate of passage, lag time, extent and site of digestion of fiber source may help to formulate the ration that maximize nutrient availability to the animal by manipulating passage rate from the rumen (Sarwar and Chaudhry, 2000). According to Blummel and Becker (1997) the *in vitro* gas production technique is a useful tool in determining the nutritional value of forages because the volume of gas produced by forage species reflects the end result of the fermentation of its substrate to volatile fatty acids (VFA), microbial biomass and neutralization of the VFA, thus demonstrating the nutritional value of such forage.

The information on rate and extent of digestion of commonly available leguminous and non leguminous forage in Ethiopia is limited. The plant species are also not tested elsewhere. Proximate, detergent, phenolics and mineral analysis in combination with *in vitro* fermentation kinetics can help in the comprehensive evaluation of the likely nutritive value of previously uninvestigated MPTS. The *in vitro* fermentation analyses also give indication of antimicrobial activity. Besides, various methods available for feed evaluation and digestibility studies through feeding experiments are expensive and require sophisticated laboratory equipment and animal keeping facilities. Thus, there is a need for more research into ways of managing browse to balance forage quality and quantity. Therefore, the present study is planned to undertake the following specific objectives:

1. To assess the chemical composition of leaves of tannin rich tree species
2. To evaluate the effects of plant species on *in vitro* fermentation characteristics with and without tannin binding agents (PEG_{4000}, PEG_{6000}, PVP and PVPP)
3. To examine the relationship between some chemical composition parameters and the *in vitro* gas production of the plants

2. LITERATURE REVIEW

This chapter describes about available feed resource of Ethiopia, studied tree species, nutrient content of feeds and fodder, estimation of nutrient value of feeds, Anti nutritional factors in the feed and their deactivation mechanisms. Also in this chapter I tried to define major terminologies which are commonly used in the current studies.

2.1. Major Livestock Feed Resources in Ethiopia

Livestock in the Sub-Saharan Africa are dependent primarily on native grasslands and crop residues (Ibrahim, 1999). According to Alemayehu (2003), Ethiopia's livestock feed resources are mainly natural grazing and browse, crop residues, improved pasture, and agro-industrial byproducts. The feeding systems include communal or private natural grazing and browsing, cut and carry feeding, hay and crop residues. At present, in the country stock are fed almost entirely on natural pasture and crop residues. Grazing is on permanent grazing areas, fallow land and cropland after harvest (Stubble). The availability and quality of forage are not favorable year round. As a result, the gains made in the wet season are totally or partially lost in the dry season (Alemayehu, 2003). Inadequate feed during the dry season is a major cause of decline in the productivity of ruminants. In Sub-Saharan Africa, human population is increasing rapidly, forcing farmers to use grazing areas for crop farming. As a result, the smallholder farmers in this part of Africa have integrated their livestock into their cropping systems and used crop residues as a main livestock feed resources (Ibrahim, 1999).

2.2. Description of the Studied Plants

Albizia gummifera, family Fabaceae, is a large deciduous tree 4.5-30 m, branches ascending to a flat top. Crown flat; bark smooth and grey. Leaves bipinnate in 5-7 pairs, leaflets dark green roughly similar in size but top pinnae in 9-16 pairs, obliquely rhombic to subfalcate, apex obtuse or acute, 10-25 by 4-12 mm, pubescent or glabrous. Two varieties are recognized var. gummifera with leaflets conspicuosly auriculate on the proximal side of the base and var. ealaensis without auriculate leaflets on the proximal side. The genus was named after Filippo del Albizzi, a Florentine nobleman who in 1749 introduced A. julibrissin into cultivation. The specific epithet

'gummifera' means the gum bearer). Trees are capable of growing rapidly. The roots develop nitrogen-fixing nodules containing Bradyrhizobium bacteria. Albizia gummifera trees live in association with arbuscular mycorrhizae.Albizia gummifera occurs in rainforest and riverine forest, sometimes also in savanna vegetation close to forest, usually at higher altitudes, up to 2500 m, but sometimes near sea-level. It is locally common. In Zimbabwe it is reportedly fire resistant and only slightly sensitive to frost. In planting experiments in Ethiopia, Albizia gummifera showed a survival rate of 94%. Young planted trees can be managed by coppicing and lopping (http://www.worldagroforestry.org).

Carissa edulis from the Apocynaceae family is a spiny, much branched, small tree, shrub or scrambler, up to 5 m in height, with a milky sap. Bark grey, smooth, young branchlets with or without hairs; spines simple, straight, 2-5 cm long, usually single. Leaves ovate to ovate-elliptic, opposite, occasionally almost circular, 2.5-6 x 1.8-3 cm, leathery, dark green above, paler green below, with or without short, soft hairs; lateral veins obscure; apex tapering, often with a bristlelike tip; base rounded to shallowly lobed; margin entire; petiole 1-4 mm long. The name Carissa is probably derived from the Sanskrit ‘corissa’, a name for one of the Indian species of the genus. The specific name, edulis, means edible. Wide spread in many parts of Africa. It grows at forest edges, in forests and woodlands where Euphorbia, Acacia, and Croton commonly occur, especially on rocky hillsides, on clay soils, especially black cotton soils, in dry and moist low- and midlands (1500-2500 m) (http://www.worldagroforestry.org).

Ficus sycomorus grouped under family Moraceae, is a large, semi-deciduous spreading savannah tree, up to 21 (max. 46) m, occasionally buttressed. Leaves broadly (ob)ovate or elliptic, base (sub)cordate, apex rounded or obtuse, margin entire or slightly repand -dentate, 2.5-13 (max. 21) x 2-10 (max. 16) cm, scabrous above, petiole 1-5 cm, 5-7 pairs of yellow lateral veins, lowest pair originating at the leaf base. Ficus is the Latin for fig, derived from the Persian ‘fica’. In Greek ‘syka’ means fig. The species name comes from the Greek ‘sykamorea’ (sycamore), used in the Gospel according to St. Luke; it was such a tree that Jesus cursed because it was barren. But the word ‘sykomorom’ had been used to denote the fruit a century before Christ. Altitude: 0-

2000 m, mean annual temperature: 0-40 deg. C, Mean annual rainfall: 500-1800 (max. 2200) mm Soil type: Prefers deep, well-drained loam to clay soil rich in nutrients. Sandy soils with a shallow groundwater level may also be suitable (http://www.worldagroforestry.org).

Millettia ferruginea is a small tree up to 13 m tall, DBH 35 cm. Young stems brownish pubescent. Leaflets 15-19, oblong, 8-9 cm long x 2-3 cm wide glabrous, except on the margins and midribs; leaf base asymmetric, tip acuminate. Birbira, *Milletia ferruginea* (Hochst), is an indigenous plant species found only in Ethiopia. It is endemic to Ethiopia and widely distributed in the country. It occurs generally between 1000-2500 m above sea level and in the region where water is easily accessible such as streams or in rain forests. (http://www.worldagroforestry.org).

Prunus africana is an evergreen tree, 10-24 (36 max.) m in height, with a stem diameter of 1 m; bark blackish-brown and rugged; branchlets dotted with breathing spots, brown and corky; twigs knobbly. Heavy, shining foliage composed of alternate, simple leaves, oval or lance shaped, 5-15 x 2-6 cm; shiny deep green on the top side, duller and lighter underside; conspicuous veins and a distinct midrib prominent on the underside, sometimes widely tapering at both ends and sometimes with a long drawn-out point, or with a round apex; margin finely toothed or untoothed; petiole 2 cm long, pink or red. Crushed leaves have a bitter-almond smell. Prunus comes from the classical Latin name of the plum tree, from the Greek ‘prunos’ (plum). The specific name means ‘of Africa’ (http://www.worldagroforestry.org).

Syzygium guineense is a leafy forest tree of the *Myrtaceae* family, found in many parts of Africa both wild and domesticated. It is a medium-sized or tall evergreen tree, 15-30 m high. Branchlets sometimes drooping. Crown rounded and heavy; stems thick and angular. Leaves narrow at both ends, length 5-17.5 cm, width 1.3-7.5 cm; simple, opposite, elliptic, lanceolate or ovate-elliptic; with margins that are untoothed and sometimes slightly wavy and rolled inward; apex obtuse to acuminate and rounded, occasionally notched; base cuneate; stalk short and grooved; midrib sunken on top, raised below, with many fine, lateral veins; glabrous, grey-green, tough, shiny; fragrant when crushed. ‘Syzygium’ is derived from the Greek ‘syzygios’ (‘paired’), on account of the leaves and twigs that in several species grow at the same point. The specific name means ‘of Guinea’, where the tree was first collected (http://www.worldagroforestry.org).

Ekebergia capensis is an evergreen or semi-deciduous, medium-sized to large tree, 7-20 (max. 35) m tall. Stem swollen at base; may be tall and fluted in forests and much shorter or unfluted in the open; it may also be buttressed. Branching is erect, then spreading and finally drooping, giving a moderately heavy, flattish crown; 2nd year branchlets slender, usually less than 6 mm in diameter, marked by old, circular leaf scars and conspicuously dotted with white lenticels. Leaves compound, 10-36 cm long, 8-18 cm broad, with common midrib, sometimes slightly winged, alternate; stalks jointed to the stem and leaving a scar on falling. The generic name, 'Ekebergia', is in honour of Captain Carl Gustaf Ekeberg (1716-1784); the specific name, 'capensis', means 'of the Cape' (http://www.worldagroforestry.org).

Draceana steudneri is a single or multi-stemmed palm-like tree, up to 15m tall, with dense terminal rosettes of leaves (http://www.zimbabweflora.co).

Table 1. Family, species and local name of the studied plant species

Family Name	**Species Name**	**Local Name(Or, Amh)**
Fabaceae	*Albizia gummifera*	Ambabessa;Sesa
Apocynaceae	*Carissa edulis*	Agamssa; Agam
Agavaceae	*Draceana steudneri*	Lankuso;Chowye,Mota
Moraceae	*Ficus sycomorus*	Odda; warka
Tiliaceae	*Grewia ferruginea*	Kechema;
Fabaceae	*Millettia ferruginea*	Askira;Brbrra
Rosaceae	*Prunus africana*	Miessa;Tqur-'nchet
Anacardiaceae	*Rhus glutinosa*	Tatessa;'Mbs
Myrtaceae	*Syzygium guineense*	Bedessa; Dokuma
Meliaceae	*Ekebergia capensis*	Sembo; Lol

Source: local community, www.worldagroforestry.org
Or, Oromo language; Amh, Amharic

2.3. Nutrient Contents of Feeds and Fodders

2.3.1. Dry matter

Dry matter (DM) is important component of feeds and fodder which is used to determine the chemical composition and nutritive value. It varies greatly with different type of feed and fodders. Therefore, DM content can be the biggest reason for variation in the composition of

feedstuff, on an “as fed basis”. For this reason, the composition of chemical constituent and biological attributes of feeds is shown on DM basis. The basic principle related on the variation in dry matter content and its relationship with digestion has been explained below (Chet and Basanta, 2006).The accumulation of dry matter in fodder plant increases as the maturity advances. In case of fodder, such as maize, accumulates large amount of starch in the grain toward the end of maturation that improves the nutritive value of plants. All dry matter from the various parts of plants is not equally digested by the animal. *Example:* among the different parts in maize plant, stalk and tassel, husk and air shank, cob and silk are least digested by the ruminant animal. The content of indigestible dry matter increases at advance stage of maturity. There is positive relationship between the dry matter accumulation and indigestible dry matter content in the fodder plants (both grasses and leguminous) (Wattiaux, 1994).

2.3.2. Crude protein and Energy

Protein is an important nutrient required to the animals. It needs to be regularly supplied with the feed. Proteins found in the grains are less soluble and more resistant to microbial degradation in the rumen than protein from forages. However, some forage contain tannin that associate with proteins and increase their resistance to ruminal degradation (Chet and Basanta, 2006).

Crude protein and energy content in the fodder leaves and forage are negatively related with the maturity of the plant. Almost similar trend exists to both leguminous and grasses in terms of CP and energy content and maturity of the plant (Schroeder, 1996). As the plants enter the reproduction phase and start flowering, total yield of dry matter continue to increase but the digestibility of fodder and forage decreases. Therefore, the most important factor that influences the nutritive value of fodder leaves, grasses or legume is the stage of maturity. With advancing maturity, the digestible dry matter, crude protein, calcium, phosphors, in the plant decreases while the fiber content increases. Lignin is indigestible and makes the carbohydrate in the fiber less available, which results in a less available energy (Chet and Basanta, 2006)

2.3.3. Crude Fiber, Acid Detergent Fiber and Neutral Detergent Fiber

Acid detergent fiber (ADF) is highly related to digestibility in the animal. Higher ADF content in the feedstuff is related to lower digestibility. Neutral detergent fiber (NDF) is related to

voluntary intake of the feed and the availability of net energy from digestible energy (Stalton, 2003).

Higher NDF content in the feedstuff is related to lower digestibility. As percentage content of ADF, and NDF increases, the quality of feedstuff decreases. A prime grade feed needs to have more than 19% CP, less than 31% ADF and less than 40% NDF; whereas feed is considered to low grade if CP is less than 8%, ADF more than 45% and NDF more than 65 percent. If forage test report has no value for NDF, the following equation can be used to calculate an expected NDF value based on the ADF as follows (Chet and Basanta, 2006):

For legumes, %NDF = 1.3 x %ADF

For grasses (including corn silage), %NDF = 1.7 x %ADF

Acid Detergent Fiber (ADF) is a measure of the amount of fiber that is not soluble in acid detergent. Many nutrition programs use ADF as the measure of the amount of fiber in the diet. The difficulty with setting an ADF requirement is that not all ADF is equal. For example, the ADF of corn silage seems to be worth more than the ADF of alfalfa, so that, for diets with corn silage as the only forage source, 15% ADF may be adequate. However, in diets with alfalfa as the only forage, 19% ADF may be optimal. The industry is demanding more feeds and low cost technology for cheaper milk production. For this reason, the information on nutrient content is necessary for accurately balancing ration. While balancing the ration, CP, ADF, NDF, Ca, and P is used as direct inputs for ruminant animal (Chet and Basanta, 2006).

2.3.4. Short chain fatty acids

Short chain fatty acids such as acetic, propionic, butyric, isobutyric, valeric, isovaleric, 2-methylbutyric, hexanoic and heptanoic acid, are produced in several parts of the gastrointestinal tract by microbial fermentation of dietary fibre. They constitute weak acids, but because the pH of the gastrointestinal tract, with the exception of the stomach, is nearly neutral, 90–99% of SCFAs are present as anions rather than as free acids. In all mammals examined, acetate is the main SCFA produced. Propionate and butyrate are also present in large concentrations, although their amounts can vary considerably with diet. The molar ratios of acetate to propionate to butyrate in mammals vary from approximately 7.5:15:10 to 40:40:20 (Bergman, 1990). An

estimated, 60 – 70% of the energy of the epithelium of the colon is derived from SCFAs, particularly from butyrate (Scheppach *et al*., 1992). Butyrate also possessed other important functions in the intestinal epithelium, such as prevention of certain types of colitis, while acetate increases colonic blood flow and enhances ileal motility (Scheppach, 1994).

SCFAs are the products of the anaerobic microbial fermentation of complex carbohydrates in the forestomach and large intestine. Acetate, propionate and butyrate, the predominant SCFAs, are readily absorbed and assimilated as a nutrient source by the ruminant (Bergman, 1990). Ruminants depend on SCFAs for up to 80% of their maintenance energy requirements (Bergman, 1990). Caecal SCFAs provide, on average, 8.6% of metabolizable energy intake in bovines (Siciliano-Jones and Murphy, 1989). Caecal and colonic fermentation accounts for 8.6 - 16.8% of total SCFAs production in ruminants (Ulyatt *et al*., 1975). In addition to their involvement as the major source of energy, the SCFAs also serve as building blocks for milk synthesis; acetate is a necessary component in the formation of milk fat, while propionate is used for glucose production, which is needed for synthesis of milk sugar (lactose). In ruminants, propionate is also the major substrate of hepatic gluconeogenesis (Herdt, 1988). Thus, effective absorption of SCFAs from the forestomach and large intestine is essential for these species. In addition, the three main SCFAs, acetate, propionate and butyrate stimulate sodium and fluid absorption in the colon and exert proliferative effects on colonocytes (Scheppach, 1994). Lactate is mostly absorbed in the small intestine (Argenzio and Southworth, 1974). In the gut, the abundance of the monocarboxylate transporter (MCT) protein in the colonic luminal membrane declines during transition from normal differentiation and proliferation in the colonic mucosa (Ritzhaupt *et al*., 1998). It has been established in the colon *in vivo* that a reduction in MCT1 expression, and hence butyrate transport, can lead to a reduction in intracellular butyrate levels (Daly *et al*., 2005). Decreased butyrate concentration results in dysfunction in the regulation of genes associated with colonic tissue homeostasis and disease prevention (Daly *et al*., 2005), thus, leading to impairment of animal welfare.

2.4. Estimation of Nutritive Value of Livestock Feeds

Evaluation of feeds should provide nutritionists with the necessary information to formulate a diet from both a physiological and an economical point of view, in order to optimize the animal

performance. The need for precise nutritive characteristics of ruminant feeds should be ever greater in the future owing to (Theodorou *et al*., 1994):

- Advances in molecular biology, particularly the development of transgenic plants;
- Crop improvement programmes;
- Environmental and economic constraints concerning the use and/or disposal of crop by-products and residues.

Laboratory methods to estimate nutritive value of feed have improved since the first ideas in 1725, when ruminant feeds were evaluated as Straw Units (Blaxter, 1986). Initially, the techniques were designed mainly to characterize nutritive value of the foodstuff rather than to predict animal performance. Improvements in the methods of food evaluation have followed new concepts in chemistry, animal physiology, rumen microbiology knowledge and related fields of science (Flatt, 1988).

Feed evaluation needs to define roughage characteristics which determine animal performance, for example live weight gain, milk yield, wool growth, etc (Blümmel *et al*., 1997a). Of particular relevance is the prediction of the animals intake, which is an important aspect related to feeding forages (Minson, 1990). In practice, the prediction of the roughage intake still presents problems (Blümmel and Becker, 1997).

The future development of feed evaluation systems should incorporate new information on the relationship between specific end-products of digestion and performance of the animals, as well as information on animal and microbial metabolism, feed composition, and effects of various factors on feed utilisation (Flatt, 1988). An adequate dietary analysis of any sort requires that the methods employed are relevant to a nutritional classification of the dietary chemical components (Van Soest and Robertson, 1985). The current achievements in this field show a very exciting new horizon in feed evaluations, with particular emphasis on roughages evaluation (Ørskov, 1993a).

In vitro methods for laboratory estimations of degraded feeds are important for ruminant nutritionists. An efficient laboratory method should be reproducible and should correlate well with actually measured *in vivo* parameters. *In vitro* methods have the advantage not only of being

less expensive and less time-consuming, but they allow one to maintain experimental conditions more precisely than do *in vivo* trials. Three major biological digestion techniques are currently available to determine the nutritive value of ruminant feeds:

- Digestion with rumen microorganisms (Tilley and Terry, 1963) or using a gas method (Menke *et al*., 1979),
- *In situ* incubation of samples in nylon bags in the rumen (mehrez and Orskov, 1977), and
- Cell-free fungal cellulase (De Boever, 1986).

These biological methods are more meaningful since microorganisms and enzymes are more sensitive to factors influencing the rate and extent of digestion than are chemical methods (Van soest, 1994).

The association between rumen fermentation and gas production has long been known, in an early attempt in 1939 was tried to record the gas production directly via the rumen cannula in sheep, the technique was too difficult to execute and of poor reproducibility (Blümmel *et al*., 1997). In 1974 Menke and Ehrensvärd (Theodorou *et al*., 1994) studying the stoichiometry of rumen fermentation with a syringe closed system noticed a very reproducible gas production data when the same substrate was incubated in the same quantity in different runs. In the system published by Menke *et al* (1979) the substrate is incubated in a calibrated gas-tight glass syringe fitted with a plunger, the gas produced over 96h period is recorded. The incubation media include rumen liquor and a buffer. The main innovation in such method was that the gas production is recorded rather than degradation. The same postulate continues until today, even when the method had been simplify and improved.

2.5. Secondary Compounds in Tropical Trees

The term secondary compounds is used to describe a group of chemical constituents in plants thought not to be involved in the biochemical processes of plant growth and reproduction (Palmer *et al.*, 1990). These secondary metabolites are thought to have a defensive role that ensures survival of the plant (Coley *et al.*, 1985), by protecting against insect predation or by restricting grazing by hervivores (Swain, 1979). These secondary compounds have been implicated in limiting the utilization of many tropical feed resources, particularly trees and

shrubs. They can inhibit digestion, have toxic effects, inhibit some enzymes and/or metabolic processes, or act as precursors of anti-nutritional compounds (Palo, 1987).

As summarized by Barry and Blaney (1987), secondary compounds can be toxic to animals or cause reduction in their productivity by reducing feed intake. These plant constituents do not affect all herbivores equally; there are examples of plants being toxic for monogastric species but not for ruminants, because the toxin is rendered harmless by the rumen bacteria (Dobson, 1959).

There are more than 1200 classes of secondary compounds. These include among others, polyphenols, cyanogenic glycosides, alkaloids, saponins, steroids, toxic proteins and amino acids, non-protein amino acids, phytohemagglutinins, triterpenes and oxalic acid (Kumar, 1992; Liener, 1980).

2.5.1. Tannin

Tannins are a diverse group of compounds that are related primarily in their ability to complex with proteins (Fahey and Jung, 1990). Thus, tannins are usually defined as water-soluble polyphenolic substances that have high molecular weight and that possess the ability to precipitate proteins. However, the definition of tannins is regularly modified in the light of new findings (Mueller- Harvey and McAllan, 1992). As a consequence, the list of polymers bound by tannins has been extended to include polysaccharides (cellulose, hemicellulose and pectin) and nucleic acids, steroids, alkaloids, and saponins (Haslam, 1986). While this working definition is useful in describing some chemical and physical characteristics of tannins, it is nevertheless vague and could complicate the research on tannins (Ayres *et al*., 1997).

According to their chemical structure and properties, tannins are divided into two main groups: hydrolysable (HT) and condensed tannins (CT) (Athanasiadou *et al,* 2001; Chaichi Semsari *et al.,* 2011; Hassanpour *et al.,* 2011; Maheri-Sis *et al.,* 2011). The characteristics of the two groups are different in molecular weight, structure and produce a different effect on the herbivorous animals especially on ruminant when ingested. According to the chemical structure of HTs (gallotannins and ellagitannins) are molecules which contain a carbohydrate, generally D-glucose, as a central core (Min and Hart, 2003). The hydrolysable groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid (Mangan, 1988; Haslem,

1989). Hydrolysable tannins are usually found in lower concentrations in plants than CTs. Hydrolysable tannins are subdivided into taragallotannins (gallic and quinic acid) and caffetannins (caffeic and quinic acid) (Mangan, 1988). They are hydrolyzed by tanninase enzymes which engage in ester bond hydrolysis. HTs can form compounds such as pyrogallol which is toxic to ruminants. Toxic compounds from more than 20% HT in the diet can cause liver necrosis, kidney damage with proximal tuberal necrosis, lesions associated with hemorrhagic gastroenteritis and high mortality, which were observed in sheep and cattle (Reed, 1995). Hydrolysable tannins can also affect monogastrics by reducing growth rates, protein utilization and causing damage to the mucosa of the digestive tract and increasing the excretion of protein and amino acids.

Condensed tannins (CT or proanthocyanidins), are the most common type of tannins found in forage legumes, trees and stems (Barry and McNabb, 1999). These types of tannins are widely distributed in legume pasture species such as *Lotus corniculatus* and in several kinds of acacia and other plant species (Degen *et al.,* 1995). CTs have a variety of chemical structures affecting their physical and biological properties (Min *et al.,* 2003). They consist of flavanoid units (flavan-3-ol) linked by carbon-carbon bonds. The complexity of CT depends on the flavanoid units which vary among constituents and within sites for interflavan bond formation. The term proanthocyanidins (PAs) is derived from the acid-catalyzed oxidation reaction producing red anthocyanidins upon heating PAs in acidic alcohol solutions. Anthocyanidin pigment is responsible for the colors observed in flowers, leaves, fruits juices and wines. The astringent taste of some leaves, fruits and wines is due to the presence of tannin.

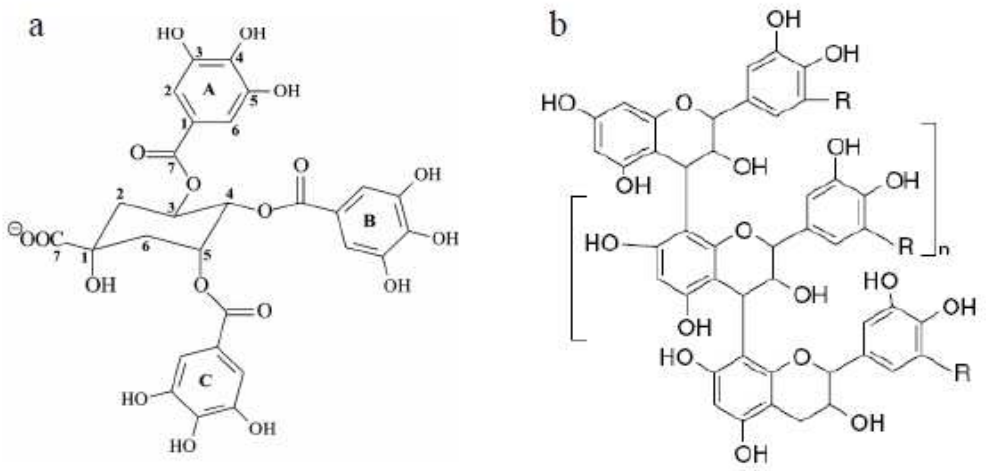

Figure 1. General structure hydrolysable (a) and condensed (b) tannin

Different plant species express different types of tannins (Foo *et al*., 1996; 1997) with different protein-binding activity (Makkar and Becker, 1998). Moreover, plants of the same species but grown in different areas can express different levels of tannins. To give an example, the sulla (*Hedysarum coronarium*) grown in a Mediterranean region contained 1.8% DM of CTs tannins (Priolo *et al*., 2005), while, if grown in New Zealand contained up to 7% DM of CTs (Stienezen *et al*., 1996). Also, the plant growth stage influences tannins expression (Makkar *et al*., 1988)

Tannins are also able to affect the growth and the activity (Min *et al*., 2005; Bossi *et al*., 2007) and to induce morphological changes (Jones *et al*., 1994) in bacteria. This inhibitory effect seems to be due to the interaction between tannins and the proteins inserted into the bacteria cell membrane, thus resulting in an impairment of microbial metabolism (Jones *et al*., 1994; Molan *et al*., 2001). Nevertheless, advances in research have shown that tannin-tolerant bacteria species exist (Brooker *et al*., 1994; Odenyo *et al*., 2001), and that some strains also display tannin hydrolyzing activity (Nelson *et al*., 1995).

When studying tannins in fodders some analytical issues arise. For example, for the quantification of tannins simple and sensitive colorimetric methods are used (Makkar, 1989): Folin-Ciocalteu, Folin-Denis, Prussian Blue for total phenols; vanillin-HCl for catechins; butanol-HCl for proanthocyanidins. However, these methods do not allow to distinguish low

molecular weight phenols from heavier polyphenols of nutritional concern. Alternatively, some assays allow quantifying tannins measuring their protein binding capacity (Makkar *et al*., 1988) in order to evaluate the biological power of tannins. However, Silanikove *et al*. (2005) have pointed out the criticism of the extraction step of tannins from plant material. In the study of these authors different extraction methods of tannins from carob pods were compared (acidic methanol; citrate phosphate buffer, pH 4.7; citrate phosphate buffer + urea either under reflux or not). It has been reported that the extraction yield obtained by the use of refluxed buffer-urea solution was four-fold higher as compared to the use of acidic methanol solution or of buffer-urea solution not refluxed.

2.5.2. Tannin effects on ruminant nutrition

Tannin effects on ruminant nutrition have been studied for several years, and are often seen only in terms of their negative impacts on intake and production: decreased nutrient utilization, particularly protein (Waghorn *et al*., 1994); decreased palatability and consequently the amount of food ingested; decreased digestibility (Silanicove *et al*., 1993, Perevolotsky *et al*., 1993 and Silanicove *et al*., 1996); volatile fatty acids production reduction, and decreased digestibility of organic matter and fiber (Ben Salem *et al*., 1997); damage of kidney and liver (Kumar and Singh, 1984); tissue damage in the rumen, intestine ulceration and morphological changes at the *microvilli* level (Hervás *et al*., 2003). However, besides these anti-nutritional and toxic effects, there is an increasing awareness of tannin's beneficial roles on animal nutrition and health (Crozier *et al*., 2009), namely influences on the cell signaling pathways (Achike *et al*., 2003), anti-oxidative effects (Koleckar *et al*., 2008), and anti-helmintic (Waghom, 1996 and Lisonbee *et al*., 2009) and anti-microbial (Buzzini *et al*., 2008) activities. In ruminants a particularly important positive effect of tannins is dietary protein protection from ruminal microflora attack (Driedger and Hatfield, 1972 and McNabb *et al*., 1996). Due to the binding of tannins to dietary protein, and also to a reduction in the activity of a large proportion of micro flora, there is an increased rate of amino acid absorption in the intestine, which improves the utilization of nitrogen by ruminants (Makkar *et al*., 1995). As well as binding to protein, tannins can also bind to carbohydrates, leading also to a reduction in ruminal gas production (Silanicove *et al*., 1993 and Makkar *et al*., 1995). Due to a combination of these activities tannins can be associated with

improvements in animal growth and productivity and consequentially minimization of effects to the environment.

The presence of high amount of plant secondary metabolites (PSMs) in certain plants may limit their intake below the animal requirements for energy and protein. Consequently herbivores tend to defend themselves by eating a variety of plant species that contain different types and levels of compounds, rather than one sole species. This is due to the fact that each of the different types of PSMs affects the organism in different ways and detoxified by different complex mechanisms. Such behavior may imply a key mechanism for reducing the toxicity associated with a particular type of metabolite (Tilman, 1982). Concerning the presence of tannins in pastures, if animals have diverse plant species available they are able to tolerate it better. It was observed that sheep eat more when offered three foods that contain predominantly terpenes, tannins and oxalates than when offered food with only one or two of such PSMs (Villalba *et al*., 2004). Comparison of intake of shrubs containing only tannins or saponins with the intake of shrubs containing both phytochemicals indicates that goats consume more biomass when fed with shrubs with both classes of compounds (Rogosic *et al*., 2006). This complementary relationship was also referred on cattle fed on tannin, saponins and alkaloids containing fodder (Lyman *et al*., 2010).

2.5.2.1. Relationships between tannin assays and their anti-nutritive properties

Considering the volume of literature on tannin assays, there is comparatively little on the effectiveness of the different assays as indicators of anti-nutritive effects. This is because such trials are difficult to conduct. The nutritive value of tanniferous feeds is due to all its components and isolating the effect of tannins is not straightforward. Tannin-binding agents have been used to inactivate tannins thus giving indications of what effects the tannins are having.

Robbins *et al*. (1987) correlated crude protein content to the proportion of digestible protein for feeds such as grasses and agriculturally produced legumes and grains with very low tannin contents. Using the correlation equation obtained to predict protein digestibility and comparing this to the measured protein digestibility of tanniferous feeds consumed by mule deer, an estimate of the reduction in digestible protein due to tannins was obtained. This was found to be

highly correlated with the activity of the tannins as assayed by a protein precipitation assay. Hanley *et al*. (1992) used predictive equations developed by Robbins *et al*. (1987) to predict successfully the protein and dry matter digestibility of tanniferous forage leaves from seven species and one sample of twigs when fed to black-tailed deer, confirming that a protein precipitation assay gave a useful estimate of the effect of tannins *in vivo*.

McKey *et al*. (1978) have also found that total phenols (by the Folin Denis assay) and extractable condensed tannins (by acid butanol) correlated negatively with the dry matter digested by rumen inoculum during 96 hours incubation. Data were quoted for 30 species of trees from which samples of mature leaves had been taken.

For 72 West African fodder trees and shrubs, Rittner and Reed (1992) found that in vitro protein degradability was negatively correlated with total phenols (by ytterbium precipitation assay) and extractable condensed tannins (by acid butanol). However, the behavior of some species deviated greatly from that indicated by the correlation studies. Wood and Plumb (1995) found strong correlations between total phenols (Prussian blue assay) and the inhibition by tannins from Bolivian fodder tree leaves of in vitro fermentation (gas production) by rumen microbes. Similar correlations were found using protein precipitation by the radial diffusion assay, but there was no significant correlation with the acid butanol assay.

Other workers have had less success in finding correlations. Makkar *et al*. (1989) were unable to find significant correlations between total phenols (Folin Denis assay), condensed tannins (by the vanillin assay) and protein precipitation with *insacco* dry matter loss for leaves of 10 species of trees from India. Khazaal and 0rskov (1994) found that the increase in gas production resulting from treating eleven leaf samples with the tannin binding agent PVPP was not related to total phenols (by Folin Denis and by a gravimetric assay), extractable condensed tannins (by acid butanol and vanillin assays) and total CTs (by acid butanol assay). Mole and Waterman (1987) found little correlation between chemical assays for total phenols (by the Folin method), CTs (by the vanillin method), HTs (by various methods) and biochemical activities as assayed by a protein precipitation method and cellulase inhibition.

2.5.2.2. Tannins and by-pass protein

Protein that is slowly degradable in the rumen may provide amino acids and peptides for microbial growth in addition to providing by-pass protein. Tannins are known to protect dietary protein against microbial attack in the rumen. Thus if a freshly harvested tropical legume given as a supplement is to provide by-pass protein then it should be selected for a relative high content of tannins, even though this may depress fibre digestibility. The benefits of including by-pass protein in the diet have been widely documented (Preston and Leng, 1987).

The tannin-protein complexes have maximum stability in the pH range 4-7. Above and below this pH range the complex is readily dissociated. In this way the tannin-protein complex, after escaping from the rumen fermentation (about pH 5-7), would be digested readily by the enzymes in the gastric (about pH 2.5) and pancreatic (about pH 8-9) secretions (Palo, 1987).

2.6. Potential Use of Tanniferous Feedstuffs

Numerous multipurpose browse trees and shrubs have been identified as having significant potential in agroforestory systems in tropical and Mediterranean-type regions (FAO, 1992; Topps, 1992). In many parts of the world, wide ranges of shrub species contribute extensively to livestock feed, mainly for sheep and goats, and contain in most cases a high level of proteins. Admittedly, protein is the most limiting nutrient in the diet of livestock, and which, because of its limited supply, should be used more efficiently to promote fermentation of roughage in the rumen to improve animal performance. Tannins are the most widely occurring anti-nutritional factors found in plants. These compounds are present in numerous tree and shrub foliages, seeds and agro-industrial by-products (Aregheore, Makkar and Becker, 1998; Makkar and Becker, 1998, 1999). The availability and usage of various unconventional feed resources, including new and lesser known ones, the presence in them of deleterious factors, and approaches to remove or inactivate these factors have been dealt with in several reviews (Kumar and Vaithiyanathan, 1990; Makkar and Becker, 1999; Makkar, 2002). Phenolic compounds particularly tannins and lignin were shown to reduce the nutritive value of feedstuffs and thereby to limit livestock production and reproductive performance (Waghorn, Red and Ndlovu, 1999). Tannins are classified into two groups: hydrolysable tannins (HTs) and condensed tannins (CTs). Both types

are hydrosoluble polymers that form soluble and insoluble complexes, mainly with proteins. They also have some affinity towards carbohydrates, amino acids and minerals. HTs, in contrast to CTs, are partly degraded in the gastro-intestinal tract and several precursors may derive from their glucose and phenolic constituents. Untransformed HTs and their phenolic degradation products may be absorbed from the gastro-intestinal tract, resulting in animal intoxication (Waghorn, Red and Ndlovu, 1999; Makkar, 2002). However, most researchers agree that CTs are not degraded in the animal gut and therefore are not toxic (Makkar, Blümmel and Becker, 1995; Makkar, 2000) under normal feeding situations when browse and tree foliage is used as a supplement. At very high level of browse and tree foliage intake, the intestinal wall may become damaged, leading to absorption of CTs and causing toxicity. However, CTs directly affect the nutritive value of feedstuffs. Until a few years ago, CTs were regarded as useless compounds with only negative effects on intake, digestion, production and reproduction in animals. Recent studies have confirmed that CTs may also have positive effects in ruminants (Barry and McNabb, 1999; Barry, McNeill and McNabb, 2001). Low CT levels in several plant species, e.g. *Acacia albida* pods (Nsahlai, Umunna and Osuji, 1999), *Lotus pedunculatus* (Barry, Manley and Duncan, 1986) and *Acacia cyanophylla* Lindl. (syn. *Acacia saligna*) foliage (Ben Salem, Nefzaoui1 and Makkar, ND) increased daily gain in sheep given protein-rich diets. This effect was ascribed to increased levels of post-ruminally available proteins.

2.7. Techniques to Deactivate Tannins

Various methods have been attempted to de-activate tannins in a wide range of browse species, grain seeds and agro-industrial by-products (Makkar, 2000). These methods have included mechanical or physical techniques (e.g. wilting, processing, ensiling, etc.), inoculation with tanninresistant bacteria (Miller *et al*., 1995; Molina *et al*., 1999) and chemical techniques (treatment with alkalis, organic solvents, precipitants, etc.).

The use of Tannin binding agent for which tannins have higher affinity than for proteins, is by far the most used reagent to neutralize these secondary compounds (Makkar *et al*., 1995; Silanikove *et al*., 2001). Consequently, it would be possible to increase the nutritive value of tannin rich plants by adding compounds such as tannin binding agent, which preferentially binds

the tannins, making plant proteins more available for digestion. This strategy is very useful in situations where foodstuffs contain high concentrations of tannins. Different means of administering Tannin binding agent have been used in the literature to assess the fodder potential of tanniferous plant species. Tannin binding agent was included either in concentrate supplement (Ben Salem *et al*., 1999a; Decandia *et al*., 2000), dissolved in drinking water (Ben Salem *et al*., 1999a), infused orally (Wang *et al*., 1996; Gilboa *et al*., 2000) or sprayed in solution on browse foliage (Ben Salem *et al*., 1999b) given to ruminant animals. The response to tannin binding agent supply in terms of intake, digestion and production varied with the mode of tannin binding agent application.

Artificial polymer such as water-soluble Polyvinyl Pyrrolidone (PVP), water in-soluble polyvinyl polypyrrolidone (PVPP), and water soluble polyethylene glycol (PEG) contain a large number of oxygen atoms capable of forming hydrogen bond with the phenolics groups in tannin and to precipitate them from solution (Jone, 1965). This property of tannin binding agents has been exploited for separation of plant metabolites in tannin rich environments (Jone, 1965).

2.8. Ruminal Fermentation

The reduction of ruminal protein degradation may be the most significant and well-known effect of tannins (McLeod, 1974; Mangan, 1988; Hagerman *et al*., 1992; Mueller-Harvey and McAllan, 1992). The affinity of tannins for these molecules is very great, and the pH of the ruminal medium favours the formation of tannin-protein complexes. In general, this reduction in protein degradation is associated with a lower production of ammonia nitrogen and a greater non-ammonia nitrogen flow to the duodenum (Barry and Manley, 1984; Waghorn *et al*., 1994b; Waghorn, 1996).

The effect of tannins on protein degradation is basically a reduction in the immediately degradable fraction, and a reduction of the fractional rate of degradation (Aharoni *et al*., 1998; Frutos *et al*., 2000; Hervás *et al*., 2000).

Though tannins mainly exert their effects on fermentation of proteins, they also have effects on carbohydrates, particularly hemicellulose, cellulose, starch and pectins (Barry and Manley, 1984;

Chiquette *et al*., 1988; Leinmüller *et al*., 1991; Schofield *et al*., 2001). For a long time, the effect of tannins on the degradation of fibre was seen as a secondary anti-nutritional effect. However, several studies have shown that fibre degradation in the rumen can be drastically reduced in animals that consume tannin-rich feeds (Barry and McNabb, 1999; McSweeney *et al*., 2001; Hervás *et al*., 2003a).

The mechanisms by which tannins reduce ruminal degradation of different dietary components are not entirely clear. Among the most accepted are substrate privation (Scalbert, 1991; McAllister *et al*., 1994b; McMahon *et al*., 2000), enzyme inhibition (Barry and Manley, 1984; Bae *et al*., 1993; Jones *et al*., 1994) and direct action on rumen microorganisms (Leinmüller *et al*., 1991; Scalbert, 1991). With respect to the first of these, several authors have reported that tannins prevent or at least interfere with the attachment of rumen microorganisms to plant cell walls, and it is well known that such attachment is essential for degradation to occur (Chiquette *et al*., 1988; McAllister *et al*., 1994a). Further, the formation of complexes with proteins and carbohydrates renders these nutrients inaccessible to microorganisms (Mangan, 1988; Mueller-Harvey and McAllan, 1992). Tannins are also chelating agents, and this could reduce the availability of certain metallic ions necessary for the metabolism of rumen microorganisms (Scalbert, 1991).

With respect to enzyme inhibition, tannins can react with microbial (both bacterial and fungal) enzymes, inhibiting their activity (Makkar *et al*., 1988; Mueller- Harvey and McAllan, 1992; McAllister *et al*., 1994b; McSweeney *et al*., 2001). Several authors (Leinmüller *et al*., 1991; O'Donovan and Brooker, 2001) indicate that tannins alter the activity of bacterial proteolytic, cellulolytic and other enzymes, but it is important to point out that the binding of tannins to enzymes – whether bacterial or endogenous does not necessarily imply their inhibition (Makkar *et al*., 1988). With respect to fibrolytic enzymes, CT more easily inhibits the activity of hemicellulases than cellulases (Waghorn, 1996). This is possibly due to the fact that the latter are associated with bacterial cell walls while the hemicellulases are extracellular and therefore more sensitive (Van Soest, 1994). This would explain why the majority of researchers report a greater reduction in the degradability of hemicellulose in the presence of tannins (Barry and Manley,

1984; Waghorn *et al*., 1994a; Hervás *et al*., 2003a). However, this can vary depending on the tannin in question (McAllister *et al*., 1994a).

Finally, tannins might have a direct effect on ruminal microorganisms, e.g., by altering the permeability of their membranes (Leinmüller *et al*., 1991; Scalbert, 1991). Nonetheless, some rumen microorganisms can tolerate tannins (Nelson *et al*., 1998; O'Donovan and Brooker, 2001). The degree of tolerance is specific to the microorganism in question, explaining the different susceptibility of bacterial strains. It also depends on the tannin, and the differences between HT and CT in this respect are notorious. Though few tolerant rumen microorganisms have been described, it is very likely that their true diversity is much greater than currently known (McSweeney *et al*., 2001).

Several species of the ruminal microbiota respond to the presence of tannins by changing their morphology (Bae *et al*., 1993; Jones *et al*., 1994; McAllister *et al*., 1994a). Chiquette *et al*. (1988) observed a thick glycocalyx on ruminal bacterial walls in response to high levels of CT from *L. corniculatus*, which did not occur when the concentration of the same compounds was lower. This phenomenon is similar to the secretion of glycoproteins in the saliva (Scalbert, 1991) for neutralizing the action of tannins.

With respect to the inhibition of enzyme activity, apart from different sensitivities at different concentrations (Jones *et al*., 1994). O'Donovan and Brooker (2001) indicate that proteolytic bacteria, which are initially sensitive to tannins, can, after a short period of adaptation, respond by modifying their metabolism. This is only one example of how ruminal bacteria with proteolytic and cellulolytic activity can continue to function when tannin levels are not too high (Jones *et al*., 1994).

Several microbial enzymes have been identified which can metabolise tannins (O'Donovan and Broker, 2001), especially HT. The degradation of CT via the cleavage of carbon-carbon bonds has not been demonstrated even *in vitro*, and it seems very unlikely that such an event could occur in the anaerobic environment of the rumen (McSweeney *et al*., 2001). Among the bacteria

able to use HT are *Streptococcus caprinus* (*S. gallolyticus*), which produces pyrogallol (a product of tannic acid degradation) when gallate decarboxylase activity increases (O'Donovan and Brooker, 2001).

2.9. Kinetics of Fermentation

In assessing nutritive value, the rate at which a feed or its chemical constituents are digested in the rumen is as important as the extent of digestion. The pattern of feed fermentation (kinetics of fermentation) is one of several factors that influence voluntary feed intake by ruminants. The rate at which different chemical constituents are fermented is a reflection of microbial growth and accessibility of the feed to microbial enzymes. By describing gas production mathematically, kinetic data can be analyzed to evaluate substrate- and media-related differences as well as the fermentability of soluble and slowly fermentable components of feeds. The gas method is an ideal technique to generate kinetics of fermentation, as it allows recording of gas produced at several times in the incubation period, which is used to predict the rate at which feed is digested (Getachew *et al.* 2004).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The chemical composition study including polyphenolic components was carried out in Animal Nutrition Laboratory of Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) campus which is located in Jimma city, south western Ethiopia. JUCAVM is situated at 7°40′N and 36°50′E latitude and longitude, respectively at an altitude of 1780 meters above sea level (http://en.wikipedia.org/wiki/Jimma). The climate of the area is characterized as semi-humid tropical with bimodal heavy rainfall which is uniform in amount and distribution, ranging from 1200 to 2800 mm per year. The ten years mean annual minimum and maximum temperature of the area was 11.3°C and 26.2°C, respectively (GOR, 2006). According to Deckers *et al*. (2008), the most common soil types around the study area are nitisols and planosols. Less common are vertisols and ferralsols.

The *in vitro* digestibility and gas production measurement was carried out in Holeta Agricultural Research Center (HARC) animal nutrition laboratory. HARC located in the West Shewa zone of the Oromia National Regional State, Central highland Ethiopia. It has a latitude and longitude of 9° 3′ 0″ N, and 38° 30′ 0″ E, respectively. The centre is located at an average altitude of 2391masl (http://en.wikipedia.org/wiki/Holeta_Genet).

3.2. Sampling Leaves of the Plant Species

Leaves of 10 browse plants species, widely known and highly preferred by the livestock farmers and have multiple uses (Yisehak *et al*., 2010), were sampled from Waktola Kebele (7°71’ N–37°04’ E, 1718 masl) of Omo Nada district, Jimma administrative zone, south west Ethiopia in the mid of autumn, 2011. Young but not old and immature leaves of plant species were collected during flowering stage. According to respondent farmers, the age of mother tree plants is about 5 years. The materials were transported to the laboratory in fresh state. The fresh material were kept on ice in ice box and transported under dark conditions. All samples were put in air tight containers and stored in dark cabinets at room temperatures until analyses. The plant species studied were *Albizia gummifera, Carissa edulis, Draceana steudneri, Ficus sycomorus, Grewia ferruginea, Millettia ferruginea, Prunus africana, Rhus glutinosa, Syzygium guineense,* and *Ekebergia*

capensis. Six individual plants per species were sampled and analyzed individually in order to have some measure for the intra-species variation and to perform statistical analyses in factorial procedure. Based on FAO/IAEA (2000) recommendations, immediately after arrival to laboratory, the samples were dried to constant weight at about 55°C using a forced air oven and ground at 1mm screen for all analysis.

3.3. Chemical Analysis

Dry matter was determined by drying the leaf samples at 105°C overnight and ash by igniting the samples in muffle furnace at 550 °C for 8 h. Content of nitrogen (N) was measured by the Kjeldhal method (AOAC, 2003). The crude protein (CP) was calculated as N x 6.25. Contents of neutral detergent fiber (NDF) and acid detergent fibre (ADF) of leave samples were determined by the method of Van Soest *et al* (1991). Concentrations of chemical parameters were expressed in g/kg DM, whereas digestible CHO, CP and EE was calculated using equations from Hveplund *et al*. (1995). The total carbohydrate (%CHO) was estimated according to Ranjhan (1997): %total CHO = 100-(% CP + %EE + %Ash+ % lignin). The content of total digestible nutrient (TDN) per kg and per kg DM of a feedstuff was calculated as follows: TDN = digestible crude protein (DCP) + 2.25* digestible ether extracts (DEE) + digestible carbohydrates (DCHO). Nitrogen free extract was calculated according to McDonald *et al*. (2002). Tilley and Terry (1963) two-stage procedure was used for IVDMD and IVOMD.

Samples were also analyzed for total phenolics (TP), total tannins (TT) and condensed tannins (CT). Dried plant material (200 mg) was extracted with acetone: water (10ml, 70:30 v/v) in an ultrasonic bath for 20 minutes. The contents were centrifuged (4°C, 10 min, 3000 rpm) and the supernatant was kept on ice until analysis (FAO/IAEA, 2000). TP (tannin phenolics and non-tannin phenolics) and TT were determined with the Folin-Ciocalteau reagent. Insoluble matrix, polyvinyl polypyrrolidone (PVPP; as this polymer binds strongly to tannins-phenolics) was used to separate tannin phenolics from non-tannin phenolics. A calibration curve was prepared using tannic acid standard. TP was calculated as tannic acid equivalent and expressed as tannic acid eq-g/kg DM. The absorbance was read at 725 nm using Spectrophotometer (Makkar *et al*., 1993). CT was measured by the Butanol-HCl-Fe method and the results were expressed as leucocyanidin equivalent (Porter *et al*., 1986). In soluble NDF-bound proanthocyanidin is

expressed as Abs_{550} absorbance units (1 cm pathlength) per gram of NDF as described by Reed *et al*. (1982).

3.4. *In vitro* Gas Production

Rumen fluid was obtained from three rumen-cannulated Boran (*Bos indicus*) x Holstein Friesian (*Bos Taurus*) crossbred bulls fed twice daily with a diet containing pasture hay (60%), and concentrate mixture (40%) and free access to water and mineral/vitamin licks. Rumen digesta was pooled together in order to achieve a homogenous inocula. A sample of rumen liquid was collected before the morning meal in pre-warmed thermos flasks and transported immediately to the adjoining laboratory where it was filtered through three layers of cheese cloth, mixed and stirred with a buffered mineral solution ($NaHCO_3$ + Na_2HPO_4 + KCl + NaCl +$MgSO_4.7H_2O$ +$CaCl_2.2H_2O$) under continuous flushing with carbon dioxide.

The preparation of buffer solutions and rumen inocula was as described by Theodorou *et al.* (1994). DM (1g) was weighed in triplicate into serum bottles kept at approximately 39ºC and flushed with CO_2 before use. Three bottles were used for each substrate with or without tannin-binding agents for one run. All the bottles were closed with rubbers stoppers, crimp sealed with aluminum capsule and incubated in an incubator set at 39°C, being shaken at regular times. The volume of gas produced in each bottle was recorded at different incubation times 2, 6, 8, 12, 24, 32, 48, 72 and 96 hours post-incubation using a pressure transducer (Theodorou *et al.*, 1994). In order to compensate for gas production in the absence of substrate and tannin binding agent containing buffer media and rumen fluid inoculum were incubated as blank. Incubations were performed in duplicated run and triplicate within the runs. Each of the tannin-binding agents (TBA) was dissolved in the buffer solution to give a concentration of 0, 0.5 and 1g TBA per gram DM of sample. Total gas values were corrected for blank and hay standards with known gas production.

The kinetics of gas production, and cumulative gas production data were fitted to the exponential model of Ørskov and McDonald, (1979):

$p = a + b\ (1-e^{-ct})$

Where:

p represents gas volume (ml) produced at time t, (a+b),

a the gas produced from soluble fraction (ml)/ initial gas produced/the intercept,

b the gas produced from insoluble but fermentable/degradable fraction (ml),

(*a+b)* the potential extent of gas production (ml), and

c the gas production rate constant for 'b' (ml/h)

t incubation time

3.5. Organic matter digestibility, Metabolizable Energy Value and Short Chain Fatty Acids

The organic matter digestibility (OMD), metabolizable energy (ME) value and the short chain fatty acids (SCFA) contents of treatments were estimated with the following equations:

OMD (%) = 0.9991 (GP) + 0.0595 (CP) + 0.0181 (CA) + 9 (Menke and Steingass, 1988);

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP (Makkar and Becker, 1996) and

SCFA (mmol) = 0.0222 (GP) – 0.00425 (Makkar, 2005),

Where, GP is gas production at 24 h of incubation, CP, EE and CA are crude protein, ether extract and crude ash content of diets, respectively.

3.6. Statistical Analysis

For the gas production parameters and their derivates, a variance analysis model with three fixed factors was used: plant species (P; 10 levels), tannin binding agents (T; 4 levels) and dosage of tannin binding agents (D; 3 levels). The linear model used was:

$$\mathbf{Y_{ijk} = \mu + P_i + T_j + D_k + (P\times T)_{ij} + (P\times D)_{ik} + (T\times D)_{jk} + \varepsilon_{ijk}}$$

where, Y_{ijk} is the observation of the i_{th} plant species, j_{th} tannin binding agent and k_{th} doses of the tannin binding agent; μ, the population mean, P_i, i_{th} plant species effect (i = 1 to 10); T_j, the j_{th} effect of tannin binding agents (j=1 to 4); D_k, the k_{th} effect of dose of the tannin binding agents; $(P\times T)_{ij}$, is the ij_{th} interaction effect between plant species and tannin binding agent; $(P\times D)_{ik}$, the ik_{th} interaction effect between plant species and doses of tannin binding agents; $(T\times D)_{jk}$, jk_{th} interaction effect between tannin binding agents and doses of the agents and ε_{jk}, the residual error.

For all other parameters, the model $Y_{ij} = \mu + P_i + \varepsilon_j$ was used for plant species evaluated without tannin binding agents. Where Y_{ijk} is the observation of the i_{th} plant species; μ, the population mean, P_i, i_{th} plant species effect (i = 1 to 10) and ε_{jk}, the residual error.

The non-linear fermentation parameters such *a*, *b* and *c* were estimated for each plant species and substrate combination using nonlinear (NLIN) procedures of SAS. Multiple run values were averaged before being analyzed and not included in the statistical model. All analyses were performed with SAS (2010 version 9.3). Differences between means were tested using Tukey test with significance declared at $P<0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance. A Pearson correlation analysis was used to establish the relationship between chemical composition and *in vitro* gas production parameters.

4. RESULTS AND DISCUSSION

This chapter deals with result and discussion of the studied plant species. In this chapter the chemical composition, in vitro gas production potential and estimated parameter of the studied plant species were presented and discussed.

4.1. Nutritive Value of the Studied Plant Species

The chemical concentration, *in vitro* digestibility and estimated nutritive value parameters of plant species are presented in Table 1. Concentration of polyphenolics varied widely among the plant species ($P<0.001$). The ash content (g/kg DM) ranged from 28 and 112. Highest mean values of CP (299 g/kg DM) was recorded for *Albizia gummifera* than the other species ($P<0.001$). There was significant difference in concentration of fiber NDF and ADF among the species ($P>0.001$). The highest IVOMD values were recorded for *Draceana steudneri* (667 g/kg*)* and *Prunus africana* (634 g/kg) as compared to the rest of plant species ($P<0.001$). *Ekebergia capensis* had the highest metabolisable energy (15.10 MJ/kg), which was significantly different to all other species ($P<0.001$). The lowest mean CT contents was determined in *Grewia ferruginea* (55 g/kg DM), whereas the highest levels were observed in *C. edulis* (175 g/kg DM, leucocyanidin tannin equivalent). *Carissa edulis* and *Grewia ferruginea* had the highest and lowest total extractable phenolic concentration, 288 and 69 g/kg DM, respectively ($P<0.001$). The NDF-bound proanthocyanidin content varied between the highest 24 AU/g (*A. gummifera*) and the lowest 7 and 8 AU/g (*G. ferruginea* and *D. steudneri*, respectively). CT was negatively correlated with IVOMD ($r=-0.692$), IVDMD ($r=-0.686$) and TDN ($r=-0.562$) ($P<0.01$).

Table 2. Effect of browse species on nutritive value of leaves of tannin-rich trees species (n=6)

Nutrients	Albizia *gummifera*	*Carissa edulis*	*Draceana steudneri*	*Ficus sycomorus*	*Grewia ferruginea*	*Millettia ferruginea*	*Prunus africana*	*Rhus glutinosa*	*Syzygium guineense*	*Ekebergia capensis*	SE	P
DM	932^{bc}	905^{d}	921^{dc}	939^{ab}	942^{ab}	951^{a}	908^{d}	932^{bc}	913^{d}	941^{ab}	4	***
CA	81^{g}	92^{d}	103^{b}	112^{a}	97^{c}	88^{e}	97^{c}	83^{gf}	84^{f}	28^{h}	4	***
CP	299^{a}	138^{f}	218^{d}	173^{e}	229^{c}	242^{b}	139^{f}	145^{e}	131^{g}	144^{f}	10	***
DCP	20^{b}	14d^{e}	21^{b}	17^{c}	21^{b}	23^{a}	11^{g}	15^{d}	12^{f}	13^{e}	0.6	***
NDF$_{-CP}$	16^{a}	10^{g}	13^{d}	12^{e}	14^{c}	15^{b}	11^{f}	11^{f}	10g	11^{f}	0.5	***
EE	45^{b}	40^{dc}	24^{g}	21^{h}	29^{f}	32^{e}	48^{a}	41^{c}	39^{d}	15^{i}	2	***
DEE	4.5^{b}	4.3^{b}	2.5^{d}	2.2^{d}	3.2^{c}	3.1^{c}	5.1^{a}	4.3^{b}	4.1^{b}	1.5^{e}	0.2	***
NDF	394^{bc}	370^{bc}	380^{bc}	416^{bc}	350^{bc}	415^{bc}	452^{ab}	408^{bc}	554^{a}	323^{c}	19	**
ADF	283^{c}	287^{c}	328^{bc}	348^{bc}	317^{bc}	380^{b}	331^{bc}	330^{bc}	465^{a}	192^{d}	16	***
ADL	121^{ab}	84^{c}	107^{bc}	73^{c}	110^{bc}	148^{a}	101^{bc}	80^{c}	127^{ab}	78^{c}	7	***
IVDMD	348^{e}	355^{e}	649^{a}	424^{c}	423^{d}	491^{c}	621^{b}	318^{f}	360^{e}	431^{d}	9	***
IVOMD	399^{d}	394^{d}	667^{a}	470^{c}	541^{b}	508^{bc}	634^{a}	363^{d}	388^{d}	513^{bc}	20	***
CHO	553^{e}	662^{c}	544^{f}	614^{d}	553^{e}	493^{g}	678^{b}	676^{b}	659c	736^{a}	1	***
DCHO	399^{g}	397^{h}	669^{b}	466^{f}	530^{d}	509^{e}	649^{c}	358^{j}	388^{i}	963a	23	***
ME	6.50^{g}	6.20^{g}	11.5^{b}	7.08^{f}	9.20^{d}	8.03^{e}	10.25^{c}	6.50^{g}	6.10g	15.10^{a}	20	***
TDN	403^{g}	398^{g}	685^{b}	467^{f}	533^{d}	513^{e}	650^{c}	358^{i}	388^{h}	964^{a}	14	***
CT	79^{d}	175^{b}	68^{e}	109^{e}	55^{f}	74de	76de	171^{b}	166^{c}	82^{d}	33	***
TT	84^{f}	204^{a}	72^{h}	113^{c}	55^{i}	75^{g}	109^{e}	191^{a}	174^{b}	120^{d}	9	***
TP	118^{g}	288^{a}	101^{i}	154^{f}	69^{j}	105^{h}	157^{e}	240^{b}	236^{c}	171^{d}	13	***
NDF$_{-bPA}$	24^{a}	12^{e}	8^{g}	10^{f}	7^{h}	22^{b}	10^{f}	13^{d}	15^{c}	11^{e}	0.4	***

DM, dry matter ; CA, crude ash ; CP, crude protein ; EE, ether extract; NDF, neutral detergent fibre assayed without a heat stable amylase and expressed inclusive of residual ash ; ADF, acid detergent fibre expressed inclusive residual ash; ADL, lignin determined by solubilisation of cellulose with sulphuric acid ; IVDMD, in vitro dry matter digestibility; IVOMD, in vitro organic matter digestibility; ME, metabolisable energy; CHO, total carbohydrates; DCHO, digestible carbohydrate; DCP, digestible crude protein; DEE, digestible ether extract; TDN, total digestible nutrients; CT, total extractable condensed tannin, TT, Total extractable tannin; TP, total extractable phenols; SE, standard errors of means; $^{a, b, c, d, e, f,g,h,i}$ *Means with different superscripts within the same row are significantly different (P<0.05);NDF*$_{-bPA}$, NDF-bound Proanthocyanidin (AU/g);***P<0.01;***P<0.001*

DM content of the plants ranged between 912 to 946 g/kg considered acceptable. Generally DM content of more than 85% in feed ingredients is considered to be suitable for optimal storage of feeds (McDonald *et al*., 2002; AOAC, 2005). The DM values recorded for all the plant parts were however comparable with those previously obtained elsewhere for browse plants (Arigbede, 1998; Topps, 1992).

Crude ash content of plant species has ranged between 28 and 112 g/kg DM. Typical ash content of forages and feeds are in the range of 79 to 126 g/kg DM Topps (1993) and Carlos *et al*., (2005), so most values in the current study which are presented in Table 2 are within the range except *E. capensis* (28g/kg DM) than ash values reported by. The result is comparable with values of 100, 91 and 81 g/kg DM reported for *Gliricidia sepium, Erythrina veriegata* and *Leucana leucocephala*, respectively by Rajaguru (1990) and 137 g/kg DM for *L. leucocephala* by Joshi and Singh (1989).

Although the CP content of *A. gummifera* was found to be higher than the wide range of data reported for MPTS in different continents of the world (Abdulrazak *et al*., 1997; Solomon *et al*., 2004; Kindu *et al*., 2006; Mekoya *et al*., 2008), the chemical composition of other plants in the present study were within the wide range of data reported for legume and non-legume browses (Balongun *et al*., 1998). The CP value of the MPTS species of the present study was higher than 13 non-leguminous browse species studied in Nigeria (Okoli *et al*., 2003). Ghanaian browse plants (presumably non-leguminous) had CP levels of 180 and 260 g/kg (Apori *et al*., 1998). The high CP content in the foliage of *A. gummifera* and *M. ferruginea* could be due to the N-fixing ability of the species. The CP content calculated for all the leaves of the MPTS is much higher than the minimum CP level (70 g CP/kg DM) required for optimum functioning of rumen (McDonald *et al*., 2002) and for adequate intake of forages (Milford and Minson (1966). The CP content of all plants species in the current study was far above the 60 g/kg DM level recommended as minimum daily requirement of ruminant animals from tropical feeds (NRC, 1984). The optimum concentration level of rumen bacteria is reached at a CP level in the diet of 130g CP/kg (=85g DCP/kg). The minimum CP content required for lactation and growth of cattle is 150 g/kg DM (Norton, 1982). The CP levels of forages correlate negatively with many desired plant components like *in vitro* digestibility and ME energy might be due to presence of considerable content of CT and other fiber fractions. Although most browse species had high CP

levels, tannins, if present, would interfere with the digestibility of the proteins and that a large proportion of the CP could be bound to the cell walls. Consequently, they concluded that a measurement of the CP concentration of topical browse is not a reliable indication of available protein in the leaves.

EE concentration of browse species in the present study is less than values recommended by Preston (1995) in that total diets do not contain EE; more than 100g/kg DM is acceptable. On the contrary, in the reports of Campbell *et al*.(2006) fats and oils are extremely rich sources of energy, although because they impede microbial fermentation, ruminant diets should be limited to about 40 g EE/kg DM.

The threshold level of NDF in tropical grass beyond which DM intake of cattle is affected is 600 g NDF/kg (Meissner *et al*., 1991) suggesting that all the MPTS leaves in the present study have acceptable NDF values (below 600 g NDF/kg DM) indicating that the MPTS are very high in feeding value in terms of NDF content. Tree forages with a low NDF content (200–350 g/kg) are usually of high digestibility (Norton, 1994). The digestibility of plant material in the rumen is related to the proportion and lignification of cell walls (Van Soest, 1994). In general, the browse species analyzed in this study and in the reported investigations had lower levels of NDF than a large variety of grass species quoted by Meissner and Paulsmeier (1995). However, although the detergent extraction techniques are used regularly when analysing foliage from trees and other browse plants (Balogun *et al*., 1998; Kallah *et al*., 2000), Makkar *et al*. (1995) pointed out that these techniques are not suitable for tannin-rich forages.

A wide range of phenolic concentration measured in the present study is consistent with the large range of values of polyphenolics reported for browse species. The range values in this study agrees with those of Kaitho (1997) and (Reed, 1986) for east Africa but higher than those of Ghanian browse species. The probable reasons for this variation in the phenolics concentration would be plant part, stage of maturity, seasonal variation and sampling site. Makkar and Becker (1998) reported relatively higher total phenolics from African forages compared to Himalian forages (157 vs. 60 g/kg DM). The CT contents were highly variable with significant ($P<0.001$) differences among the species. The effect of tannins can be either adverse or beneficial for

animals, depending on the concentration and chemical structure (Makkar, 2003; Min *et al*., 2003). It has been also reported that CT values above 50 g/kg DM can become a serious anti-nutritional factor in plant materials fed to ruminants and are even toxic (Leng, 1997). Barry and Manley (1984) explained that a forage containing more than 50 g CT/kg DM is considered tannin-rich. Higher tannin levels become highly detrimental (Barry, and Manley 1984) as they reduce digestibility of fiber in the rumen (Reed *et al*. 1985) by inhibiting the activity of bacteria (Chesson *et al*., 1982) and anaerobic fungi (Akin and Rigsby, 1985), high levels (> 50 g/kg DM) also lead to reduced intake (Merton and Ehle, 1984 cited by Leng, 1997). Brooker *et al*. (1999) also reported that a livestock consuming tannin-rich diets over 50g CT/kg DM usually develop a negative nitrogen balance and lose weight and body condition unless supplemented with non-protein nitrogen, carbohydrate and minerals.

The IVDMD values in the present study were found to be slightly higher than the values reported by Mekonnin *et al*. (2008) for MPTS species in central highlands of Ethiopia. The higher IVDMD values obtained were possibly associated with relatively the low level of NDF, ADF, and ADL in our samples. The IVDMD range of the plants in the present study (363 to 667 g/kg DM) felt within the range of 360 to 690 g/kg DM in vitro DM digestibility observed for tropical browse plants (Sawe *et al*., 1998). A feed containing over 700 g digestible DM/kg is defined as high quality (Meissner *et al*., 2000). Accordingly all of the MPTS analyzed in this study were below the minimum recommended value for IVDMD. Lower IVDMD (<700 g/kg) of the MPTS might be due to the presence of total CT which inhibited the rumen microbial enzymes (Moore and Jung, 2001; Sultan *et al.*, 2007). In reality, when IVDMD falls below 550 g/kg, physical limitations on the rate of eating, rate of digestion and passage through the gastrointestinal tract mean that intake is restricted and live weight loss is inevitable (SCA, 1990). Alimuddin (2004) also explained that a feed contain 600-700 g DDM/kg, 400-600 g DDM/kg and less than 400 g DDM/kg is considered as moderate, low and very low digestibility. It has been reported that cell wall component, NDF, ADF and lignin, were negatively correlated with IVDMD in tree leaves (Kundu and Sharma, 1988; Perveen, 1998). The negative correlation between IVDMD, IVOMD and NDF, ADF, and ADL in the present study is in line with those of Van Soest 1978, who reported poor relationship of NDF, ADF, and ADL with digestibility. In general, variations in chemical composition and *in vitro* digestibility among different foliages of the MPTS may be

partly due to genotypic factors that control accumulation of plant nutrients. Accumulation of nutrients in plants is a property of species (Minson, 1990) and varies among species and genera (Gurbuz *et al*., 2008).

4.2. *In vitro* Gas Production Potentials

Interspecies variation for *in vitro* gas production characteristics is reported in Table 3. Gas production of all the species increased significantly with the increasing incubation time (P<0.001). In 2 hr incubation time the highest and lowest gas volume was recorded for *E.capensis* (21ml) and *A. gummifera* (1ml), respectively (*P*<0.001). Although interspecies variation is significant for gas production across all the incubation times, the three digits gas volume was recorded only after 12 hours of incubation (P<0.001). However, *S. guanensis* had a three digit number gas value measured after 48h of incubation (P<0.001). At 96 hours of incubation, the highest gas volume was measured for *F. sycomorus* (260 ml), *M. ferrugenia* (237 ml), *R. glutinosa* (233 ml), *E. capensis* (252 ml) and *D. steudneri* (234 ml) whereas the lowest gas values were recorded for *S. guanensis* (157 ml) and *C. edulis* (164 ml)(P<0.001).

Table 3. Interspecies variation on gas production at different incubation hours (ml/g DM)

	Incubation times								
Plant species	**2hr**	**6hr**	**8hr**	**12hr**	**24hr**	**32hr**	**48hr**	**72hr**	**96hr**
M. ferrugenia	13^{b}	32^{bc}	53^{bc}	77^{c}	114^{bc}	145^{bc}	183^{bcd}	201^{bc}	237^{ab}
F. sycomorus	16^{b}	36^{ab}	52^{bcd}	75^{c}	115^{bc}	151^{a}	189^{abc}	227^{ab}	260^{a}
R. glutinosa	16^{b}	34^{b}	52^{bcd}	101^{ab}	122^{b}	141^{bc}	167^{cde}	196^{c}	233^{ab}
G. ferrugenia	15^{b}	31^{bc}	50^{cde}	66^{c}	97^{dc}	122^{dc}	156^{de}	185^{c}	209^{bc}
E. capensis	21^{a}	40^{a}	56^{b}	83^{bc}	133^{ab}	173^{a}	211^{a}	242^{a}	252^{a}
C. edulis	17^{b}	28^{c}	42^{f}	62^{c}	85^{d}	109^{de}	129^{gf}	152^{de}	164^{d}
S. guanensis	16^{b}	31^{bc}	45^{ef}	65^{c}	79^{d}	92^{e}	110^{g}	131^{e}	157^{d}
A. gummifera	1^{c}	21^{d}	49^{cde}	80^{c}	113^{bc}	144^{bc}	168^{cde}	186^{c}	203^{c}
P. africanus	15^{b}	31^{bc}	46^{def}	68^{c}	97^{dc}	121^{dc}	152^{ef}	175^{cd}	200^{c}
D. steudneri	15^{b}	39^{a}	69^{a}	106^{a}	145^{a}	173^{a}	203^{ab}	226^{ab}	234^{ab}
SE	0.570	0.682	0.879	2.45	2.93	3.44	3.90	4.10	4.15
P	***	***	***	***	***	***	***	***	***

$^{a, b, c, d, e, f, g}$ Means with the same letter in the columns are not significantly different

As incubation hours across the different plant species increased, the volume of *in vitro gas* production also increased (P<0.001). Similar patterns of gas production was reported by Kamalak *et al*. (2004); Chumpawadee *et al*. (2005); Njidda and Nasiru (2010); Uzman Bashir Cheema *et al*. (2011). The lower concentration of *in vitro* gas production at initial incubation hours (2 & 4 hr) in *A. gummifera* might be due to higher concentration of NDF_{-CP} that leads to low digestibility and fermentation capacity of soluble fraction during the initial phase of incubation. This is consistent with De Boever *et al*. (2005), who reported that gas production was negatively related with NDF content. Guevara-Mesa *et al*. (2011) reported low fermentation ability of feed sources due to higher concentration of NDF bound proteins. Nsahlai *et al.* (1995) found a negative relationship between gas productions with the disappearance of NDF. This might be also due lack of rapidly fermentable substrates. Many laboratories adopted a 16-24h gas production protocol for rapidly fermentable substrates as well as fermentation substrate for slowly fermentable substrates, respectively. Menke, and Steingass (1988), Theodorou *et al*. (1994) and Groot *et al*. (1996) reported that measurement of both substrates would be reasonable after certain stages of incubation.

The three digit gas production was measured at 12h incubation for only two species where as 60% and 90% of the species had a three digit gas yield at 24 and 32hours of incubation, respectively. The increment in gas production was significant for all species throughout 96hr of incubation (P<0.001). Gas production was found to be not consistent with the CP content of plant species. This could be due to binding effect of tannin with proteins that latter reduces fermentation ability of browse sources. CT could play vital role in reducing initial gas production in browse species by binding available protein for fermenting microbes. Makkar (2003) and Kamalak *et al*. (2004) reported similar findings that *in vitro* gas production and polyphenolic tannin concentrations were negatively correlated showing inverse linear relationships with either the tannin concentrations or their biological activity.

4.3. Effects of Tannin Binding Agents and the Plant Species on *In Vitro* Gas Production

Tannin treatment using tannin-binding agents significantly improved cumulative gas production in all plant species (P<0.001, Table 4). The largest improvement was measured for PEG treatment groups while the smallest gas value was recorded for the controls (P<0.001). The 1grams of both PEG_{6000} and PEG_{4000} treatments were found to be superior for their *in vitro* gas production ability over other tannin-binding agents (P<0.001). Significantly negative correlation was observed between IVGP after 2h post incubation and both CP and CT concentrations (P<0.001).

Table 4. Effect of species (ps) and different levels of tannin-binding agents (trt) on total gas production volume of tree leaves (ml/mg DM)

Plant species	Treatments(t)										
	Control	PEG_{4000}		PEG_{6000}		PVP		PVPP			
		0.5g	1.0g	0.5g	1g	0.5g	1g	0.5g	1g	SE	P
A. *gummifera*	219^{d}	255^{cd}	337^{b}	247^{cd}	434^{a}	253^{cd}	254^{cd}	262^{c}	267^{c}	5	***
C. edulis	164^{f}	314^{b}	355^{a}	264^{c}	251^{c}	209^{ed}	222^{d}	197^{e}	194^{e}	6	***
D. steudneri	234^{e}	351^{b}	391^{a}	293^{a}	393^{a}	274^{d}	337^{b}	272^{d}	297^{c}	3	***
E. capensis	251^{e}	331^{b}	368^{a}	331^{b}	358^{ab}	286^{cd}	302^{c}	253^{cd}	271^{d}	5	**
G. ferruginea	209^{f}	330^{c}	378^{b}	354^{bc}	404^{a}	303^{d}	306^{d}	259^{e}	256^{b}	5	***
M. ferruginea	237^{e}	309^{d}	430^{ab}	396^{bc}	460^{a}	270^{de}	284^{d}	312^{d}	372^{c}	6	***
P. africana	200^{f}	329^{b}	381^{ab}	315^{b}	385^{a}	267^{cde}	283^{abc}	237^{e}	243^{de}	5	***
R. glutinosa	233^{d}	409^{a}	334^{a}	335^{b}	324^{ab}	223^{d}	275^{c}	251^{de}	264^{c}	5	***
S. guineense	157^{e}	275^{b}	337^{a}	292^{b}	344^{a}	220^{cd}	233^{c}	205^{d}	200^{d}	2	***
F. sycomorus	260^{g}	401^{c}	416^{b}	420^{b}	545^{a}	340^{ed}	351^{d}	324^{f}	337^{ef}	5	***

PEG, Polyethyleneglycol; PVP, polyvinyl pyrrolidine; PVPP, polyvinyl polypyrrolidine; ^{a,b,c,d,e,f,g}Means with the same letter in the rows are not significantly different; SE, standard error of the mean

Inclusion of tannin binding agents (PEG_{4000}, PEG_{6000}, PVP and PVPP) had significant effect on *in vitro* gas production of all browse species over their control groups (P<0.001, Table 4). This is due to the high tannin content of the studied plant species and ability of tannin deactivating agent to binds to the tannins forming inert tannin deactivating agent-tannin complexes, which results in increase in gas production. Getachew *et al*. (2001), Seresinhe and Iben (2003) and Singh *et al*. (2005) reported that incubation of tanniferous feed resources with PEG improved in vitro gas production. Moreover, increments in gas production for the plant species after inclusion of

tannin binding agents clearly indicates that tannin decrease accessibility of proteins to rumen microbes by binding fermentable proteins. Kumar and Singh (1984), Makkar *et al*. (1989) and Hagerman *et al*. (1992) also reported that tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes that latter can reduce *in vitro* fermentation ability. According to Blümmel and Ørskov (1993) the increases in gas production due to incubation with tannin deactivating agent could be due to increase in SCFA production and/or changes in molar proportions of the acids. Makkar *et al.* (1995) also reported that removal of adverse effects of tannins by tannin deactivating agent increases the digestibility and energy content of the browse feeds. The increase in the gas production in the presence of tannin binding agents might possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen. The result of the current study is also in agreement with Getachew *et al*. (2002) who concluded that tropical browses with less than 45 and 20 g/kg DM of total phenols and tannin respectively are unlikely to produce significant adverse effects on ruminant livestock. In the present study we observed that increasing trend of gas production as increases in the concentration of CT in the respective plants. Further, Osuga *et al* (2005) on his study on Kenyan browse species concludes that plant species with high tannin content significantly respond positively to tannin binding agent.

In the current study both types of PEG (PEG_{4000} and PEG_{6000}) produce significantly largest amount of gas when compared with other tannin binding agents ($P<0.001$). This may be due to their lower molecular weight as compared to PVP and PVPP. Hagerman (Makkar, 2003) indicated a direct relationship between molecular weight, solubility and permeability of tannin binding agents. Although PEG MW 4000 has been the most widely used tannin-binding agent, Makkar *et al.* (2003) showed that PEG MW 6000 was more effective in overcoming the effects of tannins at neutral pH *in vitro.*

4.4. *In Vitro* Gas Production from the Soluble and Degradable Fractions of the plant Species

Gas production kinetic parameters of the tannin-rich browse species are presented in Table 5. The gas produced from soluble fraction (a) was found to be highest for *R.glutinosa* (7 ml) as

compared to the rest plants whereas the lowest intercept gas value(2ml) was recorded for *M. ferruginea*(P<0.001). The insoluble but degradable fractions (b) were also variable within the plants examined (P<0.001). On the other hand, the highest gas (68 ml) produced from insoluble but fermentable fraction (b) was recorded for *P. africana* and the lowest degradable fraction (27 ml) was for *M. ferruginea* (P<0.001). There was a significant variation (P<0.001) in the gas volume produced at every interval of time (P<0.001). The gas production rate (c) varied between 10 mlh^{-1} in *Carissa edulis* to $3mlh^{-1}$ in *M. ferruginea* and their volume of gas produced at time t(y) was highly variable. Likewise, incubation period (t) varied between 74 h for *P. africana,* where time to attain maximum rate of gas production and 24 h for *M. ferruginea* (P<0.001).

Table 5. Fermentation characteristics of leaves from 10 different tannin-rich browse species (defined by the equation: $p = a + b\ (1\text{-}exp^{-c\ t})$

Parms	***Albizia gummifera***	***Carissa edulis***	***Draceana steudneri***	***Ficus sycomorus***	***Grewia ferruginea***	***Millettia ferruginea***	***Prunus africana***	***Rhus glutinosa***	***Syzygium guineense***	***Ekebergia capensis***	**SEM**	**P**
a, ml	6.16^{c}	7.16^{b}	4.16^{e}	5.66^{c}	5.51^{d}	2.16^{g}	5.70^{c}	7.38^{b}	7.95^{a}	3.86^{f}	0.191	***
b, ml	161.72^{cd}	165.94^{c}	161.04^{cd}	161.30^{cd}	190.77^{a}	158.32^{d}	192.47^{a}	162.99^{cd}	188.17^{a}	173.12^{b}	2.695	***
C,ml/h	8.81^{c}	10.01^{b}	5.81^{e}	7.91^{d}	7.70^{d}	3.02^{g}	7.95^{d}	10.31^{b}	11.12^{a}	5.40^{f}	0.204	***
t(h)	27.90^{f}	61.54^{c}	27.35^{f}	27.57^{f}	50.43^{d}	23.94^{g}	73.93^{a}	29.43^{f}	65.70^{b}	46.59^{e}	2.870	***
pH	6.48	6.72	6.37	6.47	6.86	6.43	6.78	6.64	6.56	6.79		
R^2	0.956	0.990	0.985	0.991	0.953	0.936	0.968	0.981	0.987	0.975		

*Parms, parameter; a, gas produced from soluble fraction (ml); b, gas produced from insoluble but fermentable fraction (ml); C, rate constant of gas production during incubation (ml h^{-1}); ^{a,b,c}Means with the same letter in the rows are not significantly different. SEM: Standard error of mean. ***P<0.001*

The asymptotic gas production (a) varied across plant species (P<0.001). This might be due to variation in fermentation patterns of plant species. Kafilzadeh and Maleki (2012) observed differences in asymptotic gas production due to differences in plant species and/or variety. The higher values obtained for the potential gas production in the *C. edulis* and *R. glutinosa* might indicate a better nutrient availability for rumen microorganisms. Some species such as *G. ferruginea, P. africana* and *S. guineense* had the fastest fermentation kinetics; however, some others species had the slowest fermentation kinetics. These differences were essential for an examination of end product kinetics at different time points in relation to maximum substrate disappearance. Hence, the intake of a feed is mostly explained by the rate of gas production (*c*) which affects the passage rate of feed through the rumen, whereas the potential gas production, is associated with degradability of feed (Khazaal *et al* 1995). CT was also negatively correlated

with most of the estimated parameters. This result is consistent with findings of Frutos *et al* (2002) who found that a negative correlation between CT, a, and b. On the other hand, Larbi *et al* (1998) reported a weak relationship between CT and gas production parameters of tree leaves during wet and dry season in West Africa. A possible reason could be differences in the nature of tannins between browse species (Jackson *et al*., 1996). The negative correlation between potential gas production and NDF, ADF, CT may be a result of the reduction of microbial activity from increasingly adverse environmental conditions as incubation time progress (Abreu et.al 1998; Wood and Plumb 1995).

4.5. Effect of Plant Species and Different Levels Tannin Binding Agents on Kinetics of Fermentation

The initial gas produced from soluble fraction (a) were lowest for control and highest for all plant species treated with both types of PEG (PEG_{4000} and PEG_{6000}, Table 6) except PVPP for *P. africana* ($P<0.001$). The highest "a" value were produced by *D. studineri* (16.65ml) when treated with 1g PEG_{4000}. The insoluble but degradable fractions (b) were also variable for all treatment ($P<0.001$). Also incubation of plant species with both PEG type produce the highest gas volume from insoluble but fermentable fractions. The highest volume of gas from insoluble but degradable fraction was produced from *P. africana* (84.51ml), *S. guineense (75.76ml) and E. capensis* (71.94ml). In the current study there was significant variation on rate of gas production within plant species incubated with and without tannin binding agent ($P<0.001$). As usual both PEG types generate the highest gas production rate for all plant species except for *P. africana*. The highest rate of gas production was from *D. steudneri* receiving 1g of PEG_{4000} ($11.43mlh^{-1}$) and 1g PEG_{6000} ($2.33mlh^{-1}$). Significant variation was observed on the time required to reach the asymptote at $P<0.001$, *E. capenesis* takes the longest time duration which is 57.83h and 59.33h when treated by 1g PEG_{6000} and 0.5g PEG_{6000}, respectively while the control of *M. ferruginea* takes the shortest incubation time (15.67h).

Table 6. Effect of plant species and different levels of tannin binding agent on the kinetics of gas production

Plant species	Parameter	Treatment									SE	P
		Control	PEG_{4000}		PEG_{6000}		PVP		PVPP			
			0.5g	1g	0.5g	1g	0.5g	1g	0.5g	1g		
A. gummifera	a	4.27^{c}	5.66^{b}	8.02^{a}	6.13^{b}	7.73^{a}	5.19^{b}	6.09^{b}	6.09^{b}	6.24^{b}	0.20	***
	b	27.61^{d}	195.34^{c}	200.04^{a}	195.92^{bc}	197.34^{ab}	195.19^{c}	194.77^{c}	194.34^{c}	194.69^{c}	1.14	***
	c	4.46^{c}	7.24^{b}	7.83^{ab}	7.49^{ab}	8.35^{a}	7.18^{b}	7.19^{b}	7.22^{ab}	7.40^{ab}	0.17	***
	t	21.83^{d}	31.50^{c}	43.67^{ab}	33.67^{c}	48.00^{a}	32.50^{c}	32.50^{c}	32.50^{c}	41.50^{b}	1.21	***
C. Edulis	a	5.30^{d}	7.11^{c}	10.01^{b}	7.13^{c}	12.27^{a}	7.06^{c}	7.07^{c}	7.10^{c}	7.25^{c}	0.12	***
	b	31.87^{c}	203.54^{b}	210.38^{a}	203.85^{b}	205.37^{ab}	203.35^{b}	202.93^{b}	202.47^{b}	202.97^{b}	0.32	***
	c	8.58^{c}	9.94^{b}	10.94^{a}	9.97^{b}	10.24^{ab}	9.88^{b}	9.89^{b}	9.92^{b}	10.13^{ab}	1.03	***
	t	42.33^{c}	60.00^{b}	56.70^{a}	62.17^{b}	64.83^{ab}	60.33^{b}	61.00^{b}	59.83^{b}	60.33^{b}	1.75	***
D. steudneri	a	3.24^{d}	7.91^{c}	16.65^{a}	11.00^{c}	12.61^{b}	7.56^{c}	7.57^{c}	7.60^{c}	7.75^{c}	0.41	***
	b	26.94^{c}	196.89^{ab}	201.25^{a}	196.83^{b}	198.25^{ab}	196.39^{b}	195.97^{b}	195.54^{b}	195.89^{b}	0.62	***
	c	4.63^{c}	8.72^{b}	11.43^{a}	9.82^{ab}	12.33^{a}	7.68^{b}	7.69^{b}	7.73^{b}	7.94^{b}	0.88	***
	t	21.00^{c}	30.83^{b}	41.17^{a}	30.17^{b}	31.33^{b}	28.67^{b}	29.50^{b}	28.50^{b}	29.33^{b}	0.95	***
E. Capensis	a	2.65^{c}	5.49^{b}	5.59^{b}	6.45^{a}	6.45^{ab}	5.39^{b}	5.49^{b}	5.55^{b}	5.78^{b}	0.292	***
	b	39.54^{c}	214.11^{b}	215.60^{b}	222.11^{ab}	231.53^{a}	213.14^{b}	217.23^{b}	215.83^{b}	215.92^{b}	0.23	***
	c	3.84^{c}	6.88^{b}	7.02^{b}	9.75^{a}	8.22^{ab}	4.65^{bc}	6.87^{b}	6.73^{b}	4.16^{bc}	1.82	***
	t	29.83^{c}	49.83^{b}	51.33^{b}	57.83^{a}	59.33^{a}	47.17^{ab}	48.83^{ab}	47.83^{ab}	49.00^{b}	1.89	***
G. ferruginea	a	4.04^{c}	6.74^{b}	6.74^{b}	7.60^{ab}	8.69^{a}	6.54^{b}	6.64^{b}	6.94^{b}	7.04^{b}	0.39	***
	b	32.00^{c}	213.76^{ab}	218.66^{a}	221.76^{a}	223.25^{a}	211.26^{b}	212.75^{ab}	211.80^{b}	213.29^{ab}	0.35	***
	c	4.71^{c}	7.19^{b}	9.33^{ab}	8.39^{ab}	10.33^{a}	7.04^{b}	7.18^{b}	7.60^{b}	7.74^{b}	1.30	***
	t	17.33^{b}	47.17^{a}	49.00^{a}	55.17^{a}	57.00^{a}	48.33^{a}	46.33^{a}	45.17^{a}	47.00^{a}	1.19	***
M. ferruginea	a	1.68^{d}	4.11^{b}	4.93^{a}	4.13^{b}	4.82^{a}	3.06^{c}	3.57^{c}	3.01^{c}	3.25^{c}	0.32	***
	b	23.32^{c}	192.84^{ab}	199.05^{a}	193.63^{ab}	195.55^{ab}	191.69^{ab}	191.27^{ab}	191.34^{b}	191.69^{ab}	0.22	***
	c	2.42^{e}	4.85^{bc}	8.39^{a}	5.48^{b}	8.25^{a}	3.40^{d}	3.90^{d}	3.93^{d}	4.64^{b}	1.82	***
	t	15.67^{d}	23.50^{c}	32.17^{ab}	26.50^{abc}	33.33^{a}	25.50^{bc}	25.55^{bc}	28.17^{abc}	29.00^{abc}	2.15	***
P. Africana	a	3.99e	5.55^{bc}	5.64^{bc}	5.74^{bc}	5.87^{b}	5.17^{d}	5.22^{d}	6.67^{a}	6.72^{a}	0.15	***
	b	58.42^{e}	239.73^{dc}	241.11^{bc}	243.86^{ab}	245.92^{a}	235.61^{d}	236.30^{d}	237.11^{cd}	237.00^{cd}	0.11	***
	c	5.79^{e}	7.78^{c}	7.89^{bc}	8.02^{bc}	8.21^{b}	7.23^{d}	7.29^{d}	9.33^{a}	9.39^{a}	1.08	***
	t	54.50^{e}	71.00^{d}	77.17^{bc}	80.17^{ab}	81.17^{a}	71.67^{d}	72.00^{d}	73.00^{cd}	73.67^{cd}	1.11	***
R. glutinosa	a	5.92^{b}	7.39^{a}	7.67^{a}	7.38^{a}	7.57^{a}	7.31^{a}	7.32^{a}	7.34^{a}	7.49^{a}	0.11	***
	b	24.18^{b}	196.59^{ab}	201.29^{a}	196.87^{ab}	198.29^{a}	196.44^{ab}	196.02^{ab}	195.59^{ab}	195.94^{ab}	0.09	***
	c	8.60^{b}	10.28^{a}	10.73^{a}	10.31^{a}	10.78^{a}	10.22^{a}	10.23^{a}	10.27^{a}	10.48^{a}	0.93	***
	t	18.33^{b}	29.50^{a}	34.33^{a}	32.17^{a}	33.33^{a}	31.17^{a}	31.50^{a}	30.50^{a}	31.50^{a}	0.93	***
S. Guineense	a	3.70^{e}	5.45^{d}	10.27^{a}	7.71^{c}	8.45^{c}	9.30^{b}	10.15^{ab}	7.90^{c}	7.98^{c}	0.40	***
	b	54.47^{e}	225.73^{d}	227.38^{cd}	235.64^{ab}	237.29^{a}	229.03^{bcd}	230.69^{abcd}	232.34^{abcd}	233.99^{abc}	0.29	***
	c	5.35^{f}	7.62^{e}	14.36^{a}	10.79^{d}	11.81^{dc}	13.01^{bc}	14.19^{ab}	11.04^{d}	11.16^{d}	1.10	***
	t	50.17^{e}	61.00^{d}	62.50^{cd}	70.50^{ab}	72.00^{a}	64.00^{bdc}	65.83^{abcd}	67.50^{abcd}	69.00^{abc}	1.10	***
F. sycomorus	a	4.65^{b}	11.07^{a}	12.53^{a}	10.63^{a}	11.78^{a}	10.56^{a}	10.57^{a}	10.60^{a}	10.75^{a}	0.37	***
	b	27.19^{c}	199.84^{ab}	204.55^{a}	200.13^{b}	201.55^{ab}	199.69^{b}	199.27^{b}	198.84^{b}	199.19^{b}	0.36	***
	c	6.12^{c}	13.05^{b}	14.69^{ab}	12.87^{b}	15.07^{a}	12.78^{b}	12.79^{b}	12.82^{b}	13.05^{b}	1.30	***
	t	21.00^{c}	31.50^{b}	39.83^{a}	33.50^{b}	43.83^{a}	32.50^{b}	32.50^{b}	31.83^{b}	32.50^{b}	1.19	***

*a, gas produced from soluble fraction (ml); b, gas produced from insoluble but fermentable fraction (ml);C, rate constant of gas production during incubation (ml h^{-1}); a+b = potential gas production (ml); ^{abc}Means with the same letter in the rows are not significantly different. SEM: Standard error of mean. ***P<0.001*

Incubation of substrates with increased gas production from soluble (a), potentially degradable fractions (b) and the potential gas production (a+b) of browse species over control treatments whereas incubation of these tannin binding agents induce a decrease in rate of gas production in all plant species ($P<0.001$). This result could suggest that tannins in this case are binding to fibres, and the presence of increased microbial plant adhesion and/or the fibrolytic microbial activity. However, incubation of substrates without the PEG_{4000}, PEG_{6000}, PVP, and PVPP inclusion decreased the rate of gas production in all plant species. Similar results were also found by several authors (Getachew *et al*., 2002; Odenyo *et al*., 2003) who registered the in vitro gas production incubating several forages rich in tannin or substrates with different tannin concentrations (Makkar *et al*., 1995). Frutos *et al*. (2004) and Guimarãez-Beelen *et al*. (2006) also indicated that PEG inclusion increased in vitro fermentation products from tanniferous feed sources. The latter authors have noted that for species, which the rate of gas production is reduced, the bacteria colonization is restricted. This could suggest that complexes forming between tannins and PEG_{4000}, PEG_{6000}, PVP, and PVPP generate steric obstruction which do not permit and/or limit the fixation of adherent bacteria to the feeds. Canbolat *et al*. (2005) reported that PEG supplementation increased the gas production from the insoluble fraction (b), whereas PEG supplementation had no effect on the gas production from the immediately soluble fraction (a), and the gas production rate (c).

4.6. Metabolizable Energy, Organic Matter Digestibility and Short-Chain Fatty Acid Concentrations for the Plant species

There is significant variation between the studied plant species estimated gas production parameter ($P<0.001$). Metabolizable Energy (ME), Organic Matter Digestibility (OMD) and Short Chain Fatty Acids (SCFA) of the browse forages are presented in table 7. The value for the ME, OMD and SCFA ranged from 13.66, *S. guineense* to 26.54, *P. africana* (MJ/Kg DM), 88.69, *S. guineense* to 183.01, *P. africana* (g/kg DM) and 1.74, *S. guineense* to 3.84, *P. africana* (mmol), respectively. There were significant differences ($P<0.05$) among the MPTS in ME, OMD and SCFA.

Table 7. Estimated gas production parameter of the studied browse plant species

Estimated Parameter	Plant species										SEM	P
	A. gummifera	*C. edulis*	*D. steudneri*	*F. sycomorus*	*G. ferruginea*	*M. ferruginea*	*P. africana*	*R. glutinosa*	*S. guineense*	*E. capensis*		
SCFA	3.19^{ab}	1.89^{d}	3.22^{ab}	2.54^{bcd}	2.15^{cd}	2.52^{bcd}	3.84^{a}	2.69^{bc}	1.74^{d}	2.93^{bc}	0.25	***
ME	23.51^{ab}	14.59^{de}	23.20^{ab}	18.82^{bcd}	16.71^{cde}	19.08^{bcd}	26.54^{a}	19.57^{bc}	13.66^{e}	21.04^{bc}	1.56	***
OMD	154.96^{ab}	95.24^{de}	155.68^{ab}	125.12^{bcd}	107.61^{cde}	124.48^{bcd}	183.01^{a}	131.57^{bc}	88.69^{e}	142.28^{bc}	11.5	***

Metabolizable Energy (ME = MJ/Kg DM), Organic Matter Digestibility (OMD = g/kg DM), Short Chain Fatty Acids (SCFA = mmol)

The OMD, ME and SCFA values of the current study were much higher than values that reported from Babayemi (2006), Sallam *et al.* (2007) and Afshar Mirzaei-Aghsaghali *et al* (2011) for roughage and browse tree species. The highest and lowest SCFA were produced from *Prunus africana* (3.84mmol) and *Syzygium guineense* (1.74mmol), *respectively.* This high SCFA from *Prunus africana* might be due to high EE (48), CHO (678) and DCHO (649) and low CT (76) and NDF-bPA (10). Gas production mainly results from fermentation of CHO to SCFA. This result is in agreement with Blümmel and Ørskov, (1993) who indicated that gas production basically results from fermentation of carbohydrates to short chain fatty acids (SCFA)-mainly acetate, propionate and butyrate and from the buffering of the SCFA (CO_2 released from the bicarbonate buffer). Menke and Steingass (1988), confirmed the close relationship between SCFA production and gas volume liberated on fermentation of browse species with wide range of CP (77-300 g/Kg) and phenolic contents (Phenolic from 17- 250 g/Kg DM and TP from 7-214 g/Kg DM respectively). Getachew *et al.*, (2002) also reported the close association between SCFA and gas production *in vitro*, they used this relationship between SCFA and gas production to estimate production of SCFA from gas values. SCFA are indicators of energy availability to the animal. Similarly the highest and lowest ME (*MJ/Kg DM*) values were obtained respectively, from *Prunus africana* (26.54) and *Syzygium guineense* (13.66). This might be due to fat content of the feeds. The ether extract (EE) and Digestible ether extract (DEE) of *Prunus africana* are higher, 48 and 5.1, than the remaining browse tree species. The SCFA, ME and OMD of the studied plant species were higher than that of reported from Sallam *et al.* (2007), Afshar Mirzaei-Aghsaghali *et al* (2011) for roughage. Menke and Steingass (1988) indicated positive correlation between ME calculated from *in vitro* gas production together with CP and fat content with ME value of conventional feeds measured *in vivo*. Menke *et al.,* (1979), Steingass and Menke(1986),

Menke and Steingass (1988) and Chenost *et al.,* (1997) concluded that the prediction of ME is more accurate when based on gas and chemical constituents measurements as compared to calculations based on chemical constituents only. OMD showed significant variation (P<0.001) among the studied plant species. The highest and lowest OMD (g/kg DM) were observed for *Prunus africana* (183.01) and *Syzygium guineense* (88.69), respectively. This variation within plant species may be due to differences in species, concentration of cell wall contents, and method used for determining OMD (method used in the current study was Gas production technique). The result of the current study was lower than that of reported for semi-arid browse of north eastern Nigeria (Njidda, 2010).

4.7. Interaction Effect of plant Species and different level of Tannin Binding Agents on Organic Matter Digestibility, Metabolizable Energy and Short-Chain Fatty Acid Concentrations

The OMD, ME and SCFA contents were affected by species, treatment and their interaction (P<0.001) (Table 7). Treatment of tannin-rich plants with PEG, PVP and PVPP has significantly increased the OMD, ME and SCFA concentration (P<0.001). The highest values of SCFA, ME, OMD was recorded for PEG treatments whereas the lowest values were measured for control (P<0.001).

Table 8. Effect of tannin binding agents on organic matter digestibility, Metabolizable energy and short-chain fatty acid concentrations of browse plants

Plant species	Estimated *in vitro* parameter	Treatment									SE	P
		Control	PEG_{4000}		PEG_{6000}		PVP		PVPP			
			0.5g	1g	0.5g	1g	0.5g	1g	0.5g	1g		
A. *gummifera*	**OMD**	39.80^{b}	46.13^{ab}	47.28^{a}	44.92^{ab}	46.51^{ab}	45.78^{ab}	46.21^{ab}	47.68^{a}	48.79^{a}	0.68	***
	ME	38.81^{b}	42.17^{a}	42.76^{a}	41.33^{ab}	42.37^{a}	41.96^{a}	41.96^{a}	42.85^{a}	43.59^{a}	0.36	***
	SCFA	3.73^{b}	4.35^{a}	4.43^{a}	4.22^{ab}	4.38^{a}	4.30^{ab}	4.35^{a}	4.50^{a}	4.64^{a}	0.07	***
C. edulis	**OMD**	29.33^{b}	45.24^{a}	43.49^{a}	44.98^{a}	43.13^{a}	35.52^{b}	35.90^{b}	29.91^{b}	30.20^{b}	0.68	***
	ME	21.82^{c}	30.23^{a}	29.34^{ab}	29.75^{a}	29.32^{ab}	25.67^{ab}	25.58^{ab}	22.39^{c}	22.14^{c}	0.36	***
	SCFA	2.40^{b}	3.97^{a}	3.76^{a}	3.93^{a}	3.79^{a}	3.00^{b}	3.00^{b}	2.36^{b}	2.40^{b}	0.07	***
D. steudneri	**OMD**	43.01^{b}	52.63^{a}	50.73^{a}	51.18^{a}	51.18^{a}	48.00^{ab}	49.66^{a}	47.29^{ab}	47.92^{ab}	0.68	***
	ME	34.20^{c}	38.91^{a}	37.76^{ab}	37.98^{ab}	37.98^{ab}	36.71^{abc}	36.80^{abc}	36.19^{abc}	35.65^{bc}	0.36	***
	SCFA	3.83^{c}	4.75^{a}	4.53^{ab}	4.61^{ab}	4.61^{ab}	4.28^{abc}	4.38^{abc}	4.18^{bc}	4.23^{abc}	0.07	***
E. capensis	**OMD**	45.23^{ab}	50.39^{ab}	51.23^{ab}	51.53^{ab}	49.15^{ab}	50.84^{ab}	52.91^{a}	43.39^{b}	46.79	0.68	***
	ME	28.46^{c}	32.68^{ab}	34.45^{a}	34.90^{a}	33.81^{a}	34.31^{a}	34.60^{a}	29.48^{bc}	31.48^{abc}	0.36	***
	SCFA	3.83^{bc}	4.50^{abc}	4.62^{ab}	4.76^{a}	4.42^{abc}	4.65^{a}	4.77^{a}	3.78^{c}	4.16^{abc}	0.07	***
G. ferruginea	**OMD**	34.18^{c}	47.01^{ab}	45.27^{ab}	50.22^{a}	51.63^{a}	51.19^{a}	50.41^{a}	41.75^{b}	41.75^{b}	0.68	***
	ME	28.48^{d}	35.40^{ab}	33.49^{bc}	36.30^{ab}	37.89^{a}	36.26^{ab}	35.69^{ab}	31.77^{dc}	31.77^{dc}	0.36	***
	SCFA	2.69^{d}	4.03^{ab}	3.69^{bc}	4.23^{ab}	4.46^{a}	4.35^{ab}	4.23^{ab}	3.31^{cd}	3.31^{cd}	0.07	***
M. ferruginea	**OMD**	37.80^{c}	40.87^{ab}	45.62^{ab}	46.38^{ab}	50.65^{a}	45.35^{ab}	49.46^{a}	46.54^{ab}	50.32^{a}	0.68	***
	ME	34.70^{cd}	35.97^{cd}	38.35^{abc}	37.83^{abc}	39.71^{ab}	37.81^{abc}	40.28^{a}	37.40^{bc}	39.31^{ab}	0.36	***
	SCFA	3.22^{c}	3.48^{bc}	3.95^{ab}	3.98^{ab}	4.40^{a}	3.90^{ab}	4.42^{a}	3.89^{ab}	4.33^{a}	0.07	***
P. africana	**OMD**	33.13^{e}	49.45^{abc}	52.42^{ab}	47.29^{bc}	53.37^{a}	46.82^{c}	49.52^{abc}	37.99^{ed}	39.39^{d}	0.68	***
	ME	23.36^{b}	32.43^{a}	33.38^{a}	31.48^{a}	34.18^{a}	31.48^{a}	32.82^{a}	24.25^{b}	24.75^{b}	0.36	***
	SCFA	2.68^{d}	4.44^{abc}	4.73^{ab}	4.25^{bc}	4.84^{a}	4.20^{c}	4.45^{abc}	2.93^{d}	3.12^{d}	0.07	***
R. glutinosa	**OMD**	37.97^{d}	47.93^{abc}	55.15^{a}	49.32^{ab}	54.57^{a}	38.45^{d}	45.53^{bcd}	38.28^{d}	40.94^{cd}	0.68	***
	ME	27.06^{bc}	29.69^{abc}	33.89^{a}	31.17^{ab}	33.80^{a}	26.59^{bc}	28.70^{bc}	25.39^{c}	26.16^{bc}	0.36	***
	SCFA	3.12^{cde}	3.89^{abc}	4.64^{a}	4.18^{ab}	4.70^{a}	3.19^{cde}	3.72^{bcd}	2.87^{e}	3.04^{de}	0.07	***
S. guineense	**OMD**	25.76^{d}	36.81^{abc}	39.31^{a}	39.76^{a}	40.15^{a}	35.45^{abc}	38.04^{ab}	32.81^{bc}	31.67^{c}	0.68	***
	ME	20.67^{d}	24.77^{ab}	25.84^{a}	26.13^{a}	26.20^{a}	23.91^{abc}	25.13^{a}	22.28^{bcd}	21.51^{cd}	0.36	***
	SCFA	2.03^{d}	2.94^{ab}	3.17^{ab}	3.15^{ab}	3.20^{a}	2.69^{bc}	2.99^{bc}	2.48^{cd}	2.33^{cd}	0.07	***
F. sycomorus	**OMD**	41.98^{d}	58.02^{a}	58.47^{a}	52.87^{b}	58.45^{a}	56.69^{a}	58.87^{a}	49.10^{c}	49.49^{c}	0.68	***
	ME	27.75^{c}	35.53^{a}	35.66^{a}	32.24^{b}	35.75^{a}	35.57^{a}	35.75^{a}	30.52^{b}	30.09^{b}	0.36	***
	SCFA	3.34^{d}	4.95^{a}	4.96^{a}	4.39^{b}	5.06^{a}	4.82^{a}	5.04^{a}	3.95^{c}	3.96^{c}	0.07	***

Metabolizable Energy (ME = MJ/Kg DM), Organic Matter Digestibility (OMD = %), Short Chain Fatty Acids (SCFA = mmol)

The highest values of these parameters in treatment groups other than controls might be the fact that PEG, PVP and PVPP had able to prevent tannin-protein complex formation, its ability to release protein from such complexes related to the pH of the environment, and amount of tannin in the complex. These results are in agreement with the findings of Getachew *et al*. (2000), Getachew *et al*. (2001) and Seresinhe and Iben (2003) who estimated higher values of OMD, SCFA and ME from feedstuffs incubated with PEG with tanniferous species. This result is also in agreement with findings of Rubanza *et al*. (2005) who found that PEG supplementation resulted in the increase in OMD of leaves from Acacia species. Rubanza *et al*. (2003) also found that PEG supplementation resulted in the increase in ME of leaves from browse fodders. Priolo *et al*. (2002b) reported the greater ruminal ammonia and a VFA concentration in PEG- versus tannin-fed sheep indicates more rapid ruminal fermentation when PEG was given. Getachew *et al*. (2000) also reported that addition of PEG to tannin-containing feeds increased *in vitro* gas and SCFA production. CT was also negatively correlated with most of the estimated parameters. This result is consistent with findings of Frutos *et al* (2002). On the other hand, Larbi *et al* (1998) reported a weak relationship between TCT and gas production parameters of tree leaves during wet and dry season in West Africa. A possible reason could be differences in the nature of tannins between browse species (Jackson *et al*., 1996). The negative correlation between potential gas production and NDF, ADF, TCT, or SCT may be a result of the reduction of microbial activity from increasingly adverse environmental conditions as incubation time progress (Abreu et.al 1998; Wood and Plumb 1995). NDF and ADF explained 61.1 and 55.5 % of the variation of cumulative gas production respectively whereas the CT content explained 87.0 % of variation of cumulative gas production. Cerrillo and Juarez (2004) reported that 81 % of the variation in potential gas production could be explained by the changes in ADF content. The low predictive values in the current experiment could be due to species variation in quantity and quality of fibre as well as variation in complexation of fibre with polyphenolics (Ndlovu and Nherera, 1997).

4.8. Correlation between Some Chemical Composition Parameters and In *vitro* Gas Production

The statistical correlation between some chemical composition parameters and *in vitro* gas production from the studied tannin rich tree species is given in Table 9. The correlation between CT, TT or TP and CP, IVDMD, IVOMD, CHO, DCHO, DCP and TDN was negative and highly significant ($P<0.01$). The negative correlation was also observed between CT and *in vitro* gas production at all incubation hours except 2 h incubation ($P<0.05$). The correlation between NDF-CP and TT($r=-0.799$) or TP($r=-0.799$) and ADF-CP and TT ($r = -0.724$) was negatively strong and highly significant ($P<0.01$). This result is in agreement with findings of Khazaal and Orskov (1994), Makker and becker (1998), Getachew et al. (2000, 2002) and Kamalak et al. (2004) who reported the negative correlation between CT and *in vitro* gas production in advancing incubation hours. This result is not in agreement with findings of Abdulrazak et al. (2000). This might be due to the differences in nature of tannins between the browse species. The concentration of condensed tannin the plants tested by Abdulrazak et al. (2000) were less than 50 g/kg DM. Moreover, the correlation between TDN, IVDMD, IVOMD or DCHO and *in vitro* gas production was found to be positive and highly significant for all the indicated incubation hours ($P<0.01$). A possible reason to this fact is that gas production is an end product of digestible and potentially fermentable nutrients. Barman and Rai (2008) reported the positive associations between an *in vitro* nutrient digestibility and gas production from tannin metabolites of *Acacia nilotica*, with tannin levels ranging from 40 to 120 g/kg DM. The present study agrees with Njidda and Nasiru (2010) that digestibility and tannin concentrations were negatively correlated showing inverse linear relationships with either the tannin concentrations or their biological activity assessed using tannin binding chemicals. The findings in this experiment supported the fact that the anti-nutritive factors like tannins may also contribute to reduction of microbial activity (Makkar, 2003).

Table 9. Correlation between some chemical composition parameters and the *in vitro* gas production of the studied plants

	CT	NDF	ADF	ADL	IVOMD	IVDMD	CHO	DCHO	DCP	TDN	TT	G_{12}	G_{24}	G_{32}	G_{48}	G_{72}	G_{96}	c	a	b	t(h)
CT	1.000																				
NDF	00.187	1.000																			
ADF	00.166	0.835**	1.000																		
ADL	-00.281*	0.279*	0.336**	1.000																	
IVOMD	-0.722**	-0.017	0.030	0.094	1.000																
IVDMD	-0.669**	0.080	0.144	0.085	0.931**	1.000															
CHO	-0.454**	-0.083	-0.137	-0.552**	-0.294*	-0.264*	1.000														
DCHO	-0.662**	-0.185	-0.187	0.011	0.810**	0.811**	-0.008	1.000													
DCP	-0.427**	-0.026	-0.006	0.463**	0.278*	0.190	-0.836**	0.165	1.000												
TDN	-0.668**	-0.200	-0.192	0.021	0.823**	0.810**	-0.020	0.993**	0.163	1.000											
TT	0.849**	0.113	0.024	-0.364**	-0.694**	-0.629**	0.729**	-0.527**	-0.718**	-0.543**	1.000										
G_{12}	-0.264*	-0.104	-0.252	0.065	0.383**	0.283*	0.004	0.350**	0.027	0.356**	-0.246	1.000									
G_{24}	-0.365**	-0.213	-0.317*	0.009	0.505**	0.384**	0.036	0.530**	0.034	0.544**	-0.311*	0.942**	1.000								
G_{32}	-0.362**	-0.251	-0.329*	-0.058	0.505**	0.384**	0.074	0.569**	0.028	0.581**	-0.295*	0.884**	0.981**	1.000							
G_{48}	-0.369**	-0.243	-0.266*	-0.104	0.541**	0.431**	0.054	0.610**	0.078	0.620**	-0.329*	0.790**	0.924**	0.972**	1.000						
G_{72}	-0.331**	-0.258*	-0.257*	-0.181	0.534**	0.433**	0.080	0.616**	0.082	0.626**	-0.317*	0.711**	0.866**	0.929**	0.984**	1.000					
G_{96}	-0.239	-0.219	-0.201	-0.265*	0.498**	0.427**	0.239	0.562**	-0.095	0.568**	-0.202	0.723**	0.834**	0.882**	0.920**	0.929**	1.000				
c	0.529**	-0.104	-0.233	-0.312*	-0.492**	-0.563**	0.297*	-0.594**	-0.297*	-0.588**	0.492**	-0.054	-0.161	-0.198	-0.281*	-0.277*	-0.152	1.000			
a	0.529**	-0.104	-0.233	-0.312*	-0.492**	-0.563**	0.297*	-0.594**	-0.297*	-0.588**	0.492**	-0.054	-0.161	-0.198	-0.281*	-0.277*	-0.152	1.000**	1.000		
b	-0.054	0.085	0.095	-0.112	0.065	0.154	0.450**	0.143	-0.490**	0.149	0.103	0.039	0.030	0.011	-0.014	-0.015	0.105	0.099	0.099	1.000	
t(h)	0.032	0.041	0.011	-0.189	-0.020	0.086	0.485**	0.056	-0.564**	0.055	0.251	-0.075	-0.092	-0.107	-0.145	-0.153	-0.027	0.234	0.234	0.945**	1.000

*CT, Condensed Tannin; IVOMD, In vitro Organic Matter Digestibility; IVDMD, In vitro Organic Dry Matter Digestibility; CHO, Carbohydrate ;DCHO, Digestible Carbohydrate; DCP, Digestible Crude Protein; TDN, Total Digestible Nutrient; NDF_{CP}, NDF bounded Crude Protein; ADF_{CP}, ADL bounded Crude Protein; NDF, Nuetral detergent Fiber; ADF, Acid Detergent Fiber; ADL, Acid detergent Lignin. **P<0.01; **P<0.05*

5. SUMMERY AND CONCLUSION

The study was undertaken to evaluate nutrient value of tannin rich tropical browse species and the interaction effects of these plant species and tannin binding agents on in vitro fermentation parameters and to examine the relationship between some chemical composition parameters and the in vitro fermentation values of the plants. The studied plant species were Albizia gummifera, Carissa edulis, Draceana steudneri, Ficus sycomorus, Grewia ferruginea, Millettia ferruginea, Prunus africana, Rhus glutinosa, Syzygium guineense and Ekebergia capensis.

The species were subjected to proximate, detergent, polyphenolic and in vitro gas production analysis. Organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acids (SCFA) were estimated for 24h gas production.

A wide variability in chemical composition including polyphenolics, in vitro digestibility and fermentation and estimated parameters recorded for plants as well as interaction of plant-tannin binding agents.

Addition of PEG_{4000}, PEG_{6000}, PVP and PVPP to incubation medium increased the extent and rate of gas production all plant species as compared to species incubated without tannin binding agent. There were negative correlations between CT, NDF and ADF content of the plant species and the gas production at all incubation times.

Addition of PEG, PVP and PVPP inactivate effects of tannins and increased gas production, SCFA, ME, and OMD in all the plants. Generally, treatments received PEG_{6000} and PEG_{4000} were found to be superior over other treatments in terms of rate and extent of gas production ($p<0.001$).

Addition of PEG_{6000}, PEG_{4000}, PVP and PVPP could help to overcome adverse effects of tannins on nutrient availability as indicated by gas production parameters. This increase was noticeable in all plant species. Based on their nutrient content and fermentation kinetics as when its tannin

content is biologically inactivated by using tannin binding agents the browse species may play an important feed supplements in providing sustainable fodder for herbivores.

It was concluded that there were a significant variations in chemical composition, *in vitro* digestibility, digestible nutrients, Metabolizable energy and *in vitro* gas production characteristics of tannin rich tree leaves included in this study. The plant species included in this study could be a good protein supplements for low quality basal diets. However, the high contents polyphenolics in browse species could limit their nutritive value. The anti nutritive activity of condensed tannins could be alleviated by use of various tannin deactivating agents or chemicals such as PEG_{4000}, PEG_{6000}, PVP and PVPP.

From the tannin binding agents included in this study both types of PEG have given the highest value of *in vitro* gas followed by PVP and PVPP. Moreover, increasing inclusions of the level of tannin binding agents has also resulted a significant increase on gas production, kinetics of fermentation and other estimated parameter such as OMD, ME and SCFA.

Statistical correlations has also witnessed the influences of condensed tannins on CP, DCP, *in vitro* digestibility, digestible CHO, TDN, and *in vitro* gas production at all incubation hours.

6. RECOMMENDATION

Since this study only covered ten tree species among many, it is recommended that further studies should be carried out to evaluate more locally available browse species so that the feed value of the new potential plant species can be known.

Animal feeding experiments are also necessary to ascertain the nutritive value of the promising species in terms of palatability, intake, digestion and effect on production performance of the animals.

In addition to including tannin binding agents which are used for this particular study, researchers has to also look for more easy, cheap and locally available methods of tannin deactivation.

Since the plant species in this study are indigenous and has multipurpose, plantation strategies throughout the grazing areas should have to be encouraged.

Due to little expensiveness of tannin binding agent to farmers and pastoralists, subsidization strategy should have to be backed by government and non government organizations to improve Production and productivity of livestock.

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http://en.wikipedia.org/wiki/Jimma

http://en.wikipedia.org/wiki/Holeta_Genet

8. APPENDICES

8.1. Theodorou's gas production technique

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Background

In principle, this technique is similar to the Menke *et al*. (1889) gas production procedure using ground particulate substrates, anaerobic media and rumen fluid inoculum. It differs, however, in that incubations are conducted in gas-tight culture bottles, thus enabling gases to accumulate in the head-space as the fermentation proceeds. A pressure transducer connected to a digital readout volumeter and a gas-tight syringe assembly is then used to measure and release the accumulated gas pressures from the incubated culture bottles. By repeating the "gas-measurements + gas release" procedure at regular intervals, it is possible to construct gas accumulation profiles for feeds. The rate and extent of fermentation can also be calculated (Theodorou *et al.*, 1994).

The method was developed to get information on the fermentation kinetics of ruminant feeds based on long term end-point fermentations (166 hours). However, shorter fermentations (48 and 72 hours) have been also to evaluate tropical forages and rank them according to their fermentability. The method was developed with a nitrogen-rich (Theodorou) medium but the Menke medium can be used when a nitrogen free medium is needed. The technique has also been adapted to the biological evaluation of the effects of phenols on fermentation by adding binding agents such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) and polyvinyl-polypyrrolidone (PVPP).

Preparation of the sample

Grind substrate to pass through 1 mm dry sieve (if not already ground). Weigh out substrate. Generally use 1 g total substrate, weigh to tolerance of ±0.0020 g. Make up stock solutions for medium. Recipes for media are given below. Arrange serum bottles in order placing them on trays for easy handling.

Preparation of the medium

Make up suitable amount of medium. Stir and gas with CO2 for about 2 to 3 hours, then add a small volume of reducing agent (about 2 ml per litre buffer medium). Continue gassing until the resazurin in the medium is pink. Dispense 90 ml medium into 125 ml serum bottles using pump and gassing with CO2. Make 5 to 10 spare bottles for use in preparing the inoculum. Seal with butyl rubber stoppers, but do not crimp. Store at 4°C.

Place the samples into the bottles

Make up a suitable amount of reducing agent in fume cupboard, keeping it stirred and under an atmosphere of nitrogen. Using a small wide bore funnel transfer the substrates into their bottles and add 4 ml reducing agent. Samples are normally run in triplicate. Remember to add reducing agent to the spare bottles. Keep gassing with CO2. Reseal with butyl rubber stoppers and crimp with aluminium caps. Replace in incubator at 4°C and programme it to switch to 39°C at about 2 am.

Prepare inoculum

A minimum of 2, and preferably3, people are required to inoculate the bottles. Collect rumen fluid starting at the morning and keep it warm in a Thermos flask. Filter fluid through 4 layers of course cotton muslin and collect in beaker (with volumes marked) under atmosphere of CO_2. Keep liquid stirred (not too vigorously). Note approximate volume of filtered liquid. Transfer solids to a blender and add a volume of medium (using the spare bottles prepared earlier) approximately equal to the volume of filtered liquid. Blend for about 30 seconds and filter through muslin into the beaker with filtered liquid to pool with original filtered rumen fluid. Keep stirred and under CO_2. The inoculum is now ready for use.

Inoculation of bottles

While the inoculum is being prepared, the serum bottles must be adjusted to atmospheric pressure. This is done by using the "taking gas readings" procedure described below, but the gas volumes produced are not normally noted. Bottles are returned to the incubator at 39°C. Using a 10 ml syringe and 21 gauge 1.5 in (0.8 × 40 mm) needles, 5 ml of inoculum is injected into each bottle. Shake bottles and return to incubator. This is taken as the starting point (time = 0) of the experiment.

8.2. Theodorou Medium Preparation

Component solutions

1. Micro-mineral solution (g per 100 ml)

This is made up in 100 ml lots and stored in the refrigerator as a stock solution.

$CaCl_2.2H_2O$	13.2
$MnCl_2.4H_2O$	10.0
$CoCl_2.6H_2O$	1.0
$FeCl_3.6H_2O$	8.0

2. Buffer solution (g per liter)

This is made up in variable quantities and can be stored in a fridge. Calculate how much is required for each run.

NH_4HCO_3	4.0
$NaHCO_3$	35.0

3. Macro mineral solution (g per litre)

This is made up in variable quantities and can be stored in the refrigerator. Calculate how much is required for each run. The same volume of buffer and macro mineral solution is required in the medium.

$Na_2HPO_4.12H_2O$	9.45
KH_2PO_4	6.20
$MgSO_4.7H_2O$	0.60

4. Resazurin solution

Resazurin	0.1g/100 ml water

The medium is kept mixed and CO_2 bubbled through it.

5. Theodorou reducing agent

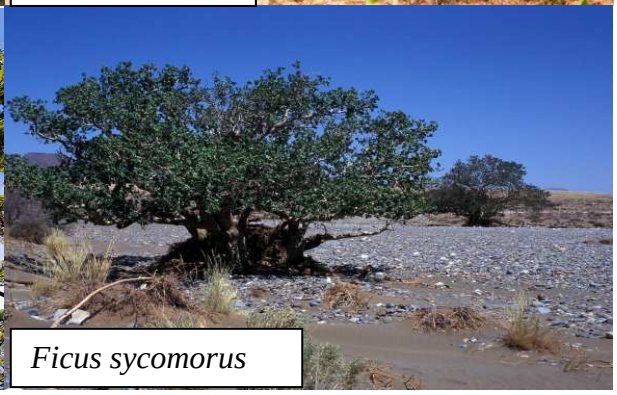
Cysteine HCl.1H_2O	625 mg
Distilled water	95 ml
1 M NaOH	4 ml
Sodium sulphide	625 mg

8.3. Plates

Albizia Gummifera

Carissa edulis

Draceana steudneri



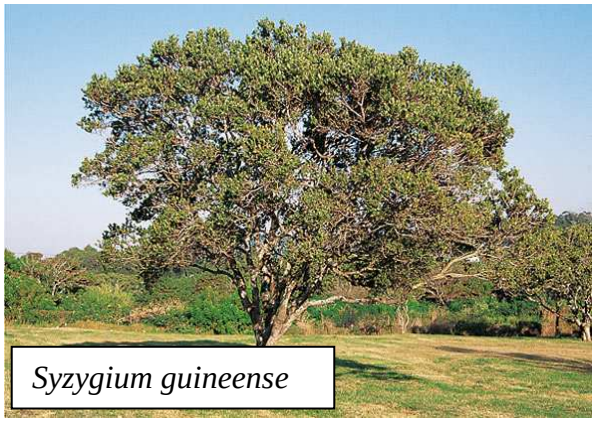
Ficus sycomorus


Grewia ferruginea


Milletia ferruginea


Prunus africana


Rhus glutinosa


Syzygium guineense


Ekebergia capensis