EFFECT OF BROWSE SPECIES, STORAGE CONDITIONS AND EXTRACTION SOLVENTS ON CONCENTRATION OF EXTRACTABLE CONDENSED TANNIN AND *IN VITRO* ANTI-*HAEMONCHUS CONTORTUS* ACTIVITY

M.SC. THESIS

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By

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APPROVAL SHEET

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As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Belay Chufamo, entitled "**Effect of browse species, storage condition and extraction solvents on concentration of extractable condensed tannin and *in vitro* anti *haemonchus contortus* activity".** We recommend that it can be submitted as fulfilling the thesis requirement.

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DEDICATION

I dedicate this thesis manuscript to my lovely mother Zewdenshe Shume, my father Chufamo Kaltebo, my sisters and brother for nursing me with affection and love and for their dedicated partnership in the success of my life.

STATEMENT OF THE AUTHOR

I declare that this thesis is my original work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in Partial Fulfillment of the Requirements for MSc Degree at Jimma University and is deposited at the university library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author was born in Debre Zeit, Eastern Showa, Ethiopia in 1979 G.C .He attended his elementary school in 1985 to1993 at Atse Lebena Dengle Elementary School. His Junior and High School education attended in Debre Zeit Compressive High School, from 1993 to 1997 and took ESLCE in 1997. Then he joined the former Jimma College of Agriculture in October 1998 and graduated with Diploma Animal Sciences in July 1999.

Between 2000 and 2003, he had worked at Ambo College of Agriculture in the Department of Animal Sciences. He was then got scholarship for a short course on International Course on Poultry Husbandry at PTC^{+} in the Netherlands and awarded a Diploma 2003. Immediately after completing his training he transferred to Dronten Agricultural University in The Netherlands where he studied form 2003/2004 to 2006. He graduated in September 2006 with Honors Degree with International Livestock Production. From September 2006 to 2008 he has been working as instructor at Ambo University in the Department of Animal Sciences.

Finally, he joined the School of Graduate Study of Jimma University in 2009/10 for his study on Degree of Master of Science in Agriculture in the specialization of Animal Production.

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LIST OF ABBREVIATIONS

AA	Amino acid
CACC	Central Agriculture Census Commission
AH	Anthelmintic
AIBPs	Agro-industrial by products
CTs	Condensed tannins
DM	Dry matter
DMSO	Dimethyl-sulfoxide
EARO	Ethiopia Agricultural Research Organization
ECT	Extractable Condensed Tannin
EEA	Ethiopian Economic Association
ESAP	Ethiopian Society of Animal Production
FEC	Fecal egg counts
FECR	Fecal egg count reduction
GLM	General linear model
GIP	Gastro intestinal parasite
HTs	Hydrolysable tannins
IAEA	International Atomic Energy Agency
ILRI	International Livestock Research Institute
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
L1	Larva stage one
L3	Larvae stage three
MoA	Ministry of Agriculture
MoARD	Ministry of Agriculture and Rural Development
PBS	Phosphate Buffered Saline
PEG	Polyethylene glycol
rpm	Rotation per minute
TRP	Tannin rich plants
VFI	Voluntary feed intake

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ABSTRACT

The study was carried out to evaluate effects of the selected indigenous tannin rich tree species, storage conditions and extraction solvents on concentration of extractable condensed tannins (ECT) and to quantify and compare the in vitro anthelmentic activity of the plant species at different storage time and extraction methods against Haemonchus contortus activity. Albizia gummifera, Carissa edulis, Ficus ovata, Maytenus obscura and Rhus glutinosa species suspected with high content of tannins were collected from their natural habitat in Omo Nada Woreda of Jimma Zone, South West Ethiopia. The plant species were subjected to extractable condensed tannin analysis. Fresh leaves, fresh leaves dried at 55 °C and ground immediately and the dried and ground plant materials that have been preserved for 1.5 years at room temperature were used. Two levels of aqueous acetone and ethanol, each having 50 and 70% v/v), were incorporated for the extraction of plant samples. The extracts were prepared to obtain 50 mg /ml concentrations and accompanied by controls: distilled water (negative control) and Albendazole (positive control). The inhibitory effects of ECT on egg hatchability, larvae development and adult mortality of Haemonchus contortus also studied. The data were subjected to a three ways analysis of variance following the general linear model procedure of statistical analysis system in a 5x4x3 factorial arrangement. Interspecies variations were significant for ECT concentration across the storage conditions ($P<0.001$). ECT concentration in dried and dried-preserved leaves of all the plants were found to be above the threshold value that affects nutrient value of feedstuffs (50 g/kg DM). ECT values obtained by both levels acetone tended to be higher than the values determined by ethanol 50 and 70%. Plant extracts induced anthelmintic effects on the three life-cycle stages of H.contortus and these effects were significantly different when they were compared to the positive and negative control group ($P<0.001$). All the plants showed anthelmintics activity in three-life cycle of the parasite. However, variations in the efficiency were observed depending on the storage conditions and plant species. The organic solvent that has high capacity to extract condensed tannins from livestock feeds would be better choices determining tannins. The potential bioactive substances used in vitro may not always correspond to in vivo bioavailability. Therefore, in vitro assays should always be accompanied by in vivo trials when used to validate anthelmintic activity of plant remedies.

Key words: *Extractable condensed tannin; extraction solvents; fodder trees and shrubs; storage condition; Haemonchus contortus; in vitro anthelmintics*

1. INTRODUCTION

Livestock contributes to the livelihoods of 60-70% of the Ethiopian population (Ayele *et al*., 2002; EEA, 2002). The availability of feed resources and their rational utilization for livestock represents possibly the most compelling task facing planners and animal scientists in the world (Holechek *et al*., 2010). Lack of adequate nutrition all year round is one of the major causes of the low productivity of ruminants in sub- Saharan Africa (Osuji *et al*., 1995). In case of Ethiopia an ever increase in human population pressure also results in shrinkage of grazing lands and feed shortages due to increased land use for cereal crop production (MoA, 1998; ILRI, 1995). Changes in nutritional status result in poor growth, productive and reproductive performances and marked fluctuations in body conditions of animals (Seyoum *et al*., 1996).

Grass dominated natural pastures and crop residues are major sources of animal feed in the highlands of Ethiopia. However, these feed resources are characterized by low digestibility, low protein content, and poor mineral composition (Seyoum and Zinash, 1989; Kafilzadeh and Maleki, 2011). Fodder trees and shrubs have the potential for alleviating some of the feed shortages and nutritional deficiencies experienced on smallholder farms (El Hassan *et al*., 2000). Integration of fodder trees and shrubs in to the cropping system could improve soil fertility as well as providing better quality forage for supplementation of low quality cereal residues mainly during dry season (Nnadi and Hague, 1985; Kindu *et al.,* 2006; Mekoya *et al*., 2009).

Fodder trees and shrubs offer considerable potential for use in mixed crop livestock production systems to alleviate and complement the low feeding value of crop residues and natural pastures that constrain livestock production in sub-Saharan Africa (Devendra, 1992; Gutteridge and Shelton, 1994). For a large part of the dry season, the forage of fodder trees and shrubs may maintain a crude protein content of more than 15 % (Yisehak *et al.,* 2010). As a result, animals with access to fodder trees and shrubs perform better than those kept on natural pasture in milk yield, weight gain, reproductive performances and survival rates (Norton, 1994b; Mohammed-Saleem *et al*.,1999).

In many small ruminant production systems, cultivated forage plants are not the main feed source because browse plants (bushes, trees or shrubs) contribute significantly to the nutrition of these animals during a prolonged dry period (Manolaraki *et al*., 2010), which occurs in tropical countries (Sanon *et al*., 2008). Tanniferous feeds are important to ruminants in many less developed countries. However, there are few published estimates on either the quantities or proportions of such feeds in the livestock diets (Devendra,1995) has noted some broad estimates of the importance of tree browses which are generally high in tannins and important in arid and semi-arid regions.

The tropical and subtropical regions have a large variety of tropical tannin rich plants (TRP) (Flores-Guido, 2001). These are an important component of the diet of ruminants (Rios and Riley 1985). Condensed tannins (CTs) found in tropical forages are thought to promote plant growth by reducing the release of leaf litter into the soil (Palm *et al*., 1991) and reducing the release of animal feces (Waghorn and McNabb, 2003). Because of the substantial benefits of CTs for ruminant health and productivity, much of research has been focused on these tannins (Waghorn and McNabb, 2003). For example, CT has been efficient at improving live-weight gain (Waghorn *et al*.1999). In sheep, they have been shown to increase milk protein concentration (Wang *et al*.1996), improve lambing percentages (Min *et al*.1999), and reduce, gastrointestinal nematode infection (Niezen *et al*.1995), incidence of fly strike (Leathwick *et al*.1995), and methanogenesis in sheep (Waghorn *et al*., 2002).

Negative effects include reduced absorption of minerals (Waghorn *et al*.,1994a), reduced rumen protein utilization (Jones and Mangan, 1977; Barry and Duncan, 1984), voluntary intake (Reed, 1995), and microbial activity in the rumen (Nuñez-Hernandez *et al*.,1991) and toxic effects reflected by damage of kidney and liver (Kumar and Singh, 1984; Vasconcelos *et al*., 2010).

In sheep (Athanasiadou *et al*.2001) and deer (Hoskin *et al*., 2000), CTs are capable of reducing parasite infection. This is dependent upon the source of the CTs used as well as the amount of CTs required to attain the desired effect (Waghorn *et al*., 2003). Although effective at reducing nematodes, this method of control is not as effective as drenching techniques, but is likely to be used as an additive to other methods of control (Waghorn *et al*., 2003).

Control of ruminant gastro intestinal parasite (GIP) over the past decades has been achieved by the use of anthelmintics drugs, but control of GIP is becoming more difficult due to the increased resistance of parasites to common anthelmintics, which has been reported in goats, sheep, and cattle (Prichard, 1994; Waller, 1994; Pomroy *et al.*, 2002). Alternative parasite management strategies using forages containing CT have recently been suggested (Niezen *et al,.*1995; Barry *et al.*, 2001; Min *et al.,*2002b).

Generally, expression of tannins as hydrolysable (HTs) and condensed tannins (CTs) seems to depend to a considerable extent on abiotic factors such as light, temperature and rainfall and also biotic factors such as nutrient availability and herbivore pressure to the plant (Waterman, 1999). Additionally, the CT-concentrations measured during the life cycle of the plant are variable, with the CT-content usually being highest in young, pre-flowering plants and lowest in the reproductive stages (Häring *et al*., 2007). Besides this, there are several factors that can influence the extraction efficiency, including extraction method, solvent type and concentration, particle size of plant materials, extraction time and temperature, solvent to solid ratio and extraction pH (Chirinos *et al*.2007).

For the extraction process, a suitable solvent is required. Generally, aqueous methanol (50%) and aqueous acetone (70%) are popular choices (Makkar, 2003). The latter has been reported by various workers to be better in extracting phenolics from tree leaves (FAO /IAEA, 2000). Still now different laboratories in different countries are using a number of organic solvents for extracting of tannins from various feed sources. There is no known official procedure developed by Association of Analytical chemists (AOAC).

Currently small-holder farmers of Jimma zone like many other sub-Saharan African countries are increasingly relying on various potential browse plants, underutilized potential feed resources, to supplement their ruminants especially in dry seasons (Yisehak *et al*., 2009). Among many indigenous fodder trees and shrubs in Jimma zone, *Albizia gummifera, Carissa edulis*, *Maytenus obscura, Ficus ovata*, and *Rhus glutinosa* are widely abundant and known as source of livestock feed (Yisehak *et al*., 2010). Very little research has been done so far on the feed value of these indigenous tree and shrub species in Ethiopia in general. However, effects of these potential tannin rich browse plants on health of animals especially CTs against GIP was not further studied using multiple storage conditions.

Besides methods of storage and extraction solvents are not compared and prioritized. This also means that indigenous knowledge of fodder tree and shrub species is not strongly supported by scientific bases.

Therefore this study was planned to evaluate the effects of selected indigenous browse species, storage conditions and extraction solvents on concentration of extractable condensed tannins and to quantify and compare the *in-vitro* anthelmentic activity of the plant species at different storage condition and extraction methods against *Haemonchus contortus.*

2. LETRATURE REVIEW

2.1. Feed resources for livestock and their nutritional value

Feed resources are classified as natural pasture, crop residue, improved pasture and forage, agro industrial by products and other by-products like food and vegetable refusal, of which the first two contribute the largest feed types (Alemayehu, 2003).

Natural pasture

Native pastures are still the most important feed source for ruminates. They account for the largest share of the land surface of numerous countries in Africa and Asia. Grazing off-takes from these lands is subject to great variations. Rapid increase of flock size as associated to the lack of appropriate management strategy (stocking rate, grazing period and duration, rangeland management, etc.) are the main causes of continuous degradation of range lands. Productivity study indicated that in the lowland areas of Ethiopia; native pasture yield 1tonne DM/ha and in seasonally water logged fertile areas 4-6 tonne DM/ha (Alemayehu, 1985). Most pasture grasses generally have DM content of 17-30 % (Enseminger *et al.,* 1990; Gashaw, 1992).

Forage yield and nutritional qualities of pasture are influenced by numerous factors representing ecological conditions and management activities. Those factors include frequency of cutting, species composition, and maturity stage of the plant, climatic conditions, soil fertility status and season of harvesting. As pasture gets mature, it is characterized by high content of fiber with a higher grade of lignifications and low protein content. The more the proportion of the legume to the grass composition, the higher the crude protein content of the mixed stand and bring better productivity on the animals (Yihalem, 2004). Browse is characteristically important as a source of feed during dry and winter periods, when either the quantity or quality of available grass is deficient. Dzowela (1993) noted that there are over 200 species native to continental Africa that has acceptable nutritional characteristics.

Trees may have CP contents up to 250 g/ Kg DM and in addition to their direct contribution to nutrient supply, may increase total DM intake and increase the digestibility of the basal

low CP diet, as discussed by Atta krah (1993) with beneficial effects on animal survival and productivity. The yield potential from fodder trees grown alone is high, with Atta Krah (1993) noted forage yields of up to 40tonne DM/ ha for *Leucaena Leucocephala* grown in Nigeria with 12 weekly cutting and 0.5 m spacing.

Crop residues

Crop residues are mainly fibrous material that is by-products of crop cultivation. Due to the intensity of and emphasis on crop production in Africa and Asia, great amounts of several by-products are produced annually. Crop residues available in the target area include, mainly, cereal straws, stubbles, fodder tree leaves and twigs. The availability of crop residues is closely related to the farming systems, the crop produced and the intensity of cultivation (Kossila, 1988).Most common crop residues (i.e. straws and stubble) have low crude protein content, in the range 2-5% on a dry matter (DM) basis. This suggests a basic limitation in the value of some of the residues (e.g. wheat and barley straw) around the border line of the 6-7 % dietary crude protein level required for promoting voluntary feed intake (VFI).Most of the residues are deficient in fermentable energy, as reflected by the relatively low organic matter digestibility, and also the limited availability of minerals

.

According to Guessous *et al.*(1989) and Outmani *et al.*(1991), the nutritive value of diet off taken by stubble grazing ewes, mainly crude protein and energy contents decreased with the number of week of grazing. CP content of stubble was below 5% DM and this crop residue was high in fiber. Consequently, the digestibility and intake of digestible nutrients are low. These deficiencies can partly be mitigated by supplementing roughage diets with feeds containing the deficient nutrients (Osuji *et a1.,*1993). With grain estimated to be 1 tons/hectare and straw to grain yield ratio being 2:1 (Conversion ratio variance 0.69), about 12 million tons of crop residues are produced annually from 6 million hectare of farmland in Ethiopia (Daniel, 1988). Alemu *et al.* (1991) further estimated that about 10.71 million tons of dry matter (DM) of crop residues are estimated to provide about 40 to 50% of annual livestock feed requirement (Daniel, 1988).

Straws from *tef*, barley and wheat form the largest component of livestock diet in the medium and highland areas, while maize, sorghum and millet Stovers constitute larger proportion of livestock feed in lower to medium altitudes (Alemayehu, 1985).

Fresh Stover's of maize and sorghum, the majority of crop residues have a DM content of 90 to 93%. Generally, the crude protein of crop residues ranges from 2.4-7% and the value of IVDMD for straw are between 34and 52% (Seyoum and Zinash, 1991and Kernan *et al.,*1979). Nevertheless, the nutritional values of crop residues vary according to the type of crop used. The better productive utilization of crop residues can be achieved either through appropriate supplementation (legumes, molasses, fruit pulps, poultry manure, urea, etc.) or chemical pretreatment (urea/ammonia treatments) both, which facilitate the microbial break down of the cell wall. Moreover, conservation and efficient or economic use of crop residues improves and enhances their utilization (Heimersen *et al.,*1984; Alemu *et al*.,1991; Chenost and Sansoucy, 1991; Getnet, 1999).

Improved pasture and forage

Quite a large number of annual and perennial forage and fodder species have been tested in the mid altitude under rain fed conditions in Ethiopia. As a result many improved herbage species have been identified for the ecology. *Chloris gayana,Panicum coloratum, Panicum maximum, Melinis minutiflora, Pennisetum purpureum, Zeamays, Sorghum vulgare, sorghum alumum, Desmodium uncinatum, Staylosanthes guanensis,Leucanea leucocephala, Dolichos lablab, (Lablab purpureus), Macroptilum atroparpurem* and *Vicia atropurpurea* are the most promising pasture and fodder species among the tested species so far and are recommended for mid altitude area (Lulseged and Alemu, 1985).

Livestock farmers are facing their biggest challenge during the dry season. Producing supplementary feed on farm by establishing grass/legume pastures would reduce their problem. For instance mixed grass legume pasture produced higher DM yields of better nutritive value than sole grass swards (Onifade and Akinola, 1986).

Agro-industrial by-products

The increasing human demands for several foods (i.e. olive oil, vegetables, wine, fruit juices, etc.) led to a considerable increase of lands occupied by crops producing these feeds. Consequently, huge amounts of agro-industrial by-products are available in numerous African and Asian countries (e.g. molasses, wheat bran, wheat middling, winery marc, etc.), which are still not fully utilized in livestock feeding. Most of these AIBPs are low in, and/or not balanced for, main nutrients. Moreover, the difficulty of the use of these feed sources as fresh material for extended periods and the lack of efficient ways for their integration in feeding calendars may account for their under-utilization.

2.2. Browse species and tannins

2.2.1 Tannins definition

Tannins are secondary plant polyphenols with great diversity (Jayanegara *et al.,* 2011). They are produced by a wide range of plant species with some variability with respect to the plant organ. Tannins are functionally defined by their capacity to bind proteins, which was traditionally exploited by the leather industry to preserve (tan) leather (Mueller Harvey and McAllan, 1992). Tannins are categorized into two major structural groups Hydrolysable and Condensed tannins. Hydrolysable tannins (HTs) are gallic or ellagic acid esters of sugars (Mueller Harvey, 2001). When they are consumed by ruminants, they can be degraded into gallic acid, which is readily absorbed from both the rumen and the small intestine and has been associated with liver and kidney lesions in sheep (Zhu *et al.,*1992).

Hydrolyzable tannins can be further metabolized to compounds such as pyrogallol (Murdiati *et al.,*1992), which are potentially toxic to ruminants (Dollahite *et al.,*1962). Some rumen bacteria involved in this degradative pathways include *Eubacterium oxidoreducens*, *Streptococcus bovis*, *Syntrophococcus sucromutans,* and *Coprococcus* spp. (Tsai *et al.,* 1976; Krumholz and Bryant 1986a,b).Plants that are considered to be toxic due to HT include *Clidemia hirta* (harendog; Murdiati *et al.,*1991), *Quercus ilex* (oak; Camp *et al.*, 1967), *Terminalia oblongata* (yellow wood; Doig *et al.,*1990) and *Ventilago viminolis* (supplejack; Pryor *et al.,*1972).

Condensed tannins (CTs) are polyphenolic oligomers and polymers of catechin (flavan-3 ols). The depolymerisation products of CTs are cyanidin and prodelphinidin and CTs have therefore been further classified as procyanidins and prodelphinidins (Waterman, 1999). Only a low degree of absorption of CTs by the digestive tract of ruminants has been reported (Terrill *et al.,* 1994). One of their most important chemical properties is the ability to form soluble and insoluble complexes with macromolecules, such as protein, fiber and starch (Luck *et al.,* 1994, Haslam, 1996).

The CT-protein interactions are most frequently based on hydrophobic and hydrogen bonding and are determined by the molecular mass and the molecular structure of both the tannin and the protein. Tannin-protein binding is usually reversible and acidic or alkaline pH or treatment with organic solvents can result in the dissociation of the complexes. For example, Jones and Mangan (1977) reported that CTs can bind with protein at pH 3.5-7.5 (i.e., ruminal and small intestinal conditions) to form CT-protein complexes, which dissociate and release protein at pH below 3.5 (i.e., abomasal conditions) or above 7.5. Thus, CT-containing plants can protect dietary protein against degradation in the rumen and increase AA supply to the abomasum and small intestine, resulting in an improved nutritional status of the animal.

Barry and Forss (1983) defined CT associated with plant protein after mastication as bound CT, and the CT remaining in the supernatant after high-speed centrifugation as free CT. It has been suggested that high concentrations of free CT in the rumen can react with other sources of protein after chewing by animals, such as enzymes secreted by rumen bacteria, and so inhibit rumen carbohydrate fermentation (Barry and Manley, 1986).Even if fodder trees have important nutritional merits, there are also reports (McNabb *et al.,*1993; Wang *et al.,*1994; Silanikove *et al.,* 1996; Kaitho *et al.,*1998; Du Plessis *et al.,*1999; Norton, 2000; Solomon, 2002) which indicate that anti-nutritional factors found in fodder trees such as tannins, saponins, non-protein amino acids, phyto-estrogens can affect growth, onset of puberty and reproductive functions via direct toxicity, interference in the metabolic process or reduction of nutrient availability or a combination of these pathways.

According to Yisehak *et al*. (2010) the feed quality of the studied plant species are presented in Table 1. As it might be clearly indicated that the plant species are looks quality feed sources for livestock species. The average values of each plant species for all the indicated nutrient parameters are averaged from the dry and wet season data of the same author.

Table 1.The nutritive value of leaves of tannin-rich trees species include in this study

Nutrients	**Albizia *gummifera***	***Carissa edulis***	***Maytenus obscura***	***Ficus ovata***	***Rhus glutinosa***
DM	932	905	913	914	932
CA	81	92	123	123	83
CP	299	138	151	186	145
EE	45	40	35	127	41
NDF	394	370	408	444	408
ADF	283	287	345	302	330
ADL	121	84	115	99	80
IVDMD	348	355	453	456	318
IVOMD	399	394	387	414	363
CHO	553	662	631	621	676
DCHO	399	397	453	464	358
DCP	20	14	14	17	15
DEE	4.5	4.3	3	1	4.3
ME	6.50	6.20	7	7	6.50
TDN	403	398	455	525	358

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;

2.3. Factors affecting extraction of tannins

Extraction process is widely used as a process of separation to obtain a crude extract of photochemical from the plant materials (Chirinos *et al.*,2007; Tabart *et al*.,2007). However, owing to each plant material has its unique properties in term of phenolic extraction; different plants may require different extraction conditions to achieve maximum recovery of phenolic compounds (Chirinos *et al.,* 2007). There are few factors that would contribute in influencing the rate of extraction and quality of extracted bioactive phenolic compounds, including type of extraction solvent, solvent concentration, particle size of medicinal plants, temperature and pH of extraction and extraction time (Liyana-Patthirana and Shahidi, 2004; Nobre *et al*., 2005).

2.4. Condensed tannins and gastrointestinal nematodes

Fodder trees and shrubs have high potential value as a source of feed for domestic livestock and wildlife. They can be successfully integrated into production systems to provide additional feed resources for use in mixed diets of livestock, fuel and mulch, to control erosion when planted as wind breaks and to maintain or rehabilitate degraded areas of rangelands. Grazing ruminants, goats and sheep are particularly known to consume a wide range of browse foliages and are reported to select those that meet their nutritional needs and avoid those that can be toxic (Ngwa *et al.,*2003). Feed selection by animals depends on the palatability of the feeds. Palatability is a complex phenomenon being determined by both plant and animal factors (Marten 1978). Browse foliages have been reported to contain tannins with varying concentrations (Abdulrazak *et al*., 2000; Osuga *et al.,* 2006, 2007). The tannins in the foliages exhibit anti-nutritional effects or positive nutritional merits.

The low protein content of low-quality tropical feeds that limits their digestion. This limitation can be overcome by supplementing a protein source e.g. fodder trees or shrubs. Sufficient literature exists on the nutrient content of several fodder trees and shrubs (Le Houerou, 1980; Topps, 1992; Siaw *et al.,*1993). Though the reported crude protein (CP = 6.25 * the nitrogen (N) content) is variable, it is within the range of 12-30%, and several exceed alfalfa hay in protein content.

Therefore, most fodder trees would be good protein supplements, provided they are degraded adequately in the rumen to make the protein available to the animal and non-toxic (Leng, 1997). Fodder trees contain significant fiber, but *in-vitro* digestion studies indicated that the fiber was as digestible as that of alfalfa hay, and much better than that of cereal straws (El Hassan *et al.,* 2000). Macro- and micro- mineral content of fodder trees are usually adequate to cover animal requirements (Smith, 1992).

When ruminants are fed on high-quality fresh forages containing high concentrations of N (25 to 35 g of N/kg of DM), carbohydrate digestion in the rumen is efficient; however, degradation of forage N is excessive, resulting in surplus levels of ammonia (20 to 35%) in the rumen and absorption of that ammonia from the rumen, which is ultimately excreted as urea in the urine (Ulyatt *et al.,* 1975). Therefore, a reduction of protein degradation in the rumen will increase the quantity of protein digested in the small intestine, potentially increasing animal production. It has been reported that CT in forages markedly reduces protein solubilization and degradation in the rumen and reduces ruminal proteolytic activity, but may inhibit extracellular microbial enzymes(proteinases, cellulases, and hemicellulases; Chung *et al.*,1998a,b; Min *et al.*,2001a; 2002a).

Dependent on the tanniferous plant species and its CT-concentration the consumption of tanniferous plants with high concentrations of CTs (> 60 g kg-1 DM) has been associated with a number of detrimental effects on the metabolic nutrient supply and performance of ruminants, including reduction in voluntary feed intake, growth inhibition and interference with the morphology and the proteolytic activity of microbes in the rumen or depressed growth rates. (McNabb *et al.,*1996, Waghorn and McNabb, 2003). Low or moderate CT-concentrations (< 60 g kg-1 DM) have resulted in positive effects on sheep (Min *et al.,*2003a). For example, increased live weight gain, reduced carcass fat content as well as increased milk and wool production have all been associated with the consumption of CTs (Waghorn and McNabb, 2003).

The consumption of plant species with high CT contents (generally > 50 g kg-1 of dry matter, DM) significantly reduces voluntary feed intake, while medium or low consumption (< 50 g kg-1 DM) seems not to affect it (Barry and Duncan, 1984; Barry and Manley, 1984; Waghorn *et al.,* 1994a).

Barry and McNabb (1999) indicated that the negative effect of consuming *Lotus pedunculatus* (CT content > 50 g kg-1 DM) on voluntary feed intake in grazing sheep is not seen when the same animals consume *L. corniculatus* (which has only 34-44 g CT kg-1 DM). Hervás *et al.*(2003c) dosed sheep intraruminally with different quantities of quebracho CT extract (0, 0.5, 1.5 and 3.0 g kg-1 live weight, LW, per day, equivalent to 0, 28, 83 and 166 g kg-1 DM consumed in the diet) and found that all the animals ate everything offered them, except for those that had received the highest dose (eq. 166 g kg-1 DM).In these sheep, voluntary feed intake was practically nil after 5 or 6 days. The tannin-proline-rich protein complexes formed, unlike other protein-tannin complexes, are stable across the whole pH range of the digestive tract. This might cancel their negative effect on palatability, and therefore on feed intake, and improve the digestion of tannin- rich feeds (Robbins *et al.,*1987; Austin *et al.*,1989; McArthur *et al.,*1995; Narjisse *et al.,* 1995).

2.4.1. The ecology of gastrointestinal nematodes

The nematodes of major importance for sheep production are *Haemonchus contortus, Teladorsargia circumcincta* and *Trichostrongylus spp.* because they are the most profuse and cause severe losses in production. *H. contortus* is the most important species in regions with summer-dominant rainfall or tropical and subtropical areas where warm, moist pasture conditions are conducive to growth and survival of its larval stages (O'Connor *et al.,*2006). *H.contortus*, also known as barber pole worm, is the larger stomach worm found in the abomasum of ruminants. This parasite belongs to Family *Trichostrongylidae* and has been reported worldwide, particularly being a problem in areas with a hot/moist climate. Sheep, especially lambs, are more consistently susceptible to the adverse effects of worms than mature animals, and clinical disease is more common with that species as well (Merck, 2010).

The disease caused by this parasite, known as haemonchosis, is characterized by anemia due to hemorrhage originating from the feeding behavior (Dunn, 1978). A single worm is responsible for the removal of approximately 0.05 ml of blood a day. This parasite also utilizes calcium, phosphorus, cobalt, copper and vitamins for nutrients and the mobilization of iron and proteins for compensatory erythropoiesis due to anemia may lead to animal death (Urquhart *et al.,* 1996). The females of *H.contortus* are very prolific and able to deposit around 5,000 to 10,000 eggs per day that will be released into the environment through sheep feces, thus contaminating the pasture and propitiating the life cycle for this parasite (Urquhart *et al*., 2007). As for infection, a fecal egg count of 10,000 eggs per gram of faeces is indicative of trichostrongyle infection, which concurrently with lower packed cell volumes, characterizes the pathogeny by *H. contortus* (Mehlhorn, 2008).

A typical nematode life cycle includes egg, 4 larval stages and adult. The third larval stage is usually the infective stage (Urquhart *et al.,* 1996). The minimal mean temperature for eggs hatch is 18ºC. Below this temperature, larvae will not develop. Eggs will not survive at a mean temperature of 21ºC for more than 20 days. However, if an infected animal defecates in humid places with temperatures above 18ºC, eggs will eclode normally in 14 to 20 hours. Knowing the ecology of parasites is of great importance in understanding development, survival and transmission.

2.4.2. Anti-parasitic Plants on the Control Strategy of Gastrointestinal Parasite

Anitparasitic plants have long been used as a method to control gastrointestinal nematodes. Renewed interest in the use of these traditional medicines has stemmed from the cost of drugs, resistance to anthelmintics, and the poor availability of veterinary services in underdeveloped countries, such as Kenya (Anon.1996; Monteiro *et al.,*1998; Wanyama, 1997ab; Wanyangu *et al.,*1996). Recently, there has been much interest in feeding plants containing condensed tannins to animals to reduce the effects of infection with gastrointestinal nematodes. Parasitism of the abomasums and small intestine causes extensive protein losses in the digestive tract of sheep (Kimambo *et al.,* 1998). Alternative, nondrug parasite-control strategies have recently been suggested based on using forages that contain CTs (Niezen *et al*., 1995; Barry *et al.,* 2001).

CTs may have direct effects on internal parasites themselves or may indirectly control the parasites by increasing the resistance and resilience of animals to GIP infections through improved protein nutrition. Possible direct effects could be mediated through CT-nematode interactions, which reduce nematode viability. It has been reported (Niezen *et al*.,1995) that direct effects of CTs on GIP may account for reduced fecal egg counts (FEC) and nematode burdens in lambs that grazed *Hedysarum coronarium* compared to *M. sativa* in New Zealand. Evidence in support of the direct effect of CT was provided by Molan *et al*.(2000) who demonstrated that the CT extracted from *L. pedunculatus, L. corniculatus, H. coronarium, and O. viciifolia* forages reduced the rate of larval development (eggs to L_3 larvae) by 91%, reduced the number of eggs hatching by 34%, and decreased the mobility of L_3 larvae by 30%. CT containing forage legumes have been shown to reduce parasite burdens in both immature and adult sheep and goats. Niezen *et al.* (1995) used lambs carrying a high parasite load grazing tannin-containing *sulla (Hedysarum coronarium)* with *alfalfa*.

The *sulla* pasture treatment significantly reduced faecal egg count numbers, and increased live weight gain by 50% after 28 days compared to lambs grazing alfalfa. In a second experiment involving a lower parasite burden, sulla eliminated parasite-induced anorexia and decreased worm count after 42 days. Moore *et al.* (2008) later established that feeding *Sericia lespedeza (Chinese bush clover)* containing tannin concentrations of 87-181 g/kg to young male goats reduced abomasmal worm counts by 37% over non-tannin containing *Bermuda grass (Cynodon dactylon).*

The FEC and parasite burdens at slaughter were considerably lower for lambs grazing *H. coronarium* (CT-containing forage) than for lambs grazing *M. sativa* (Niezen *et al.,* 1995; 1998a,b). Dewormed lambs grew at similar rates when grazing *H. coronarium or M. sativa*. However, non-dewormed lambs grew much better on the *H. coronarium,* indicating a reduced need for anthelmintic drugs to control GIP in grazing lambs. Recently, Min *et al*.(2003b) showed that GIP were controlled when Angora does were grazed (81 days) on *L. cuneata* (52 g of CT/kg of DM) in spring and summer, but not when goats were grazed on control forages (Crabgrass/tall fescue; 2.0 g of CT/kg of DM). Tracer goats that grazed *L. cuneata* had a 76% reduction in total adult worm burdens compared with the control.

The *L. cuneata* diet was also associated with a reduction in the numbers of Haemonchus (94%) and *Teladorsagia spp.* (100%) in the abomasum and *Trichostrogylus* (45%) in the small intestine. CT may enhance resistance of GIP infection through increases in protein supply, which are prioritized for tissue repair and immune response (Barry *et al*.2001; Niezen *et al*., 2002). The CT could complex with nutrients and inhibit nutrient availability for larval growth or decrease GIP metabolism directly through inhibition of oxidative phosphorylation (Scalbert, 1991), causing larval death (Athanasiadou *et al.,* 2001).

The *in vitro* study by Mihreteab *et al.*(2011) suggested that *the Rhus glutinosa, Syzygium glutinosa* and *Albizia glutinosa* CTs plant species has one of the alternative in the packages towards the control of haemonchosis in sheep. Research by Molan *et al.*(2000) and Paolini *et al.* (2003) suggest that since L_3 stage of infective larvae are strongly inhibited by tannins, CT containing forages may interrupt parasite life cycles and reduce pasture contamination with infective nematodes. In vitro studies, such as those by Barrau *et al.*(2005) and Brunet *et al.* (2008) have supported an alternative hypothesis that CT exerts direct toxic effects on parasitic nematodes. Therefore, chemical composition and molecular size of CT, as well as concentration, may be factors in GIP control.

2. 5. Methods used to alleviate deleterious effects of tannins in browse trees

Various methods have been used to alleviate deleterious effects of 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 tannin resistant bacteria (Miller *et al*., 1995) and chemical techniques (treatment with alkalis, (e.g. urea, ammonia, calcium hydroxide, sodium hydroxide, potassium hydroxide) Makkar and Singh, 1993; Vitti *et al*., 2005), organic solvents, precipitants (Ben Salem *et al.,* 2007); chelating metal ions (Price *et al*., 1979), and oxidising agents (e.g. potassium dichromate, potassium permanganate) (Makkar and Singh, 1993).

Although being effective for overcoming toxic effects of tannins (Mueller-Harvey, 2006), alkalis, metal ions and oxidising agents require expertise, result in large losses of soluble nutrients and are corrosive (Vitti *et al*., 2005). Moreover, if mismanaged, they can be poisonous to people and animals and are not environmentally friendly (Vitti *et al*., 2005).

Other de-tanninification approaches involve use of microbial enzymes (McSweeney *et al*., 2001) and tannin binding compounds such as polyethylene glycol and polyvinyl-pyrrolidone (Priolo *et al*., 2005; Mlambo *et al*., 2007). The use of polyethylene glycol (PEG; MW 4 000 or 6 000), for which tannins have higher affinity than for proteins, is by far the most used reagent to neutralize these secondary compounds (Provenza, 2001). Consequently, it would be possible to increase the nutritive value of tannin rich browse by adding compounds such as PEG, 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. However, the limited availability and high cost of microbial enzymes and tannin binding compounds makes their application impractical and unprofitable under low input cattle production systems (Ben Salem *et al*., 2005a). Oven, freeze, and sun air drying techniques have also been used to lessen the adverse effects of phenolics in browse legumes (Dzowela *et al*., 1995; Stewart *et al*., 2000).

3. MATERIRAL AND METHODS

3.1. The study areas

The quantification of extractable condensed tannin (ECT) was conducted at Jimma University in nutrition laboratory, south western Ethiopia located at 7°40′N and 36°50′E and at an altitude of 1780 m above sea level (http://en.wikipedia.org/wiki/Jimma_Zone). The climate is characterized as 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. Farmers in the area carry out mixed crop-livestock agriculture. Foliages of fodder trees and shrubs (Yisehak *et al*., 2010; Yisehak and Belay, 2011) are becoming potential supplements of ruminants especially in the dry season.

The effects of ECT on egg inhibition, larval development and adult motility for *Haemonchus contortus* activities was done at Addis Ababa University Faculty of Veterinary Medicine Parasitology and pathology laboratory, Debre Zeit, Ethiopia located 47 km south of Addis Ababa, in Oromia National Regional State. Topographically the city is located in tepid to cool sub-moist mid highland at an altitude of about 1920 meters above sea level with moderate weather condition The absolute location of Debre Zeit is 8°45′N 38°59′E8.75°N 38.983°E (http://www.bishoftu.org)

3.2. Experimental procedures and data collection

3.2.1. Collection of plant materials and Extraction protocol

The plant species such as *Albizia gummnifera, Carissa edulis, Ficus ovata, Maytenus obscure* and *Rhus glutinosa* were collected from their natural habitat, Omo Nada *Woreda* of Jimma zone southwest, Ethiopia. Fresh leaves, fresh leaves dried and ground immediately and the plant materials samples that have been preserved for 1.5 years at room temperature were used for the study. The preparation of samples for the extraction was proceed as follows.

The fresh leaves samples were transported to the laboratory in fresh state in a plastic bag kept on ice and transported under dark conditions. After arriving at the laboratory immediately for fresh storage sample using a chopper and sieved with 1mm and for dried sample dried to constant weight at 55°C.

Aqueous ethanol (50%) and extraction of each plant was performed by decoction. Dried and finely ground plant material (200 mg) is taken in a glass beaker of approximately 25 ml capacity. For each samples 10ml of aqueous ethanol (50%) was added and the beaker was suspended in an ultrasonic water bath and subjected to ultrasonic treatment for 20 min at room temperature. Then, the content of the beaker was transferred to centrifuge tubes, cooling by keep on ice and subject to centrifugation for 15 minute at 2000 rpm. And the extract was stored at 4°C until used. The same procedure was applied on an aqueous ethanol, (70%) and for aqueous acetone (50%, 70%).

3.2.2. Quantification of extractable condensed tannins

The determination of extractable condensed tannin (CT) was based on the oxidative de-polymerization of plant extracts in butanol-HCl reagent using 2% ferric ammonium sulfate in 2N HCl catalyst (Porter *et al*.1986).

3.2.2.1. Collection of adult parasites and egg recovery technique

To collect adult female parasites of *H. contortus*, the abomasa of naturally infected sheep from Debre Zeit Municipal Abattoir. Incised along the curvature the abomasa and washed slowly under tap water several times. Then, adult worms was picked manually using forceps and put in a universal bottle containing phosphate buffered saline(PBS, pH: 7.2) and transported in cold chain (4 ^{0}C) to Addia Ababa University Faculty of Agriculture and Veterinary Medicine, Parasitology and pathology laboratory. The egg recovery was performed according to the method described by (Jabbar *et al*., 2006) female adult worms were crushed using pestle and mortar. After liberation, the eggs were culture in a 250ml jar filled with autoclaved sheep feces for eight days at room temperature.

3.2.2.2. Infected sheep with *H. contortus* larvae

About 2000 to 3000 larvae inoculated to six de-wormed Adda *woreda* sheep that be maintained in a partitioned animal house of the faculty of Agriculture and Veterinary Medicine, Addis Ababa University to be served as donor of *H. contortus* eggs for the *in-vitro* tests.

3.2.2.3. Collecting and counting of eggs from donor sheep

Feces collected from *H. contortus* egg donor sheep was processed and then centrifuged in the test tubes for 1 minute then 2000 rpm and supernatant were discarded. Tubes then agitate on a vortex mixer to loosen the sediment and saturated sodium chloride solution was added until a meniscus formed above the tube. A cover slip placed and plucked off carefully after 5 minutes from tubes and eggs washed off into a conical glass centrifuge tube. The tube was filling with water and centrifuged for 1 minute at 2000 rpm. The supernatant were decanted and eggs were re-suspended in saline solution with by McMaster technique counting of egg was done. The result was reported as egg per gram (epg) as according to Coles *et al.* (1992).

3.2..3. Egg hatchability inhibition test

The egg hatchability inhibition test was conducted according to the procedure described by Coles *et al.* (1992) with little modifications. Extracts of CTs from the five plant species was be used as the test treatments. Albendazole dissolved in Dimethyl-Sulfoxide (DMSO) and diluted in distilled water used as a positive control while untreated eggs in distilled water will be used as negative control. The test was conducted in 10ml test tubes. In the assay, approximately 170-270 eggs in 1.5ml of water were place in each test tube. A Concentration 50 mg/ml of each plant extract was added. The test tubes were be covered with aluminum foil making 15 to 20 holes for air circulation and kept in an incubator at 27 ^{0}C for 48 hrs. The experiment was repeated three times. Hatched larvae and unhatched eggs were then counted under dissecting microscope at 40× magnification.

The anthelmintic efficacy of *H. contortus* was evaluated calculated according to (Coles *et al*.,1992; Taylor *et al*. 2002; Coles *et al.,* 2006).

The percent faecal egg count reduction was calculated using the following formula:

$$\% \text{ FECR} = \frac{a-b}{a} \times 100$$

Where, a = EPG pre-treatment and b = EPG post treatment.

3.2.4. Larval development inhibition assay

The larval development inhibition test was conducted with a modification of the technique described by Costa *et al*. (2006). CTs extracts from the five plant species were used as the test treatments. Albendazole (Anti biotic) 10mg/ml dissolved in DMSO and diluted in distilled water used as positive control while untreated eggs in distilled water were used as negative control. After incubating the eggs at 27 ^{0}C for 24 hours, an aliquot of 1.5ml, containing 100-150 first stage larvae (L_1) of *H. contortus* was mixed with 10gm of feces that collected from a de-wormed sheep free of gastrointestinal nematodes. A 50mg/ml concentration of each tannin extract were added on a sheep feces which containing L_1. The test materials were incubated for 6 days at room temperature. At the end of the 6th day the wall of each cup containing the sample was thoroughly rinsed with 10ml of water to collect the larvae. Then one drop of Lugol's iodine solution was added and all (L_3) stage larvae were counted under stereomicroscope.

3.2.5. Adult motility test

Adult motility test was performed according to Petersen *et al*. (1997). Accordingly 50 mg/ml of each CTs extracts was diluted with distilled water. Albendazole dissolved in Dimethyl-Sulfoxide (DMSO) and diluted in distilled water used as a positive control while distilled water was used as negative control. For each treatment 10 adult parasites were used. The same concentration form each plant extracts (50mg/ml) was placed in Petridis and the parasites were immersed, the times of mortality were recorded.

Table 2. The lists of main factors included in this study

Factors		
Plant species (5 levels)	**Method of extraction (4 levels)**	**Storage time (3 levels)**
Albizia gummifera	Aqueous acetone (70%)-Butanol-HCl-Fe	Fresh leaves immediately grinded to 1mm screen
Carissa edulis, *Ficus ovata*	Aqueous acetone (50%) Acetone-HCl-Fe	Dried leaves; fresh leaves dried to constant weight at 55°C
Maytenus obscura	Aqueous methanol (70%)-butanol-HCl-Fe	Dried-preserved; dried to constant weight at 55°C and preserved for 1.5 years at room temperature
Rhus glutinosa	Aqueous methanol (50%)-butanol-HCl-Fe	

3.3 Statistical Analysis

The data were subjected to a three ways-analysis of variance using the general linear model (GLM) procedure of statistical analysis system (SAS, 2010 version 9.3) in a 5 x 4 x 3 factorial arrangement. The GLM model used was:

$$Y_{ijkl}=\mu + p_i + m_j + s_k + (p\times m)_{ij} + (p\times s)_{ik} + (m\times s)_{jk} + (p\times m\times s)_{ijk} + \varepsilon_{ijkl}$$

Where: Y_{ijkl}, is total observation, μ, population mean, p_i, i^{th} plant species effect (i = 1 to 5), m_j, j^{th} method of extraction (j = 1 to 4); s_k, the k^{th} effect of storage condition (k = 1 to 3); $(p\times m)_{ij}$, interaction effect between plant species and method of extraction; $(p\times s)_{ik}$ is the interaction effect between plant species and storage condition; $(m\times s)_{jk}$, the interaction effect between method of extraction and storage condition; $(p\times m\times s)_{ijk}$ was the interaction effect of all fixed factors and e_{ijkl} was the residual error.

4. RESULTS AND DISCUSSION

4.1. The Effect of Plant Species and Storage Conditions on the Concentration of Extractable Condensed Tannins Extracted with Acetone 50% and 70%

The least square mean values (%) of the concentration of ECT analyzed for the leaves of various plant species at three different storage conditions following acetone 50 and 70% extraction procedures are presented Table 3. The concentration of ECT with 50% acetone extraction in *A. gummifera* and *M. obscura* leaves showed the same value at fresh condition (6%); however, the values of ECT decreased from dried to dried preserved conditions (18.8 to 15.9% in *A. gummifera* and 18.6 to 13.9% in *M. obscura*) ($P<0.001$). On the contrary, significant difference was not observed for ECT value in the leaves of *C. edulis* and *R. glutinosa* at dried and dried-preserved conditions ($P>0.001$). The ECT concentration of *A. gummifera* and *F. ovata* leaves showed nearly similar value at fresh state (5%) and increased from dried (12.8 and 9.5%) to and dry preserved had (19 and 13.6%), respectively in acetone 70% extraction. Similar to 50% acetone extraction, fresh leaves had the smallest ECT concentration across the plant species compared to the dried leaves ($P<0.001$). The increasing trends of CT concentration as advances in storage time and drying conditions might be indication of having a new molecular bond between condensed tannins and new chemical substance produced during heating and storage time.

Variations in the ECT value across the plants and storage conditions might be associated to differences in the length of storage on top of differences in plant species. The highest value of ECT in both dried and dried-preserved conditions across plant species as compared to fresh plant material might be due to oxidation and /or polymerization of condensed tannins with other chemical constituents during drying and length of storage after drying. According to Ferreira *et al.* (2003), tannin quantification has to be done immediately after harvesting to avoid formation of protein complexes or polymerization. Moreover, Lin *et al.* (2010) also reported rapid loss of total phenolics and extractable condensed tannin (ECT) from pericarps of tanniferous plants during the dry storage.

He also confirmed that during the dry storage, most of the ECT of hypocotyls formed complexes with multiple phenolic hydroxyl groups, and may form complexes with proteins, metal ions, amino acids and polysaccharides. Norton and Ahn (1997); Dalzell *et al*. (1998), Hove *et al.* (2003), Makkar (2003), Boudhrioua *et al.*(2008) and Villamor *et al*. (2009 also observed polymerisation effects of drying tanniferous feedstuffs on the concentration of ECT. Hättenschwiler *et al.* (2010) reported that inter-specific variation in ECT concentration due to the chemical structure of ECT, such as the polymerization degree; therefore, there is wide variation in structure and concentration of leaf ECT among tropical tree.

The concentration of condensed tannins in most of the plant species across all the storage conditions reached the level that affects intake, digestibility and absorption of feedstuffs for livestock species (average more than 50 g/kg DM). The variation of concentration of ECT values plants could be variation in plant species. Kaitho *et al*. (1998), Ozturk *et al*. (2006), Arigbede *et al*.(2011) reported similar findings that species variation has significant effect on chemical composition of browse plants.

Table 3.Least square means of ECT determined for different plant species extracted by acetone 50 and 70% at different storage conditions

Plant species	Extraction solvent	Storage condition	ECT content	SE	P
Albizia gummifera	Acetone 50%	F	6.0[c]		
		D	18.8[a]	0.52	***
		DP	15.9[b]		
Carissa edulis		F	5.1[b]		
		D	15.1[a]	0.71	***
		DP	15.4[a]		
Ficus ovata		F	6.0[c]		
		D	12.0[b]	0.78	***
		DP	16.3[a]		
Maytenus obscura		F	6.0[c]		
		D	18.6[a]	0.67	***
		DP	13.9[b]		
Rhus glutinosa		F	6.8[b]		
		D	16.0[a]	0.35	***
		DP	16.6[a]		
Albizia gummifera	Acetone 70%	F	4.9[c]		
		D	12.8[b]	0.66	***
		DP	19.0[a]		
Carissa edulis		F	5.8[b]		
		D	9.1[ab]	0.61	***
		DP	12[a]		
Ficus ovata		F	4.6[c]		
		D	9.5[b]	0.61	***
		DP	13.6[a]		
Maytenus obscura		F	6.8[b]		
		D	15.3[a]	1.57	***
		DP	13.6[a]		
Rhus glutinosa		F	5.6[b]		
		D	18.5[a]	0.60	***
		DP	19.6[a]		

*F, fresh; D, dried to constant weight at 55°C; dried to constant weight at 55°C and preserved for 1.5 years; ECT, extractable condensed tannin; SE, standard error of means; ***P<0.001*

4.2. The Effect of Plant Species and Storage Conditions on the Concentration of Extractable Condensed Tannins Extracted with 50% and 70% Ethanol

The effect of plant species and storage conditions on the concentration of extractable condensed tannins extracted with 50% and 70% Ethanol is presented in Table 4. Similar to acetone 50 and 70% extraction, the least values of ECT was determined for the fresh plant leaf samples as compared to ECT values in both dried and dried-preserved ($P<0.001$). These differences could be associated with differences in dry matter content of the plant species. The present study is in agreement with Kamalak *et al*. (2004) that the chemical composition of plants varies with variations in the plant species diversity. Haslam (1998) reported the chemical complexity and heterogeneity of plant tannins. Kelman and Tanner (1990), Ayres *et al*.(1997) and Ozturk *et al*.(2006) also reported major difference in tannin structure between plant species. On the contrary to acetone extraction, significant difference was not observed between dried and dried-preserved samples of all the plants in Ethanol 50% extraction ($P>0.001$). This variation might be due to differences in the tannin removing ability of the solvents from the same plant species. Makkar (2003), Liyana-Patthirana and Shahidi (2004) and Nobre *et al*.(2005) reported different tannin removing abilities of organic solvents such as acetone, methanol and ethanol that would contribute in influencing the rate of extraction and quality of extracted bioactive phenolic compounds.

On top of plant species variation, the present study also investigated the influences of storage condition on concentration of ECT. According to Ferreira *et al.* (2003), different storage times showed significant differences for phenolic substances, indicating that tannin quantification should be done immediately after harvesting plant to avoid formation of protein complexes or polymerization which leaded to exaggerated results.

Mueller – Harvey (2006) reported the wide range of different tannin structures between plant species, varieties and even within plant parts. Makkar Singh (1991a, 1993) also confirmed the relative degree of polymerization of tannin in stored leaves. Makkar (2003) reported absences of decreasing tannin inactivation either by steaming or autoclaving plant leaves.

With 70% ethanol extraction, *A. gummifera, C. edulis,* and *F. ovata* leaves had a highly significant difference for ECT concentration in each storage conditions ($P<0.001$). ECT concentration didn't vary in both dried and dried-preserved storage conditions for *M. obscura* and *R. glutinosa* leaves ($P>0.001$).

The smallest concentration of ECT in the fresh leaves as compared to the dried and dried-preserved storage conditions might be due to variation in plant species as well as the dry matter content of the plant species. The high concentration of tannins in dried and dry preserved plants as compared to fresh plants indicated the presence of polymerization of tannins with byproducts of heating feed stuffs. Also tannin concentration varies with variations in plant species and extraction solvents, the literature sources cited for the previous table (Table 3) can also be considered for comparison of the results indicated in Table 4.

Table 4.Least square means of ECT determined for different plant species extracted by ethanol 50 and 70% at different storage condition

Plant species	Extraction solvent	Storage condition	ECT content	SE	P
Albizia gummnifera	Ethanol 50%	F	5.6[b]	1.1	***
		D	15.5[a]		
		DP	16.0[a]		
Carissa edulis		F	6.9[b]	0.90	***
		D	12.8[a]		
		DP	14.9[a]		
Ficus ovata		F	6.0[b]	0.90	***
		D	13.0[a]		
		DP	13.4[a]		
Maytenus obscura		F	7.2[b]	1.2	***
		D	16.0[a]		
		DP	16.2[a]		
Rhus glutinosa		F	7.2[b]	0.34	***
		D	15.3[a]		
		DP	15.3[a]		
Albizia gummnifera	Ethanol 70%	F	2.5[c]	0.51	***
		D	10.2[a]		
		DP	8.2[b]		
Carissa edulis		F	4.2[c]	1.47	***
		D	14.0[a]		
		DP	7.8[b]		
Ficus ovata		F	3.2[c]	0.34	***
		D	9.3[a]		
		DP	6.5[b]		
Maytenus obscura		F	5.8[b]	1.0	***
		D	11.8[a]		
		DP	11.7[a]		
Rhus glutinosa		F	5.1[b]	0.48	***
		D	8.1[a]		
		DP	6.5[ab]		

*F, fresh; D, dried to constant weight at 55°C; dried to constant weight at 55°C and preserved for 1.5 years; CT, extractable condensed tannin; SE, standard error of means; ***P<0.001*

4. 3. The Interaction effects of Plant Species, Extraction Solvents and Storage Conditions on the Concentrations of Extractable Condensed Tannin

The combined effect of plant species and storage time on concentration of ECT compared for each extraction solvent is presented in Table 5. For each extraction solvent, interspecies and variations in storage time had a significant effect on concentration of ECT ($P<0.001$). For each plant species and extraction solvent the least concentration of ECT was observed in fresh leaf samples as compared to the ECT values recorded in other two storage conditions ($P<0.001$). In general, it was observed that the concentration of ECT in fresh leaves for all plants across the extraction solvents was not more that 7.2%; however, the CT values in both fresh dried and stored after dried leaves ranged from 6.5 to 19.6%.

Further more, the ECT content was found to be the highest (18.8%) in *A.gummifera* extracted with acetone 50% at dried condition where as the lowest ECT value (5.1%) was observed in *C. edulis* at fresh leaf samples ($P<0.001$). On the other hand with 70% acetone extraction, the highest and lowest values of ECT were recorded in *R. glutinosa* (19.6% at fresh dried condition) and *A. gummifera* (4.9%) or *F. ovata* (4.6%)leaves determined in fresh condition, respectively ($P<0.001$). The dried and preserved leaves *of M. obscura* had the highest ECT concentration (16.2%) with ethanol 50% extraction as compared to the rest of plants in various storage conditions. On the contrary, among the plant species included in this study, the ECT concentration in fresh leaves of *A.gummifera* extracted with both Ethanol 50 and 70% levels showed the least value (5.5 and 2.4%)ECT, respectively as compared to the rest of plant species and storage conditions ($P<0.001$).

Table 5.Least square means for the effects of plant species, extraction solvents and storage time on concentration of extractable condensed tannin

	Albizia gummifera			*Carissa Edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*				
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	SE	P
A 50%	6^{j}	18.8^{a}	15.9^{e}	5.1^{k}	15.5^{f}	15.4^{f}	6.0^{j}	12.0^{h}	16.3^{d}	6.0^{j}	18.6^{b}	13.9^{g}	6.8^{i}	16.0^{e}	16.6^{c}	0.05	***
A 70%	4.9^{n}	12.8^{f}	19.0^{b}	5.8^{k}	9.1^{i}	12.0^{f}	4.6^{n}	9.5^{h}	13.6^{e}	6.7^{j}	15.3^{d}	13.6^{e}	5.6^{l}	19.6^{a}	18.5^{c}	0.05	***
E 50%	5.5^{k}	15.5^{c}	16.0^{b}	6.9^{i}	12.8^{g}	14.9^{e}	6.0^{j}	12.9^{g}	13.4^{f}	7.2^{h}	16.0^{b}	16.2^{a}	7.2^{h}	15.3^{d}	15.3^{d}	0.05	***
E 70%	2.4^{m}	10.2^{c}	8.2^{e}	4.2^{k}	14.0^{a}	7.8^{g}	3.2^{l}	9.3^{d}	6.5^{h}	5.8^{j}	11.9^{b}	11.9^{b}	5.9^{i}	8.1^{f}	6.5^{h}	0.05	***

*F, fresh leaves; FD, dried; DP, dried-preserved; ES., extraction solvent; ST, storage time; A, acetone; E, ethanol; SE, standard error of mean;***P<0.001*

The combined effects of plant species and extraction solvents on concentration of ECT compared for each storage condition is presented in Table 5. For each storage condition, differences in plant species and extraction solvents had a significant variation the concentration of ECT ($P<0.001$). The tannin extracting ability of different concentration levels of acetone and ethanol varied with variations in the plant species and storage condition ($P<0.001$). Ethanol 50% showed better ability of extracting condensed tannin in fresh samples than the rest of extraction solvents ($P<0.001$). In general, it was observed that the concentration of ECT extracted by 70% ethanol showed the least value for all plants and for all storage conditions. This might be due to differences in the biological activity of the plant species, the molecular mass and the type of phenolic compounds of the solvent.

According to Downey and Hanlin (2010), acetone extracts more condensed tannin than ethanol from grape skin; however, in the present study we observed variations in tannin extracting capacity of acetone and ethanol which have been tending to vary with storage time and plant species. Shu-Dong *et al.* (2011) reported that the acetone-water (1:1, v/v) was more effective solvent for extracting total phenolics and extractable condensed tannins from *Machilus pauhoi* leaves than methanol, ethanol, acetone, water, methanol water (1:1, v/v), and ethanol-water (1:1, v/v).However in the present study the tannin extracting ability of the various solvents varied with the storage conditions and plant species.

Table 6.Least square means for the effects of plant species and extraction solvents on concentration of extractable condensed tannin compared for each storage type

ES	*Albizia gummifera*			*Carissa Edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*		
	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP
A50%	6^{a}	18.8^{a}	15.9^{b}	5.1^{c}	15.5^{a}	15.4^{a}	6.0^{a}	12.0^{b}	16.3^{a}	6.0^{c}	18.6^{a}	13.9^{b}	6.8^{b}	16.0^{b}	16.6^{b}
A70%	4.9^{c}	12.8^{c}	19.0^{a}	5.8^{b}	9.1^{d}	12.0^{c}	4.6^{b}	9.5^{c}	13.6^{b}	6.7^{b}	15.3^{c}	13.6^{c}	5.6^{d}	19.6^{a}	18.5^{a}
E50%	5.5^{b}	15.5^{b}	16.0^{b}	6.9^{a}	12.8^{c}	14.9^{c}	6.0^{a}	12.9^{a}	13.4^{c}	7.2^{a}	16.0^{b}	16.2^{a}	7.2^{a}	15.3^{c}	15.3^{c}
E70%	2.4^{c}	10.2^{d}	8.2^{c}	4.2^{d}	14.0^{b}	7.8^{d}	3.2^{c}	9.3^{d}	6.5^{d}	5.8^{d}	11.9^{d}	11.9^{d}	5.9^{c}	8.1^{d}	6.5^{d}
SE	0.10	0.04	0.08	0.05	0.03	0.05	0.04	0.05	0.06	0.05	0.05	0.05	0.02	0.05	0.07
P	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***

*F ,fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; SE, standard error of mean;***P<0.001*

4.4. The effect of Plant Species in the different Storage Conditions and Extraction Solvents on % egg Hatchability Inhibition

The experiment was carried out with exposure of *H. contortus* eggs to extracts of various experimental plant species using 50 mg/ml dose for egg inhibition test (Table 7). The mean inhibition percentage obtained for each extraction solvent was significantly different with differences in storage condition and plant species ($P<0.001$). The mean inhibition percentage of the five plant extracts for acetone 50% varied from lowest inhibition effect at dried *R. glutinosa* (13.1%)to the highest inhibition effect of preserved *F. ovata* (76.6%) were significant different along the three different storage times ($P<0.001$). Comparing the inhibition effect of acetone 50%, for fresh extract of the leaves *M. obscura* presented the highest inhibition activity (60.9%) than the leaves of *C. edulis* (52.3%), *R. glutinosa* (44.6%), *F. ovata* (40.9%), and *A. gummifera* (37.4%) ($P<0.001$). On the other hand, acetone 70% extract for *C. edulis* (55.3%) and *M. obscura* (9.1%) observed the highest and lowest values of egg inhibition effect across the storage conditions and plant species, respectively ($P<0.001$). Whereas, the highest (75.4%) and lowest (21.3%) % egg inhibition were recorded for *R. glutinosa* and *A. gummifera* leaves at fresh state extracted by 50% ethanol, respectively ($P<0.001$). Further, the highest % egg inhibitions by ethanol 70% extracts was determined for *C. edulis* and *F. ovata* leaves at fresh condition whereas the lowest % egg inhibition effect was recorded for fresh dried leaves of *C. edulis* ($P<0.001$).

Generally from Table 7, we observed different values of % egg hatching inhibition for the plant species at various storage conditions. The % egg hatching values varies within the plant species at different storage conditions. This variation might be correlated with variation in storage system, genetic factors and biochemical activity of the plant. Mihreteab *et al.*(2010) reported that the condensed tannin inhibiting egg hatching most potently was *R. glutinosa* and *A. gummifera*; of the plant suggested that the three condensed tannin extracts exhibited various potencies to inhibit the egg hatching. In different study in Brazil, Maciel *et al.* (2006) also found 50% inhibition of *H.contortus* egg hatching using *Melia azedarach* ethanol extracts of leaves at 2.2 mg/ml concentration. Tanniferous plant extracts the assumption is that condensed tannins complex with the sheath proteins of nematodes, which have high proline content and prevent exsheathment (Brunt*et al.,* 2007).

On the other hand, Gilani *et al*. (1996), Cowan (1999) and Maqbool *et al*. (2004) suggested that the anthelmintic activity of *F. parviflora* may be due to the alkaloids of *F. parviflora,* which have ability to intercalate with DNA synthesis of parasites. And also based on the direct and indirect effects of tannins, have been proposed to explain their antiparasitic effect. However, the exact mechanisms of action of these metabolites remain obscure and could differ depending on the parasite, its stage of development and possibly, the biochemical characteristics of the forage species (Hoste *et al.,*2006). Pessoa (2001), Costa *et al.* (2002) reported 95.66% inhibition of *H. contortus* egg hatchability in a dose dependent manner when using extract from seeds of *Mangifera indica*. Dose dependent ovicidal and larvicidal activity of crude extracts of *Maesa lanceolata* and *Plectranthus punctatus* against *H. contortus* was also observed for both aqueous and hydro-alcoholic extracts (Tadesse *et al*., 2009).

Despite some positive findings from different studies investigating the effect of tannins and tanniniferous fodder in a nematode parasitized host it is still equivocal on how these effects are brought about. While some studies have shown that the effects of tannins are due to their ability to interact and protect degradation of ruminal proteins (Niezen e*t al.,*1993; Wang *et al.,*1996), others have demonstrated direct toxic effects of tannins on nematodes (Athanasiadou *et al.,* 2000; Butter *et al.,* 2000; Max *et al*., 2005a). Findings from similar studies can therefore vary widely depending on the predominant mode of action. It has also been suggested that chemical structure of CT could be more important than their actual concentration as far as biological responses are concerned (McNabb *et al*., 1998). Results from the *in vitro* trials can be influenced and confounded by the presence, in the plants, of other unknown bioactive substances together with differences in nutritional values in addition of the different extraction solvents.

Table 7.Least square means for the effects of storage condition of plant species and extraction solvents on % egg inhibition compared for each extraction solvent

	Albizia Gummnifera			***Carissa edulis***			***Ficus ovata***			***Maytenus obscura***			***Rhus glutinosa***				
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	SE	P
A 50%	37.4^{j}	42.6^{h}	36.0^{k}	52.3^{f}	57.4^{e}	59.4^{d}	40.9^{i}	61.4^{b}	76.6^{a}	60.9^{c}	15.2^{n}	32.9^{l}	44.6^{g}	13.1^{o}	30^{m}	0.02	***
A 70%	49.1^{b}	48.9^{b}	47.7^{c}	14^{m}	55.3^{a}	34.3^{i}	41.7^{e}	41.1^{f}	38^{g}	20.6^{l}	23.1^{k}	9.1^{n}	44.9^{d}	32.9^{j}	35.7^{h}	0.22	***
E 50%	44.3^{c}	21.3^{o}	34.5^{f}	28^{j}	34.9^{e}	28.6^{i}	38.6^{d}	27.1^{l}	23.4^{n}	33.2^{h}	33.7^{g}	27.4^{k}	75.4^{a}	25.7^{m}	61.1^{b}	0.02	***
E 70%	42.3^{d}	27.7^{g}	12.9^{k}	50.9^{a}	6.6^{l}	18.6^{j}	57.4^{a}	23.1^{i}	20^{j}	26^{h}	46.9^{b}	38.3^{e}	44.6^{c}	22.3^{i}	32.3^{f}	0.58	***

*F ,fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; SE, standard error of mean;***P<0.001*

Effects of storage conditions of the plant species and extraction solvents on %egg inhibition are presented in Table 8. The highest % egg inhibition of *A.gummifera* was observed at 70% acetone extraction procedures across the storage conditions of the same plant as compared to the rest of organic solvents ($P<0.001$). On the other hand, across the storage condition in *C. edulis* the highest % egg inhibition was recorded for 50% acetone as compared to the rest of the extraction solvents ($P<0.001$). In the resent study, the egg inhibition capacity of different plants showed differences across the storage conditions and extraction solvents. This could be associated with concentration of condensed tannin in the plant species which are also affected by storage condition and extraction solvents. Molan *et al*.(2002) confirmed the differences in the effectiveness of inhibiting egg hatchability by tannin extracts from different tanniferous plant species.

Even though Albendazole, a positive control, inhibited egg hatchability by 100%, it has clearly observed in the present study that the tannin extracts from various tannin rich plants had ability to inhibit egg hatchability to maximum 75.4%. This implies that effects of tannins, cheaper source from locally available plants, can be compared with artificial anthelmintics drugs. The tannins could also bind with feed nutrients and possibly prevent bacterial growth in the faeces (larva feed on bacteria) and so limit the feed available for larval growth, or in some other way inhibit larvae growth and movement.

Table 8.Least square means for the effects of storage condition of plant species and extraction solvents on % egg inhibition compared between extraction solvents

	Albizia gummnifera			***Carissa edulis***			***Ficus ovata***			***Maytenus obscura***			***Rhus glutinosa***		
ES	**F**	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**
A 50%	37.4^{d}	42.6^{c}	36.0^{b}	52.3a	57.4^{a}	59.4^{a}	40.9^{c}	61.4^{a}	76.6^{a}	60.9^{a}	15.2^{d}	32.9^{b}	44.6^{c}	13.1^{d}	30^{d}
A 70%	49.1^{a}	48.9^{a}	47.7^{a}	14^{d}	55.3^{b}	34.3^{b}	41.7^{b}	41.1^{b}	38^{b}	20.6^{d}	23.1^{c}	9.1^{d}	44.9^{b}	32.9^{a}	35.7^{b}
E 50%	44.3^{b}	21.3^{d}	34.5^{c}	28^{c}	34.9^{c}	28.6^{c}	38.6^{d}	27.1^{c}	23.4^{c}	33.2^{b}	33.7^{b}	27.4^{c}	75.4^{a}	25.7^{b}	61.1^{a}
E 70%	42.3^{c}	27.7^{c}	12.9^{d}	50.9^{a}	6.6^{d}	18.6^{d}	57.4^{a}	23.1^{d}	20^{d}	26^{c}	46.9^{a}	38.3^{a}	44.6^{c}	22.3^{c}	32.3^{c}
SE	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.30	0.02	0.02	0.02	0.02	0.02	0.02
P	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
PC	100														
NG	5.5														

*F ,fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; PC, positive control; NG, negative control; SE, standard error of mean;***P<0.001*

4.5. The effect of Plant Species in the different Storage Conditions and Extraction Solvents on Larval Development inhibition

The experiment was done with exposure of *H. contortus* $L_1$to the extracts of the experimental plant species in a 50 mg/ml dose for larva development test (Table 9). ECT extracted with 50% acetone from fresh leaves of the plant species inhibited larval development by 100%. In addition, in *C. edulis* and *R .glutinosa* at all storage conditions the effectiveness of ECT on larval development was also by 100%. It was also observed in most of storage conditions in the plant species that the larval development inhibition of the ECT was ranged from 2 to 100% in 70% acetone extraction. In comparison to acetone 50 and 70%, ECT extracted with Ethanol 50% inhibited larval development to the maximum 100% only in six out of total fifteen storage conditions.

ECT extracted with 70% ethanol from *A.gummifera* inhibited larval development by 100% in all storage conditions as compared to other plant species ($P<0.001$). This might be in conjunction with the concentration of tannin in each plants species and also the anthelmintic activity of plant extracts on larvae could be attributed to tannins capacity to bind to proteins and could operate via several mechanisms. Condensed tannins may bind to the cuticle of larvae, which is highly in glycoprotein and cause their death. *In vitro* experiments showed that prodelphinidin monomers and flavan-3-ol gallates were more active on the egg hatching, larvae motility and exsheathment of *H. contortus*, the procyanidins monomers and flavan-3-ol (Molan*et al.,* 2003; Brunet; Hoste, 2006).

Plant extracts contain flavonoids, saponins and tannins which are known to possess anthelmintic activity. However some studies have evaluated the anthelmintic properties of the other biochemical compounds in the plant extracts. The flavonoids and tannins contained in the polar fraction of *Leuceana leucocephala* (Ademola *et al.*,2005) and the flavonol glycosides in *sainfoin* (*Onobrychis viciifolia)* (Barrau *et al.*, 2005) have been demonstrated to have effects on the third stage larvae (L_3) of *H. contortus*.

The study by Alonso-Díaz *et al.* (2008b) demonstrated 90% efficacy on *H. contortus* larval exsheathment using 1,200 μg.mL^{-1} of *L. leucocephala* leaf extract, while this study obtained100% efficiency on larval exsheathment using 300 μg.mL^{-1}. This difference could be also attributed to the plant chemical composition. Genetic factors, climate, soil, harvest time and solar radiation can influence the composition and concentration of secondary metabolites (Goboo-Neto; Lopes, 2007).

Table 9.Least square means for the effects of storage condition of the plant species compared separately for each extraction solvent on % larva development inhibition

ES	*Albizia gummifera*			*Carissa edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*			SE	P
	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP		
A 50%	100^{a}	100^{a}	3.3^{d}	100^{a}	100^{a}	100^{a}	100^{a}	$83,3^{b}$	100^{a}	100a	17.3^{c}	100^{a}	100^{a}	100^{a}	100^{a}	0.50	***
A 70%	9.3^{e}	100^{a}	100^{a}	100^{a}	100^{a}	4.7^{f}	100^{a}	2^{g}	100^{a}	100^{a}	87.3^{c}	90.9^{b}	64.7^{d}	100^{a}	2.7^{g}	0.37	***
E 50%	62^{e}	16.7^{g}	100^{a}	12^{i}	14.7^{h}	80^{c}	100^{a}	87^{b}	100^{a}	6^{j}	48.7^{f}	100^{a}	74^{d}	100^{a}	100^{a}	0.49	***
E 70%	100^{a}	100^{a}	100^{a}	100^{a}	12^{e}	100^{a}	100^{a}	100^{a}	3.3^{g}	6.7^{f}	100^{a}	41.3^{c}	43.3^{b}	30.7^{c}	100^{a}	0.38	***

*F ,fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; SE, standard error of mean;***P<0.001*

Least square means for the effects of storage condition of the plant species compared separately for extraction solvents on % larval development inhibition is presented in Table 10. ECT from fresh leaves extracted with acetone 50% and ethanol 70% inhibited larval development by 100% as compared to acetone 70% and ethanol 50% in *A. gummifera* ($P<0.001$). However, the effects of extraction solvents didn't vary in inhibiting larval development activity in the fresh leaves of *F.ovata.* The larval development inhibiting ability of ECT extracted by various organic solvents varied with differences in plant species, in most of the plants the 100% inhibition was recorded for fresh while for the others the highest value was recorded at dried-preserved storage conditions. In general, different species subjected to different storage conditions and extraction solvent showed different larval development inhibition ability. Larval inhibition was thus consecutively associated with ECT concentration.

Salminen (2003) investigated the effects of different methods of sample drying and storage, and the choice of extraction solvent and analysis method on the concentrations of 14 individual hydrolyzable tannins (HTs), and insoluble ellagitannins in birch (*Betula pubescens*) leaves. Freeze- and vacuum-drying of birch leaves were found to provide more reliable results than air- or oven-drying. Hossain (2010) investigated the changes in total phenols (TP) in six Lamiaceae herbs after three drying days at 20 °C and compared to fresh treatments (air-, freeze- and vacuum oven-drying) stored for 60 days. Harbourne *et al*. (2009) also investigated the effect of drying conditions on the phenolic constituents in tanniferous feeds. Further, Chew *et al*. (2011) reported significant effects of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant, capacity of *Orthosiphon stamineus* extracts. Molan *et al*. (1999) reported reduced the development of L_1 larvae to L_3 larvae and decreased the motility of L_3 larvae when assessed by the larval migration inhibition assay and this may reduce their infective capacity condensed tannins. In the present study, the anti-larval activity of tannin extracts were popular at fresh condition which disagrees with the works of Hoste *et al*.(2009) that the most consistent results of anti-laraval development activities were found with the plant extracts possessing the highest tannin content of various tanniferous plants.

Table 10.Least square means for the effects of storage condition of the plant species compared separately for extraction solvents on % larva development inhibition

		Albizia gummifera			***Carissa edulis***			***Ficus ovata***			***Maytenus obscura***			***Rhus glutinosa***		
ES		**F**	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**
A 50%		100^{a}	100^{a}	3.3^{b}	100^{a}	100^{a}	100^{a}	100	$83{,}3^{c}$	100^{a}	100^{a}	17.3^{c}	100^{a}	100^{a}	100^{a}	100^{a}
A 70%		9.3^{c}	100^{a}	100^{a}	100^{a}	100^{a}	4.7^{c}	100	2^{d}	100^{a}	100^{a}	87.3^{b}	90.9^{b}	64.7^{c}	100^{a}	2.7^{b}
E 50%		62^{b}	16.7^{b}	100^{a}	12^{b}	14.7^{b}	80^{b}	100	87^{b}	100^{a}	6^{b}	48.7^{c}	100^{a}	74^{b}	100^{a}	100^{a}
E 70%		100^{a}	100^{a}	100^{a}	100^{a}	12^{c}	100^{a}	100	100^{a}	3.3^{b}	6.7^{b}	100^{a}	41.3^{c}	43.3^{d}	30.7^{b}	100^{a}
	SE	0.43	0.43	0.43	0.43	0.35	0.35	0.43	0.37	0.43	0.5	0.25	0.35	0.25	0.43	0.5
	P	***	***	***	***	***	***	NS	***	***	***	***	***	***	***	***
	PC	100														
	NC	4.6														

*F ,fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; PC, positive control; NG, negative control; SE, standard error of mean;***P<0.001*

4.6. Effects of Plant Species in the different Storage Conditions and Extraction Solvents on Adult motility

The experiment was done with exposure of 10 adult *H. contortus*, parasite, to the experimental plant species using 50 mg/ml dose for adult motility test (Table 11). This table presents adult parasite mortalities in hours; the larger number of hours indicated the slower mortality rate of the parasite. In other words, the lower time indicates the fastest motility rate. The effects of plant species and storage condition showed different adult motility rate across the extraction solvents ($P<0.001$). The fastest adult motility rate was observed in *F. ovata* at fresh dried storage condition (1:45 h) where as the slowest adult motility rate was recorded in *A. gummifera* at fresh storage condition with acetone 50% extraction ($P<0.001$). On the other hand, the fastest rate of adult mortality was observed in *M. obscura* (1:45 h) at fresh dried storage condition whereas the slowest mortality rate (4:27 h) was recorded for *A. gummifera* leaves extracted with acetone 70% at fresh condition. ECT with ethanol 50 extraction, the fastest adult motility rate was recorded from the fresh leaves of *M. obscura* (1:30 h) and the slowest mortality (3:50 h) was observed in *C. edulis* at fresh condition ($P<0.001$). The ECT values extracted with Ethanol 70% resulted the fastest adult motility rate in both *F. ovata* (1:50 h) and *M. obscura* (1:50 h) at fresh dried storage condition where as the slowest mortality was recorded in *R. glutinosa* (3:52 h).

The anthelmintic effects followed a dose dependent manner and all the worms were found dead between 1:30 to 4:15hr post-exposure at 50 mg/ml in each extracts. Mortality of worms was comparable to that with Albendazole (positive control) at 10mg /ml at 2:30 h post-exposure. The commercial anthelmintic Albendazole (positive control) exhibited the highest anthelmintic activity on reduction of FEC of animals infected naturally with gastrointestinal nematodes and the efficacy rate was observed as 100%. Albendazole acts as cholinergic agonist on neuromuscular junctions in nematode parasites, which causes paralysis of the worm, leading to their death or expels them from the host (McKellar and Jackson, 2004). There was no mortality of worms in PBS (negative control) till 5h. The results also revealed that ethanolic extract were slightly more effective as compared to aqueous extract. Transcuticular diffusion is a common means of entry into helminth parasites for non-nutrient and non-electrolyte substances in nematodes.

A study reported by Eguale *et al*., (2007) has shown that this route is predominant for the uptake of major broad spectrum anthelmintics by different nematode, cestode and trematode parasites as opposed to oral ingestion. This might be the direct or indirect effect of CTs. Some researchers believe that the plant tannins may affect parasites either directly or indirectly (or both). Tannins may react directly with adult worms by attaching to their "skin", causing them distress, or in directly by improving protein nutrition of the host and boosting the immune system (Min *et al*., 2005).

Table 11.Least square means for the effects of plant species, extraction solvents and storage time on adult motility

ES	*Albizia Gummnifera*			*Carissa edulis*			*Ficus Ovata*			*Maytenus obscura*			*Rhus glutinosa*			SE	P
	F	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**	**F**	**FD**	**DP**		
A 50%	$4:25^{a}$	$2:38^{k}$	$3:50^{c}$	$3:40^{e}$	4.20^{b}	$2:40^{j}$	$2:52^{h}$	$1:45^{n}$	$2:08^{m}$	$2:45^{i}$	$2:.30^{l}$	$3:40^{e}$	$3:05^{g}$	$3:46^{d}$	$3:15^{f}$	0.005	***
A 70%	$4:27^{a}$	$2:30^{j}$	$3:45^{d}$	$4:08^{c}$	$4:20^{b}$	$2:46^{h}$	$2:20^{l}$	$2:35^{i}$	$2:25^{k}$	$2:35^{i}$	$1:45^{m}$	$3:35^{e}$	$2:50^{g}$	$3:45^{d}$	$3:25^{f}$	0.005	***
E 50%	$2:08^{i}$	$2:25^{g}$	$3:47^{b}$	$3;50^{a}$	$2:35^{f}$	$2:45^{e}$	$2:10^{h}$	$1:46^{j}$	$2:25^{g}$	$1:30^{k}$	$1:45^{j}$	$3:30^{c}$	$2:50^{d}$	$2:45^{e}$	$2:50^{d}$	0.005	***
E 70%	$2:08^{h}$	$2:10^{g}$	$2:35^{d}$	$3:10^{g}$	$2:36^{d}$	$2:36^{d}$	$2:22^{e}$	$1:50^{i}$	$2:08^{h}$	$2:20^{f}$	$1:50^{i}$	$3:05^{b}$	$2:45^{c}$	$2:35^{d}$	$3:52^{a}$	0.004	***

*F ,fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; PC, SE, standard error of mean;***P<0.001*

The effects of ECT due to variations of storage conditions and extraction solvents on the time of adult motility are presented in Table 12. Significant difference was observed in adult mortality time across the storage conditions and extraction solvents ($P<0.001$). The fastest and the slowest mortality rate was recorded for Ethanol (2:08) and acetone (4:25 to 4:27 h) levels in the fresh leaves of *A. gummifera*, respectively ($P<0.001$). In general in *C. edulis*, the adult motility rate ranged from 2:35 to 4:20; for *F. ovata*, the highest and the lowest adult mortality rate was recorded. In fresh (2:52h) and fresh dried (1:45 h)($P<0.001$). In general, the adult motility rate was associated with the concentration of condensed tannin in various storage conditions and plant species. The results of present study agrees with the reports of Brunet e*t al*. (2009) who reported that tanniferous reach plants have antihelmentic properties which may therefore represent a possible alternative option to control nematodes such as *H. contotus*. Max *et al*. (2007) confirmed similar impression that anthelmintic activity of the ECT in parasite reductions can have practical epidemiological implications in reducing pasture larval contamination.

Table 12.Least square means for the effects of plant species, extraction solvents and storage time on adult motility

ES	*Albizia gummifera*			*Carissa edulis*			*Ficus ovata*			*Maytenus obscura*			*Rhus glutinosa*		
	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	
A 50%	4:25^{b}	2:38^{a}	3:50^{a}	3:40^{a}	4:20^{a}	2:40^{c}	2:52^{a}	1:45^{d}	2:08^{b}	2;45^{a}	2:30^{a}	3:40^{a}	3:05^{a}	3:46^{a}	3:15^{c}
A 70%	4:27^{a}	2:30^{b}	3:45^{c}	4:08^{a}	4;20^{a}	2:46^{a}	2:20^{c}	2:35^{a}	2:25^{a}	2:35^{b}	1:45^{c}	3:35^{b}	2:50^{b}	3:45^{b}	3:25^{b}
E 50%	2:08^{c}	2:25^{c}	3:47^{b}	3:50^{b}	2:35^{c}	2:45^{b}	2:10^{d}	1:46^{c}	2:25^{a}	1:30^{d}	1:45^{c}	3:30^{c}	2:50^{b}	2:45^{c}	2:50^{d}
E 70%	2:08^{c}	2:10^{d}	2:35^{d}	3:10^{d}	2:36^{b}	2:36d	2:22^{b}	1:50^{b}	2:08^{b}	2:20^{c}	1:50^{b}	3:05^{d}	2:45^{c}	2:35^{d}	3:52^{a}
SE	0.0025	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
P	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
PC	2:33														
NG	5:40														

*F ,fresh leaves; FD, dried; DP, preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; PC, positive control; NG, negative control; SE, standard error of mean;***P<0.001*

5. CONCLUSION AND RECOMMENDATION

There is a clear justification for building up feed resources, since there is a major gap between the requirements and supplies of nutrients for small ruminants. Nutrient deficiencies (particularly protein, energy and minerals) and parasite infestations are common in ruminant livestock raised by smallholders. Thus the use of tanniniferous feeds for livestock feeding a replacement to concentrate diets could represent a strategy to face the rising production costs. Therefore the study was conducted in order to determine the effects the effect of plant species in different storage conditions and extraction solvents.

In general, it was observed that the concentration of CT in fresh leaves for all plants was not more that 7%, however, the CT values in both fresh dried and stored after drying the leaves ranged from 7 to 19%. The smallest concentration of ECT in the fresh leaves as compared to the dried and dried-preserved storage conditions might be due to variation in plant species as well as the dry matter content of the plant species. The high concentration of tannins in dried and dry preserved plants as compared to fresh plants indicated the presence of polymerization of tannins with byproducts of heating feed stuffs. Also tannin concentration varies with variations in plant species and extraction solvents

Ethanol 50% showed the better ability of extracting condensed tannin in fresh samples than the rest of extraction solvents. In general, it was observed that the concentration of ECT extracted by 70% ethanol showed the least value for all plants and for all storage condition.

In conclusion, our results suggest that the antiparasitic effects of tanniferous forages in general are achieved. Anthelmintic effects observed in response to tanniferous fodder *in vitro* are believed to be associated to condensed tannins (CTs), contained in the plants. Chemically these molecules belong to the polyphenols and are expressed by a range of fodder plants together with other secondary metabolites. Across experiments, the most consistently observed anthelmintic effect is a reduction in parasite egg hatchability, larva development and adult motility.

The main advantages of using *in vitro* tests/assays to screen the anti-parasitic properties of the plants and plant extracts are low costs and rapid turnover, which allow screening large number of plants. An additional advantage is that these tests measure the effect of anthelmintic activity directly on the processes as such hatching, development and motility of parasites without interference of internal physiological functions of the host.

Since first being reported, anthelmintic resistance has progressively become a crisis in some sectors of livestock production, especially regarding small ruminant production in tropical and subtropical areas, where in some cases resistance to all anthelmintic groups has been reported. Furthermore, the high cost of these drugs, residual concern in food animals and environmental pollution have awaken interest in medicinal plants as an alternative source of anthelmintic drugs. More investigation is required by using a serial dilution to allocate an effective and efficient dose. In spite of the advantages of *in vitro* tests on evaluation anthelmintic activity of plants, considerations should be made those potential bioactive substances used *in vitro* may not always correspond to *in vivo* bioavailability. Therefore, *in vitro* assays should always be accompanied by *in vivo* trials when used to validate anthelmintic activity of plant remedies.

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