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Chemical Composition, *In vitro* Gas Production, Digestibility and Phenolic Compounds of Indigenous Multipurpose Fodder Trees and Shrubs Harvested during Dry and Wet Seasons from three Districts of Sidama Zone Southern Ethiopia

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Abstract: A study was conducted to evaluate the fodder value of Mulberry and other indigenous multi-purpose trees (MPTs) collected from selected farmers who own Mulberry plant from Shebedino, Dale and Chuko districts of Sidama Zone southern Ethiopia. In total 15 MPTs (14 indigenous and 1 exotic) were identified. From these, five indigenous and 1exotic which are used as a fodder plant (*B. aegyptica, C. africana, E. cymosa, M. ferruginea, M. alba* and *V. amygdalina)* were selected. The chemical composition, *in-vitro* organic matter digestibility (IVOMD), *in-vitro* gas production (IVGP), total tannin (TT), total phenolics (TP) and condensed tannin (CT) were analyzed. The highest ($P<0.05$) number was recorded for *M. ferruginea* followed by *C. africana* and *V.amygdalina* from Chuko while the lowest was for *B. aegyptica* from Dale district. The highest ($P<0.05$) crude protein (20.73%DM) was recorded for *M. alba*during wet season. The lowest neutral detergent fiber ($P<0.05$) content was for *B. aegyptica* followed by *V. amygdalina* and *M. alba* during wet season, while the highest ($p<0.05$) was from *C. africana* during dry season. The highest ($P<0.05$) acid detergent fiber content (38.08%DM) was for *E.cymosa*and the lowest ($p<0.05$) was for *B. aegyptiaca* and *M. alba*in dry season and the lowest (15.15%DM) was for *V. amygdalina* in wet season. The highest ($P<0.05$) acid detergent lignin content (22.22%) was recorded for *C. africana* during dry season and the lowest ($p<0.05$) was for *B. aeygptica* and *V. amygdalina* followed by *M. alba* in wet season. The highest ($P<0.05$) IVOMD (84.83%) was recorded for *V. amygdalina* followed by *M. alba* and *M. ferruginea* in wet season. The lowest IVOMD (57.25%) was recorded from *C. africana* during dry season. The highest ($P<0.05$) IVGP were recorded for *M. alba* during the whole incubation period in wet season and the lowest ($p<0.05$) was for *C. africana* in dry season. The highest ($P<0.05$) organic matter digestibility, metabolizable energy and short chain fatty acids at 24hr of incubation was recorded for *M. alba* in wet season and while the lowest was for *C. africana* in dry season. The lowest ($P<0.05$) TP, TT and CT in dry season was for *M. alba.* The highest ($p<0.05$) methane gas production (10.33 and 15.08ml) was for *V. amygdalina* and *B. aegyptiaca* during wet season at 24 and 72hr of incubation period respectively. While the lowest for *M. alba* during wet season. Therefore, high CP content and digestibility, low methane production and low phenolic compound in *M. alba* helps to incorporate the plant as a supplementary feed and it can be integrated with other indigenous MPTs in the agro-forestry system of the region.

Key words: Chemical Composition・Digestibility・*Invitro* Gas Production・Methane・Multi-Purpose Trees・Phenol Compounds

INTRODUCTION

Feed shortage is the main problem of livestock sector in Ethiopia. The major feed resources are crop residues and natural pasture. Inadequate feed supply is a major constraint to ruminant production during the dry season in Ethiopia which is one of the reasons for poor performance of livestock. In many rural areas, the

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available feeds from grazing are not sufficient to meet the maintenance requirements of grazing animals during dry periods [1]. Because of population pressure the available grazing land is shrinking which could lead to land degradation and reduced feed availability [2]. Thus there is a need to fill the feed gap especially with protein source feed through increased utilization of agro-industrial by products. However, agro-industrial by-products are not affordable or feasible for most of the farmers either it is expensive or not available especially for small farmers [3]. Thus, in an effort to alleviate the animal feed supply problem, looking for alternative potential feed resources, particularly those which survive during the dry season, cheap and which can be used as a substitute for commercial concentrate deserves due attention [4].

Indigenous multipurpose fodder trees is a potentially cheap protein supplement for ruminants and highly valued by farmers particularly during the critical periods of the year when the quantity and quality of herbage is limited and considered as a more reliable feed resource of high quality to develop sustainable feeding systems. Indigenous fodder trees and shrubs have been used for generations as a multipurpose resource and farmers preferred them for high biomass production, multi-functionality, life span and compatibility to the cropping system [5]. The main features of multipurpose trees as a feed resource is the high crude protein content ranging from 10 to 25% DM and may be considered as a more reliable feed resource of high quality to develop sustainable feeding systems and increasing livestock productivity [5, 6].

Mulberry is also under multi-purpose tree which was introduced to the region long time ago. It is considered as high quality forage plant due to its high protein and low fiber content and it is comparable with leguminous multi-purpose trees as a feed for ruminants [7]. It is also one of fast growing plant and propagated easily through stem cutting and it was used as shade tree and could be intercropped with other crops in the agro-forestry system [8].

Hence, the use of multipurpose tree leaves is regarded as an option for increasing the availability of feeds for smallholder farmers and improving the feeding value of low quality roughages [9]. Therefore, this study was designed to evaluate the fodder value of Mulberry as compared with other indigenous multipurpose trees.

MATERIALS AND METHODS

Description of the Study Areas: The samples were collected from three districts of Sidama Zone, Southern Nation Nationalities and Peoples Regional state (SNNPRS), Ethiopia; Namely AletaChuko, Dale and Shebedino. The districts were selected from seven surveyed districts for sample collection based on the potential use of multi-purpose trees in agro forestry farming systems. Aletachuko district is located at approximately 6°27'20"E - 6°40'14"N latitude and 38°12'31"E–38°25'33"E longitude and has two agro-climatic zones, which are *Kolla* (lowland) situated at an altitude between 1400 to 2300m.a.s.l and *WeyinaDega* (midland) at an altitude between 1500 to 3027m.a.s.l. A mean annual rainfall of the district is 1100 -1400 mm. Dale district is located at 6.45N and 38.23E and at an altitude range of 1500m to 2300m.a.s.l. and the mean annual rainfall is 1314 mm [10]. Shebedino district is located approximately at 7°00’-7°06N and 38°-37’ E. The average annual rainfall is 1300-1500mm and temperature is between 18-25°C [11].

Identification and Selection of Indigenous Multipurpose Trees: Twenty (20) households (HHs) from each districts and totally 60 (sixty) HHs from three districts were asked purposively who own Mulberry plant to identify different indigenous multipurpose trees (MPTs) on the basis of their utilization. The households identified fourteen (14) indigenous multi-purpose tree (MPTs) from the farms and their scientific name was adopted from Abiot and Zebene [12]. Out of them the 6 (Six) MPTs were selected which were used as fodder plants by respondents.

Sample Collection: Samples were collected from farmers who own Mulberry (*Morus alba)* and different multi-purpose tree (MPTs) species. Structured and semi-structured questionnaires were used to collect information from purposively selected respondents who own Mulberry plant during survey work on types of MPTs, species available, vernacular names, parts of plants eaten by animals. Sampling of forage material was conducted once in each of the two major seasons, dry season (Dece – Jan) and main rainy seasons (July – Aug) in 2016. Ten plant leaf samples were collected for each species from each district. Samples of the same species were pooled and bulked and sub samples were taken and air dried. Finally oven-dried at 60°C for 48hr and ground to pass through 1mm sieve size and kept in air-tight containers for the determination of chemical composition, *in vitro* digestibility and gas production.

Chemical Composition: The dry matter (DM), ash, ether extract (EE) and Kjeldahl nitrogen analyses were performed according to AOAC [13] and CP was calculated as N x 6.25. Neutral detergent fiber (NDF) was determined by the method of Van Soest, Robertson and Lewis, [14] whereas acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest and Robertson [15] using ANKOM fiber analyzer (ANKOM Technology®, Macedon, NY, USA). For the determination of phenolic the samples were extracted using 70% aqueous acetone and total extractable phenolic (TP) was determined using FolinCiocalteu procedures as described by Rubanza *et al.* [16]. The TP concentration was calculated using the regression equation of tannic acid standard. Total extractable tannins (TT) was estimated indirectly after being absorbed to insoluble polyvinyl pyrrolidone (PVP) and the concentration calculated by subtracting the TP remaining after PVP treatment. Condensed tannins were determined by using Butanol-HCl procedures and were expressed as leucocyanidin equivalent (% of DM). The concentrations of condensed tannins were calculated by the formula:-

$$\frac{\text{Absorbance at 550 nm} \times 78.26 \times \text{Dilution factor}}{\text{\% DM}}$$

***In vitro* Digestibility Experiment:** *In vitro* organic matter digestibility was determined by collecting ruminal fluid from two local rams by inserting suction tubes into the rumen through esophagus in the morning before they were offered Rhodes grass hay and added into a pre-warmed thermos flask [17]. The rumen fluid was strained through double layers of cheese cloth and added into a Jar. McDougall's buffer solution was prepared and approximately 0.5 gram of milled forage sample was weighed and sealed into fiber bags. The buffer solution with rumen fluid and 26 bags (24bags containing feed sample and 2bags without feed samples) were incubated in each jar for 48h at 39°C. Fermentation was stopped after 48h and acidified pepsin was added. Then bags were incubated in a Jar for another 48h at 39°C. The contents were then filtered and the residue dried and weighed. After drying, residue was ashed. *In vitro* ruminal organic matter digestibility (IVOMD) was determined as the quantity of organic matter (OM) lost during fermentation and subsequent pepsin digestion.

***In vitro* Gas Production:** *In-vitro* gas production was analyzed according to the procedure of Menke and Steingas [18]. Ruminal fluid was collected by inserting a suction tube through esophagus from two local rams in the morning before they were offered Rhodes grass hay. Then the rumen fluid was added into a pre-warmed thermos flask [17]. The fluid was strained through double layers of cheese cloth before adding into glass syringe. The syringes were pre-warmed at 39°C before addition of 30ml of buffer mixture and rumen liquor into each syringe. McDougall's buffer solution was prepared and about 200 mg of dry sample was incubated with rumen fluid in a calibrated glass syringe of 100 ml in duplicate. The syringes were shaken gently 30 min after the start of incubation and every hour for the first 10h of incubation [19].

Syringes with buffered rumen fluid without feed sample were also included in duplicate as a control. All the syringes were incubated in a water bath maintained at 39°C. Gas production was recorded after 3, 6, 12, 24, 48, 72 and 96h of incubation. The gas production characteristics were estimated by fitting the mean gas volumes to the exponential equation $G = a + b\,(l\text{-}e^{-ct})$, Makkar HPS [19]

where

G = Is the volume of gas produced (ml/200mg OM) at time t,

a = The gas production from the immediately soluble fraction (ml),

b = The gas production from the insoluble but degradable fraction (ml),

$a + b$ = Is the potential gas production (ml) and

c = Is the rate constant of gas production (Fraction/h)

The OMD (Organic matter digestibility), ME (metabolizable energy) and SCFA (short chain fatty acid) were estimated according the equation of Menke and Steingas [18]. The OMD was calculated from the equation:- OMD (%) = 14.88 + 0.889G24 + 0.45CP where:- OMD = organic matter digestibility at 24 hours; CP = Crude protein content of feed samples and G24 = is gas production value (ml/200mg) at 24hrs of incubation. The ME was calculated from equation: ME (KJ/gDM) = 2.2 + 0.136G24 + 0.057CP where:-G24 = Gas production value (ml/200mg) at 24hrs of incubation; CP = Crude protein content of feed samples. The SCFA were estimated as: SCFA = 0.0239G24 - 0.0601where:- G24 = Gas production value (ml/200mg) at 24hrs of incubation.

Measurement of Methane Production: Methane (CH4) production after 24 and 72 hours of incubation was measured by connecting lower end of syringe with another syringe containing 4.0ml of NaOH (10M) which was then introduced latter into incubated contents, thereby avoiding gas escape [21]. Mixing of contents with NaOH allowed absorption of CO_2 and the volume remaining in syringe is considered to be CH4. Net methane and gas productions were calculated by differences of methane and total gas in test syringe and corresponding blank. Methane concentration was calculated as net methane production per net gas production [22].

Statistical Analysis: Statistical analyses were performed using general linear model (GLM) procedure of Statistical Analysis System (SAS) [23]. The model used for analysis was:- $Yijk = \mu + Ai + Bj + A^* B(ij) + e(ijk)$: *w*here *y* is the parameter studiedμ is overall mean, *Ai* is fixed plant species (i=1,2,3,4,5,6) *Bj* is fixed season (j=1,2,), *A*B(ij)* is interaction between species and season and *e(ijk)* is error term. Mean were compared using fisher least significance difference (LSD) and significance was declared at $p < 0.05$. A simple correlation analysis was used to establish the relationship between anti-nutritional concentration, *in vitro* gas production and *in vitro* digestibility.

RESULTS AND DISCUSSION

Multipurpose trees (MPTs) identified from selected districts are presented in Table 1. Totally 15(fifteen) MPTs including Mulberry were identified from the three surveyed districts. Among the identified 15 species, respondents selected six plant species including mulberry plant which were used as livestock feed Table 2. These plants were grown in the garden with cash crops and food crops (coffee, enset, etc). In addition use of the MPTs as feed for the animals the plants were grown for shade, shelter, improving soil fertility, land sustainability, fuel wood, construction and medicine [24]. All parts of these plants are consumed by animals except stem.

Number of Multipurpose Fodder Trees and Shrubs available at Household Level: Number of multipurpose trees in house hold level used as animal feed is presented in Table 3. The highest ($P<0.05$) number of *B. aegyptiaca* and *E. cymosa*, grown per HH was recorded from Chuko districts.

The highest ($p<0.05$) number of *M. fergunia* and *V. amygdalina* was recorded from Chuko and Dale districts, while the lowest was from Shebedino per HH. There was no difference among the districts on the mean value for *C. africana* and *M. alba.* The low number of MPTs per household could be due to shortage of the land. Because, most portion of the land was used for food and cash crop production. However, *C. Africana, M. fergunia* and *V.amygdalina* are the common plant observed in large number in the study districts in the current study. Because, these plants are highly used for shade for coffee and other plants. Farmers used multi-purpose trees to solve feed shortage problem in the dry season and sometimes in wet season. The use of MPTs in dry period is the common practice to solve feed shortage by farmers around Sodo southern Ethiopia [25].

Chemical Composition and *In-vitro* Organic Matter Digestibility: Chemical composition and *in vitro* organic matter digestibility of multipurpose fodder trees are presented in Table 4. The highest ($p<0.05$) ash content was recorded for *M. alba* in dry and wet season while the lowest ($p<0.05$) was for *E. cymosa* in the dry season. The current ash content for *M. alba* was shown higher result than reported earlier for *G. latifolum*, *A. indica* and *V. amygdalina* [26-28]. However, the ash

Table 1: Types of multipurpose tree and shrubs identified from the three districts

No	Species name	Local name(Sidamic)	Family name	Origin
1	*Millettiaferrugnea (Hochyst, Baker)*	Hengedicho	Papilionoideae	Indigenous
2	*Vernoniaamygdalina*	Hecho	Asteriaceae	Indigenous
3	*Cordiaafricana (Lam)*	Wadicho	Boraginaceae	Indigenous
4	*Croton macrostachyus(Hochst. Ex. Del.)*	Masincho	Euphorbiaceae	Indigenous
5	*Albiziagumifera (G.F.Gmel.C.A.Sm.)*	Maticho	Mimosaseae	Indigenous
6	*Bersamaabyssinica (Fres.)*	Xeberako	Melianthaceae	Indigenous
7	*Ficussur (Forssk.)*	Oddako	Moraceae	Indigenous
8	*Ehretiacymosa (Thonn.)*	Gidincho	Boraginaceae	Indigenous
9	Maesalanceolata (Forssk)	Gobacho	Myrsinaceae	Indigenous
10	*Prunusafricana (Hook.f.Kalkm)*	Garbicho	Rosaceae	Indigenous
11	*Syzygiumguineense (Wild.D.C)*	Duwancho	Myrtaceae	Indigenous
12	*Afrocarpusfalcatus (Thunb)*	Dagucho	Podocarpaceae	Indigenous
13	*Vernoniaauriculifera (Heim)*	Rejicho	Asteriaceae	Indigenous
14	*Balanitesaegyptiaca*	Bedena	Fabaceae	Indigenous
15	*Morus alba*	Go'ra	Moraceae	Exotic

Table 2: Selected plants from identified multipurpose tree (MPT) used as a forage plant

Name of species	Favored plant parts	Animal species
B. aegyptiacaC. africana	Leaf, podsBerries, Leaf	Goats, sheep & cattleGoats, Sheep & Cattle
E. cymosa	Leaf, twigs	Cattle, Sheep & Goats
M. ferrugnea	Leaf, flower, twigs	Cattle, sheep & goats
M. alba	Berries, Leaf and Edible branch	Goats, sheep & Cattle
V. amygdalina	Leaf, twigs flower,	Cattle, sheep & goats

Table 3: Number of selected multipurpose trees in household level in study districts (Mean ± SD)

	Districts				
Species	Chuko (Mean ± SD)	Dale(Mean ± SD)	Shebedino (Mean ± SD)	Over all(Mean ± SE)	P - value
B. aegyptiaca	2.1 ± 1.3a	1.17 ± 0.65b	1.1 ± 1.07b	1.3 ± 1.06	0.0003
C. africana	4.3 ± 2.1	5.0 ± 1.4	3.6 ± 2.8	4.3 ± 2.19	0.1386
E. cymosa	2.6 ± 1.53a	1.15 ± 0.87b	1.2 ± 1.19b	1.65 ± 1.23	0.0004
M. ferrugnea	5.6 ± 1.9a	4.55 ± 1.7a	3.3 ± 1.45b	4.48 ± 1.7	0.0003
M. alba	1.15 ± 0.36b	1.4 ± 0.59ab	1.5 ± 0.51a	1.35 ± 0.50	0.0845
V.amygdalina	3.85 ± 1.38a	3.55 ± 1.23a	2.4 ± 1.09b	3.26 ± 1.24	0.0012

SD = Standard deviation, SE = standard error

Table 4: Chemical composition and *in vitro* organic matter digestibility of selected multi-purpose fodder trees

		Chemical composition and *in vitro* organic matter digestibility (%DM)							
Season	Species	DM%	Ash	EE	CP	NDF	ADF	ADL	IVOMD
Dry	*B. aegyptiaca*	97.25c	16.87b	1.05d	17.04b	34.97e	20.21f	11.95e	71.17c
	C.africana	98.15b	15.53c	1.42c	10.68e	61.29a	35.07b	22.22a	57.25e
	E. cymosa	98.47a	11.45e	4.32a	13.68d	53.46b	38.08a	17.39b	62.33d
	M.ferruginea	95.24d	12.27d	1.68c	16.57b	50.92c	32.41c	14.11c	76.10b
	M.alba	98.46a	21.13a	1.76b	19.23a	42.03d	20.67e	12.19d	81.32a
	V.amygdalina	95.67d	12.16d	0.74e	14.38c	43.14d	21.01d	10.26f	81.52a
Wet	*B. aegyptiaca*	95.83d	16.9b	1.44b	17.92b	29.86e	17.14e	8.61e	79.15c
	C.africana	97.89c	11.53c	1.26e	13.34d	56.96a	32.81a	18.09a	65.65e
	E. cymosa	98.08b	11.63c	4.63a	16.57c	50.99b	32.23b	17.37b	70.20d
	M.ferruginea	94.94e	11.99c	1.35c	20.15a	50.64b	28.55c	15.28c	83.27b
	M.alba	96.34c	18.63a	1.35c	20.73a	40.07c	21.25d	11.83d	83.18b
	V.amygdalina	94.95e	10.64d	1.30d	17.39b	36.23d	15.15f	8.72e	84.83a
	Species	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
P-value	Season	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Species * Season	0.0001	<.0001	0.7048	<.0001	0.0849	0.0167	0.0035	<.0001

Different letters in the column shows significant ($P < 0.05$) difference DM = Dry matter, OM = Organic matter, EE = Ether extract, CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, IVOMD=*In-vitro* organic matter digestibility

content observed for *E.cymosa*, *M. ferruginea*. and *V. amygdalina* was comparable with the result reported earlier [25, 26, 28] but higher than the result reported by Belachew *et al.* [29] for *E. capensis, F.sycomorus, M.lanceolata* and *R.glutinosa*. The highest ($p<0.05$) CP content was for *M.alba* during dry and wet season and *M.ferruginea* during wet season while the lowest ($p<0.05$) was for *C. africana* in the dry season.

Crude protein content for *M. alba* in wet season is comparable with the result reported by Aynalem Haile and TayeTolemariam [5] for *B. polystachya* and *M. lanceolata* but higher than the result reported earlier [25, 27] for *V.amygdalina, E.cymosa* and *C. africana* and [28] for *E. capensis, F.sycomorus, M. lanceolata* and *R. glutinosa.* However, it was lower than the result reported for *V. amygdalina* and *G. latifolum* [26]. The CP content for *C. africana* and *V. amygdalina* in the current study was lower than the result reported by Shenkute *et al.* and AmsaluSisay, TegeneNegesse and AjebuNurfeta [30, 31]. The CP content for *B.aegyptiaca* during dry period was higher than the result reported by Shenkute *et al.* [30] but it is comparable with the result reported for *B. aegyptiaca* and other MPTs in the dry season [32].

E.cymosa and *C.africana* shown the highest (p<0.05) NDF content and the lowest (p<0.05) was recorded for *B.aegyptiaca* during dry and wet season. The NDF content observed for *B.aegyptiaca* in the current study was shown lower result than the result reported by AssenEbrahim *et al.* [33] for *M. alba* leaf collected during dry period and for *C.africana* reported by AmsaluSisay, TegeneNegesse and AjebuNurfeta [31]. The NDF content for *C.africana*in the current study is higher but the NDF content for *B .aegyptiaca*is lower than the result reported by TakeleGeta, Lisanework N igatu and GetachewAnimut and Shenkute *et al.* and AmsaluSisay, TegeneNegesse and AjebuNurfeta [25, 30, 31]. However; the NDF content for *C. africana* is comparable with the value reported for *F. sycomorus* and *M. lanceolata* by Belachew *et al.* [29]

The lowest (P<0.05) ADF value was recorded for *B.aegyptiaca* during dry season and *V.amygdalina* during wet season. The highest (p<0.05) ADF was recorded for *E.cymosa* and *C.africana* during dry and wet season. The ADF content observed in the current study for *M.alba* in dry and wet season was lower than the result reported earlier for *V. amygdalina*, *E.cymosa* and *C.africana* by Aynalem Haile and TayeTolemariam and TakeleGeta, Lisanework N igatu and Getachew Animut [5, 25] for *B. polystachya, M. lanceolata* and *V. amygdalina.* The lowest ADL content was observed for *V.amygdalina*. The ADL content recorded in this study for *B. aegyptiaca* and *V. amygdalina* in wet season was lower than the result reported earlier [5] for *B.polystachya, M. lanceolata* and *V. amygdalina* and within the range of the result reported by TakeleGeta, Lisanework Nigatu and GetachewAnimut [25] for *V. amygdalina, E. cymosa* and *C.africana*. The highest (P<0.05) IVOMD was recorded for *M.alba* and *V.amygdalina* during dry season. The IVOMD value for *M.alba*, *V.amygdalina* and *M.ferrgunea* in the current is higher than the result reported by Aynalem Haile and TayeTolemariam [5] for *B. polystachya, M. lanceolata* and *V.amygdalina*, AssenEbrahim *et al.* [33] for *M. alba* leaf harvested in wet season and EtanaDebela and AdugnaTolera [34] for leaf, twig, pod and whole forage of *M.oleifera* and *M. stenppetala*.

***In-vitro* Gas Production and gas production Characteristics:** Total gas production and gas production characteristics are presented on Fig 1, Fig 2 and Table 5. The highest (P<0.05) gas production was recorded for *M. alba* from dry and wet season and the lowest was for *C.africana* from dry and wet season. *In-vitro* gas production presented in this study during wet season were higher than the result reported earlier AmsaluSisay, TegeneNegesse and AjebuNurfeta and Njidda, Olatunji and Garba and Okunade *et al.* [31, 32, 35] for *M. ferruginea, V.amygdalina* and *C. africana* and for selected semi- arid multi-purpose browses.

The "a" fraction of gas production was shown negative value for all species. However the highest value was recorded for *C.africana* followed with *M. ferrugnea* during wet season. The highest (P<0.05) "b" fraction was recorded for *M.alba* in wet and dry season and *V. amygdalina* shown the highest "b" fraction during dry season. The gas production potential (a+b) was higher (p<0.05) for *M. alba* and *V.amygdalina*in wet season and dry season and the lowest a+b was for *B. aegyptiaca*., *C. africana* and *E. cymosa* in the dry season.

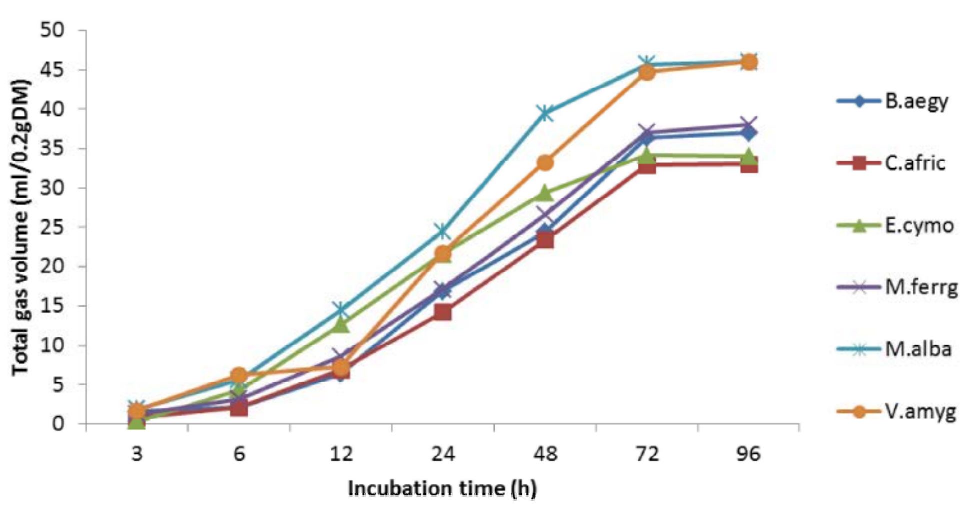

Fig. 1: Gas production in dry season

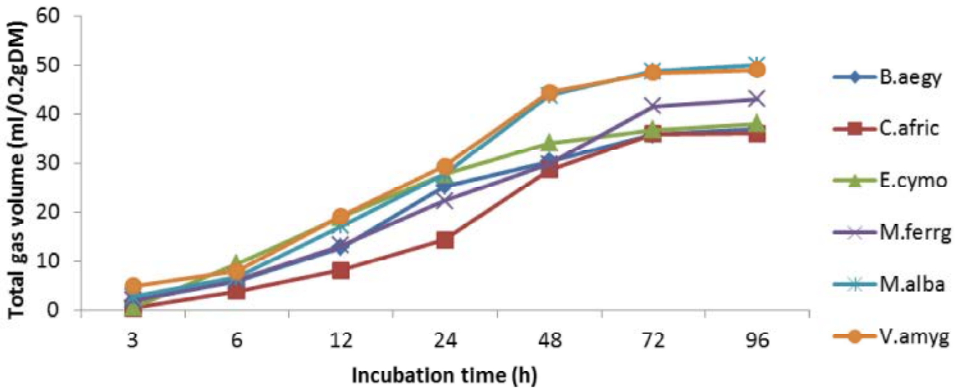

Fig. 2: Gas production in wet season

Table 5: *In-vitro* gas production constants of selected multipurpose trees harvested during dry and wet season

		In-vitro gas production parameters				
Season	Species	a	b	a+b	c	L
Dry	*B. aegyptiaca*	-6.1a	38d	31.9c	0.037bc	4.7ab
	C.africana	-6.1a	38.6cd	32.5c	0.032c	5.3a
	E. cymosa	-8.1b	41.4c	33.4c	0.051a	4.3bc
	M. ferruginea	-5.5a	44.5b	39.0b	0.033c	3.9c
	M. alba	-8.3b	50.6a	42.2a	0.042b	4.2bc
	V .amygdalina	-6.8ab	49.6a	42.8a	0.034bc	4.3bc
Wet	*B. aegyptiaca*	-6.6a	40.8d	34.2b	0.054ab	3.2
	C.africana	-4.2a	41.3d	37.1b	0.027c	3.9
	E. cymosa	-9.9b	46.6cb	36.6b	0.064a	3.8
	M. ferruginea	-5.1a	44.1cd	38.9b	0.039c	3.1
	M .alba	-7.3ab	57.2a	49.9a	0.041bc	3.3
	V. amygdalina	-6.3a	51.2b	45.2a	0.041bc	3.1
	Species	0.0264	<.0001	<.0001	0.0021	0.4776
P-value	*Season*	0.6786	0.1261	0.0766	0.1192	<.0001
	*Species*season*	0.0003	<.0001	<.0001	<.0001	0.233

*Means within a column with different superscripts letter differ significantly (p<0.05), A = Gas production from the immediately soluble fraction (ml), B = gas production from the insoluble but degradable fraction (ml), a + b = potential gas production (ml), C = the rate constant of gas production (fraction/hr), L = lag time, SEM = standard error of mean

The rate constant of gas production (C) was highest for *E. cymosa* during dry and wet season. The highest lag time (L) was recorded for *C. africana* during the dry season. The constant (a) was negative and low for all MPTs which is similar with the result reported [36] for selected browse species in the rift valley of Ethiopia. However (b) and (a+b) fractions in the current study were higher as compared with the result presented by Amsalu Sisay, TegeneNegesse and AjebuNurfeta [36]. In the other hand the lag time (L) in this study was lower than the result reported earlier by Njidda, Olatunji and Garba [32]. The (a), (b) and (a+b) fraction in this study were lower than the result reported earlier by MergaBayssa, TegeneNegesse and AdugnaTolera [37] for treated *A. tortolis* and *P. juliflora* leaf and pod. The *in vitro* gas production technique is a relatively simple method for evaluating feeds, as large numbers of samples can be incubated and analyzed at the same time [37].

Organic Matter Digestibility, Metabolizable Energy, Short Chain Fatty Acids, Totalphenol, Total Tannin and Condensed Tannin: Organic matter digestibility, ME, SCFA at 24hr of incubation period and TP, TT and CT content of MPFTs are presented in Table 6. The highest (P<0.05) OMD, ME and SCFA were recorded for *M. alba* during dry and wet season and the lowest for *C. africana* in the dry season. The SCFA content of *M. alba* was comparable with the result reported by AmsaluSisay, TegeneNegesse and AjebuNurfeta [36] for selected browse species from rift valley and lower than the result

Table 6: Organic matter digestibility, metabolizable energy, short chain fatty acids, total phenol, total tannin and condensed tannin content of multi-purpose trees

Season	Species	OMD	ME(MJ/kgDM)	SCFA(μmol/g DM)	TP (%)	TT (%)	CT (%)
Dry	B. aegyptiaca	38.59e	5.62e	0.37e	12.03b	8.55b	7.64b
	C.africana	33.09f	4.86f	0.30f	15.05a	12.41a	11.34a
	E. cymosa	40.26c	5.93c	0.46c	9.61c	7.65c	5.88c
	M.ferruginea	39.69d	5.79d	0.41d	8.92d	6.29d	5.37c
	M.alba	45.55a	6.66a	0.53a	1.79f	1.36f	0.89e
	V.amygdalina	41.41b	6.08b	0.48b	6.64e	4.58e	3.11d
Wet	B. aegyptiaca	45.38d	6.65d	0.54c	13.75b	11.11b	9.76b
	C.africana	33.90f	4.96f	0.29e	16.53a	14.61a	13.36a
	E. cymosa	46.72c	6.87c	0.59b	11.89c	10.76b	7.6c
	M.ferruginea	43.25e	6.30e	0.46d	10.63d	8.51c	8.1c
	M.alba	50.95a	7.47a	0.66a	2.72f	2.48e	1.05e
	V.amygdalina	46.95b	6.89b	0.59b	8.21e	6.39d	4.79d
P-value	Season	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Species	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Season * species	0.0288	0.0322	0.0094	<.0001	<.0001	<.0001

reported by AmsaluSisay, TegeneNegesse and AjebuNurfeta [36] for *A. tortolis* and *P. juliflora* and higher than the result reported by Okunade *et al.* [35].

The OMD observed at 24hr of incubation periods in this study ranged from 33.09 to 45.55% in dry season and 33.9 to 50.95% in wet season. The OMD obtained in this study during wet season was comparable with the result (32.11- 49.06% and 34.84-49.31) reported for different browse species by Njidda, Olatunji and Garba and Okunade *et al.* [32,35] respectively and it is in line with the result (33.7 to 58.34%) reported by AmsaluSisay, TegeneNegesse and AjebuNurfeta [31] for *M. ferruginea, V. amygdalina* and *C. africana*.

However, it was lower than the result reported for different MPT species [37] and [39]. The ME recorded from *M. alba* was significantly higher (p<0.05) than other MPTs in dry and wet season. The ME content for *M. alba* in this study was comparable with the result reported earlier [35, 36, 39]. The lowest TP, TT and CT% were recorded for *M. alba* as compared with other indigenous MPTs from dry season and the highest from *C. africana* from the wet period. Mulberry leaf contained considerably lower levels of total phenolics compared with leaves and pods of most native browse trees and shrubs [40].

The TP contents of MPFTs except *C. africana* were lower than the result (15.3 to 39.0%) reported by MergaBayssa, TegeneNegesse and AdugnaTolera [37] for untreated *A.tortolis*and *P.juliflora* leaf and pods. The TT and CT contents of *A. tortolis* leaf and pods reported by MergaBayssa, TegeneNegesse and AdugnaTolera [37] were higher as compared with the result in this study. However, CT content reported for *M. ferruginea*, *V. amiygdalina* and *C. africana* by [31] were lower than the results presented for multipurpose fodder trees (MPTs) in this study. The TT presented in this study during dry and wet period was higher for most species except *M. alba* which shown lower content in dry and wet season. The TT content for *M. alba*in wet season recorded in this study is comparable with the result reported by ChanderDatt, Datta and Singh [39]. In general the TP, TT and CT contents of MPFTs which were collected during wet season were higher (p<0.05) than those collected during dry season.

Methane Production and Concentration: Methane production (ml/0.2gDM) and concentration (ml/total gas volume) of MPFTs incubated at 24 and 72hr of incubation were significantly (P<0.05) different among MPFTs species, between dry and wet season and (Table 7). The highest (P<0.05) methane production at 24hr of incubation period was from *V. amygdalina* and *M. ferruginea* in wet season and the lowest was from *M. ferrugnea* in dry season. Methane production in 24hr of incubation period in this study was lower compared with earlier reports [31, 35]. The highest (P<0.05) gas volume at 72hr of incubation was from *B. aegyptiaca*. The highest methane gas concentration at 24hr of incubation period was from *C. africana* in wet season and the highest gas concentration at 72hr of incubation period was for *B. aegyptiaca* in wet season. As reported by MergaBayssa, TegeneNegesse and AdugnaTolera [37] the highest methane gas production for treated *A .tortolis* and *P. juliflora* leaf and pods than the result presented for MPTs in the current study. Methane gas production was increased with increasing of incubation time as observed in this study.

Table 7: Methane production and concentration of multipurpose at 24 and 72hr of incubation time

		24hr		72hr	
Season	Species	Methane production (ml/0.2gDM)	Gas concentration (ml/total gas volume)	Methane production (ml/0.2gDM)	Gas concentration (ml/total gas volume)
Dry	*B. aegyptiaca*	6.75[a]	0.38[a]	13.75[a]	0.39[a]
	C. africana	6.0[ab]	0.40[a]	13.5[a]	0.40[a]
	E. cymosa	5.75[ab]	0.26[b]	8.5[b]	0.23[b]
	M .ferruginea	4.17[c]	0.21[b]	8.75[b]	0.22[bc]
	M. alba	5.5[b]	0.22[b]	8.58[b]	0.18[d]
	V. amygdalina	5.75[ab]	0.26[b]	8.83[b]	0.19[cd]
Wet	*B. aegyptiaca*	7.08[c]	0.29[d]	15.08[a]	0.41[a]
	C. africana	8.42[b]	0.57[a]	12.58[c]	0.34[b]
	E. cymosa	6.5[c]	0.23[d]	10.41[d]	0.26[c]
	M. ferruginea	10.08[a]	0.46[b]	13.83[b]	0.33[b]
	M. alba	6.58[c]	0.22[d]	11.5[cd]	0.23[d]
	V. amygdalina	10.33[a]	0.38[c]	12.08[c]	0.26[cd]
	Species	<.0001	<.0001	<.0001	<.0001
P-value	Season	<.0001	<.0001	<.0001	<.0001
	Season*species	<.0001	<.0001	<.0001	<.0001

Table 8: Pearson correlation coefficient (r) matrix among TG, OMD, ME, SCFA, TP, TT and CT

	TG	OMD	ME	SCFA	TP	TT	CT
TG	1.00						
OMD	0.99*	1.00					
ME	0.99*	0.99*	1.00				
SCFA	0.99*	0.98*	0.99*	1.00			
TP	-0.78	-0.82*	-0.81*	-0.78	1.00		
TT	-0.77	-0.83*	-0.82*	-0.79	0.99	1.00	
CT	-0.82*	-0.89*	-0.89*	-0.87*	0.98*	0.97*	1.00

TG =total gas, OMD = organic matter digestibility, ME = metabolizable energy SCFA = short chain fatty acids, TP = total phenol, TT = total tannin and CT= condensed tannin.

Correlation among Total Gas, Organic Matter Digestibility and Metabolizable Energy, Short Chain Fatty Acid, Total Phenol, Total Tannin and Condensed Tannin: Correlation among total gas (TGP), organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA), total phenol (TP) total tannin (TT) and condensed tannin (CT) is presented in Table 8. Total phenol (TP), TT and CT were negatively correlated ($P<0.05$) with TG, OMD, ME and SCFA. This indicates that presence of TP, TT and CT affects the activities of microorganisms and it affects digestibility of the feed during fermentation process and reduces TGP, OMD, ME and SCFA. However, TGP was positively correlated ($P<0.05$) with OMD, ME and SCFA. Total gas production is as a source of fatty acids for energy production.

CONCLUSION

The nutritive value of mulberry was comparable with *M. ferruginea* and *V. amygdalina* which are the commonly used indigenous multipurpose fodder trees by farmers and it was higher than the other multipurpose tree evaluated in this study. The high crude protein content, higher organic matter digestibility, less methane production, less total tannin and condensed tannin makes mulberry more preferable as fodder tree and it could be integrated with other indigenous multi-purpose tree and crops in the agro forestry production system of the districts.

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REFERENCES

1. Matlebyane, M., J.W. Ng'ambi and E.M.Aregheore, 2010. Indigenous knowledge ranking of available bro,wse and grass species and some shrubs used in medicinal and ethno-veterinary practices in ruminant livestock production in Limpopo province, South Africa. Journal of Livestock Research for Rural Development 22(3).

2. Berhanu Gebreme dhin, Adane Hirpha and Kahsay Berhe, 2009. Feed marketing in Ethiopia: Results of rapid market appraisal. Improving Productivity and Market Success (IPMS) of Ethiopian farmers project. ILRI (International Livestock Research Institute), Nairobi, Kenya, pp: 64.
3. Adugna, T., 2007. Feed resources for producing export quality meat and livestock in Ethiopia, examples from selected Woredas in Oromia and SNNP regional states. Consultation report submitted to the Ethiopia Sanitary and Phytosanitary Standards and Meat Marketing Program (SPS-LMM) Texas agricultural experiment station (TAES) Texas A & M University, Addis Ababa, Ethiopia.
4. AbebeMekoya, 2008. Multipurpose fodder trees in Ethiopia; farmers' perception, constraints to adoption and effects of long-term supplementation on sheep performance, PhD thesis, Wageningen University, the Netherlands, ISBN: 978-90-8504-818-3
5. Aynalem Haile and Taye Tolemariam, 2008. The feed values of indigenous multipurpose trees for sheep in Ethiopia: The case of *Vernonia amygdalina, Buddle japolystachya* and *Maesal anceolata*, Jimma University, College of Agriculture and Veterinary Medicine, Jimma, Ethiopia, Livestock Research for Rural Development, 20(3).
6. Zewdie Wondatir, 2010. Livestock production systems in relation with feed availability in the Highlands and central rift valley of Ethiopia, MSc. Thesis presented to Haramaya University.
7. Kabi, F. and F.B. Bareeba, 2008. Herbage bio-mass production and nutritive value of Mulberry (*Morus alba*) and *Caliandra calothyrsus* harvested at different cutting frequencies. J. Animal Feed Science and Technology, 140(1): 178-190.
8. Tsegaye, E., N. Tegene and N. Ajebu, 2018. Assessment of feed Resources and Mulberry (*Morus spp*.) plant use and production status in Coffee Ensete (*E. ventricosum*) Based Production System of Selected Districts of Sidama and Gedeo Zones, Southern Ethiopia, Hawassa University College of Agriculture School of Animal and Range Science, Journal of Agricultural Science and Food Technology, http:// pearlresearchjournals.org/ journals/ jasft/index.html.
9. Ajebu Nurfeta, Abebe Berecha and Aberra Melesse, 2013. The Effects of Tagasaste (*Chymancytisus palmensis*) Leaf Meal Supplementation on Feed Intake, Growth Performance and Carcass Characteristics of Rhode Island Red Chicks, In: Conference Proceeding of Agricultural development within the rural-urban continuum, Tropentag, Stuttgart-Hohenheim, September 17-19, 2013.
10. Amare Seifu and Daniel Fitamo, 2016. Diversity of Enset landraces (EnsetVentricosum (Cheesman) in AletaChuko District, Sidama Zone Southern Nation Nationality and Peoples Regional state (SNNPR), Ethiopia, Journal of Plant Sciences, 4(1): 1-7. doi:10.11648/j.jps.20160401.11
11. Negassa, S., 2005. Rapid Health, Nutrition and Food Security Assessment of ShebedinoWoreda Sidama, SNNPR, Ethiopia.
12. Abiot, M. and A. Zebene, 2014. Woody species diversity under natural forest patches and adjacent enset coffee based agro forestry in the midland of Sidama Zone, International Journal of Biodiversity and Conservation, 6(10): 708-723, http:// www.academicjournals.org/ IJBC
13. AOAC, 2005. Official Methods of Analysis of the Official Analytical Chemists, 18th Ed. (Horwitz, W., Eds.) Association of Official Analytical Chemists, Washington DC.
14. Van Soest, P.J., J.B. Robertson and B. Lewis, 1991. Method of dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition, Journal Dairy Sciences, 74: 3583-3597.
15. Van Soest, P.J. and J.B. Robertson, 1985. Analysis of Forages and Fibrous Foods. A Laboratory Manual for Animal Science 613. Cornel University, Ithaca. New York, USA, pp: 202.
16. Rubanza, C.D.K., M.N. Shem, R. Otsyina, S.S. Bakengesa, T. Ichinohe and T. Fujihara, 2005. Polyphenolics and tannin effect on *in- vitro* digestibility of selected Acacia species leaves. Animal Feed Science and Technology, 119: 129-142.
17. Tilley, J.M.A. and R.A. Terry, 1963. A two stage technique for the *in vitro* digestion of forage crops. Journal of British Grassland Society, 18: 104-111.
18. Menke, K.H. and H. Steingas, 1988. Estimation of the energetic feed value from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development, 28: 7-55.

19. Makkar, H.P.S., 2002. Application of the in vitro gas method in the evaluation of feed resources and enhancement of nutritional value of tannin-rich tree/browse leaves and agro-industrial by-products. Development and Field Evaluation of Animal Feed: 23–39. Supplementation Packages, IAEA-TECDOC-1294, IAEA, Vienna.
20. Blümmel, M. and E.R. Ørskov, 1993. Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Animal Feed Science and Technology, 40: 109-119.
21. Fievez, V., O.J. Babayemi and D. Demeyer, 2005. Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid production that requires minimal laboratory facilities. Animal Feed Science and Technology, 123-124: 197-210.
22. Jayanegara, A., N. Togtokhbayar, H.P.S. Makkar and K. Becker, 2009. Tannins determined by various methods as predictors of methane production reduction potential of plants by in vitro rumen fermentation system. Animal Feed Science and Technology, 150: 230-237.
23. SAS, Institute Inc, 2005. SAS/STAT User's Guide: Version 9. 1st edition. SAS Institute Inc., Cary, NC, USA.
24. YitebituMoges, 2004. Recommended Agro forestry/ Multipurpose trees for Borana lowlands, Middle land and their production techniques. Forestry Research Center, Dryland Forestry research project coordinator. Addis Ababa, Ethiopia.
25. Takele Geta, Lisanework Nigatu and Getachew Animut, 2014. Evaluation of Potential Yield and Chemical Composition of Selected Indigenous Multi-Purpose Fodder Trees in Three Districts of Wolayta Zone, Southern Ethiopia, Jigjiga University, Ethiopia, World Applied Sciences Journal, 31(3): 399-405, ISSN 1818-4952.
26. Atangwho, I.J., P.E. Ebong, E.U. Eyong, I.O. Williams, M.U. Eteng and G.E. Egbung, 2009. Comparative chemical composition of leaves of some ant diabetic medicinal plants: *Azadirachta indica,Vernonia amygdalina* and *Gongronema latifolium*, Department of Biochemistry, University of Calabar, P. M. B. 1115, Calabar, Nigeria. African Journal of Biotechnology, 8(18): 4685-4689, ISSN 1684-531.
27. Okpe Oche, Attah Rosemary, Ojowu John1, Edenta Chidi, Samuel M. Rebecca and Upev A. Vincent, 2017. Chemical Constituents and Nutrient Composition of *Carica papaya* and *Vernonia amygdalina*. Leaf Extracts, Department of Biochemistry, University of Agriculture, Makurdi, Nigeria, Journal of Complementary and Alternative Medical Research 2(1): 1-8; Article no.JOCAMR.29402
28. Sodamade, A., 2013. Proximate Analysis, Mineral Content, Amino Acid Composition and Functional Properties of *V. amygdalina* Vegetable Leaf Protein Concentrates, Department of Chemistry, Emmanuel Alayande College of Education, Greener Journal of Agricultural Sciences, ISSN: 2276-7770, 3(3): 204-210, March 2013.
29. Belachew, Z., K. Yisehak, T. Taye and G.P.J. Janssens, 2013. Chemical composition and in saccoruminal degradation of tropical trees rich in condensed tannins, Department of animal sciences, college of agriculture and veterinary medicine, Jimma university Ethiopia, Czech Journal of Animal Sciences, 58(4): 176-192
30. Shenkute, B., A. Hassen, T. Assafa, N. Amen and A. Ebro, 2012. Identification and Nutritive value of potential fodder trees and shrubs in the mid rift valley of Ethiopia, Journal of Animal & Plant Sciences, 22(4): 2012, 1126-1132 ISSN: 1018-7081
31. Amsalu Sisay, Tegene Negesse and Ajebu Nurfeta, 2017. Methane Production Potential and Nutritive Value of Indigenous Browses from mid Rift Valley of Ethiopia, School of Animal and Range Sciences, College of Agriculture, Hawassa University, Ethiopia. Pakistan Journal of Nutrition ISSN 1680-5194 DOI: 10.3923/pjn.2017.789.796.
32. Njidda, A.A., E.A. Olatunji and M.G. Garba, 2013. In sacco and In-vitro Organic Matter Degradablity of Selected Semi- Arid Browse Forages, Department of Animal Sciences, Bayero University, Kano, P.M.B.3011,Kano State, Nigeria. Journal of Agricultural and Veterinary sciences p-ISSN:2319-2372. Volume 3, www.iosrjournals.org
33. AssenEbrahim, GetachewAnimut, Mengistu Urge and AkliluHailemichael, 2016. Nutritional value of mulberry (*Morus alba*) forage harvested in dry and wet seasons in Northern Ethiopia. Department of Animal Production and Technology, Aksum University, Shire, Ethiopia Indian Journal of Animal Nutrition 33 (1): 45-53.

34. Etana Debela and Adugna Tolera, 2013. Nutritive value of botanical fractions of *Moringa oleifera* and *Moringa stenoptala* grown in the mid-rift valley of southern Ethiopia, an international journal incorporating agro forestry forum, Agro forest system, 87: 1147-1155DOI10.1007/s10457-013-9626-9.
35. Okunade, S.A., O.A. Isah, R.Y. Aderinboye and O.A. Olafadehan, 2014. Assessment of Chemical Composition and In-vitro degradation profile of some guinea savannah browse plants of Nigeria, Tropical and Subtropical Agro Ecosystem, 17: 529-538.
36. Amsalu Sisay, Tegene Negesse and Ajebu Nurfeta, 2018. Short Chain Fatty Acid Production, Organic Matter Digestibility and Metabolizable Energy Content of Indigenous Browses from Ethiopian Rift Valley School of Animal and Range Sciences, College of Agriculture, Hawassa University, Ethiopia. Journal of Agriculture and Veterinary Science, ISSN: 2319-2380, Volume 11, PP 61-68 www.iosrjournals.org.
37. Merga Bayssa, Tegene Negesse and Adugna Tolera, 2016. Effect of Chemical and Biological Treatments on Nutrient and Phenolic Composition, in sacco Degradability, *in vitro* Gas and Methane Production Kinetics of *Acacia tortilis* and *Prosopis juliflora* Leaves and Pods, School of Animal and Range Sciences, Hawassa University, Middle-East Journal of Science and Research, 24(4): 1236-1251.
38. Getachew, G., De peters, Edward J. Robinson and H. Peter, 2004. *In-vitro* gas production provides effective method for assessing ruminant feed, Uc Agricultural & Natural Resources, Journal of Calfornia Agriculture, 58(1): ISSN 2160-8091.
39. Chander Datt, M. Datta and N.P. Singh, 2008. Assessment of fodder quality of leaves of multipurpose trees in sub-tropical humid climate of India, ICAR research complex for NEH region, Lmbuchera, Tripura 799 210, India Journal of Forestry Research 19(3):209-214. DOI 10.1007/s11676-008-0035-2.
40. Shayo, C.M. and P. Udén, 1999. Nutritional uniformity of neutral detergent soluble in some tropical browse leaf and pod diets. Animal Feed Science and Technology, 82: 141-151.