

ORIGINAL ARTICLE

Chemical composition, *In vitro* gas production and tannin biological activity of *Prosopis juliflora* pods varying with harvesting seasonAsmamaw Zafu^{1,2*}, Adugna Tolera², Ajebu Nurfeta², Yisehak Kocho³¹Samara University, College of Dry land Agriculture, P.O. Box, 132, Samara, Ethiopia²Hawassa University, College of Agriculture, P.O. Box 5, Hawassa, Ethiopia³Areba Minch University, College of Agricultural Sciences, P.O. Box 21, Areba Minch, Ethiopia*Corresponding author: chameasme@gmail.com**ABSTRACT**

This study was conducted to evaluate the effect of harvesting season on chemical composition, *in vitro* gas production and tannin biological activity of *Prosopis juliflora* pod. *P. juliflora* pods were collected during dry (May/June) and wet (September/October) seasons from three districts (Asayta, Dubti and Amibara) of Afar region, Ethiopia. Dry matter, ash, neutral detergent fiber, acid detergent fiber, acid detergent lignin, total phenols, total tannin and condensed tannin content were higher ($P<0.05$) for pods collected during dry season while crude protein content was lower ($P<0.05$) for dry season pods. Gas production at 24h incubation, gas production from immediately fermentable fraction (a), gas production from water insoluble but potentially fermentable fraction (b), potential gas production (a+b), the rate constant of gas production (c) were higher ($P<0.05$) for pods collected during wet season whereas, lag time (L) of *P. juliflora* pod were higher ($P<0.05$) during the dry season. Lower ($P<0.05$) organic matter digestibility, metabolizable energy and short chain fatty acids contents were recorded at 24h of incubation of pods collected during dry season than those of wet season. The tannin activity was higher ($P<0.05$) for pods collected in the dry season (7.76%) than in the wet season (4.16 %). The overall results of this study revealed a decline in crude protein, organic matter digestibility, short chain fatty acid and metabolizable energy of *P. juliflora* pods during the dry season. Therefore, it is recommended to use different strategies such as supplementation of pod by mixing with better quality feeds or application of physical and chemical treatments to reduce the impacts of dry season on nutritional quality of *P. juliflora* pod.

Keywords: Chemical composition, *in vitro* gas production, tannin biological activity

INTRODUCTION

The use of browse and multi-purpose tree species as green forage for ruminants is becoming increasingly important in many parts of the tropics, including Ethiopia (Berhan and Getachew, 2009; Camacho *et al.*, 2010). In free-ranging livestock-production systems, browse species are important supplements to low quality roughages to alleviate dry season feed shortages and to improve both the quantity and quality of feed. *Prosopis juliflora* is an invasive multi-purpose tree species widely distributed throughout most of the arid and semi-arid pastoral areas of Ethiopia (Ameha, 2006).

P. juliflora bears pods in both dry and wet seasons (Wakie *et al.*, 2016). The pod serves as an important feed resource for ruminants (Ahmed *et al.*, 2012). However, evaluation of feed quality is required for the prediction of animal performance (Tatli Seven and Cerci, 2006), since there is close correspondence between seasonal variation in the quality of feeds and animal productivity (El Aich *et al.*, 2007; Sanon *et al.*, 2007).

Nutritional compositions including secondary metabolites have been reported in *P. juliflora* pod (Pasicznik *et al.*, 2013; Marga *et al.*, 2016). However, there is limited information on changes in nutritional composition of *P. juliflora* pod due to seasonal variation. Therefore the objective of this study was to evaluate the effect of harvesting season on chemical composition, *in vitro* gas production and tannin biological activity of *P. juliflora* pod. **MATERIALS AND METHODS**

Description of study area

This study was conducted in three districts of Afar Regional State, Ethiopia; Namely Asayta, Dubti and Amibara. The Afar Regional State is located in the northeastern part of Ethiopia. It is located between 39°34' and 42°28' East Longitude and 8°49' and 14°30' North Latitude. The altitude of the region ranges from 116 meter below sea level to 1600 meters above sea level. The temperature of the region varies from 25°C during the wet season (September/October) to 48°C during the dry season (May/June). Rainfall is bimodal throughout the region with a mean annual rainfall below 500 mm in the semi-arid western escarpments decreasing to 150 mm in the arid zones to the east (EMA, 2015).

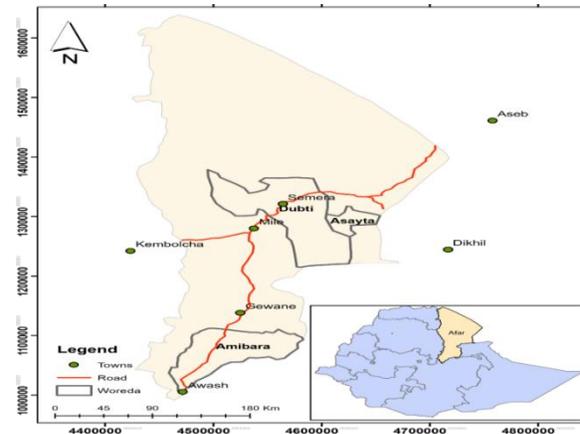


Figure 1. Map of the study area

Sample collection and preparation

Pods of *Prosopis juliflora* were collected both in dry (May/June) and wet (September/October) seasons (Wakie *et al.*, 2016) from three districts of Afar Region (Asayta, Dubti and Amibara). These districts were selected for sample collection based on the intensity of *P. juliflora* encroachment. Five representative kebeles (peasant associations) were purposively selected from each of the three selected districts based on the distribution of *P. juliflora* and five transect lines were randomly laid in each study Kebeles. Furthermore, five sampling plots of 20mx20m area at 200m interval or at 50 meters altitudinal drops were arranged for sampling of *P. juliflora* trees. The pods were collected using hooked sticks at early morning after a night windshake as the trees were large. The samples were air dried in the shade to minimize changes in tannins content and activity (Yousef and Rouzbehan, 2008). Sample of each transect lines were composited and stored in plastic bags. The samples were oven-dried at 60°C for 48h and ground to pass through 1mm sieve size and kept in airtight containers for the determination of chemical composition and gas production.

Chemical analysis

The dry matter (DM), ash and Kjeldahl nitrogen analyses were performed according to (AOAC, 2005) and crude protein (CP) was calculated as $N \times 6.25$. Neutral detergent fiber (NDF) was determined by the method of Van Soest *et al.* (1991) whereas acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest and Robertson (1985). Phenolics were extracted using 70% aqueous acetone and total extractable phenolics (TP) was determined using Folin Ciocalteu procedures as described by Makkar (2003b). The TP concentration were calculated using the regression equation of tannic acid standard. Total concentration of extractable tannins (TT) was estimated indirectly after being adsorbed to insoluble Poly Vinyl Poly Pyrolidone (PVPP) and the concentration calculated by subtracting

the TP remaining after PVP treatment. Condensed tannins were determined by using Butanol-HCl procedures and expressed as leucocyanidin equivalent (% of DM). The concentrations of condensed tannins were calculated by the formula:

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

The dilution factor was equal to 1 if no 70% acetone is added or 0.5 ml per volume if the extract was taken (Makkar, 2003b).

***In vitro* gas production**

In vitro gas production of *P.juliflora* pod samples was determined according to the procedures of Menke and Steingass (1988). Rumen fluid was obtained from three male adult Adilo sheep breed by suction tube before the morning feeding. The animals were fed with 40% concentrate feed/day (wheat bran (40%), maize grain (32%) and linseed cake (27%) and common salt (1%)) and Rhodes grass *adlibitum*. A sample of about 200 mg DM was weighed into a 100 ml capacity glass syringe calibrated for gas measurement and incubated in triplicate at 39°C using 30 ml buffered rumen fluid. The gas production was measured at 3, 6, 12, 24, 48, 72 and 96h. Total gas values were corrected for blank incubation which contains only rumen fluid. The volume of gas production characteristics were estimated using the equation $Y = a + b(1 - e^{-ct})$ (Ørskov and McDonald, 1979). Where, Y= volume of gas produced at time t; a= gas production from the immediately soluble fraction (ml); b= gas production from the insoluble fraction (ml); c= gas production rate constant (ml h⁻¹); a + b= potential gas production (ml); t= incubation time (h). Organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acid (SCFA) contents were estimated according to the equation of Menke and Steingass (1988). The OMD was calculated from the equation: - OMD (%) = 14.88 + 0.889G24 + 0.45CP. Where, OMD = organic matter digestibility at 24h ; CP = Crude protein content of feed samples and G24 = gas production value (ml/200mg) at 24h 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 24h of incubation; CP = Crude protein content of feed samples. The SCFA were estimated as: - SCFA = 0.0239G24 - 0.0601. Where, G24 = Gas production value (ml/200mg) at 24h of incubation

Tannins bioassay using polyethylene glycol (PEG)

The gas production technique described by Makkar, (2003b) was used for biological assay of browse

samples. Incubations were carried out in 100ml calibrated syringes with or without the addition of 750 mg PEG (peg, mw 4000) in water bath at 39°C. Thirty milliliter inoculum containing strained rumen liquor and buffer were added to 375mg sample in the syringe under continuous flushing with CO₂. Syringes with buffered rumen fluid and PEG (without feed sample) were included in duplicate as a blank. The syringes were shaken every 30 min during the first 4 hours and once every hour thereafter as suggested by Makkar, (2003b). The gas production was measured at 3, 6, 12 and 24h of incubation. Percentage of tannin activity was calculated as the difference between gas production with and without PEG (Makkar, 2003b).

Statistical analysis

All the data collected were subjected to the analysis of variance, using General Linear Model (GLM) procedure of SPSS version 24. Mean comparisons were carried out using Tukey's honestly significant difference test, at P< 0.05. The statistical model used for analysis of data was

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where Y_{ij} is the parameter studied; μ is overall mean, A_i is season (i=1, 2) and e_{ij} is error term.

RESULTS AND DISCUSSION

Chemical and phenolic compositions

The chemical and phenolic composition of *P.juliflora* pod collected during both dry and wet seasons are presented in Table 1. Crude protein (CP), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), total phenols (TP), total tannin (TT) and condensed tannin (CT) contents of *P.juliflora* pod were significantly higher (P<0.05) for the pods collected during dry season while CP content was lower (P<0.05) for the pods collected during dry season. The chemical composition of *P.juliflora* pod in this study lie within the ranges reported under similar conditions in northeastern Ethiopia (Marga *et al.*, 2016) and elsewhere (Pasicznic *et al.*, 2013). The significant effect of season on the chemical composition might be due to reduced uptake of essential nutrients from the soil and reduced photosynthetic activities of the plants induced by environmental stress during the dry season. The higher DM content of *P.juliflora* pods collected during the dry season might be due to the lower moisture levels experienced during the dry season relative to wet seasons (Anelea *et al.*, 2009).

Table 1: Chemical and phenolic compositions of *P.juliflora* pods

Components DM (%)	Season		SEM	SL
	Dry season	Wet season		
Dry matter	95.40 ^a	91.19 ^b	0.28	***
Crude protein	9.39 ^b	10.77 ^a	0.21	***
Neutral detergent fiber	26.91 ^a	23.61 ^b	0.50	***
Acid detergent fiber	17.31 ^a	13.78 ^b	0.47	***
Acid detergent lignin	4.62 ^a	3.16 ^b	0.14	***
Ash	4.66 ^a	3.15 ^b	0.14	***
Total phenol	11.77 ^a	10.55 ^b	0.44	**
Total tannin	8.76 ^a	7.35 ^b	0.31	**
Condensed tannin	6.29 ^a	5.83 ^b	0.12	**

DM= dry matter; SEM= standard error mean; SL= significance level; ***= (P<0.001); **= (P<0.05); ns = no significant; ^{ab}Mean in the same row with a diverse letter are significantly different (P<0.05)

The lower CP content of *P.juliflora* pods harvested during dry season as compared to the wet season could be due to advancement in age of the plant associated with maturity, lower moisture content of the soil and availability of less nitrogen, and higher proportion of fibre fraction (Belachew *et al.*, 2013). On the other hand, a higher CP content during the wet season compared to dry season might be due to higher photosynthetic rate and higher moisture content which increase nitrogen uptake (Anele *et al.*, 2009). Thus, the low CP during the dry season is associated with low water availability and slow rate of photosynthesis. The findings conform to the reports of previous studies (Yayneshet *et al.*, 2009; Aster *et al.*, 2012) who reported seasonal variations in nutritional value of browse species, with lower values of CP reported for plants with advanced age and during moisture stress. Higher NDF, ADF and ADL content in *P.juliflora* pod collected during dry season was in accordance with other reports recorded in tropical and sub-tropical regions (Camacho *et al.*, 2010; Safari *et al.*, 2011) which could be attributed to increased cell wall lignification during dry season (Anelea *et al.*, 2009). Higher content of phenolic compounds (TP, TT and CT) in *P.juliflora* pod collected during dry season was also in line with the results of other studies (Salem *et al.*, 2006; Camacho *et al.*, 2010) which was due to thermal stress during the dry season (Cabiddu *et al.*, 2000). The more the plant is stressed, the more it produces tannins as a self-defense mechanism (Makkar, 2003a)

***In-vitro* gas production**

In-vitro gas production characteristics of *P.juliflora* pods are presented in Table 2. Gas production at 24 h incubation (GP₂₄), gas production from immediately fermentable fraction (a), gas production from water insoluble but potentially fermentable fraction (b), and potential gas production (a+b), the rate constant of gas production (c), were significantly higher (P<0.05) for pods collected during the wet season whereas, lag time (L) of *P.juliflora* pod was significantly higher (P<0.05)

for pods collected during the dry season. The amount of gas produced in the rumen is a reflection of the extent to which the feed is degraded and fermented (Getachew *et al.*, 2004) and can vary between the same feedstuffs due to its harvesting season (Tsegaye *et al.*, 2019). Thus, gas volume can be considered as a good reflection of substrate fermented to VFAs and an estimate of potential digestibility in the rumen (Getachew *et al.*, 2000).

The lower gas production volume at 24h incubation and lower values of calculated parameters (a, b, a+b, and c) of *P.juliflora* pod collected during dry season was consistent with the results of other studies (Tsegaye *et al.*, 2019), which could be due to higher phenolic compounds and fiber contents in the pods collected during dry season (Camacho *et al.*, 2010; Safari *et al.*, 2011). Getachew *et al.* (2000) noted that the presence of tannins in forages depresses the *in vitro* gas production since gas production resulted from fermentation of feed OM and buffering of the short chain fatty acids by the bicarbonates in the rumen. The fiber contents also reduce feed digestibility through their interwoven matrix of polymers which creates barriers against the microbial invasion and limits their access to digestible cell wall components (McDonald *et al.*, 2011).

Table 2: *In-vitro* gas production characteristics of *P.juliflora* pods

Parameters	Season		SEM	SL
	Dry	Wet		
Gas volume(ml/200mg)				
24h	58.64 ^b	67.18 ^a	1.11	***
Gas production constants				
a (ml)	19.70 ^b	25.27 ^a	0.46	***
b (ml)	67.98 ^b	72.60 ^a	0.55	***
a+b (ml)	87.67 ^b	97.87 ^a	0.83	***
c(hr ⁻¹)	0.03 ^b	0.04 ^a	0.00	***
L (h)	4.66 ^a	3.15 ^b	0.14	***

a= gas production from immediately soluble component (ml); b=gas production from insoluble but potential degradable portion (ml); a+b=potential gas production (ml); c= the rate constant of gas production (fraction/h); L= lag time; SEM= standard error mean; SL= significance level; ***= (P<0.001); ^{ab}Mean in the same row with a diverse letter are significantly different (P<0.05)

The higher lag time recorded from *P.juliflora* pod in the current study during dry season might be attributed to the effect of either high tannin concentration in the pods that impede the activity of rumen microbial fermentation or high level of fiber fraction (NDF) of the pods which would have hindered the penetration of rumen microorganisms to the fermentable carbohydrate fraction for rapid fermentation rate

Organic matter digestibility, metabolizable energy and short chain fatty acids contents at 24h of incubation were significantly higher (P<0.05) for pods collected

during the wet season compared to those collected during dry season (Table 3). The lowest gas volume at 24h of incubation of the pods collected during dry season was reflected in reduced concentration of short chain fatty acids and ME production from *P.juliflora* pod. The presence of higher NDF and phenolic compounds of the pods collected during dry season influenced the amount of substrate of OM fermented and the short chain fatty acid produced upon fermentation and the ME estimated from gas production (Osunga *et al.*, 2006).

Table 3: Organic matter digestibility and, metabolizable energy and short chain fatty acids content of *P.juliflora* pods

Parameters	Season		SEM	SL
	Dry	Wet		
Organic matter digestibility (%)	71.24 ^b	79.45 ^a	1.05	***
Metabolizable energy (MJ/kg DM)	10.71 ^b	11.95 ^a	0.16	***
Short chain fatty acids (μmol/g DM)	1.34 ^b	1.54 ^a	0.03	***

SEM= standard error mean; SL= significance level; ***= (P<0.001); ^{ab}Mean in the same row with a diverse letter are significantly different (P<0.05)

Tannin biological activities

The results of tannin biological activities of the pods treated with PEG are presented in Table 4. The tannin biological activity was higher (P<0.05) for pods collected during dry season. Tannin activity is measured as the percent increase of gas produced in the gas production test with the addition of polyethylene

glycol (PEG) compared to samples without PEG. The increase in the gas production in the presence of polyethylene glycol (PEG) is possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen. McSweeney *et al.* (2001) showed that the addition of PEG caused a significant and marked increase in the rate and extent of ammonia production in the rumen

Table 4: Tannin biological activities of *P.juliflora* pods treated with PEG

Parameters	Season		SEM	SL
	Dry	Wet		
Gas volume at 24 hr.(ml/200mg)				
PEG ⁺	68.72 ^b	78.38 ^a	0.97	***
PEG ⁻	60.96 ^b	74.21 ^a	1.14	***
Tannin biological activity (%)	7.76 ^a	4.16 ^b	0.53	***

PEG⁺= with polyethylene glycol; PEG⁻= without polyethylene glycol; SL= significance level; ***= (P<0.001); ^{ab}Mean in the same row with a diverse letter are significantly different (P<0.05)

In this experiment high percentage of tannin biological activity was recorded from the pods collected during dry season, which was due to higher concentration of phenolic compounds in the pods collected during dry season (Camacho *et al.*, 2010). Higher content of phenolic compounds affect the activity of microorganisms and digestibility of *P.juliflora* pod during fermentation process and reduce gas production at 24 h incubation, OMD, ME and SCFA production. The antinutrient effect of tannin in the browse leaves was removed by addition of PEG which was demonstrated by increase in gas production, SCFA, methane and ME concentration and this could be due to release of tannin bound soluble nutrients to the fermentation media (Marga *et al.*, 2016)

CONCLUSION

Results of the present study showed that there were variation in chemical composition (CP, NDF, ADF and ADL), phenolic compound (TP, TT and CT), in vitro gas production, OMD, SCFA content, ME and tannin biological activity between seasons. During the dry season, the NDF, ADF, ADL, TP, TT, CT content and tannin biological activity generally were higher, while CP, in vitro gas production, OMD, SCFA and ME contents were low, which shows a decline in overall nutritive value. Therefore, it is recommended that livestock producers use different strategies to reduce the impacts of dry season on nutritional quality of *P.juliflora* pod. One of the possible strategies could be the supplementation of the pod to the animals after application of either physical or chemical treatment.

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