Anti-nutrient contents and methane reduction potential of medicinal plants from maize stover based diet

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A B S T R A C T

Greenhouse gas emissions from Ethiopian agriculture are significantly increasing, with the largest share is from enteric fermentation and manure left on pasture. An investigation was conducted to evaluate the anti-nutrient composition and effect of commonly used medicinal plant extracts on enteric methane emission from fibrous feeds using maize stover as substrate feed. Total phenols, flavonoid, tannin and essential oil contents were analyzed using established standards. Effects of leaf extracts of *Acacia nilotica, Azadirachta indica*, three varieties of *Cymbopogon citratus* (*Cymbopogon citratus-I, Cymbopogon citratus java* and *Cymbopogon citratus upper awash*), *Leucaena leucocephala, Moringa stenopetala*, three varieties of *Rosmarinus officinalis* (*Rosmarinus officinalis I, Rosmarinus officinalis II* and *Rosmarinus officinalis III*) and *Thyme schimperi*, seed of three *Coriandrum sativum* varieties (*Coriandrum sativum* Batu, *Coriandrum sativum* Tulu and *Coriandrum sativum* Waltai) and root of *Echinops kebericho* on total gas production, digestibility and methane production of maize stover were investigated at different doses using the standard procedures. The results indicated that leaf extracts of *Acacia nilotica* had the highest (P < 0.001) total phenolic and total tannin contents. Compared to other evaluated plant species, all varieties of *Cymbopogon citratus* had the highest (P < 0.001) flavonoid content. Significantly high (P < 0.001) essential oil content was observed in *Rosmarinus officinalis II* than other varieties of *Rosmarinus officinalis* and other plant species. Significant reduction (P < 0.001) of methane production was observed with extracts of *Cymbopoon citratus java* (22.5 % less methane than the control) and *thyme schimperi* (16.7 % less methane than the control) at dose of 50 mg/kg DM. There was also significant (P < 0.001) interaction effect between plant species and dose rates at 50 mg/kg DM for both plant species. It can be concluded that the use of 50 mg/kg DM of *Cymbopoon citratus java* and *Thyme schimperi* extract to maize stover reduced methane production without negatively affecting feed digestibility. Further studies are necessary to examine the storability of the extracts in different time durations and evaluate their effects *in vivo* with animals.

1. Introduction

Global greenhouse gas (GHG) emissions from agriculture are significantly increasing. The greenhouse gas emissions from crop and livestock activities contribute to about 5 billion metric tons of CO_2eq to the atmosphere each year [1]. Similarly, GHG emission from

Ethiopian agriculture was highly increasing from 47984 to 102933 Gg CO_2eq per year (114.5 % growth) for the period of 1993–2017, in which enteric fermentation (51.3 %) and manure left on pasture (36 %) contribute the largest share [2]. Regarding the production systems, ruminants in mixed crop-livestock production system contributed more enteric methane emission in Ethiopia compared to other production systems [3]. Enteric methane emission in ruminants represents a loss of 2–20 % of gross energy intake of the animals [4]. Beside energy wastage through CH_4 formation, it traps the atmospheric heat and it is considered as a GHG that plays a vital role in the global warming with negative consequences on the worldwide environment [5].

In Ethiopia, maize (*Zea mays*) stover is one of the major crop residues used as basal feed under mixed crop-livestock production system. About 56 % of maize stover biomass is used as feed under mixed crop-livestock system in Ethiopia [6]. However, maize stover is characterized by high concentration of structural carbohydrates, low voluntary intakes, slow digestibility, low concentration of protein and energy [7]. *In vitro* studies in Ethiopia indicated that methane emission from maize stover is substantially high (18–32 % of the total gas) compared to other fibrous feed resources [8,9]. Therefore, research is needed to find a suitable alternative to mitigate rumen CH_4 production from fibrous feeds for better environment and improved livestock production.

Plant secondary metabolites are sometimes regarded as anti-nutritional due to their potential to adversely affect feed intake and nutrient utilization; however, when administered at optimum concentrations extracts from medicinal plants can beneficially alter ruminal fermentation [10], improve microbial protein synthesis [11] and suppress methanogenesis [10,12]. Different species and variety of plants can have various phytochemical contents. Therefore, knowledge of phenolic content and its effect on fermentation patterns is needed for each individual species in order to fully attach its potential to improve livestock production [13]. There is also a need to identify natural feed additives with potential to modify rumen fermentation, thereby enhancing the efficiency of feed energy utilization while decreasing rumen methanogenesis. In Ethiopia there are several native and foreign medicinal plants species traditionally used to treat several human and livestock diseases [14,15], serve as source of food, feed, beverage and spices [16]. Despite their diverse use, there is little information on their anti-nutritional contents, *anti*-methanogenic potentials and inclusion rate to be used as feed additives. Therefore, this study was aimed to evaluate the anti-nutrient composition and *anti*-methanogenic potential of selected medicinal plants and to determine the optimum inclusion rate of extracts into roughage feed to be used as additives.

2. Materials and methods

2.1. Selection and collection of experimental plants

Fifteen experimental plants were selected based on the information of previous studies conducted on the utilization and potential of medicinal plants in Ethiopia [14,16,17]. Samples were collected from the South-eastern part of Ethiopia, situated at 8°54′N and 38°28′ to 39°27′ E. The altitude ranged from 1715 to 4377 m above sea level. The mean annual rainfall of the study sites ranges from 760 to 1500 mm. The mean minimum and maximum temperatures were 14 and 28 °C, respectively.

About 500 g fresh leaves of *Acacia nilotica* (AN), *Azadirachita indica* (AZ), three variety of *Cymbopogon citratus* (CC) namely: *Cymbopogon citratus* -upper awash (CC-UA), *Cymbopogon citratus*-Java (CC-Java) and *Cymbopogon citratus*-I (CC–I), *Moringa stenopetala*
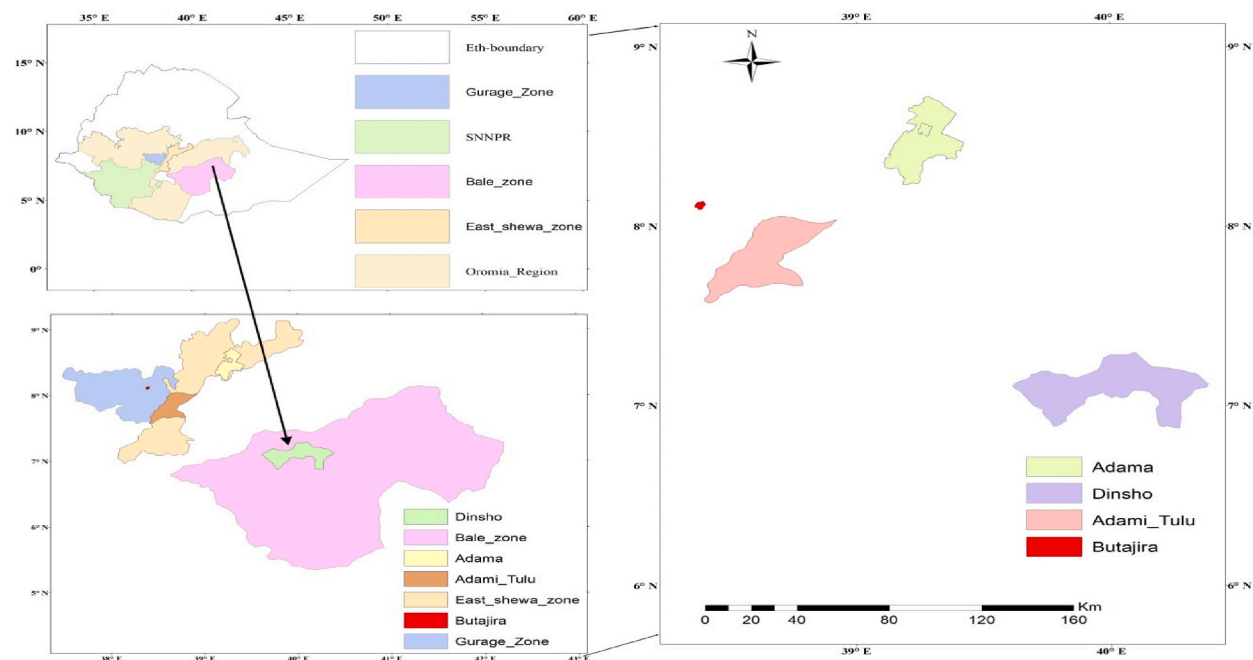

Fig. 1. Map of the study area.

(MS), *Leucaena leucocephala* (LL), three variety of *Rosmarinus officinalis* (RO) namely: WG-rosemary-I (RO-I), WG-rosemary-II (RO-II), and WG-rosemary-III (RO-III), *Thyme schimperi* (TS), seeds of three *Coriandrum sativum* (CS) varieties (Batu, Tulu and Waltai) and root of *Echinops kebericho* (EK) were collected from different sites (Fig. 1).

2.2. Preparation of extracts and determination of anti-nutrient contents

The collected sample from each plant species was washed with distilled water to remove debris and dust particles and then air-dried at room temperature under shade for 72 h. The average daily temperature and relative humidity of the drying environment were 30 °C and 25 %, respectively, which were measured using digital hygrometer [18]. The air dried sample was ground to pass through 1 mm (2 mm for tannin assay) sieve size and kept in air-tight plastic container and stored at 4 °C in a dry, dark and cool place to prevent phenolic degradation until used.

Extraction was done by adding 1500 mL 100 % methanol to 150 g of powdered plant materials in a glass flask following the procedure of Kirby and Schmidt [19]. The mixture was placed on a mechanical shaker at 150 rpm, a temperature of 25 °C and allowed to soften for 96 h. After 96 h, the mixture was filtered using a Buckner funnel and Whatman 1 filter paper. The filtrate was concentrated to dryness in a rotary evaporator (Büchi Labortechnik, Germany) under reduced pressure and controlled temperature (50 °C) to remove the solvents and give final extracts. The weight of the dried sample was reconstituted by dissolving with methanol in the ratio of 1 mg into10 mL and stored at 4 °C in a light proof glass container until used. Total phenols and tannins were determined according to Makkar [20]. The absorbance of each extract was read by using a UV–Vis spectrophotometer (UV–Vis spectrophotometer, U-1800, 5930482, High Technology Corporation, Tokyo, Japan) with a wavelength of 724 nm.

Finally, the dried extract of each plant was reconstituted by dissolving, 2.5, 5.0 and 7.5 mg in 1000 ml of distilled water separately to give three levels of concentrations: 25 mg, 50 mg and 75 mg/kg DM, respectively [12] to be used as additive. All plant extracts were kept under refrigeration at 4 °C until further use.

Essential oil content was determined by steam extraction following the procedure of Guenther [21]. Accordingly, leaves of *CC, RO, TS*, seed of CS and root of EK were collected from respective study areas. Briefly, 250 g of fresh leaves were cut into small pieces and placed in a 1L flask containing 400 mL of distilled water. It was hydro distilled for 3 h at 50 °C using a Clevenger-type apparatus. Similarly seeds and root were milled into small pieces and distilled as described above. The essential oil contents of plants were calculated based on the formula described below:

$$\text{Oil content} \frac{\text{V}}{\text{W}} \% = \frac{Volume\ of\ extracted\ oil\ (ml)}{mass\ of\ sample\ (g)} x100$$

2.3. Proximate analysis of substrate feed

Maize (Corn) stover (variety MH-130) sample was collected from Adami Tulu Research Center, dried in a forced-air oven at 60 °C for 48 h and ground to pass through a 1-mm sieve using a laboratory Wiley mill. The ground samples were kept in sealed plastic bags at room temperature pending analysis. Dry matter, ash and crude protein contents of substrate feed were determined according to the procedure described by AOAC [22]. Neutral detergent fiber (NDF) was determined according to Van Soest et al. [23] using an Ankom 220 fiber analyzer (Ankom Technology, Macedon, NY). Acid detergent fiber and Acid detergent lignin (ADL) were determined according to Van Soest and Robertson [24].

The chemical composition of maize (*Zea mays* L.) stover indicates the amount of ash was 8 %. Crude protein stands at 3.6 % and the neutral detergent fiber, acid detergent fiber and acid detergent lignin were 32 %, 76.2 %, 47 % and 6.6 %, respectively.

2.4. In vitro gas and methane production measurement

2.4.1. Rumen fluid collection and in-vitro rumen fermentation procedures

Rumen fluid was collected from slaughtered sheep following the procedure of Wang et al. [25]. On each experimental day, fresh rumen fluid was collected from two Arsi-Bale sheep slaughtered in abattoir. The donor animals were fed grass hay for 7 days before slaughter. Rumen fluid was collected early in the morning immediately after slaughter and collected into a pre-warmed thermos flask (39 °C) and immediately transported to laboratory. The pooled rumen fluid was strained through 4 layers of gauze then mixed with Menke's buffer (incubation medium) in a 1:2 ratio (v/v). The buffer solution was prepared using the method described by Menke and Steingass [26]. All handling was carried out under continuous flushing with CO_2 to minimize change in microbial population.

In vitro rumen incubation was done using a 100 ml glass syringe. Prior to incubation, 200 mg of dry and milled (1 mm sieve size) maize stover sample was weighed into each glass syringe and 2 ml of the already prepared concentrations of leaf extracts of AN, AZ, CC, LL, MS, RO, and TS, root of EK, seeds of CS, were added to syringes containing maize stover in triplicate in two separate runs/replications [12]. In both runs, each sample was tested with 3 replications of controls (syringes with samples and without plant extracts) and three blanks (syringes incubated with rumen fluid + buffer solution alone). The syringes containing feed samples, pre-warmed at 39 °C overnight and their pistons lubricated with vaseline to ease movement and prevent gas from escaping, then 30 ml of rumen fluid was added (rumen fluid + buffer mixture) under continuous CO_2 flushing. The syringes were incubated at 39 °C in a water bath and shaken manually every hour for an initial 8 h (including 0 h) of incubation [27] and then at each recording time [28]. Gas volume was recorded before incubation (0 h) and after 3, 9, 12, 24, 48, 72 and 96 h of incubation. Total gas volumes were corrected for readings of the blank syringes and initial volume at each time.

2.4.2. Determination of total gas and methane production

Total gas production was determined by reading the position of the piston at each time following the procedure of Menke and Steingass [26]. Net gas production (ml/200 mg) at t hours was calculated as: $G_t = [(V_t–V_0–G_0) \times 200]/W_S$. Where: G_t = gas production value (ml/200 mg) at t hours, G_0 = gas production of blank syringes (ml), V_0 = initial volume in ml, V_t = volume in ml at t hours, W_S = weight of dried sample in mg.

In-vitro organic matter digestibility and metabolizable energy of the samples were calculated using the equations of Menke and Steingass [26] as follows:

$$OMD(\%)= 14.88 + 0.889GP+0.45CP+0:0651XA$$

Where: OMD = Organic matter digestibility at 24 h.

CP = Crude protein content of feed sample (% DM),

GP = Gas production (corrected for blank at 24 h of incubation), XA = ash content.

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057CP

Where: GP= Gas production at 24 h of incubation (ml per 200 mg DM).

CP= Crude protein content of feed sample (% DM).

ME = Metabolizable enrgy.

Short-chain fatty acids (SCFA) were estimated as:

SCFA (mmol/g DM) = (0.0239 GP) - 0.0601 Getachew et al. [29].

Where: GP = Gas Production at 48 h of incubation.

Methane Production: Methane production at 24 h of incubation was measured using the procedure described by Fievez et al. [30]. For measuring methane production at the end of incubations and after recording the final gas volume, the lower end of the syringe was connected to the lower end of another syringe containing 4.0 ml of 10 M sodium hydroxide (NaOH). Sodium hydroxide was then introduced from the latter into the incubated contents, thereby avoiding gas escape. Mixing of the contents with NaOH allowed absorption of CO_2 and the gas volume remaining in the syringe was considered to be CH_4.

Net methane production was calculated by the differences of the methane in the syringe and the equivalent blank. The methane concentration was calculated according to Jayanegara et al. [31]:

$$\text{Methane concentration }(\%)= \left(\frac{\text{Net methane production}}{\text{Net gas production}} \times 100 \right)$$

2.5. Statistical analysis

Data on anti-nutrient composition were analyzed using SAS ANOVA procedure SAS [32], based on the model:

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

Table 1
Concentrations of total phenols, total flavonoid, total tannins and essential oils in extracts of selected medicinal plant species in Ethiopia (mean ± SE).

Plant parts and species	TP (mg GAE/g dry extract wt.)	TF (mg QE/g dry extract wt.)	Total tannin g/kg	Condensed tannins (g/kg)	Hydrolysable tannins (g/kg)	Essential oil (% on fresh wt. base)
Leaves						
AN	471.7 ± 3.5[a]	36.5 ± 1.2[i]	151.5 ± 1.2[a]	89.0 ± 0.9[a]	62.5 ± 1.4[a]	ND
AZ	52.8 ± 1.7[h]	3.7 ± 0.1[l]	48.8 ± 0.9[c]	25.0 ± 2.3[d]	23.8 ± 0.8[d]	ND
CC-I	63.3 ± 2.0[f]	130.5 ± 1.8[b]	45.0 ± 0.9[d]	19.3 ± 0.9[f]	25.7 ± 1.1[c]	0.34 ± 0.0[hi]
CC-Java	131.8 ± 3.7[b]	143.6 ± 1.7[a]	49.4 ± 2.6[bc]	19.8 ± 0.5[f]	29.6 ± 0.8[b]	0.65 ± 0.0[c]
CC-UA	123.0 ± 1.7[c]	129.0 ± 3.0[b]	51.3 ± 1.1[b]	43.2 ± 1.0[b]	8.05 ± 1.1[hi]	0.56 ± 0.0[e]
LL	92.0 ± 3.0[e]	38.0 ± 1.0[i]	44.6 ± 1.0[de]	19.2 ± 0.1[f]	25.3 ± 0.6[cd]	ND
MS	97.4 ± 1.5[d]	65.5 ± 1.5[d]	25.9 ± 1.6[h]	16.2 ± 1.4[g]	9.7 ± 0.8[gh]	ND
RO-I	64.0 ± 3.4[f]	47.0 ± 2.9[g]	42.7 ± 0.4[e]	29.8 ± 1.0[c]	12.9 ± 0.1[f]	0.62 ± 0.0[d]
RO-II	58.1 ± 2.2[g]	53.0 ± 1.0[f]	34.8 ± 0.2[g]	19.3 ± 1.4[f]	15.5 ± 1.0[e]	1.02 ± 0.0[a]
RO-III	57.3 ± 1.6[g]	43.3 ± 1.5[h]	33.3 ± 1.5[g]	20.3 ± 2.0[ef]	12.9 ± 1.6[f]	0.76 ± 0.0[b]
TS	134.0 ± 2.6[b]	75.0 ± 1.8[c]	39.9 ± 0.6[f]	22.6 ± 2.6[e]	17.3 ± 0.8[e]	0.45 ± 0.0[f]
Seeds						
CSB	26.0 ± 2.0[j]	5.7 ± 0.35[l]	21.5 ± 0.6[i]	13.9 ± 0.7[gh]	7.6 ± 1.4[i]	0.36 ± 0.0[h]
CST	27.4 ± 2.0[j]	9.6 ± 0.65[j]	21.4 ± 0.5[i]	12.6 ± 1.0[h]	8.8 ± 0.5[hi]	0.32 ± 0.0[i]
CSW	27.1 ± 2.0[j]	8.9 ± 0.95[jk]	23.7 ± 1.1[h]	19.3 ± 0.9[f]	4.4 ± 1.0[j]	0.42 ± 0.0[g]
Root						
EK	36.4 ± 1.5[i]	6.3 ± 1.0[kl]	25.4 ± 2.0[h]	14.8 ± 0.9[gh]	10.6 ± 1.3[g]	0.1 ± 0.0[j]

Means with different letters across the columns for plant species are significantly (P < 0.05) different; TP = Total Phenol; TF = total flavonoid, GAE = gallic acid equivalents; QE = quercetin equivalents; ND = not detected; AN: *Acacia nilotica*; AZ: *Azadirachta indica*; CSB: *Coriandrum sativum* Batu; CST: *Coriandrum sativum Tulu;* CSW: *Coriandrum sativum Waltai;*CC-I: *Cymbopogon citratus* I; CC-Java: *Cymbopogon citratus Java;* CC-UA: *Cymbopogon citratus* Upper Awash; EK: *Echinops kebericho*; LL: *Leucaena leucocephala*; MS: *Moringa stenopetala*; RO-I: *Rosmarinus officinalis* I; RO-II: *Rosmarinus officinalis II*; RO-III: *Rosmarinus officinalis III*; TS: Thyme schimperi.

Where Y_{ij} the dependent variable, μ = the overall mean; α_i = the fixed effect of plant species and e_{ij} = the effect of random error. Means of anti-nutrient (total phenol, total flavonoid, total tannin and essential oil) composition data were compared using the least square means procedure. Means were considered significantly different at P < 0.05. Values for the total phenolic contents were obtained from a calibration curve (y = 0.0114x + 0.0563, R^2 = 0.9791) of gallic acid (0–150 µg/mL) and expressed in gallic acid equivalents (GAE) per gram dry extract weight. The values of total flavonoid were derived from the calibration curve y = 10.031x + 0.0775, R^2 = 0.9613 of quercetin (0–125 µg/mL) and expressed in quercetin equivalents (QE) per gram dry extract weight.

A 4 by 15 factorial arrangement was used for the analysis of total gas, methane, IVOMD, ME and short chain fatty acids using PROC GLM (general linear model) procedure of SAS [32] 9.4 (SAS Institute Inc., Cary, NC, USA) based on the model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ij} is the dependent variable, μ is the least squares mean, α_i is the effect of ith plant, β_j is the effect of jth dose and $\alpha\beta_{ij}$ are the interaction effect of ith plant and jth dose rate and ε_{ijk} is the residual effect. Means were considered significantly different at P < 0.05.

3. Results

3.1. Anti-nutrient contents of medicinal plant extracts

Concentrations of total phenols, total flavonoid, total tannins and essential oils in extracts of selected medicinal plant species are presented in Table 1. Large variations were observed in total phenolic (TP), total flavonoid (TF) and total tannin (TT) contents of selected plant species and varieties. The content of TP in methanol extracts ranged from 26.06 to 471.7 mg GAE/g, demonstrating an approximate eighteen-fold variation. Leaf extract of *Acacia nilotica* had the highest (P < 0.001) TP content.

The TF content in methanol extracts of medicinal plant species ranged from 3.60 to 143.6 mg QE/g, indicating more than forty-fold variation. Extracts of three *Cymbopogon citratus* (CC) varieties had the highest TF compared to other plant species while, *CC-Java*, revealed the highest (P < 0.001) flavonoid content. The smallest contents of flavonoids were found in *Azadirachta indica*. The highest (P < 0.001) total tannin concentration was observed in the leaf extract of *Acacia nilotica* while the lowest was observed in the extracts of three *Coriandrum sativum*. The highest (P < 0.001) essential oil concentration was observed in the leaf of *Rosmarinus officinalis*, variety II (1.02 %) while, the lowest essential oil content was observed in the root of *Echinops kebericho* (0.1 %).

3.2. Total gas and methane production of maize stover treated with plant extracts

Estimated total gas production (TGP) and methane emission of maize stover treated with crude plant extracts at different doses are shown in Table 2. Compared to the control treatment there was no significant difference (P > 0.05) in TGP among plant species at the dose of 25 mg/kg DM for all plant species. Compared to the control treatment TGP was increased (P < 0.001) by 2–31 % for different

Table 2
Estimated in vitro gas production and methane emission of maize stover treated with crude plant extracts at different doses (mg/kg DM) at South east Ethiopia.

Source of extracts	Parameters and mean values											
	48 h total gas production (ml) at different doses						24 h methane volume (ml) at different doses					
	Control	25	50	75	SEM	P value	Control	25	50	75	SEM	P value
AN	51.1^a	52.0^a	42.1^{Db}	41.9^b	1.6	0.003	12.0^a	12.0^a	11.0^{Cb}	10.0^{Cc}	0.25	<0.001
AZ	51.1^b	52.0^b	67.0^{Aa}	42.0^c	2.7	<0.001	12.0^a	12.0^a	12.0^{Ba}	11.0^{Bb}	0.13	<0.001
CC I	51.0^b	52.0^b	55.0^{Ba}	42.0^c	1.5	<0.001	12.0^a	12.0^a	12.3^{Ba}	11.0^{Bb}	0.17	0.002
CC Java	51.2^b	52.0^b	55.0^{Ba}	42.0^c	1.4	<0.001	12.0^a	11.9^a	9.3^{Ec}	11.0^{Bb}	0.40	<0.001
CC UA	51.3^b	52.2^b	55.0^{Ba}	42.0^c	1.5	<0.001	12.0	12.0	12.0^B	12.3^A	0.08	0.4
CSB	51.1^b	52.0^b	55.0^{Ba}	42.1^c	1.5	<0.001	12.0	12.0	12.0^B	12.3^A	0.07	0.5
CST	51.1^b	52.0^b	55.0^{Ba}	42.2^c	1.5	<0.001	12.0	12.0	12.3^B	12.3^A	0.11	0.6
CSW	51.0^b	52.0^b	55.0^{Ba}	42.2^c	1.4	<0.001	12.0	12.0	12.3^B	12.3^A	0.12	0.5
EKM	51.0^a	51.7^b	52.1^{Ca}	42.0^b	1.2	<0.001	12.0	12.2	12.0^B	12.0^A	0.08	0.44
LL	51.1^b	52.0^b	67.0^{Aa}	42.2^c	2.7	<0.001	12.0	12.0	12.3^B	12.3^A	0.11	0.6
MS	51.0^b	52.0^b	67.0^{Aa}	42.0^c	2.6	<0.001	12.0^b	12.0^b	14.0^{Aa}	12.3^{Ab}	0.26	<0.001
RO I	51.1^b	52.2^b	55.0^{Ba}	42.0^c	1.4	<0.001	12.0	12.1	12.7^B	12.3^A	0.13	0.2
RO II	51.0^b	52.2^b	55.0^{Ba}	42.2^c	1.6	<0.001	12.0	12.0	12.0^B	11.7^{AB}	0.08	0.4
RO III	51.1^b	52.0^b	55.0^{Ba}	42.1^c	1.5	<0.001	12.0	12.0	12.3^B	11.7^{AB}	0.15	0.3
TS	51.0^b	52.2^b	55.0^{Ba}	42.0^c	1.4	<0.001	12.0^a	11.9^a	10.0^{Db}	11.7^{ABa}	0.28	0.005
SEM	0.01	0.05	0.61	0.07	–	–	0.02	0.02	0.09	0.12	–	–
P value	0.08	0.76	<0.001	0.51	–	–	0.65	0.09	<0.001	<0.001	–	–
P × D	0.75	0.91	<0.001	0.61	–	–	0.82	0.72	<0.001	0.65	–	–

Means with different upper case letters across the columns for plant species are significantly different; Means with different lower case letters across the row for doses are significantly different; SEM: Standard error of mean. P × D: Interaction between plant extracts and doses. Key for plant species are given as a footnote to Table 1.

species at the dose of 50 mg/kg DM except *Acacia nilotica*. In contrast, all plant extracts significantly reduced ($P < 0.001$) TGP at the dose of 75 mg/kg DM compared with the control treatment. Among the evaluated plant species, *Azadirachta indica*, *Leucaena leucocephala* and *Moringa stenopetala* increased TGP by 31 % at the doses of 50 mg/kg DM. There was also a significant interaction effect between plant species and doses on TG production at the dose of 50 mg/kg DM.

Generally, a significant methane reduction ($P < 0.001$) was observed for extracts of *Cymbopogon citratus Java* and *Thyme schimperi*, at the dose of 50 mg/kg DM. Compared to the control, extracts from *Cymbopogon citratus Java* and *Thyme schimperi* reduced methane production by 22.5 and 16.7 % of methane, respectively, at the dose rate of 50 mg/kg DM. There was significant ($P < 0.001$) interaction between plant species and doses for methane volume at doses of 50 mg/kg DM. Compared with the control treatment, plant extracts from other tested plants did not reduce methane production at all dose levels except *Acacia nilotica* at 50 and *Azadirachta indica*, *Rosmarinus officinalis* II, *Rosmarinus officinalis* III and *Thyme schimperi* at the doses of 75 mg/kg DM.

3.3. In vitro digestibility and metabolizable energy of maize stover treated with plant extracts

Estimated in vitro organic matter digestibility and metabolizabe energy contents of maize stover treated with different doses of crude plant extracts are shown in Table 3. Inclusion of AZ, CC Java, LL, MS and TS extracts improved (P < 0.001) the IVOMD of maize stover, when used at the dose of 50 mg/kg DM, which ranges from 8 to 16 % compared with the control. Compared to the control treatment there was no significant difference (P > 0.05) in IVOMD at the dose of 25 mg/kg DM in all plant species. There was also (P < 0.001) interaction effect between plant species at the dose of 50 mg/kg DM. However, all plant extracts reduced the digestibility of maize stover at 75 mg/kg DM feed except CC I, EKM and RO I.

Inclusion of extracts at the dose of 25 mg/kg DM did not affect the estimated ME content of substrate feed in all plant species. Compared to the control treatment inclusion of AZ, CC Java, LL, MS and TS extracts increased the estimated ME content of the substrate feed at the dose rate of 50 mg/kg DM. The improvement in the calculated ME ranged from 6.7 to 26.7 % with the highest (P < 0.001) increase was observed in *Moringa stenopetala* (26.7 %) at the dose of 50 mg/kg DM. The estimated ME of the substrate feed was decreased (P < 0.001) by 6.7 % with the addition of *Acacia nilotica* extract at 50 mg/kg DM. The estimated ME content was decreased by 6.7–13.3 % with addition of extracts from other plants at dose of 75 mg/kg DM except EKM. Compared to the control treatment, no change was observed in the estimated ME content with the addition of extracts from *Echinops kebericho* at all doses.

3.4. Concentration of methane and short chain fatty acids

Methane concentration and short chain fatty acid of maize stover treated using different doses of plant extracts are shown in Table 4. The lowest CH_4 concentration was observed with the inclusion of CC Java (23.3 %) and TS (25 %) extracts at the dose of 50 mg/kg DM of substrate feed. There was interaction (P < 0.001) effect between plant species and dose at 50 mg/kg DM for methane concentration. Inclusion of extracts at the dose of 25 mg/kg DM did not affect CH4 concentration in plant species. There was high CH_4 concentration at the dose of 75 mg/kg DM in all plant species.

Significantly higher (P < 0.0001) SCFA concentration (1.1 mmol/g DM) was obtained when maize stover was incubated with

Table 3
Estimated in vitro organic matter digestibility (%) and metabolizabe energy (MJ/kg DM) contents of maize stover treated with different doses of crude plant extracts (at 24 h of incubation).

Source of extracts	Parameters and mean values											
	IVOMD						ME					
	Control	25	50	75	SEM	P value	Control	25	50	75	SEM	P value
AN	50.0[a]	50.1[a]	48.0[Ea]	44.0[Bb]	0.73	<0.001	7.5[a]	7.5[a]	7.0[Db]	6.5[Cc]	0.12	<0.001
AZ	50.0[b]	50.0[b]	55.0[Ba]	44.6[Bc]	1.1	<0.001	7.5[b]	7.5[b]	8.1[Ba]	6.5[Cc]	0.17	<0.001
CC I	50.0	50.0	52.0[C]	48.0[A]	0.44	0.25	7.4[a]	7.5[a]	7.7[Ba]	7.0[Bb]	0.07	<0.001
CC Java	50.0[b]	50.0[b]	54.0[Ba]	48.0[Ab]	0.40	<0.001	7.5[b]	7.5[b]	7.9[Ba]	7.0[Bc]	0.07	<0.001
CC UA	50.0[a]	50.0[a]	52.0[Ca]	47.0[Ab]	0.54	<0.001	7.5[a]	7.5[a]	7.7[Ca]	7.0[Bb]	0.08	<0.001
CSB	50.2[a]	50.0[a]	52.0[Ca]	46.0[Ab]	0.64	<0.001	7.5[a]	7.5[a]	7.7[Ca]	7.0[Bb]	0.08	<0.001
CST	50.0[a]	50.0[a]	52.0[Ca]	47.0[Ab]	0.7	<0.001	7.5[a]	7.5[a]	7.7[Ca]	7.0[Bb]	0.07	<0.001
CSW	50.0[a]	50.0[a]	52.0[Ca]	47.0[Ab]	0.6	<0.001	7.4[a]	7.5[a]	7.7[Ca]	7.0[Bb]	0.08	<0.001
EKM	50.0	50.0	50.0[C]	49.7[A]	0.14	0.88	7.5	7.5	7.5[C]	7.5[A]	0.02	0.08
LL	50.2[b]	52.0[b]	55.0[Ab]	48.0[Ab]	0.80	<0.001	7.4[b]	7.6[b]	8.0[Ba]	7.0[Bc]	0.11	<0.001
MS	50.0[b]	50.0[b]	58.0[Aa]	49.7[Ab]	1.0	<0.001	7.5[b]	7.5[b]	8.6[Aa]	7.0[Bc]	0.15	<0.001
RO I	50.2	50.0	52.0[C]	48.0[A]	0.46	0.008	7.5[a]	7.5[a]	7.7[Ca]	7.2[Bb]	0.07	0.003
RO II	50.0[a]	50.2[a]	52.0[Ca]	47.0[Ab]	0.5	<0.001	7.5[a]	7.5[a]	7.7[Ca]	7.0[Bb]	0.07	0.002
RO III	50.0[a]	50.0[a]	52.0[Ca]	47.0[Ab]	0.6	<0.001	7.5[a]	7.5[a]	7.7[Ca]	7.0[Bb]	0.06	<0.001
TS	50.1[b]	50.0[b]	54.0[Ba]	47.0[Ac]	0.58	<0.001	7.5[b]	7.5[b]	7.9[Ba]	7.1[Bc]	0.05	<0.001
SEM	0.001	0.03	0.23	0.24	–	–	0.001	0.012	0.03	0.03	–	–
P value	0.089	0.086	<0.001	<0.001	–	–	0.68	0.59	<0.001	<0.001	–	–
P × D	0.075	0.082	<0.001	0.089	–	–	0.75	0.45	<0.001	0.069	–	–

Means with different upper case letters across the columns for plant species are significantly different; Means with different lower case letters across the row for doses are significantly different; SEM: standard error of mean; Key for plant species are given as a footnote to Table 1.

Table 4

Methane concentration/24 h GP (%) and short chain fatty acid (mmol/L) of maize stover treated using different doses (mg/kg DM) of plant extracts.

Source of extracts	Parameters and mean values											
	CH$_4$ concentration						SCFA (mmol/g DM)					
	Control	25	50	75	SEM	P value	Control	25	50	75	SEM	P value
AN	31.5[a]	31.5[a]	30.8[Ab]	32.0[Ba]	0.14	<0.001	0.84[a]	0.80[a]	0.77[Eb]	0.68[Cc]	0.02	0.003
AZ	31.4[b]	31.5[b]	30.7[Ab]	33.0[Ba]	0.65	<0.001	0.83[b]	0.85[b]	0.98[Ba]	0.70[Cc]	0.03	<0.001
CC I	31.5	31.5	31.5[A]	31.0[B]	0.19	0.14	0.84[b]	0.85[b]	0.90[Ca]	0.80[Bc]	0.01	<0.001
CC Java	31.5[a]	31.3[a]	23.3[Cc]	28.0[Cb]	1.0	<0.001	0.85[b]	0.85[b]	0.90[Ca]	0.77[Bc]	0.01	<0.001
CC UA	31.3[b]	31.4[b]	30.8[Ab]	35.0[Aa]	0.54	<0.001	0.85[b]	0.85[b]	0.90[Ca]	0.77[Bc]	0.007	<0.001
CSB	31.5[b]	31.3[b]	31.0[Ab]	35.0[Aa]	0.68	0.01	0.85[b]	0.85[b]	0.90[Ca]	0.80[Bc]	0.01	<0.001
CST	31.5[b]	31.4[b]	30.8[Ab]	35.0[Aa]	0.67	0.05	0.85[b]	0.85[b]	0.90[Ca]	0.77[Bc]	0.01	<0.001
CSW	31.3[b]	31.2[b]	30.8[Ab]	35.0[Aa]	0.62	0.02	0.85[b]	0.84[b]	0.90[Ca]	0.77[Bc]	0.02	<0.01
EKM	31.5	31.4	31.8[A]	32.0[B]	0.09	0.92	0.85	0.85	0.85[D]	0.84[A]	0.01	0.072
LL	31.4[b]	31.5[b]	30.5[Ab]	34.0[Aa]	0.70	<0.001	0.85[b]	0.85[b]	0.98[Ba]	0.76[Bc]	0.02	0.002
MS	31.5[b]	31.6[b]	30.8[Ab]	34.0[Aa]	0.48	<0.001	0.85[b]	0.83[b]	1.1[Aa]	0.77[Bc]	0.03	0.001
RO I	31.5[b]	31.2[b]	31.7[Ab]	34.0[Aa]	0.40	0.005	0.85[b]	0.85[b]	0.90[Ca]	0.77[Bc]	0.04	<0.001
RO II	31.3[b]	31.7[b]	30.9[Ab]	34.0[Aa]	0.55	0.04	0.82[b]	0.84[b]	0.90[Ca]	0.77[Bc]	0.001	<0.001
RO III	31.5	31.3	30.8[A]	32.0[B]	0.21	0.59	0.83	0.83	0.94[C]	0.77[B]	0.06	<0.001
TS	31.5[a]	31.5[a]	25.0[Bc]	28.0[Cb]	1.0	<0.001	0.85[b]	0.84[b]	0.94[Ca]	0.77[Bc]	0.02	<0.001
SEM	0.001	0.90	0.25	0.34	–	–	0.41	0.02	0.06	0.07	–	–
P value	0.82	0.67	<0.001	0.003	–	–	0.09	0.09	<0.001	<0.001	–	–
P × D	0.75	0.45	<0.001	0.52	–	–	0.56	0.068	<0.001	0.08	–	–

Means with different upper case letters across the columns for plant species are significantly different; Means with different lower case letters across the row for doses are significantly different; mmol: micromole; SEM: Standard error of mean; SCFA: short chain fatty acid. Key for plant species are given as a footnote to Table 1.

extracts of *Moringa stenopetala*, *Leucaena leucocephala*, and *Azadirachta indica* at the dose of 50 mg/kg DM. In the other plant species the improvement ranges from 9.3 % to 29.4 % at dose rate of 50 mg/kg. However, inclusion of extracts at the dose of 25 mg/kg DM did not affect the concentration of SCFA in all plant species. Extracts from all plant species significantly (P < 0.001) reduced the concentration of SCFAs at the dose of 75 mg/kg DM, indicating that the fermentation (fiber digestibility) was adversely affected by the extracts when dose rate exceeds 50 mg/kg DM.

4. Discussion

4.1. Anti-nutrient composition of medicinal plants

The current result of the total phenolic content in *Acacia nilotica* is in line with the report of Melesse et al. [33], who reported high concentration of total phenolic content in the leaf powder of *Acacia nilotica* in Ethiopia. However, a lower total phenolic content of 136.5 ± 2.5 mg GAE/g was reported for ethanol extracted leaf of *Acacia nilotica* [34]. Compared to the current result, Bhatta et al. [35] reported higher content of TP (108 g/kg DM) from *Azadirachta indica* sampled from both matured and over matured leaves in India. The variation might be related to difference in location and weather conditions.

Similar with the current finding, Safaei-Ghomi et al. [36] also reported comparable total phenolic contents for Iranian *Thymus caramanicus*. However, contrary to the current finding, a lower value of total phenolic content was reported for *Thyme schimperi* in northern Ethiopia [37]. On the other hand, the value of total phenol, total flavonoid and total tannin observed for *Cymbopogon citratus* varieties in the current study were higher compared to the values reported by Uraku et al. [38] for *Cymbopogon citratus* variety in West Africa.

The variations in the compositions of different anti-nutrients in the current findings and previous reports might be related to the variety of analyzed plants, the type of soil from which the samples were collected, location difference and extraction solvents used. Kumar et al. [39] reported phytochemical composition of plants is greatly influenced by different agro-climatic conditions. Plants produce more phytochemicals during stress to withstand the adverse conditions. Studies conducted by Kaplan et al. [40] showed higher production of flavonoids, anthocyanins and mucilaginous substances when plants are in high temperature stress. Kumar et al. [39] also reported that lower temperature leads to higher production of phenolics and vice versa. Generally, lower total phenol, total flavonoid and total tannin contents were identified in all tested varieties of *Coriandrum sativum* in the current study.

4.2. Total gas and methane production

The lowest gas production observed by addition of leaf extracts of *Acacia nilotica* at the doses of 50 and all plants species at 75 mg/ kg DM might be attributed to the TP and TT contents of the plants which could have depressed rumen microbial fermentation at high doses. Similarly Bhatta et al. [35] reported reduction of total gas production from total mixed ration incubated with plant samples containing higher TP and TT contents. Hydrolysable and condensed tannins are toxic to rumen micro-organisms when large quantities are included due to their higher protein-binding affinities [41]. Therefore, the effect might be associated to the binding ability to cell

membranes, resulting in the prevention of nutrient transport into the cell and inhibition of microbial growth [42].

Significant methane reduction observed for extracts of *Cymbopogon citratus Java* and *Thyme schimperi* at the dose of 50 mg/kg DM might be related to the higher flavonoid and essential oil composition of these plant species. The reduction of methane production observed in this study was in agreement with the result of Oskoueian et al. [43] who demonstrated the potential of flavonoid to decrease protozoa and methanogens population in vitro. Seradj et al. [44] also demonstrated depressed methanogenic archaea communities by flavonoid compounds when added at dose of 200 μg/g dry matter. Previous reports also indicated that essential oils and flavonoids directly inhibit methanogens and reduce methane production [45,46]. Similarly, Broudiscou et al. [47] indicated a reduction of 8–14 % methanogenesis in screening 13 flavonoid-rich plant extracts added to a 50:50 hay + barley grain. Bodas et al. [48] also observed a 15 % CH_4 reduction with six out of 450 plant species screened for their effects on CH_4 production with no adverse effects on feed digestibility, total gas and VFA production. Generally, the decrease in methane production upon addition of plant extracts rich in total phenols, flavonoids and essential oils in the current study could be attributed to the antimethanogenic action of the compounds. On the other hand, the reduction of methane volume observed from extracts of *Acacia nilotica* at 50 and *Azadirachta indica*, *Rosmarinus officinalis* II, *Rosmarinus officinalis* III and *Thyme schimperi* at the doses of 75 mg/kg DM might be related to reduction in TGP and IVOMD. The result of the current study is in line with the observation that forage diets with a low *in vivo* passage rate (long resident time in the rumen) are likely to produce high CH_4 than those with a high passage rate (Waghorn et al. [49]).

4.3. In vitro organic matter digestibility and meabolizable energy

The increase in IVOMD of maize stover by addition of AZ, CC Java, LL, MS and TS extracts at 50 mg/kg DM as observed in this study is in line with the previous reports on the potential of plant phytochemicals to improve the digestibility of low quality roughages. Akanmu et al. [12] also reported increased IVOMD of *Eragrostis* by addition of *Aloe vera* extract due to the presence of phytochemicals such as diastase and amylase which facilitate the breakdown of long chain polysaccharides. So the increased IVOMD in the current study might be due to presence of phytochemicals in total phenolic compounds which facilitate the hydrolysis of long chain activity of polysaccharides. Nasser et al. [50] also reported a close correlation between in vitro gas production and digestibility in Berseem hay.

4.4. Concentration of methane and short chain fatty acids

The reduction in methane concentration by addition of extracts from *Cymbopogon citratus Java* and *Thyme schimperi* at the dose of 50 mg/kg DM in the current study make these plants promising species to reduce methane production at in vitro level. Methane as a proportion of total gas might be used as an indicator to determine the capacity of a feed additive to reduce methane production in vitro [10]. Lower methane to gas proportion indicate that a particular candidate feed additive would be better as a rumen modifier for methane reduction than those yielding higher percentages [10]. Similar to the current finding Berhanu et al. [9] reported lower concentration of methane produced from forage species rich in secondary metabolites. Low methane to total gas production ratio (MTGR) can be used as an index to evaluate the CH_4 reduction potential of the species, as it shows the amount of CH_4 produced per unit OM degraded [12].

Methane reduction by extracts from *Cymbopogon citratus Java* and *Thyme schimperi* at dose of 50 mg/kg DM without affecting the volume of total SCFA in the current study could be due to the direct effect of phenolic contents in the plant species on methanogen. Production and composition of SCFA might be affected by various factors. Hristov et al. [51] reported that tannic acids reduced total SCFA production, whereas, inhibition of fiber degradation shift SCFA composition away from acetate and hence lower production of hydrogen and methane fermentation. Bhatta et al. [35] indicated that the effects of phytochemical feed additives on ruminal fermentation are desirable if, they lead to an increase or do not alter the VFA concentration, and decrease methane production. The result of the current finding is in agreement with the finding of Bhatta et al. [35], who reported up to 20 % reduction of methane concentration without affecting the concentration SCFAs when including *Azardirachta indica*, *Autocarpus integrifolis* powders at level of 2.5 % in mixed ration.

5. Conclusion

In conclusion, this study revealed that the methanolic extracts of leaves of *Acacia nilotica,* contain high levels of total phenolic and total tannin contents, while leaf extracts of *Thyme schimperi* and two *Cymbopogon citratus* varieties (*Cymbopogon citratus* -Java and *Cymbopogon citratus-upper awash*) contain high levels of flavonoid contents. It can be concluded that crude extracts of *Cymbopoon citratus java* and *Thyme schimperi* can reduce methane production without negatively affecting the digestibility of maize (zea mays) stover at dose of 50 mg/kg DM. Therefore, further studies are necessary to examine stability of phenolic compounds in plant extracts under different storage durations and evaluate their effects *in vivo* with animals.

6. Limitation of the study

The limitation of this study is that it was conducted in vitro and not verified by *in vivo* study.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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