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SCOPE OF RUMEN MANIPULATION USING MEDICINAL PLANTS TO MITIGATE METHANE PRODUCTION

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Abstract- Microbial fermentation of feed in the rumen may result in considerable dietary protein and energy losses as ammonia nitrogen and methane. Many plants contains secondary metabolites such as phenolic compounds, essential oils, flavonoids and saponins that affect the microbial activity and reduced the ruminal methane production, ammonia nitrogen concentration and ciliate protozoal population resulting in better dietary energy and protein utilization. A large number of plant extracts were tested for their potential to inhibit methanogenesis and ciliate protozoa in an *in vitro* gas production test using cattle rumen liquor as inoculum. Leaves of several medicinal plants were collected, dried at 45-50°C and ground to pass through 2 mm sieve. Extracts of each tested medicinal plants were prepared in three solvents e.g., in water, 50% methanol and 50% ethanol at the rate of 10 g dried and ground plant material per 100 ml solvent. The effect of these medicinal plant extracts on ruminal methanogenesis and protozoal population, were tested using rumen liquor of cattle under *in vitro* condition. Among the 96 tested plant extracts of 32 plants, only 29 plant extracts (30.2%) were found to be effective (reduce more than 20% methane) against *in vitro* methanogenesis. Out of these 29 plant extracts, 24 were ethanol extracts and 05 were methanol extracts. None of the water extracts was effective to reduce *in vitro* ruminal methanogenesis significantly. Ruminal methane production decreased about 71.5 and 65.9% due to inclusion of ethanol extracts of *Piper betle* and *Psidium guajawa* leaves in incubation media, respectively. Rumen ciliate protozoa present in the collected rumen liquor as well as incubation media is the B-type population due to presence of *Polyplastron multivesiculatum* protozoa. Among the 32 tested medicinal plants, maximum anti-protozoal activities were observed in the ethanol extracts of *Piper betle* leaves followed by ethanol extracts of *Jatropha gossipifolia*, *Costus spaciosus*, *Solanam nigram* and *Psidium guajawa* leaves in the incubation media. It was noticed that ethanol extracts of these tested medicinal plants were more effective to reduce ruminal methanogenesis and ciliate protozoal population than the methanol extract. Whereas, water extracts of tested medicinal plants had no effect on ruminal methanogenesis and ciliate protozoal population. *In vitro* true dry matter digestibility (IVTDMD) was suppressed by all the tested extract.
Keywords- Methane, plant extract, plant secondary metabolites, rumen protozoa

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Introduction

Ruminants e.g., cattle, buffalo, sheep and goats, are foregut fermenters and their stomach has four distinct compartments consisting of rumen, reticulum, omasum and abomasum. The rumen, which is located at the beginning of the tract, contain different types of microbes mainly, bacteria, protozoa and fungi, and plays a major role in the digestion of ligno-cellulosic food which are not consumed by the human being. Ruminal microbial activity is essential for the use of structural carbohydrate and the synthesis of high quality microbial protein. Anaerobic fermentation of feed in the rumen is the result of physical and microbiological activities which convert components of the diet to products which are useful (VFA and microbial protein) and useless (methane and carbon dioxide) to the host animals. Methane is produced as an by-product of organic matter fermentation in the rumen and represents about 7-9% loss of gross energy intake. The concentration of methane in the atmosphere has increased at a rate of 10 nL/L per year, since the preindustrial revolution (Moss *et al*., 2000). Domesticated ruminants are estimated to produce about 80 Tg of methane annually (1 Tg = 1 million metric tons), accounting for

about 22% of methane emission from human-related activities. Methane has 21 times more global warming potential than carbon dioxide (IPCC, 2007). As evidence for global warming becomes prevalent, there is growing consensus that the emissions of green house gases into our atmosphere must be mitigated. For the livestock industry, decreasing methane losses can represent an improvement in feed efficiency. Thus mitigating methane loses from cattle has implications not only for global environmental protection (long term), but also for efficient animal production (short term). Diet modification can help mitigation of methane emission from livestock. Dietary manipulation reduce methane emission by decreasing the fermentation of organic matter in the rumen, shifting the site of digestion from the rumen to intestine, diverting hydrogen away from methane production during ruminal fermentation, or by inhibiting methanogenesis by ruminal bacteria.

Modification of rumen microbial fermentation to decrease ruminal methane, ciliate protozoa and ammonia nitrogen production by using feed additives is a useful strategy to improve production efficiency of ruminant animals [1]. Recently, there has been an increase interest to use natural products containing plant secondary compounds instead of chemical feed additives such as ionophore, antibiotic and anti-methanogenic compounds to modify rumen fermentation for improving feed utilization and productive performances of ruminant animals. Plants containing secondary metabolites are known to have anti-micrbial compounds and are commonly used as (i) medicines for treating ailments, (ii) spices in food preparation, (iii) detergent for making soaps, shampoos and cosmetics. Several thousands of different plant secondary metabolites have been reported which include saponins, terpenoids, phenolics, phenolic glycosides, tannins, lignins, alkaloids, poly saccharides (gums), essential oils etc. The secondary metabolites in plants protect them from insect predation or grazing by the herbivores due to their anti-microbial activity. This specificity of plant secondary metabolites against microbial groups can be used for the selective inhibition of some undesirable microbes in the rumen. The methanogens classified as Archae have a distinct different cell wall structure from true rumen bacteria. Thus, there exists a possibility that some of the plant secondary metabolites might act as a selective inhibitor of methanogens and can be used as a feed additive for the manipulation of rumen fermentation to achieve inhibition in methane emission by the livestock. The plant containing high amount of saponins such as *Sapindus saponaria, Yucca schidigear, Enterolobium ciclocarpum and Sessbania sesban* have been reported to have a potential to suppress rumen protozoal populations, increase bacterial and fungal populations, propionate production, microbial yield, microbial protein synthesis and decreased ruminal methanogenesis and ammonia production which leads to better productivity of ruminant animals [11,19,29,33]. Similarly, condensed tannin containing plants reduced dietary protein degradation and methanogenesis in the rumen and increased microbial protein synthesis [29,32]. However, effectiveness of plant to manipulate rumen fermentation varied depending upon the source and type of secondary metabolites present in these plants. Therefore, locally available some medicinal plants were tested to observe its potential for reducing rumnial methanogenesis. The overviews summaries of a series of experiments conducted at ERS, NDRI, Kalyani under externally funded NFBSRA project to screen a large number of plant extracts for their potential to inhibit methanogenesis and ciliate protozoa in cattle rumen liquor in *in vitro* gas production test are presented in this manuscript.

Preparation of plant extract

The plant part (leaf) obtained were dried at 45-50°C in a forced hot air oven. The plant material was then ground to pass through 1 mm sieve. Extracts of each tested plant were prepared in three solvents e.g., in water, 50% methanol and 50% ethanol at the rate of 10 g dried and ground plant material per 100 ml solvent. After mixing the known quantity of plant material with solvent in a 250 ml flask, it was closed with a stopper and incubated at 39°C in an orbital shaker at 120 rpm for 18 h. After shaking, the content of the flask is squeezed through four layers of muslin cloth. The filtrate obtained from water extracts was centrifuged at 3500 rpm for 20 minutes and then filtered through Whatman filter paper No. 1 while methanol and ethanol extracts directly filtered using Whatman No 1 filter paper. The filtrates were collected and stored at -20°C for further use.

Preparation of inoculums/incubation medium

Rumen liquor was collected by using stomach tube from cattle fed on a diet containing paddy straw and concentrates mixture in 1:1 ratio, which was same as the substrate used for the *in vitro* gas production test. Feed was offered twice daily in the morning and evening to the animals. Rumen liquor was collected just before morning feeding and transported in thermo flask under anaerobic condition to the laboratory. In the laboratory, collected rumen liquor was filtered through three layer of muslin cloth immediately and mixed with buffer in the ratio of 1:2. Buffer solutions and rumen liquor for *in vitro* test were prepared as described by Menke and Steingass (1988).

***In vitro* gas production test**

For *in vitro* gas production test, 200 $\pm$ 5 mg of substrate was weighed in glass syringes of 100 ml capacity. The substrate comprising mixture of air dried milled (<1.0 mm) paddy straw and concentrate mixture (crushed maize grain 30%, groundnut cake 22%, wheat bran 45%, mineral mixture 2% and common salt 1%) in 1:1 ratio. 30 ml of incubation medium was dispensed anaerobically in each syringe. Syringes were incubated at 39°C for 24 h in a water bath. Each plant extracts was tested in four replicate. Three syringes containing only incubation medium (with out substrate) kept as blank and three syringes containing incubation medium and maize green fodder (air dried and passed through 1 mm sieve) as substrate considered as control account for variation among days.

Estimation of gas and methane production

After 24 h incubation gas production was estimated by the displacement of the piston of syringe during incubation. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe (containing no substrate, but only the inoculum and buffer) from the total gas produced in the syringe containing substrate and inoculum. The gas produced in standard syringe (containing standard maize hay) was used to check day to day variation in the quality of inoculum. For methane estimation, 1.0 ml gas was sampled from the head space of syringe in an airtight syringe and injected in to Netel Ultima 2100 gas

chromatograph equipped with dual flame ionizing detector (FID) and stainless steel column packed with Porapak-Q. The gas flow rates for nitrogen, hydrogen and air were 30, 30 and 320 ml per minute, respectively. Temperature of injector oven, column oven and detector were 120, 50 and 120°C, respectively. A 31.8:68.2 mixture of methane and carbon dioxide was used as standard. The peak of methane gas was identified on the basis of retention time to standard methane gas and the response factor obtained was used to calculate methane percentage in the gas sample. The methane produced from substrate during 24 hours incubation was corrected for the blank values. The volume of methane produced was calculated as follows:

$$\text{Methane production (ml)} = \text{Total gas produced (ml)} \times \text{\% methane in the sample}$$

Staining and counting of rumen ciliate protozoa

At the end of incubation (24 h), 1 ml of incubation medium was transferred with a wide orifice pipette into a screw-capped test tube containing 1 ml formalinized physiological saline with brilliant green dye (0.85% w/v sodium chloride solution containing 20% w/v formaldehyde and 2% w/v brilliant green dye). The content of the test tube mixed thoroughly and allowed to stand overnight at room temperature before counting was done. Total and differential count of rumen protozoa were made in 20 microscopic fields at a magnification of 100x. Ciliates were identified according to the method of Hungate (1996) and counted as described by Veira *et al*. (1983).

Estimation of *in vitro* true dry matter digestibility (IVTDMD)

For the estimation of IVTDMD, the content of syringe was transferred quantitatively to spoutless beaker by repeated washing with 100 ml neutral detergent solution. The content was refluxed for 1 h and filtered through pre-weighed gooch crucible (Grade G1). The DM of the residue was weighed and IVTDMD of feed was calculated as follows [40].

$$\text{True dry matter digestibility (TDMD)} = \frac{\text{(DM of feed taken for incubation - NDF residue)} \times 100}{\text{DM of feed taken for incubation}}$$

Effect of plant extracts on ruminal methanogenesis *in vitro*

Among the 96 tested plant extracts of 32 plants, only 29 plant extracts (30.2%) were found to be effective (reduce more than 20%) against *in vitro* methanogenesis (Table 1). Out of these 29 plant extracts, 24 were ethanol extracts and 05 were methanol extracts. None of the water extracts were effective to reduce *in vitro* ruminal methanogenesis significantly. These indicating that majority of plant secondary metabolites were alcohol soluble [20,30]. Cowan (1999) also reported that a large number of known plant secondary metabolites were extracted either in ethanol or methanol and only a few of these metabolites were soluble in other organic solvents.

The extracts of 11 plants [*Solanam nigram* (ethanol and methanol extract), *Jatropha gossipifolia* (ethanol extract), *Saurauia nepalensis* (ethanol extract), *Eclipta alba* (ethanol extract), *Alstonia scholaris* (ethanol extract), *Nyctonthes arbortristis* (ethanol extract), *Abroma augusta* (ethanol extracts), *Clerodrendron indicum* (ethanol extract), *Vitex negundo* (ethanol extracts), *Piper betle* (ethanol extracts and methanol extracts) and *Psidium guajawa* (ethanol extracts and methanol extracts)] leaves, inhibited ruminal methane production more than 30%. The plants tested either their leaves rich in tannin and phenolics compounds or essential oils. All theses three groups of plants secondary metabolites are known for their anti microbial activity [31,36]. Among the different extracts tested, the ethanol extracts of *Piper betle* leaves showed the maximum reduction (71.5%) in methane production followed by the ethanol extracts of *Psidium guajawa* leaves (65.9%). *Piper betle* leaves (Bangla variety) contains 0.08 to 0.2 g essential oil / 100 g fresh leaf and this essential oils is constituted by a mixture of about twenty-one different compounds of which eugenol is the chief ingredient constituting about 29.5 g /100 g of the oil [9]. These essential oils have been reported to have a range of antimicrobial properties [4,28]. These antimicrobial effects of extracts of *Piper betle* leaves might modulate the rumen microbial ecosystem, resulting in a decrease in methane production. Evans and Martin (2000) observed that thymol (400 mg/l), a main component of essential oil derived from *Thymus* and *Origanum* plants, was a strong inhibitor of methane *in vitro*. In another study, essential oils from *Origanum vulgare* and its component, thymol caused a suppression of methane to the extent of 99% at 6 mM concentration [22]. The *in vivo* study of Wang *et al*. (2009) showed that inclusion of 0.25 g/day of essential oil mixture from oregano plants in the diet of sheep for 15 days lowered methane, while the *in vivo* study of Beauchemin and McGinn (2006) with essential oil fed to beef cattle (1 g/day) for 21 days did not reveal any effect on methanogenesis, which might be due to the addition of low dose of essential oils.

Leaves of *Psidium guajawa* are rich in tannin and flavonoids (Kamra *et al*., 2008) which might be responsible for reduction in methane production. Tannins are nutritionally important plant secondary compounds and are complex phenolic organic molecules with molecular weight ranging from 500 to 3000 kDa. They occur in many nutritionally important forage trees, shrubs and legumes, fruits, cereals and grains. Tannins are usually classified into two groups: hydrolysable tannins and condensed tannins. The adverse effect of tannins on methane production by rumen microbes has been reported earlier too [19]. Tavendale *et al.* (2005) first demonstrated the inhibitory effect of tannin on the growth of a pure culture of rumen methanogens. Bhatta *et al.* (2009) reported that *Quebracho* tannins inhibited the methane production linearly (13-45%) with increasing doses (5-25% of substrate). Similarly in *in vivo* experiments, reduced methanogenesis was demonstrated by strategic supplementation of tannin containing feeds. Male Muzaffarnagari lambs fed “Gamberin” (a product containing 49% condensed tannin prepared as a solidified extracts of the leaves of *Uncaria gambir*) supplemented diet showed significantly lower methane energy loss as a percentage of gross energy and digestible energy [37]. Friesian dairy cows had lower methane emission when fed on *Lotus corniculatus* (a condensed tannin containing legumes) silage as compared with the cows fed on rye grass silage [41]. For tannin containing plants, the antimethanogenic activity has been attributed mainly to condensed tannins [32]. Tavendale *et al*. (2005) suggested two modes of action of tannins on methanogenesis: first, directly affecting methanogens resulting in lower methane emission and second, indirectly by reduced hydro-

gen production due to lower feed degradation. Ethanol extracts of *Piper betle* as well as *Psidium guajava* leaves was more effective than methanol extracts to reduce ruminal methanogenesis *in vitro* indicated that plant secondary metabolites extracted more efficiently in ethanol than methanol.

Table 1- Effect of plant extracts on inhibition of methanogenesis in vitro

Sr. No.	Botanical name	Common name	Inhibition of methanogenesis (%)		
			Water extract	Methanol extract	Ethanol extract
1	*Solanam nigram*	Kakmachi	1.7	31.2	40.7
2	*Jatropha gossipifolia*	Lalvarenda	3.9	22.6	31.4
3	*Saurauia nepalensis*	Gogun	1.9	18.9	43.8
4	*Asparagus racemosus*	Satamuli	4.7	18.2	22.7
5	*Costus spaciosus*	Keu	7.3	14.8	28.3
6	*Ocimum basilicum*	Sweetbasil	3.9	16.2	21.4
7	*Cinnamomum zeylanicum*	Dalchini	5.3	18.9	25.7
8	*Chlorophytum borivillium*	Safedmusli	-0.66	5.7	8.3
9	*Withania somnifera*	Aswagandha	8.3	12.7	19.2
10	*Ricinus communis*	Castor-oil	1.4	9.8	16.5
11	*Eclipta alba*	Kesuth	5.9	18.7	39.2
12	*Alstonia scholaris*	Chattim	3.7	19.4	41.3
13	*Saraca asoka*	Asoka	1.9	17.8	21.3
14	*Mesua ferra*	Nagkeshar	3.8	19.2	28.6
15	*Butea monosperma*	Palas	2.1	16.8	26.3
16	*Lagerstroemia speciosa*	Jarul	2.9	18.7	29.2
17	*Barringtonia racemosa*	Hijal	1.3	14.6	21.7
18	*Biksa orellana*	Bixa	2.4	13.5	23.8
19	*Nyctonthes arbortristis*	Siuli	4.7	19.3	31.4
20	*Strychnos nux-vomica*	Kuchila	-0.9	8.9	13.1
21	*Cinnamomum camphora*	Karpur	3.7	14.9	25.8
22	*Abroma augusta*	Ulatkambal	5.1	17.9	31.2
23	*Clerodrendron indicum*	Bamanhati	7.3	21.5	39.8
24	*Desmodium gangeticum*	Salpani	1.3	10.5	17.3
25	*Vitex negundo*	Nishinda	5.1	19.4	38.6
26	*Piper longum*	Pipul	-1.5	-2.3	-1.7
27	*Paederia foetida*	Gandal	1.6	13.8	21.5
28	*Artemisia vulgaris*	Nagdona	0.9	8.7	15.3
29	*Eupatorium ayapana*	Ayapana	-0.5	-1.2	4.5
30	*Psoralia corylifolia*	Babchi	2.7	13.6	21.9
31	*Piper betle*	Betel	6.8	49.3	71.5
32	*Psidium guajawa*	Guava	4.7	35.7	65.9

Effect of plant extracts on rumen protozoal population

The *in vitro* system used in the present experiment was a short term batch culture. Ciliate protozoa present in the collected rumen liquor from cattle as well as incubation medium after completion of incubation period, belonged to a B-type population due to presence of *Epidinium* sp. and the absence of *Polyplastron multivesiculatum* [6]. The ciliate protozoal population present in the incubation medium after completion of incubation period was almost entirely *Entodinium* sp. (90% of total protozoal count) with *Isotricha* sp. about 8% which is also similar to the earlier finding [34,35]. The maximum anti-protozoal activities were observed on addition of ethanol extracts of *Piper betle* leaves followed by ethanol extracts of *Jatropha gossipifolia*, *Costus spaciosus*, *Solanam nigram* and *Psidium guajava* leaves in the incubation media. Rumen protozoa have a negative role on utilization of dietary nitrogen by ruminants. Protozoa engulf and digest large number of ruminal bacteria thereby decreasing net microbial protein flow from the rumen to the duodenum [17]. Rumen protozoa also possess proteolytic and deaminating activities [43].

Table 2- Effect of plant extracts on rumen protozoal population (x 103 /ml) in vitro

Sr. No.	Botanical name	Common name	Rumen protozoa number		
			Water extract	Methanol extract	Ethanol extract
1	*Solanam nigram*	Kakmachi	9.6	5.9	4.9
2	*Jatropha gossipifolia*	Lalvarenda	8.2	5.6	4.6
3	*Saurauia nepalensis*	Gogun	9.7	8.1	7.9
4	*Asparagus racemosus*	Satamuli	8.9	7.4	6.6
5	*Costus spaciosus*	Keu	9.3	6.2	4.6
6	*Ocimum basilicum*	Sweetbasil	10.1	6.9	4.8
7	*Cinnamomum zeylanicum*	Dalchini	11.2	7.3	6.7
8	*Chlorophytum borivillium*	Safedmusli	9.8	6.1	5.4
9	*Withania somnifera*	Aswagandha	13.2	11.9	10.8
10	*Ricinus communis*	Castor-oil	10.8	9.7	8.4
11	*Eclipta alba*	Kesuth	9.1	7.5	6.4
12	*Alstonia scholaris*	Chattim	10.7	8.3	6.8
13	*Saraca asoka*	Asoka	10.8	9.9	8.5
14	*Mesua ferra*	Nagkeshar	9.9	9.1	7.5
15	*Butea monosperma*	Palas	12.3	11.7	10.9
16	*Lagerstroemia speciosa*	Jarul	13.7	12.8	11.6
17	*Barringtonia racemosa*	Hijal	10.2	9.1	7.8
18	*Biksa orellana*	Bixa	10.9	9.2	8.3
19	*Nyctonthes arbortristis*	Siuli	9.7	6.1	4.8
20	*Strychnos nux-vomica*	Kuchila	14.8	13.2	11.9
21	*Cinnamomum camphora*	Karpur	9.3	6.3	5.6
22	*Abroma augusta*	Ulatkambal	10.8	9.5	8.3
23	*Clerodrendron indicum*	Bamanhati	8.7	7.2	6.8
24	*Desmodium gangeticum*	Salpani	15.9	14.8	13.2
25	*Vitex negundo*	Nishinda	9.5	7.2	6.7
26	*Piper longum*	Pipul	11.3	10.1	9.2
27	*Paederia foetida*	Gandal	10.1	10.9	9.2
28	*Artemisia vulgaris*	Nagdona	12.7	11.2	10.6
29	*Eupatorium ayapana*	Ayapana	10.8	9.1	8.7
30	*Psoralia corylifolia*	Babchi	12.6	11.9	11.4
31	*Piper betle*	Betel	9.2	6.7	4.2
32	*Psidium guajawa*	Guava	8.9	6.9	5.1
Control (70:30 roughage)		Standard	9.5	9.1	8.8

Thus, removal of protozoa from the rumen (i.e., defaunation) prevents recycling of microbial nitrogen from the rumen. For instance, bacterial protein flow to the intestine in defaunated sheep was 35% higher than the microbial protein flow in faunated sheep [16]. Increased bacterial protein synthesis in the rumen due to defaunation could benefit the host by supplying additional amino acids for absorption. Moreover, improved efficiency of nitrogen metabolism in the rumen could reduce nitrogen loss in faeces and urine. Due to lack of suitable defaunating agent, and spontaneous refaunation, defaunation has not been practical in commercial ruminant production system. However, a considerable reduction in rumen protozoal population may also be beneficial for improving the productive performance of the animals as it increased milk yield in dairy cows and growth rate of young ruminants [18]. Anti ciliate protozal effect of *Piper betle* leaves extracts (as we observed in our laboratory) might be due to presence of essential oils. Ando *et al.* (2003) reported that feeding 200 g / day (i.e., 30 g/kg total dietary DM) of peppermint (*Menthaxpiperita* L.) to Holstein steers decreased the total number of protozoa. The ethanol and methanol extracts of *Psidium guajawa* leaves, rich in flavonoids and tannin also exhibited anti protozoal activity in the *in vitro* gas production test. The reports from literature indicate that phenolic acid have been found to be toxic for many of the rumen microbes, especially ciliate protozoa [13]. Extracts of *Jatropha gossipifolia*, *Costus spaciosus* and *Ocimum basilicum* also inhibit the

growth of rumen protozoal population in *in vito* due to presence of tannin and saponin in their extracts. Decreased rumen protozoal counts with supplementation of saponins rich extracts [12] or saponin rich forage [27] or fruits [11] have been reported. The sensitivity of protozoa towards saponins may be explained by the presence of sterols in protozoa but not in bacterial, membranes [43]. Saponins possibly bind with sterol of cell membrane of protozoa and change the permeability of cell membrane resulting in lysis of the protozoal cell membrane [10].

Table 3- Effect of plant extracts on in vitro true dry-matter digestibility (IVTDMD)

Sr. No.	Botanical name	Common name	IVTDMD (%)		
			Water extract	Methanol extract	Ethanol extract
1	*Solanam nigram*	Kakmachi	55.9	54.8	52.2
2	*Jatropha gossipifolia*	Lalvarenda	56.3	54.9	53.2
3	*Saurauia nepalensis*	Gogun	55.9	54.6	52.2
4	*Asparagus racemosus*	Satamuli	57.8	55.3	54.8
5	*Costus spaciosus*	Keu	58.2	56.7	55.1
6	*Ocimum basilicum*	Sweetbasil	55.2	54.7	53.3
7	*Cinnamomum zeylanicum*	Dalchini	58.4	57.6	57.2
8	*Chlorophytum borivillium*	Safedmusli	56.8	54.9	53.2
9	*Withania somnifera*	Aswagandha	58.9	56.2	54.3
10	*Ricinus communis*	Castor-oil	56.3	54.8	52.9
11	*Eclipta alba*	Kesuth	57.2	53.9	52.2
12	*Alstonia scholaris*	Chattim	57.9	54.7	52.4
13	*Saraca asoka*	Asoka	58.3	56.9	55.2
14	*Mesua ferra*	Nagkeshar	56.2	54.5	53.9
15	*Butea monosperma*	Palas	55.9	53.8	52.9
16	*Lagerstroemia speciosa*	Jarul	56.8	55.7	54.1
17	*Barringtonia racemosa*	Hijal	55.7	56.9	55.6
18	*Biksa orellana*	Bixa	56.8	54.3	52.5
19	*Nyctonthes arbortristis*	Siuli	54.5	56.9	53.2
20	*Strychnos nux-vomica*	Kuchila	56.2	55.7	54.1
21	*Cinnamomum camphora*	Karpur	57.9	56.2	54.8
22	*Abroma augusta*	Ulatkambal	58.6	57.3	56.6
23	*Clerodrendron indicum*	Bamanhati	57.9	57.2	55.6
24	*Desmodium gangeticum*	Salpani	56.8	55.9	54.2
25	*Vitex negundo*	Nishinda	57.6	56.2	54.8
26	*Piper longum*	Pipul	58.1	56.9	55.7
27	*Paederia foetida*	Gandal	58.6	57.3	56.9
28	*Artemisia vulgaris*	Nagdona	58.3	57.4	56.1
29	*Eupatorium ayapana*	Ayapana	57.9	55.2	54.9
30	*Psoralia corylifolia*	Babchi	58.2	56.9	55.8
31	*Piper betle*	Betel	59.2	57.1	56.6
32	*Psidium guajawa*	Guava	57.9	56.2	55.9
Control (70:30 roughage)		Standard	60.7	59.4	59.1

Effect of plant extracts on degradability of feeds

In vitro true dry matter digestibility (IVTDMD) was suppressed by all the extracts tested (Table 3). The suppression in digestibilty varied only between 2 to 7% in comparison to control. It seems that these extracts had some secondary metabolites which might be detrimental to one or the other important rumen microbes. Lila *et al*. (2003) and Patra *et al*. (2010) also observed that degradability of feed become decreased due to inclusion of different plant extracts in incubation media. The tested plant extracts contained various secondary metabolites e.g., tannin, saponin, flavonoids or essential oils and published literature suggested that these plant secondary metabolites reduced the ruminal feed degradability. Tannins have been implicated for their inhibitory effect on feed digestion, microbial population and enzyme activity in many experiments [13,23,24].

Conclusion

The plant extracts appear to have a potential to manipulate rumen fermentation favorably to reduce ruminal methane emission and ciliate protozoal population for efficient utilization of dietary energy and protein. Since these extracts also suppressed ruminal feed degradability, various levels of the extracts should be tested to find out a suitable dose to get maximum inhibition in methane emission without adversely affecting feed degradability. Further, the studies conducted with plant extracts are limited to *in vitro* screening for the desired characteristics (antimethanogenic and antiprotozoal activities). There is a need to study the adaptation of rumen microbes to the antimicrobials present in these secondary metabolites on prolonged feeding to the animals. Moreover, these plant extracts should be tested by *in vivo* animal experiment and suitable dose for inhibiting methane emission and ciliate protozoal population with minimum adverse effect on ruminal fermentation of feed so that these can be practically explore for economic and ecologically friendly livestock production.

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