

Preliminary *in vitro* screening of some spices and medicinal plants from Edo and Rivers States, Nigeria for reducing enteric methane production in ruminants

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Abstract

Methane gas produced by ruminants during feed fermentation contributed to global warming as well as poor efficiency of food utilization. Mitigating ruminal methane production through anti-microbial feed additive has serious health implications. A preliminary study of the potential of medicinal plants and spices from Edo and Rivers State, as possible replacement to antibiotic feed additives was carried out. Medicinal plants and spices were purposefully sampled, dried at 40 °C, milled and 10 mg was added with 190 mg of substrate in a pre-weighed nylon incubation bags. Bags and their contents were incubated using 30 mL of buffered rumen liquor in 100 mL syringes following the standard procedure of *in vitro* fermentation. Variables monitored were 24h total gas volume, short chain fatty acids (SCFA), methane (CH₄) gas, dry matter degradability (DMD) as well as some chemical, cell wall and anti-nutritive components. Results revealed that adding the medicinal plants and spices as feed additives did not depress gas production in most cases (19.5 – 29.50 mL, 17.75 – 30.00 mL and 12.00 – 29.00 mL for Edo medicinal plants, Edo spices and Rivers medicinal plants respectively) and also did not depress DMD (> 50%). *Azadirachta indica* stem reduced CH₄ production mostly by 53.56% while *Dennttia tripetala* did by 47.69% among the Edo medicinal plants and spices, respectively. In Rivers State, *Amaranthus spinosus* gave the highest value of 60.33% CH₄ reduction. In the same bracket of similar and good CH₄ reduction potentials were *Alstonia boonei* leaves (49.06%), *Newbouldia laevis* root (41.50%), *Euphorbia heterophylla* (37.97%), *Allium crispum* (47.31%), *Allium cepa* (46.80%), *Aframomum mueguata* (46.54%), *Capsicum annum* (47.32%), *Ageratum conyzoides* (56.52%), *Lagenaria breviflora* (48.03%) and *Centrosema molle* (45.08%). All the medicinal plant and spices with potential for CH₄ reduction had corresponding high SCFA values (0.46 – 0.65 Mmol) with tannin (%) and saponin (%) contents of 0.011 – 17.50 and 0.01 – 2.40 respectively. Conclusively, the medicinal plants and spices demonstrated good potentials for reducing rumen methane production *in vitro* and may be subjected to further *in vivo* studies.

Keywords: *in vitro*, spices, medicinal plants, methane production, ruminants

Introduction

There is an increasing problem of global warming in many countries of the world due to atmospheric accumulation of greenhouse gas (GHG). Central to this is the enteric methane production by ruminants during feed fermentation in the gut. This represents 18% of global greenhouse gas (GHG) emissions (Steinfeld *et al.*, 2006)

and has a 9 – 10 year atmospheric lifetime with a 25 fold higher warming potential than CO₂ (Lassey, 2008; Unger *et al.*, 2010). Enteric methane emissions from ruminant will continue to increase, as demand for livestock products rises in response to the predicted human population reaching 9 billion by 2050 (UN, 2008; McAllister, 2011). Ruminant methane production apart

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from contributing to global warming also represents loss of between 2 – 15% of the gross energy intake to the animal (Johnson and Johnson, 1995; Crosson *et al.*, 2011). Thus reducing enteric methane emissions from ruminants will translate to nutritional and environmental benefits to man. Techniques already developed to address this problem involved the use anti-microbial feed additives like ionophores (Van Nevel and Demeyer, 1995) and chemicals like halogenated compounds (Clapperton, 1977) and they have created more serious health problems in humans. While chloroform usage is implicated for being hepatotoxic and carcinogenic (Golden *et al.*, 1997; Plaa, 2000), antimicrobial feed additives have been blamed for the development of resistance to antibiotics in human beings. This development has necessitated the search for safer alternatives in which the potential of plants with secondary compounds and metabolites is being considered. The objective of the study was to produce an inventory of some spices and medicinal plants in Southern Nigeria and determine their chemical composition as well as potential to suppress methane production *in vitro*.

Materials and methods

Spices and medicinal plant parts were collected using purposeful sampling technique and identified through the assistant of botanists and ethno-botanists. They were oven-dried at 40° C to constant weight, milled to pass through 1 mm screen and kept in airtight containers until they were used for the study. *In vitro* fermentation gas study was done using randomized complete block design with each incubation batch representing the block. The modified *in vitro* fermentation procedure of Navarro-Villa *et al.* (2011) was adopted. A phosphate – bicarbonate buffer (Mould *et al.*, 2005) used (g/L) were:

1.985 Na₂HPO₄·12H₂O, 1.302 KH₂PO₄, 0.105 MgCl₂·6H₂O, 1.407 NH₄HCO₃, 5.418 NaHCO₃, 0.390 cysteine HCl and 0.100 NaOH. Rumen fluid was obtained from three fistulated goats before morning feeding into a thermos flask and taken to the laboratory where it was strained through four layers of Cheesecloth under continuous flushing with CO₂. Inoculum for incubation was prepared using ratio of rumen fluid to buffer of 1:2. 190 mg of substrate (mixture of equal proportion ground maize, Guinea grass and *Centrosema molle*) was weighed with 10 mg of the test material (spice or medicinal plants) into nylon bags, sealed and incubated using 30 ml of inoculum in 100 mL graduated syringes at 30° C for 24hrs. Syringes containing only the substrate (i.e. without the test plants) and those containing only the inoculum (i.e. without sample) represent the control and blank respectively. Gas production (i.e. accumulated gas in the head space of each fermentation syringe) was read 3 hourly for 24 hours to know the volume of gas produced. At the end of incubation, 4mL of 10M NaOH was introduced into the headspace of the syringes for methane determination. The bags with the residue were removed from the syringes, rinsed thoroughly with water and dried at 100° C for 24 h, and then to constant weight to determine *in vitro* dry matter disappearance (IVDMD). Standard methods of Van-Burden and Robinson (1981), Obadoni and Ochuko (2011) and Van Soest *et al.* (1991) were used for the determination of tannin, saponin and cell wall fractions respectively while crude protein, organic matter, ash and NDF were determined by AOAC (1990). Metabolisable energy (ME) and organic matter digestibility (OMD%) were estimated using Menke and Steingass (1988) equation while short chain fatty acid (SCFA) was estimated with equation of Getachew *et al.* (1999). Data were analysed, using the Proc GLM procedure of SAS

(2004).

Results and discussion

The additive effect of some medicinal plants and spices from Edo and River States on *in vitro* rumen fermentation parameters is presented in Tables 1, 2 and 3. In most of the medicinal plants and spices in both states, their inclusions into the substrate at the 10% level did not significantly depress volume of gas produced. High cumulative 24h gas production is an indication of efficient fermentation and high organic matter digestibility (Tefera *et al.*, 2008). Carbohydrate digestion is central to gas production in an *in vitro* fermentation study while the contribution from protein is very small. The incorporation of the medicinal plant and spices as additives in the ruminant feed may not adversely affect the fermentation of the carbohydrate contents. This is further evident in the dry matter digestibility (DMD) values, which were above 50% in all the medicinal plants and spices studied in Edo State except for *Psidium guajava* leaves, which had a value of 41.15%. About 66% of the medicinal plants studied in Rivers State also had DMD of greater than 50%. The DMD values in this study are in agreement with the range values reported for the medicinal plants in the work of Bodas *et al.* (2008) and Medjekal *et al.* (2017).

In Edo State, the medicinal plants influenced methane production in different ways with significant variations. The absolute volume of methane ranged from 5.0 mL in *Alstonia boonei* to 10.75 mL in the control. However, on expressing the methane production as a percentage of the total gas produced, the value was greatest in the control (47.99%) and least in *Azadirachta indica* stem (22.22%).

Consequently, *A. indica* stem gave the highest percentage methane reduction of 53.56% over the control and was similar with the methane reduction ability expressed by *Alstonia boonei* leaves (49.06%), *Newbouldia laevis* root (41.50%), *Euphorbia heterophylla* (37.89%), *Newbouldia laevis* bark (37.21%), *Carica papaya* seed (34.69%), *Aloe barbadensis* (34.59%), *Psidium guajava* leaves (34.52%), *Euphorbia hirta* (33.97%) and *Azadirachta indica* bark (33.81%). A lower methane-to-total gas ratio has been demonstrated with the leaves of multipurpose trees (MPTs) such as *Acacia spp* and *Leucaena spp* as well as medicinal plants like *A. indica* and *Myrsine africana* leaves (Soliva *et al.*, 2008).

Among the Edo spices, *Dennttia tripetala* had the highest methane reduction ability (47.69%) while the least was from *Piper nigrum* (23.71%). The potential displayed by *Dennttia tripetala* was however, not significantly different from those of *Allium crispum* (47.31%), *Allium cepa* (46.80%), *Aframomum muegueta* (46.54%), *Capsicum annum* (46.43%), *Cymbopogon citratus* (43.77%), *Vernonia amygdalina* (39.81%), *Occimum gratissimum* (37.49%), *Allium sativum* (34.44%), *Gongronema latifolium* (33.66%) and *Syzgium aromaticum* (32.14%). Among the medicinal plants of Rivers State, *Amaranthus spinosus* gave the highest methane reduction (60.33%) and was similar to the reduction ability obtained for *Ageratum conyzoides*, *Lagenaria breviflora*, *Centrosema molle*, *Delonix regia*, *Synedrella nodiflora*, *Chromolaena odorata*, *Stachytarpheta cayennensis*, and *Laportea aestuans* with percentage values of 56.52, 48.03, 45.08, 45.08, 44.89, 44.33, 43.12 and 42.15, respectively.

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Table 1: Additive effect of some medicinal plants from Edo State on gas volume, dry matter degradability (DMD), methane gas and short chain fatty acids (SCFA) production

Medicinal Plants	24h gas volume	SCFA (Mmol)	CH ₄ (Ml)	CH ₄ (%)	CH ₄ Red (%)	DMD (%)
Control	22.75 ^{abc}	0.50 ^{abc}	10.75 ^a	47.99 ^a	0.00 ^g	83.27 ^{abc}
<i>Sida acuta</i> leaves	29.50 ^a	0.65 ^a	9.75 ^{ab}	34.05 ^{cde}	28.82 ^{bcde}	63.80 ^{def}
<i>Jatropha gossipifolia</i>	25.25 ^{abc}	0.56 ^{abc}	9.00 ^{abc}	35.74 ^{bcd}	25.28 ^{ede}	78.15 ^{abcd}
<i>Aspilia Africana</i>	21.50 ^{abc}	0.47 ^{abc}	9.00 ^{abc}	43.58 ^{abc}	8.89 ^{efg}	58.27 ^{ef}
<i>Psidium guajava</i> stem	19.50 ^c	0.43 ^c	9.00 ^{abc}	45.74 ^{ab}	4.36 ^{fg}	87.69 ^{2a}
<i>Bauhinia monandra</i>	23.50 ^{abc}	0.52 ^{abc}	8.75 ^{abcd}	38.66 ^{abcd}	19.18 ^{defg}	77.50 ^{abcd}
<i>Carica papaya</i> seed	28.50 ^{ab}	0.63 ^{ab}	8.75 ^{abcd}	31.24 ^{def}	34.69 ^{abcd}	71.54 ^{bcde}
<i>Euphorbia heterophylla</i>	28.75 ^{ab}	0.63 ^{ab}	8.50 ^{bcd}	29.70 ^{def}	37.89 ^{abcd}	84.62 ^{ab}
<i>Alstonia boonei</i> root	22.50 ^{abc}	0.50 ^{abc}	8.25 ^{bcde}	36.93 ^{bcd}	22.79 ^{edef}	78.97 ^{abcd}
<i>Azadirachta indica</i> bark	26.75 ^{abc}	0.59 ^{abc}	8.00 ^{bcde}	31.66 ^{def}	33.81 ^{abcd}	71.79 ^{bcde}
<i>Newbouldia laevis</i> root	29.50 ^a	0.65 ^a	8.00 ^{bcde}	27.98 ^{def}	41.50 ^{abc}	71.79 ^{bcde}
<i>Jatropha curcas</i>	23.25 ^{abc}	0.51 ^{abc}	8.00 ^{bcde}	34.32 ^{cde}	28.25 ^{bcde}	70.77 ^{bcdef}
<i>Hura crepitans</i>	21.25 ^{abc}	0.47 ^{abc}	7.75 ^{bcde}	37.65 ^{bcd}	21.28 ^{cdef}	68.21 ^{cdef}
<i>Euphorbia hirta</i>	24.75 ^{abc}	0.55 ^{abc}	7.75 ^{bcde}	31.58 ^{def}	33.97 ^{abcd}	74.24 ^{abcd}
<i>Psidium guajava</i> leaves	23.75 ^{abc}	0.52 ^{abc}	7.50 ^{bcde}	31.32 ^{def}	34.52 ^{abcd}	41.15 ^g
<i>Spondias mombin</i>	21.75 ^{abc}	0.48 ^{abc}	7.50 ^{bcde}	35.53 ^{bcd}	25.71 ^{cde}	56.03 ^f
<i>Cynodon dactylon</i>	22.25 ^{abc}	0.49 ^{abc}	7.50 ^{bcde}	33.54 ^{cde}	29.89 ^{bcde}	69.79 ^{bcdef}
<i>Sida acuta</i> root	29.50 ^a	0.46 ^{bc}	7.25 ^{cde}	34.54 ^{cde}	27.78 ^{bcde}	75.64 ^{abcd}
<i>Spondia mombin</i> seed	21.25 ^{abc}	0.47 ^{abc}	7.00 ^{cdef}	32.86 ^{cdef}	31.30 ^{bcd}	78.46 ^{abcd}
<i>Aloe barbadensis</i>	22.50 ^{abc}	0.50 ^{abc}	7.00 ^{cdef}	31.29 ^{def}	34.59 ^{abcd}	65.58 ^{def}
<i>Newbouldia Laevis</i> bark	22.50 ^{abc}	0.05 ^{abc}	6.50 ^{def}	30.04 ^{def}	37.21 ^{abcd}	66.92 ^{def}
<i>Azadirachta indica</i> stem	28.00 ^{ab}	0.62 ^{ab}	6.00 ^{ef}	22.22 ^f	53.56 ^a	69.78 ^{bcdef}
<i>Alstonia boonei</i> leaves	21.00 ^{bc}	0.46 ^{bc}	5.00 ^f	24.36 ^{ef}	49.06 ^{ab}	75.89 ^{abcd}
SEM	2.39	0.07	0.05	3.27	6.36	4.07

abcd^{efg} = Means on the same column with the same letters are not significant different (P > 0.05), CH₄Red – Methane reduction.

The ability of the medicinal plants and spices to reduce *in vitro* methanogenesis is linked to their antimicrobial property and activities on the methanogens and protozoa that have been linked with methane gas production in the rumen of ruminants. The inhibitory action of *P. guajava* leaves for example has been traced to its phytochemical constituents such as alkaloids, saponins and steroidal rings (Elekwa *et al.*, 2009). The methane reduction ability displayed by these plants, compared well with values reported for monensin in some studies (Schelling, 1984; Rumpler *et al.*, 1986) which is an ionophore most commonly used as feed additive in

ruminant to improve rumen fermentation and functioning including the reduction of methane production. There was a corresponding high value of short chain fatty acids (SCFA) for most of the medicinal plants and spices in Edo and Rivers States that had the greatest methane reduction ability. The few exceptions to this were the SCFA values for *Alstonia boonei* leaves, *Allium sativum*, *Allium crispum*, *Ageratum conyzoides*, and *Stachytarpheta cayennensis*. The SCFA are essentially volatile fatty acids (VFA) of three to six carbon atom and the major one concerned in *in-vitro* and *in vivo* rumen studies are the acetic, propionic and butyric acids.

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Table 2: Additive effect of some spices in Edo State on gas volume, dry matter degradability (DMD), methane (CH₄) concentration and short chain fatty acid (SCFA)

Spices	24h gas Volume (ml)	SCFA (Mmol)	CH ₄ (ml)	CH ₄ (%)	CH ₄ (%)	Red (%)	DMD (%)
Control	22.75 ^{abcde}	0.50 ^{abcde}	10.15 ^a	47.99 ^a	0.00 ^e	83.27 ^{bcd}	
<i>Ocimum gratissimum</i>	27.00 ^{abc}	0.60 ^{abc}	8.00 ^{bcde}	29.90 ^{bcd}	37.49 ^{abcde}	79.7 ^{abcd}	
<i>Aframomum muegueta</i>	24.50 ^{abcd}	0.54 ^{abcde}	6.25 ^{ef}	25.56 ^{8d}	46.54 ^{abc}	73.88 ^{de}	
<i>Allium cepa</i>	24.50 ^{abcd}	0.54 ^{abcde}	6.25 ^{ef}	25.45 ^d	46.80 ^{ab}	72.82 ^{de}	
<i>Vernonia amygdalina</i>	26.00 ^{abcd}	0.57 ^{abcd}	7.25 ^{cdef}	28.7 ^{abcd}	39.81 ^{abcde}	61.54 ^f	
<i>Piper guineense</i>	22.00 ^{bcde}	0.48 ^{bcde}	7.25 ^{cdef}	33.86 ^{bcd}	29.21 ^{cde}	74.15 ^{de}	
<i>Allium ascalonicum</i>	27.50 ^{abc}	0.61 ^{abc}	9.00 ^{abc}	33.55 ^{bcd}	29.87 ^{cde}	91.03 ^{ab}	
<i>Piper nigrum</i>	23.38 ^{abcde}	0.51 ^{abcde}	8.50 ^{bcd}	36.49 ^b	23.71 ^e	85.02 ^{abcd}	
<i>Allium crispum</i>	22.00 ^{bcde}	0.48 ^{bcde}	5.50 ^f	25.20 ^d	47.31 ^{ab}	74.62 ^{de}	
<i>Native salt</i>	25.75 ^{abcd}	0.57 ^{abcd}	8.50 ^{bcd}	33.16 ^{bcd}	30.68 ^{bcde}	79.23 ^{bcd}	
<i>Shea butter</i>	18.25 ^{de}	0.40 ^{de}	6.50 ^{def}	35.8333 ^{bc}	25.08 ^e	96.15 ^a	
<i>Cymbopogon citratus</i>	26.00 ^{abcd}	0.57 ^{abcd}	6.75 ^{def}	26.89 ^{cd}	43.77 ^{abcd}	79.12 ^{bcd}	
<i>Murraya koenigii</i>	19.75 ^{cde}	0.43 ^{cde}	6.75 ^{def}	33.67 ^{bcd}	29.60 ^{de}	75.77 ^{de}	
<i>Dennittia tripetala</i>	30.00 ^a	0.66 ^a	7.50 ^{cdef}	25.02 ^d	47.69 ^a	74.96 ^{de}	
<i>Allium sativum</i>	20.75 ^{bcde}	0.46 ^{bcde}	6.50 ^{def}	31.36 ^{bcd}	34.44 ^{abcde}	65.64 ^{ef}	
<i>Syzygium aromaticum</i>	21.75 ^{bcde}	0.48 ^{bcde}	7.00 ^{cdef}	32.46 ^{bcd}	32.14 ^{abcde}	89.00 ^{abc}	
<i>Allium cepa</i> (white)	28.00 ^{ab}	0.62 ^{ab}	9.75 ^{ab}	36.79 ^b	23.08 ^e	79.23 ^{bcd}	
<i>Gongronema latifolium</i>	17.75 ^c	0.39 ^e	5.50 ^f	31.73 ^{bcd}	33.66 ^{abcde}	80.19 ^{bcd}	
<i>Capsicum annum</i>	27.25 ^{abc}	0.60 ^{abc}	7.00 ^{cdef}	25.62 ^d	46.43 ^{abc}	77.10 ^{cde}	
SEM	2.36	0.063	0.05	2.75	5.10	2.97	

abcdef = means on the same column with the same letters are not significantly different (P>0.05)

Methane formation is an avenue to prevent H₂ accumulation and acidic condition in the rumen. This is dependent on VFA production, which determines the amount of excess H₂ in the rumen that is converted to CH₄ by the methanogens (Bodas *et al.*, 2012).

In previous studies, a close relationship between the production of methane and total VFA as well as acetate-to-propionate ratio has been reported (Babayemi *et al.*, 2004; Fievez *et al.*, 2005). Hydrogen accumulation hinders the pathways for acetic acids synthesis and favours propionic acid production (Van Nevel and Demeyer, 1996) thereby resulting in lower C₂: C₃ ratios. The improvement in the VFA (represented by the SCFA value) along with the methane reduction by the medicinal plants is an advantage and this is consistent

with earlier reports (Garcia-Gonzalez *et al.*, 2008; Medjekal *et al.*, 2017). The implication of this is that the medicinal plants have stimulated rumen microbial metabolism rather than been antagonistic or lethal to them, unlike what is frequently experienced with monensin. Monensin's mode of action involves inhibition of some specific microorganisms (Scharen *et al.*, 2017) leading to reduction in ruminal fermentation and digestibility. Ruminal production of VFA contributes about 70% of the energy needs of the ruminant animals (Medjekal *et al.*, 2017). Specifically, the antimicrobial and/or the antimethanogenic properties of some of the medicinal plants and spices have been reported in literature. *Sida acuta* is known to have high levels of alkaloids and flavonoids and has been used as feed additive in livestock and poultry

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Table 3: Additive effect of some medicinal plants in Rivers State on gas volume, dry matter degradability (DMD), methane (CH₄) gas and short chain fatty acids (SCFA)

Plants	24 hr gas				DMD (%)	SCFA (Mmol)
	Volume(ml)	CH ₄ (ml)	CH ₄ %	CH ₄ Red		
Control	21.00 ^{abcd}	15.00 ^a	72.83 ^{ab}	0.00 ^{ij}	67.92 ^a	0.46 ^{abcd}
<i>Euphorbia lateriflora</i>	29.00 ^a	14.00 ^{ab}	48.53 ^{defg}	33.37 ^{defgh}	51.94 ^{abcd}	0.64 ^a
<i>Costus afer</i>	21.34 ^{abcd}	12.00 ^{abc}	56.11 ^{cdef}	23.00 ^{efgh}	49.17 ^{abcd}	0.46 ^{abcd}
<i>Sida acuta</i>	24.00 ^{abc}	12.00 ^{abc}	50.00 ^{defg}	31.35 ^{cdefgh}	46.39 ^{abcd}	0.52 ^{abc}
<i>Harungana madagascariensis</i>	26.60 ^{ab}	12.00 ^{abc}	44.17 ^{efghi}	39.36 ^{bcdef}	54.72 ^{abcd}	0.60 ^{ab}
<i>Euphorbia hirta</i>	24.66 ^{abc}	12.00 ^{abc}	47.78 ^{defg}	34.40 ^{cdef}	55.56 ^{abcd}	0.54 ^{abc}
<i>Ludwigia repens</i>	14.00 ^{cd}	12.00 ^{abc}	84.44 ^a	15.95 ^j	46.94 ^{abcd}	0.30 ^{cd}
<i>Anthonotua macrophylla</i>	20.00 ^{abcd}	11.34 ^{abcd}	56.70 ^{cde}	22.15 ^{fgh}	66.67 ^{ab}	0.44 ^{abcd}
<i>Lagenaria breviflora</i>	29.00 ^a	11.00 ^{abcd}	37.86 ^{ghi}	48.02 ^{abc}	46.39 ^{abcd}	0.64 ^a
<i>Tithonia diversifolia</i>	22.00 ^{abcd}	11.00 ^{abcde}	50.86 ^{defg}	30.17 ^{cdefgh}	48.33 ^{abcd}	0.48 ^{abcd}
<i>Mitracarpus scaber</i>	15.34 ^{bcd}	10.66 ^{abcde}	68.98 ^{bc}	11.32 ^{hi}	48.33 ^{abcd}	0.34 ^{bcd}
<i>Newbouldia laevis</i>	19.34 ^{abcd}	10.66 ^{abcde}	54.76 ^{cdef}	24.81 ^{defgh}	52.22 ^{abcd}	0.42 ^{abcd}
<i>Alchornea chordate</i>	18.00 ^{abcd}	10.00 ^{abcde}	55.56 ^{cdef}	23.71 ^{efgh}	42.50 ^{cd}	0.40 ^{abcd}
<i>Ipomoea involucrate</i>	22.00 ^{abcd}	10.00 ^{abcde}	49.11 ^{defg}	32.57 ^{cdefg}	36.67 ^d	0.48 ^{abcd}
<i>Cyothula prostrate</i>	20.66 ^{abcd}	10.00 ^{abcde}	49.93 ^{defg}	31.44 ^{cdefgh}	49.72 ^{abcd}	0.46 ^{abcd}
<i>Triumfetta rhomboidea</i>	15.34 ^{bcd}	10.00 ^{abcde}	63.29 ^{bcd}	13.10 ^{ghi}	54.44 ^{abcd}	0.34 ^{bcd}
<i>Aspillia Africana</i>	18.00 ^{abcd}	10.00 ^{abcde}	55.71 ^{cdef}	25.50 ^{efgh}	52.22 ^{abcd}	0.40 ^{abcd}
<i>Calapogonium mucunoides</i>	21.34 ^{abcd}	10.00 ^{abcde}	46.67 ^{efgh}	35.92 ^{cdef}	54.72 ^{abcd}	0.46 ^{abcd}
<i>Chromolaena odorata</i>	22.00 ^{abcd}	9.00 ^{abcde}	40.18 ^{fghi}	44.83 ^{abcd}	52.22 ^{abcd}	0.48 ^{abcd}
<i>Urena lobata</i>	17.34 ^{abcd}	8.66 ^{bcde}	50.00 ^{defg}	31.35 ^{cdefg}	56.11 ^{abcd}	0.38 ^{abcd}
<i>Alchornea acutifolia</i>	19.34 ^{abcd}	8.66 ^{bcde}	46.27 ^{efgh}	36.47 ^{bcdef}	57.78 ^{abc}	0.42 ^{abcd}
<i>Centrosema molle</i>	20.00 ^{abcd}	8.00 ^{bcde}	40.00 ^{fghi}	45.08 ^{abcd}	43.06 ^{bc}	0.44 ^{abcd}
<i>Delonix regia</i>	20.00 ^{abcd}	8.00 ^{bcde}	40.00 ^{fghi}	45.08 ^{abcd}	62.50 ^{abc}	0.44 ^{abcd}
<i>Synedrella nodiflora</i>	20.00 ^{abcd}	8.00 ^{bcde}	40.14 ^{fghi}	44.89 ^{abcd}	55.00 ^{abcd}	0.44 ^{abcd}
<i>Laportea aestuans</i>	19.34 ^{abcd}	8.00 ^{bcde}	42.13 ^{efghi}	42.15 ^{abcdef}	51.67 ^{abcd}	0.42 ^{abcd}
<i>Alchornea codifolia</i>	16.66 ^{bcd}	7.34 ^{cde}	46.27 ^{efgh}	35.71 ^{cdef}	47.22 ^{abcd}	0.36 ^{bcd}
<i>Amaranthus spinosus</i>	18.66 ^{abcd}	5.34 ^{cde}	28.89 ⁱ	60.33 ^a	50.00 ^{abcd}	0.40 ^{abcd}
<i>Ageratum conyzoides</i>	16.00 ^{bcd}	5.00 ^e	31.67 ^{hi}	56.52 ^{ab}	49.44 ^{abcd}	0.34 ^{bcd}
<i>Stachytarpheta cayennensis</i>	12.00 ^d	5.00 ^e	41.43 ^{efghi}	43.12 ^{abcde}	41.67 ^{cd}	0.26 ^d
SEM	3.048	1.634	4.243	5.421	5.952	0.068

abcd = Means on the same column with the same superscripts are not significantly different (P>0.05), CH₄ Red - Methane Reduction

(Egunjobi, 1969). *Psidium guajava* due to its content of secondary compounds reduced ruminal methanogenesis in the work of Kamara *et al.* (2006). Soliva *et al.* (2006) did not find methane suppression activity for *A indica* seeds. *Allium cepa* and *Allium sativum* extracts have

demonstrated strong antimicrobial agents (Benkeblia, 2004) while *A sativum* decreased methane production and acetate to propionate ratio in previous study (Garcia-Gonzalez *et al.*, 2008). The chemical and cell wall composition of the medicinal plants and spices in the two states

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that showed relatively high potential for reducing methane production are presented in Tables 4, 5 and 6. Significant variations existed in the contents of crude protein (CP), ash, organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), tannin and saponin of the medicinal plants and spices in the two States. These variations are expected on the ground of

phenological portion and developmental stages of the plant used. However, among the chemical components, the secondary compounds have been reported to have more influence on *in vitro* fermentation (Ngwa *et al.*, 2001). Among the secondary compounds, tannins, saponins, flavonoids and essential oils have been suggested to be responsible.

Table 4: Chemical and cell wall composition (%) of some medicinal plants in Edo State that showed potential to reduce *in vitro* rumen methane production

Plants	CP	Ash	OM	NDF	ADF	Tannin	Saponin
<i>A. indica</i> leaves	31.50a	6.75ab	93.28bc	62.40c	52.2a	0.050b	0.490a
<i>A. boonei</i> leaves	21.00b	6.40ab	93.60bc	36.50f	31.95c	0.017f	0.404b
<i>C. papaya</i> seed	14.85c	6.49ab	93.52bc	34.45g	32.00bc	0.012g	0.391c
<i>A. boonei</i> root	8.75d	-	-	-	-	0.028c	0.245d
<i>S. acuta</i> root	8.65d	7.16ab	92.85bc	71.40a	39.00b	0.022de	0.230e
<i>N. laevis</i> root	8.00d	5.27c	94.74a	47.15d	31.50c	0.019ef	0.228e
<i>N. laevis</i> bark	-	-	-	-	-	0.015fg	0.224e
<i>S. acuta</i> leaves	7.18e	7.20a	92.80c	37.15c	12.75f	0.011g	0.210f
<i>E. heterophylla</i>	7.10e	6.00bc	94.00ab	30.93h	17.70e	0.024d	0.191g
<i>C. dactylon</i>	6.13e	7.10ab	92.90bc	67.40b	25.10d	0.057a	0.010h
SEM	0.41	0.35	0.35	5.13	0.84	0.0012	0.0017

abcdefg = Means on the same column with different letters are significantly different (P < 0.05). CP – Crude protein, OM – Organic matter, NDF – Neutral detergent fibre, ADF – Acid detergent fibre

Table 5: Chemical composition and cell wall contents (%) of some of the spices in Edo State that showed potency for *in vitro* methane production mitigation

Spices	CP	ASH	OM	NDF	ADF	Tannin	Saponin
<i>Allium ascalonicum</i>	14.00 ^c	2.86 ^{ab}	97.14 ^{ab}	14.43 ^d	6.25 ^g	0.014 ⁱ	0.002 ⁱ
<i>Allium crispum</i>	19.50 ^c	2.39 ^b	97.98 ^a	43.25 ^{ab}	4.25 ^h	0.040 ^f	0.006 ⁱ
<i>Allium cepa</i>	24.50 ^a	4.22 ^a	95.77 ^b	47.25 ^a	36.0 ^b	0.081 ^c	0.140 ^f
<i>Dennttia tripetala</i>	20.13 ^{bc}	3.24 ^{ab}	96.73 ^{ab}	43.75 ^{ab}	25.2 ^d	0.093 ^b	0.200 ^d
<i>Capsicum annum</i>	16.63 ^d	3.66 ^{ab}	96.43 ^b	52.00 ^a	40.5 ^a	0.102 ^a	0.216 ^c
<i>Aframomum melegueta</i>	12.63 ^{ef}	3.93 ^a	95.98 ^b	46.25 ^a	23.00 ^e	0.067 ^c	0.120 ^g
<i>Gongronema latifolium</i>	11.19 ^f	3.48 ^{ab}	96.53 ^b	25.50 ^c	10.50 ^f	0.020 ^h	0.239 ^b
<i>Murraya koenigii</i>	24.50 ^a	3.94 ^a	96.04 ^b	27.75 ^c	21.50 ^c	0.025 ^g	0.247 ^a
<i>Allium cepa</i> (white)	18.38 ^{cd}	3.81 ^a	96.20 ^b	33.25 ^{bc}	30.00 ^c	0.074 ^d	0.100 ^h
<i>Piper nigrum</i>	21.88 ^b	3.81 ^a	96.30 ^b	33.75 ^{bc}	29.5 ^c	0.022 ^{gh}	0.191 ^e
SEM	0.72	0.42	0.43	3.35	0.62	0.0015	0.0020

abcdefghi. = means on the same column with the same letters are not significantly different (P>0.05)

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Table 6: Chemical and cell wall composition (%) of some medicinal plants in Port Harcourt that showed potential for *in vitro* methane reduction

Plants	Ash	CP	OM	NDF	ADF	Tannin	Saponin
<i>Laportea aestuans</i>	18.57 ^b	24.13 ^a	81.43 ⁱ	19.60 ^j	17.63 ^h	1.96 ^l	0.06 ^h
<i>Cyothula prostrate</i>	17.95 ^{bc}	24.02 ^a	82.06 ^{hi}	33.10 ^{figh}	28.25 ^{cdef}	4.68 ^{hi}	0.91 ^{de}
<i>Chromolaena odorata</i>	11.44 ^c	21.11 ^d	88.56 ^e	24.79 ^{ij}	19.90 ^{gh}	8.68 ^e	0.15 ^{gh}
<i>Amaranthus spinosus</i>	13.79 ^{dc}	19.86 ^{bc}	86.2 ^{lf}	40.64 ^{def}	20.69 ^{figh}	3.03 ^k	1.00 ^d
<i>Calopogonium mucunoides</i>	7.95 ^{fg}	19.76 ^{bc}	92.05 ^{cd}	54.46 ^a	28.47 ^{cde}	3.74 ^j	0.55 ^{defg}
<i>Centrosema molle</i>	7.92 ^{fg}	19.38 ^{bc}	93.33 ^{bc}	53.12 ^a	33.27 ^{bcde}	4.26 ^{ij}	1.50 ^c
<i>Delonix regia</i>	4.40 ^h	19.17 ^{cd}	95.60 ^a	39.00 ^{defg}	36.04 ^{bc}	15.60 ^e	0.59 ^{defg}
<i>Ageratum conyzoides</i>	11.55 ^e	17.52 ^{cd}	88.46 ^e	31.84 ^{hgi}	27.75 ^{def}	-	-
<i>Alchornea acutifolia</i>	9.03 ^f	16.81 ^{ef}	90.98 ^d	44.55 ^{bcd}	35.36 ^{bc}	7.68 ^f	0.54 ^{efg}
<i>Alchornea chordate</i>	5.59 ^{gh}	15.19 ^{fg}	94.42 ^{ab}	27.45 ^{hi}	19.52 ^h	17.50 ^a	0.50 ^{efgh}
<i>Stachytarpheta cayennensis</i>	7.19 ^{fg}	14.77 ^{gh}	92.82 ^{bcd}	39.13 ^{defg}	32.97 ^{bcde}	9.37 ^{dc}	1.97 ^b
<i>Synedrella nodiflora</i>	14.28 ^d	13.37 ^{ghi}	85.72 ^{figh}	48.48 ^{abc}	28.64 ^{cde}	5.69 ^g	0.65 ^{def}
<i>Harungana madagascariensis</i>	4.35 ^h	13.10 ^{hij}	95.65 ^a	50.87 ^{ab}	46.75 ^a	16.60 ^b	1.49 ^c
<i>Lagenaria breviflora</i>	31.31 ^a	12.69 ^{ij}	68.26 ^j	34.79 ^{efgh}	29.24 ^{cdc}	5.29 ^{gh}	0.21 ^{figh}
<i>Ipomoea involucrate</i>	16.05 ^{cd}	11.38 ⁱ	83.95 ^{gh}	40.23 ^{def}	39.44 ^{bc}	9.51 ^d	1.56 ^{bc}
<i>Euphorbia lateriflora</i>	7.98 ^{fg}	11.33 ^j	92.02 ^{cd}	41.34 ^{cde}	27.09 ^{efg}	8.72 ^e	2.40 ^a
SEM	0.712	0.676	0.676	2.413	2.34	0.238	0.141

abedefghijklm = Means on the same column with the same letters are not significantly different ($P > 0.05$). OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, SEM = standard error of the mean difference

The tannin content ranged from 0.011% in *S. acuta* leaves to 0.057% in *C dactylon* for the Edo medicinal plants, from 0.014% in *A. ascalonicum* to 0.102% in *Capsicum annum* for Edo spices and from 1.96% in *Laportea aestuans* to 16.60% in *Harungana madagascariensis* for Rivers State medicinal plants. For the saponin content, the value varied among the Edo medicinal plants from 0.010% in *C. dactylon* to 0.490% in *A indica* while for the Edo spices it varied from 0.002% in *A. ascalonicum* to 0.247% in *Murraya koenigii*. In the medicinal plant of Rivers State, *Laportea aestuans* had the lowest saponin value of 0.06% while *Euphorbia lateriflora* had the highest (2.40%). The tannin and saponin contents of these medicinal plants and spices further explain the abilities they have displayed in reducing *in-vitro* enteric methane production. Hess *et al.* (2003) attributed the methane

reduction ability of plants rich in saponin to their anti-protozoa effects while Rira *et al.* (2014) reported tannin's reduction of methanogenesis in sheep through its direct effects on the activity of the methanogenic archaea.

Conclusion

Conclusively, the medicinal plants and spices have demonstrated different degrees of ability to modulate *in vitro* rumen fermentation to reduce enteric methane production in ruminants. They have therefore shown potential for the replacement of antibiotic feed additives in ruminant feeding and should be subjected to further studies.

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Received: 17th October, 2018

Accepted: 19th February, 2019