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Dietary supplementation of *Justicia* secunda oil: effects on growth performance, apparent digestibility, ruminal fermentation and microbiome population of Jamunapari goats

Alagbe John Olujimi<sup>1,2</sup>\*

## **ABSTRACT**

This experiment is aimed at accessing the effect of dietary supplementation of *Justicia* secunda oil on growth performance, apparent digestibility, ruminal fermentation and microbiome population of Jamunapari goats. Thirty Jamunapari bucks with a mean body weight of 35.77 ± 0.23 kg were used in a completely randomized design for a 60 days feeding period. Experimental treatments included: treatment 1 (control) - basal diet without essential oil; treatment 2: basal diet + 200 mg Justicia secunda oil/kg feed/day and treatment 3 - basal diet + 400 mg Justicia secunda oil/kg feed/day. GC-MS analysis of Justicia secunda oil revealed that it contained 29 bioactive compounds dominated by 9,12-Octadecadienoic acid (17.11 %), Hexadecanoic acid (10.89 %), Hexane 1- hexyloxy-5-methyl (9.18 %), Ethyl palmitate (9.07 %), Nonane -3 methyl (8.04 %), 1-Tricosanol (7.66 %), Hexahydrofarnesyl acetone (6.55 %), 10-Azido-1decanethiol (5.81 %) and 2,4-Di butyl phenol (5.70 %). Dietary supplementation with Justicia secunda oil improved (p < 0.05) average daily body weight gain, feed intake and decreased feed conversion ratio. Justicia secunda oil supplementation also enhanced (p<0.05) apparent dry matter, organic matter, crude protein, ether extract, acid detergent fibre and neutral detergent fibre digestibility. Crude coriander oil supplementation linearly enhanced (p<0.05) the apparent digestibilities of dry matter, organic matter, non-structural carbohydrates, and fibers, while the high crude coriander oil supplementation level increased apparent crude protein digestibility. Justicia secunda oil supplementation decreased ruminal ammonia and protozoal population, and increased the concentrations (p<0.05) of total volatile fatty acids, fungi, bacteria, acetate, propionate and butyrate. However, pH, isobutyrate, valerate and isovalerate concentrations were not affected (p>0.05). In conclusion, Justicia secunda oil up to 400 mg/kg feed/ day had no negative effect on the general performance of goats. Therefore, high dietary supplementation is recommended.

**Keywords:** *Justicia secunda*, fermentation, antibiotics, sustainability, performance, growth



## 1. INTRODUCTION

Since 2006, the sub therapeutic use of antibiotics was banned by many countries due to the increasing cases of antimicrobial resistance. The presence of toxic residues in animal products as well as danger of environmental pollution has necessitated the need for natural alternative to antibiotics (Shittu and Alagbe, 2020). Medicinal plants and their extracts have been used for decades for therapeutic agents for the treatment of human and animals because they contain compounds with interesting pharmacological activities (Alagbe, 2022). They have been also reported to be environmental friendly, safe and with absence of residues with deleterious effect (Alagbe et al., 2022; Hernandez and Alagbe, 2025b). Essential oils contain volatile compounds with medicinal properties and have been found to be one of the numerous alternative to replace antibiotic growth promoters, improve the level of production and has no withdrawal period (Hernandez and Alagbe, 2025a). In ruminants, it has been shown that essential oils can function effectively by inhibiting the activities of some bacterial population in the rumen (Yang et al., 2010a).

Essential oils from *Justicia secunda* have several medicinal properties and can effectively replace antibiotics because of its potential. The plant belongs to the family Acanthaceae and is widely distributed in Africa, South America, Europe and some parts of Asia (Day et al., 2000; Sabbaraju et al., 2004). Phytochemical examination of *Justicia secunda* leaves reveals the presence of: tannins, phenolic compounds, flavonoids, alkaloids, saponins, terpenes, glycosides, leuco-anthocyanins and anthocyanins which possess different biological effects and can be utilized for the treatment of diseases in animals (Jimenez et al., 2001). Previous studies by (Akens and Stephens, 2017) have also shown that essential oil from the leaves of *Justicia secunda* contains, 9, 12, 15- Octadecatrienoic acid, methyl ester (36.56%), 9-Octadecenamide (7.12%), E-14-Hexadecenal (6.3%), Trifluoroacetoxy hexadecane (6.00%) and 1-Heptadecene (5.68%) as major bioactive compounds. These compounds have antimicrobial, antioxidant, antiangiogenic, antiviral, anti-leishmanial, antihelminthic, antifungal, hypolipidemic (Kavitha et al., 2003), cardiotonic, gastro-protective, anti-tumor, anti-depressant, analgesic, hepatoprotective, cytotoxic, immuno-stimulatory activities, antiplatelet, anti-asthmatic and anti-diarrheal properties (Ruysschaert et al., 2009).

In the ayurvedic medicine ethanolic extracts from *Justicia secunda* has the been employed in the treatment of headache, snakebite, blennorrhagia, boil, cancer, cold, colic, conjunctivitis, convulsion, cough, diarrhea, fever, gastritis, gonorrhea and jaundice (Kanchanapoom et al., 2004). The roots of *Justicia secunda* are useful for the treatment of jaundice, malaria, night-blindness, ophthalmia, rheumatism, diabetes and chronic nephritis (Agra et al., 2007; Jimenez et al., 2001). Aqueous extract from the leaves can be used as a remedy from snake bite, gastro-intestinal disorder, skin infection and sexually transmitted infections (Kanchanapoom et al., 2004; Day et al., 2000).

Although the practice of using essential oils as feed additives in ruminant nutrition is a novel concept and their effects on ruminant fermentation as well as general nutritional effects are still not well defined. However, previous studies have shown that essential oil can be used to modulate ruminal metabolism (Yang et al., 2010b), selectively inhibit rumen methanogenesis due to the presence of bioactive compounds (Wallace, 2005; Wang et al., 2018), improve intake and growth, nutrient digestibility, reduce rumen ammonia concentrations which leads to more efficient utilization of diets (Tager and Krause, 2011), improve blood constituents and inhibit the proliferation of pathogenic organisms in the gut (Canbolat et al., 2018). Supplementation of the oil at high dose can be toxic to animals due to the variation in their phyto-compounds. This research will not only help to establish a safe dose for goats but will also help to promote sustainability in livestock production.

# 2. MATERIALS AND METHODS

The research was carried out at the Ruminant Unit, Ganhi College of Agriculture, Rajasthan situated in the North-Western part of India which lies between longitude  $23^{\circ}03'$  to  $30^{\circ}12'$  North and latitude  $69^{\circ}30'$  and  $78^{\circ}17'$  East.

#### Animals management and experimental design adopted

Thirty Jamunapari bucks with a mean body weight of 35.77 ± 0.23 kg, 7 months of age were used in a completely randomized design for a 2 months (60 days) feeding period preceded by a 14-days initial adjustment period. All animals used in the experiment were cared for according to applicable recommendations of Indian Society of Animal Production and Management. After an initial adjustment of 14 days, body weights were taken fortnightly for 60 days. Animals were vaccinated with Goat pox vaccine (Uttarkashi strain) and Enterotoxaemia vaccine and stratified based on their weights into three dietary groups with 10 goats per treatment, each goat was a replicate in each treatment. Animals were housed in individual pens measuring (2.5m²/goat) equipped with feeders and drinkers. Experimental treatments included: treatment 1 (control) – basal diet without essential oil; treatment 2 and treatment 3 – basal diet

supplemented with 200 mg and 400 mg *Justicia secunda* oil per kg DM feed daily. The basal diet meets the standard nutrient requirements for goats according to Nutritional Research Council recommendations (2012) and its proximate analysis of was carried out using standard procedures according to Association of Analytical Chemist (2019). Animals were fed twice daily between at 07:30 and 16:00 h and had unrestricted access to clean water. Cleaning of the pens and washing of drinkers was done daily. Feed intake was estimated as the difference between feed offered and leftover. Body weight gain was determined as the difference between final body weight and their initial weight while feed conversion ratio was calculated as by dividing feed intake by weight gain.

## Justicia secunda leaf collection and oil extraction

Fresh leaves of *Justicia secunda* were collected from Gujarat and sent to the Crop Science department, Gandhi College in Rajasthan for proper for proper assessment and authentication before it was assigned a reference number NV/08GH/2023. The collected leaves were rinsed with running water followed by distilled water to remove dirt's before it was shade dried for 13 days. Dried *Justicia secunda* leaves was pulverized into powder using electric blender and stored in an air tight container prior to extraction. Extraction of oil was carried out using steam distillation technique using Clavenger apparatus as earlier described by Hernandez and Alagbe (2025). 200 g of *Justicia secunda* powder was transferred into a round bottom flask filled with 800 mL water heated in a water bath at a temperature of 70°C for 15 minutes, steam collected passes through the condenser and collected in a beaker. The mixture of steam and water was separated in a separator, stored in the refrigerator at a temperature of 4 °C.

#### Gas chromatography-mass spectrometry analysis

GC-MS analysis of *Justicia secunda* oil was performed on an Agilent Model 7890A Gas Chromatography interfaced to an Agilent 7000 GC/MS Triple Quad. The equipment carrier gas was helium which was maintained at a pressure, temperature and average velocity of 1.500 psi, 300 °C and 43.11 cm/sec respectively. The MS was operated at an ionization voltage of 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C. Components identification The components of the essential oil were identified on the basis of their retention indices. Identification confirmation of reference compounds from the Library of National Institute of Standard and Technology database (2011); Adams (2007).

#### **Apparent Nutrient Digestibility Trial**

At the end of the experiment, three goats were selected per treatment for the seven days' digestibility trial. Animals were kept in a specially constructed metabolic cage (to allow easy collection of urine and faeces) for 2 days to allow goats to acclimatize. Feed intake per animal was recorded daily. Internal indigestibility marker was mixed with feed supplied to each animals, fecal droppings from each animal were collected separately every morning before feeding and were weighed and recorded daily for five days. Each day's total faecal output was weighed and about 10% of it was saved, oven dried at 60°C for 48 hours and coefficients of apparent digestion was calculated. Proximate composition of faecal droppings was carried out according to the procedure outlined by AOAC (2019).

Apparent nutrient digestibility (%) = <u>Nutrient intake (DM) - Nutrient in faeces (DM)</u> x 100 Nutrient Intake (DM)

Concentrations (% DM) of non-structural carbohydrates = 100 - (Neutral detergent fibre (NDF) + crude protein (CP) + EE + ash)

Cellulose = Acid detergent fibre (ADF) – Acid detergent Lignin (ADL)

Hemicellulose = NDF – ADF

Organic matter = 100 – Ash

## **Analysis of Rumen Fluid**

On the last day of the study (60th day), rumen liquor was collected from 2 hours after morning feeding from five randomly selected animals per treatment to determine volatile fatty acid and ammonia nitrogen concentrations. Collection was done using a stomach tube and a vacuum pump linked to a flask. pH was immediately determined using a digital hand pH meter from 100 mL fluid collected from each animal. The collected sample were stained with 4 times volume of methyl-green formalin saline solution. One tube of the filtrate with 250 g/L (w/v) metaphosphoric acid (8: 2, v/v) was stored (-20°C) to detect the volatile fatty acids (VFA), and another tube of 10 mL filtrate was preserved by adding 2 mL of 20 g/L (w/v) Tetraoxosulphate (VI) acid for ammonia nitrogen determination (AOAC, 2019). Concentrations of volatile fatty acids (acetate, butyrate and propionate) were determined by RuG Gas-liquid chromatography

(Model AAD/07/2011, Netherlands) which uses helium as carrier gas and set at a column temperature of 100 °C for 1 minutes and gradually increased to 20 °C/min to 140 °C, injector temperature (200 °C) and detector temperature (250 °C). Identification of ruminal bacteria, fungi and protozoa population was done according to the method earlier described by Zhou et al. (2020); Dehority (1993).

## **Statistical Analysis**

All data collected were analyzed separately using a one-way analysis of variance (ANOVA). Means showing significant differences were separated using Turkey's Multiple Range Test. Probability values of p < 0.05 were declared significant.

#### 3. RESULTS

Table 1 reveals the composition of experimental diet. Diet was formulated according to the nutritional requirement for ruminants according to NRC (2007).

Table 2 represents the 29 bioactive compounds identified in *Justicia secunda* oil by GC-MS representing 88.38 %. The most dominant compounds recorded were; 9,12-Octadecadienoic acid (17.11 %), Hexadecanoic acid (10.89 %), Hexane 1- hexyloxy-5-methyl (9.18 %), Ethyl palmitate (9.07 %), Nonane -3 methyl (8.04 %), 1-Tricosanol (7.66 %), Hexahydrofarnesyl acetone (6.55 %), 10-Azido-1-decanethiol (5.81 %) and 2,4-Di butyl phenol (5.70 %) while the minor compounds were; 1-methylbutylidene (0.02 %), cis-Vaccenic acid (0.03 %), 1-Fluorooctane (0.10 %), Phthalic acid (0.11 %), 2-Chloropropionic acid (0.11 %), 2,2-dimethyl- Cyclohexanol (0.14 %) amongst others.

Table 3 reveals the growth performance of Jamunapari goats as affected by dietary supplementation of *Justicia secunda* oil. Dietary supplementation of *Justicia secunda* oil increased (p<0.05) average daily weight gain and average daily feed intake values which varied from (0.15 - 0.24 kg) and (1.33 - 1.39 kg) compared with the control. *Justicia secunda* oil decreased (p<0.05) feed conversion ratio (5.82 - 7.98) compared with control (treatment 1).

Table 4 represents the apparent digestibility of Jamunapari goats as affected by dietary supplementation of *Justicia secunda* oil. Dry matter digestibility was higher in treatment 2 (71.90 %) and treatment 3 (72.33 %) than in treatment 1 (66.77 %) (p<0.05). Similarly, dietary supplementation with *Justicia secunda* oil increased (p<0.05) organic matter, crude protein, ether extract, neutral detergent fibre and acid detergent fibre digestibility compared with the control (p<0.05).

Table 5 represents ruminal fermentation of Jamunapari goats as affected by dietary supplementation of *Justicia secunda* oil. Dietary supplementation of *Justicia secunda* oil increased (p<0.05) total volatile fatty acids, acetate, propionate and butyrate. However, higher concentration of ammonia nitrogen was observed in control (treatment 1) relative to the other group (p<0.05). Isobutyrate, valerate and isovalerate values varied from 1.72 - 1.92 mmol/L, 1.55 - 1.68 mmol/L and 1.67 - 1.75 mmol/L respectively. However, outcome was not influenced by the dietary supplementation of *Justicia secunda* oil (p>0.05). pH values were not significantly influenced (p>0.05) by the treatment.

Table 6 represents the ruminal microbiome of Jamnapari goats as affected by dietary supplementation of *Justicia secunda* oiln. Dietary supplementation with *Justicia secunda* oil increased (p<0.05) total ruminal bacteria and fungi count compared with the control (treatment 1). Conversely, protozoa population decreased as the level of *Justicia secunda* oil supplementation increased across the treatments (p<0.05).

**Table 1:** Ingredient and chemical composition of basal diet (% DM)

Components	Quantity (% DM)
Corn	30.00
Corn barn	20.00
Cowpea husk	20.00
Groundnut cake	15.50
Soya bean meal	8.00
Mineral/Vitamin Premix	2.50
Limestone	2.50
Salt	2.00
Total	100.0
Chemical composition (% dry matter - DM)	
Dry matter	93.00

Organic matter	92.00
Crude protein	14.55
Ether extract	2.10
Ash	8.00
Acid detergent lignin	12.60
Non-structural carbohydrate	39.65
Acid detergent fibre	28.70
Neutral detergent fibre	37.00
Cellulose	16.10
Hemicellulose	8.30
Energy (Kcal/kg)	2681.2

<sup>2.5</sup> kg Growers premix contained: 15,000 I.U. vitamin A; 8000 mg vitamin B1; 3000 I.U. vitamin D3; 60.0 mg vitamin E; 15 mg Choline; 0.96 mg Cobalt; 2.00 mg I; 50 manganese Mn; 0.50 mg Selenium; 250 mg.

Table 2: Compounds identified in Justicia secunda oil by GC-MS

S/N	Compounds	Retention time (min)	Percentage Area
1	9,12-Octadecadienoic acid	6.88	17.11
2	Nonane -3 methyl	7.07	8.04
3	Ethyl palmitate	7.11	9.07
4	Hexadecanoic acid	7.85	10.89
5	9,12-Octadecadienal	8.09	0.35
6	Hexane 1- hexyloxy-5-methyl	9.08	9.18
7	Hexahydrofarnesyl acetone	9.23	6.55
8	2,4-Di butyl phenol	9.66	5.70
9	2-Dimethylsilyloxytetradecane	10.41	0.55
10	1-Fluorooctane	10.58	0.10
11	2,2-dimethyl- Cyclohexanol	10.90	0.14
12	10-Azido-1-decanethiol	11.28	5.81
13	Cyclopentaneundecanoic acid	11.55	0.16
14	Isopropylcyclobutane	11.97	0.30
15	2- Chloropropionic acid	12.01	0.11
16	Triflouro acetoxy hexadecane	13.37	1.80
17	cis-Vaccenic acid	13.89	0.03
18	β-Cyclocitral	14.07	0.44
19	13-Octadecenal	15.22	0.59
20	Phthalic acid	15.50	0.11
21	Thiomorpholine	15.92	0.40
22	2-Dimethylsilyloxytetradecane	16.07	0.34
23	β-Elemene	17.11	1.92
24	2-hydroxy- 2-Propen-1-amine	17.84	0.15
25	1-methylbutylidene	18.68	0.02
26	Isophytol	19.04	0.17
27	γ-Palmito acetone	20.40	0.18
28	Dimethyl Urethane	21.88	0.51
29	1 – Tricosanol	22.50	7.66
	Total		88.38

Table 3: Growth performance of Jamunapari goats as affected by dietary supplementation of Justicia secunda oil

-	_				
Parameters	Treatment 1	Treatment 2	Treatment 3	SEM	p-value
Initial body weight (kg)	36.00	35.90	35.77	0.01	0.001
Final body weight (kg)	45.00b	49.80a	50.09a	1.93	0.02
Body weight gain (kg)	9.00b	13.90a	14.32a	0.42	0.01
Average daily weight gain (kg)	0.15b	0.23a	0.24a	0.02	0.01
Total feed intake (kg)	80.00b	82.80a	83.40a	2.09	0.07
Average daily feed intake (kg)	1.33b	1.38a	1.39a	0.02	0.01
FCR	7.98a	5.95b	5.82b	0.03	0.01

 $a_0b_2$ : Means within a row with different superscripts are significantly different (p<0.05); Treatment 1: basal diet without oil; Treatment 2 and 3:basal diet with supplemented with 200 mg and 400 mg *Justicia secunda* oil per kg DM feed daily; FCR: feed conversion ratio; Average daily weight gain = body weight gain/60days; Average daily feed intake = total feed intake/60days.

Table 4: Apparent digestibility of Jamunapari goats as affected by dietary supplementation of Justicia secunda oil

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Parameters	Treatment 1	Treatment 2	Treatment 3	SEM	p-value
Apparent nutrient digestibility (%)					
Dry matter	66.77b	71.90a	72.33a	2.94	0.08
Organic matter	60.90b	68.45a	68.99a	2.11	0.06
Crude protein	68.62b	71.73a	72.09a	2.55	0.04
Ether extract	59.86b	65.08a	66.96a	1.96	0.02
Neutral detergent fibre	41.61b	47.35a	48.01a	0.95	0.03
Acid detergent fibre	33.15b	39.07a	39.44a	0.41	0.03

<sup>&</sup>lt;sup>a,b</sup>: Means within a row with different superscripts are significantly different (p<0.05); Treatment 1: basal diet without oil; Treatment 2 and 3:basal diet with supplemented with 200 mg and 400 mg *Justicia secunda* oil per kg DM feed daily

Table 5: Ruminal fermentation of Jamunapari goats as affected by dietary supplementation of Justicia secunda oil

Parameters	Treatment 1	Treatment 2	Treatment 3	SEM	p-value
рН	6.08	6.11	6.13	0.12	0.03
NH3-N (mg/dL)	18.33a	13.98 <sup>b</sup>	11.78°	1.52	0.02
TVFA (mmol/L)	92.14 <sup>b</sup>	113.17ª	115.33ª	12.7	0.05
Acetate	54.70 <sup>b</sup>	66.12a	67.08a	4.08	0.03
Propionate	22.80 <sup>b</sup>	30.18a	30.02a	1.15	0.02
Butyrate	9.66b	12.67ª	12.88a	0.47	0.01
Isobutyrate	1.76	1.86	1.92	0.02	0.01
Valerate	1.55	1.61	1.68	0.02	0.01
Isovalerate	1.67	1.73	1.75	0.02	0.01

a,b,c: Means within a row with different superscripts are significantly different (p<0.05); Treatment 1: basal diet without oil; Treatment 2 and 3:basal diet with supplemented with 200 mg and 400 mg *Justicia secunda* oil per kg DM feed daily

Table 6: Ruminal microbiome of Jamunapari goats as affected by dietary supplementation of Justicia secunda oil

Parameters	Treatment 1	Treatment 2	Treatment 3	SEM	p-value
Total ruminal bacteria count (×10 <sup>10</sup> cell /mL)	5.04b	8.00a	8.29a	0.83	0.02
Ruminococcus spp	1.08b	2.00a	2.01a	0.51	0.03
Prevotella spp	0.62b	0.78a	0.82a	0.35	0.05
Streptococcus spp	0.44b	0.51a	0.54a	0.67	0.21

0.31b	0.41a	0.43a	0.90	0.18
1.01b	2.04a	2.11a	0.66	0.03
0.02b	0.05a	0.06a	0.19	0.05
0.18b	0.22a	0.25a	0.36	0.01
0.03b	0.06a	0.07a	0.87	0.02
0.15b	0.17a	0.19a	0.22	0.01
0.38b	0.52a	0.55a	0.17	0.03
0.66b	0.94a	0.96a	0.11	0.04
0.16b	0.27a	0.30a	0.19	0.01
1.46b	2.14a	2.26a	0.69	0.02
0.02b	0.05a	0.07a	0.77	0.01
0.18b	0.25a	0.28a	0.68	0.02
0.51b	0.83a	0.88a	0.12	0.04
0.75b	1.01a	1.03a	0.57	0.03
5.94a	3.30b	3.09b	0.46	0.21
1.21a		0.94b	0.55	0.05
0.84a	0.50b	0.44b	0.86	0.02
0.93a	0.60b	0.51b	0.12	0.01
1.67a	0.91b	0.90b	0.11	0.01
0.72a	0.20b	0.18c	0.27	0.06
0.57a	0.11b	0.13b	0.15	0.01
	1.01b 0.02b 0.18b 0.03b 0.15b 0.38b 0.66b 0.16b  1.46b  0.02b 0.18b 0.51b 0.75b  5.94a 1.21a 0.84a 0.93a 1.67a 0.72a	1.01b     2.04a       0.02b     0.05a       0.18b     0.22a       0.03b     0.06a       0.15b     0.17a       0.38b     0.52a       0.66b     0.94a       0.16b     0.27a       1.46b     2.14a       0.02b     0.05a       0.18b     0.25a       0.51b     0.83a       0.75b     1.01a       5.94a     3.30b       1.21a     0.98b       0.84a     0.50b       0.93a     0.60b       1.67a     0.91b       0.72a     0.20b	1.01b       2.04a       2.11a         0.02b       0.05a       0.06a         0.18b       0.22a       0.25a         0.03b       0.06a       0.07a         0.15b       0.17a       0.19a         0.38b       0.52a       0.55a         0.66b       0.94a       0.96a         0.16b       0.27a       0.30a         1.46b       2.14a       2.26a         0.02b       0.05a       0.07a         0.18b       0.25a       0.28a         0.51b       0.83a       0.88a         0.75b       1.01a       1.03a         5.94a       3.30b       3.09b         1.21a       0.98b       0.94b         0.84a       0.50b       0.44b         0.93a       0.60b       0.51b         1.67a       0.91b       0.90b         0.72a       0.20b       0.18c	1.01b       2.04a       2.11a       0.66         0.02b       0.05a       0.06a       0.19         0.18b       0.22a       0.25a       0.36         0.03b       0.06a       0.07a       0.87         0.15b       0.17a       0.19a       0.22         0.38b       0.52a       0.55a       0.17         0.66b       0.94a       0.96a       0.11         0.16b       0.27a       0.30a       0.19         1.46b       2.14a       2.26a       0.69         0.02b       0.05a       0.07a       0.77         0.18b       0.25a       0.28a       0.68         0.51b       0.83a       0.88a       0.12         0.75b       1.01a       1.03a       0.57         5.94a       3.30b       3.09b       0.46         1.21a       0.98b       0.94b       0.55         0.84a       0.50b       0.44b       0.86         0.93a       0.60b       0.51b       0.12         1.67a       0.91b       0.90b       0.11         0.72a       0.20b       0.18c       0.27

<sup>,</sup>a,b,c: Means within a row with different superscripts are significantly different (p<0.05); Treatment 1: basal diet without oil; Treatment 2 and 3:basal diet with supplemented with 200 mg and 400 mg *Justicia secunda* oil per kg DM feed daily

#### 4. DISCUSSION

Bioactive compounds in Justicia secunda oil suggests that it have several medicinal properties and can be used in the treatment of skin infections, tooth ache, gastro-intestinal problems, cough, pyrexia, anorexia, nasal congestion amongst others (Musa et al., 2020; Adewale et al., 2021). They also possess anti-inflammatory (Singh et al., 2021), gastro-protective (John, 2024a), cardio-protective, antioxidant (Ojediran et al., 2024a), analgesics, antibacterial (Ojediran et al., 2024b), antiviral, anti-tumor, anti-helminthic (John, 2024c; Oluwafemi et al., 2022), antidiabetic, anti-cancer, cytotoxic, antidiarrheal (Agubosi et al., 2022a; Daniel et al., 2023), antimicrobial, hypolipidemic (Agubosi et al., 2022b), immuno-modulatory (John, 2024d) and hepato-protective properties (Singh et al., 2022). It has also been established the presence of these bioactive compounds enables *Justicia secunda* oil to inhibit the activities of some pathogenic organisms which includes, Aspergillus flavus, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus anginosus, Bacillus subtilis, Corynebacterium spp, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Candida albicans amongst others (Theiler et al., 2017; Mpiana et al., 2010). The presence of 9,12-Octadecadienoic acid, Hexadecanoic acid and Hexane 1- hexyloxy-5-methyl shows that Justicia secunda oil have antimicrobial, anti-serotonergic, anti-malarial, anti-migraine, antitumor activities (Hernandez and Alagbe, 2025a; Bazie et al., 2014). Hernandez and Alagbe (2025b); Aworinde et al. (2016) reported that Nonane -3 methyl have antioxidant and anti-helminthic property (Sharma, 2012). 1-Tricosanol, Hexahydrofarnesyl acetone and 10-Azido-1-decanethiol have been suggested to possess antifungal (Gawali and Jadhav, 2011), antimicrobial, anti-tumor and gastroprotective activities (Prabhu and Guruvayoorappan, 2012). The GC-MS result obtained for *Justicia secunda* oil in this experiment agrees with the report of Swiatek et al. (2023).

Results on growth performance showed that *Justicia secunda* oil have antimicrobial property thus promoting a well-balanced gut flora and its stabilization to permit improved ssutilization and absorption capacity of nutrient (dry matter, crude protein, ether extract, organic matter, neutral detergent fibre and acid detergent fibre) translating to better weight gain and health status of animals (Ike et al., 2024). The oil also has the capacity to increase the secretion of saliva and bile production due to stabilization in the rumen pH to facilitate better activities of rumen microbes (Kholif et al., 2017). Yaxing et al. (2022) reported an increased body weight gain of sheep fed diet supplemented with *Allium mongolicum* Regel essential oil. Mahmoud et al. (2025) also recorded improved body weight gain of Holstein dairy cows fed a diet supplemented with rosemary and ginger essential oils with positive effect on feed conversion ratio. Supplementation of *Justicia secunda* oil also improved palatability of diet via enhanced flavor and odor. Similar result was recorded by Benchaar et al. (2006) when different dose of essential oil was fed to beef cattle.

Volatile fatty acids which are by-products of ruminal fermentation and are the main source of energy for goats and other ruminants (Benchaar et al., 2007a). In this current study, *Justicia secunda* oil supplementation in the diet of goats improved the total volatile fatty acid as a result of increased population in ruminal bacteria and fungi (Benchaar et al., 2007b). Increase in acetate, butyrate and propionic acid is associated increase in the concentration of volatile fatty acid (Benchaar et al., 2007b). The amount and proportion of volatile fatty acids (acetate, butyrate and propionate) produced in the rumen depends directly on the profile of the microbial population fermenting the feedstuff (Hernandez and Alagbe, 2025b). Most of the acetic acid and all propionic acid is transported to the liver. Most of the propionic acid will be metabolized in the liver where it will be oxidized and will be used as a glucose precursor (McBride and Kelly 1990; Kwon et al., 2022). A reduction in ruminal ammonia nitrogen is due to the significant elimination of protozoa in the rumen. The result suggests that *Justicia secunda* oil has the ability to suppress protozoa which live in the symbiotic relationship with methanogenic archaea. The result obtained is in consonance with the reports of Zhang et al. (2021) when oregano essential oil was supplemented in the diet of beef cattle. Similar observation was made by Naseri et al. (2022) who recorded a significant reduction in the population of rumen protozoa population when *Pistacia atlantica* gum essential oil was added to the diet of sheep.

## 5. CONCLUSION

It was concluded that *Justicia secunda* oil contains numerous bioactive compounds which enables it exhibit different biological effects. Dietary supplementation of *Justicia secunda* oil up to 400 mg/kg diet significantly improved average body weight gain and feed intake. Ruminal microbial population of bacteria, fungi are enhanced with improvement in the concentrations of volatile fatty acid. It was concluded that higher supplementation causes no deleterious effect on the general performance of goats.

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#### Conflict of interest

The author declares that there are no conflicts of interests.

#### Ethical approval

In this article, the animal regulations are followed as per the ethical committee guidelines of Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India; the authors observed the *Justicia secunda* oil effects on growth performance, apparent digestibility, ruminal fermentation and microbiome population of Jamunapari goats. The Animal ethical guidelines are followed in the study for observation, identification & experimentation.

#### Informed consent

Not applicable.

## Data availability

All data associated with this work are present in the paper.

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