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Research Article

Efficacy Of Trichilia Monadelpha Stem Bark Extracts on The Growth Performance of Growing Rabbits

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Abstract

The use of medicinal plants has been gaining increasing attention due to antibiotic resistance and the need to promote a healthy environment as well as food safety. Secondary metabolites in Trichilia monadelpha stem bark extract (TMSBE) was analyzed using gas chromatography mass spectrometry (GC/MS) system while proximate analysis of experimental diet was carried out using Near Infra-red Spectrophotometer kit (NIR). 40 growing New Zealand white × Chinchilla crossbred rabbits of 6-7 weeks age (472.6 ± 6.2 g) were used for the experiment. Rabbits were randomly assigned to 4 groups; each treatment was further divided into 5 replicates consisting of 2 rabbits each. Group 1 (G1): Basal diet with no TMSBE, G2: Basal diet plus 3 mL TMSBE per rabbit/day, G3: Basal diet plus 6 mL TMSBE per rabbit/day and G4: Basal diet plus 9 mL TMSBE per rabbit/day. Experimental diet was formulated to meet the nutrient requirement standards for growing rabbits according to Nutritional Research Council standards (1977). GC/MS analysis of TMSBE revealed that it is abundant in coapene (25.40 %) followed by azulene (18.29 %), δ-cadinene (15.39 %), α-cubebene (10.61 %) and α-longipinene (10.11 %) respectively other compounds were less than 5 %. Average weekly weight gain (AWWG), average weekly feed intake (AWFI) and feed conversion ratio (FCR) were significantly (P<0.05) different among the treatments. AWWG and AWFI follow similar trend and were highest in G3 and G4, intermediate in G2, lowest in G1 (P<0.05). Better FCR were recorded among rabbits in G4 compared to the other groups (P<0.05). It was concluded that feeding TMSBE at 9 mL/day improved feed intake and enhance performance in rabbits.

Keywords: Trichilia monadelpha, Rabbits, Phytochemicals, Performance, Spectrophotometer

Introduction

Livestock farmers are transitioning to a production technique that uses few or no synthetic antibiotics at all in a new era. This is due to the increasing rate of antimicrobial resistance and more frequent notation of multidrug resistance strains (IPA, 2003). Environmental pollution, presence of toxic residue in the carcass leading to death of animal has also been identified as one of the disadvantages on indiscriminate use of antibiotics (Alagbe, 2019; Hyun *et al*., 2018). The use of phytogenics (plant extracts) have been reported as the possible solution to promote food safety and boost livestock production especially after the European Union in 2006 placed a ban on the use of antibiotics in animal nutrition. Plant extracts contain phytochemicals which exhibit different biological properties in animal.

Phytochemicals are present in herbs, spices, and their extracts and serve a variety of purposes for plants, including pigmentation, growth, reproduction, disease resistance, and so on (Ankri and Mirelman, 1999; Sivropoulou *et al*., 1996). *In vitro* and *in vivo* studies have demonstrated the antibacterial, anti-cancer, anti-inflammatory, antimicrobial, antihelminthic, cytotoxic, immune-modulatory, hypolipidemic, hepato-protective and anti-oxidant benefits of these compounds (Teissedre and Waterhouse, 2000; Shittu *et al*., 2022). Examples of phytochemicals (secondary metabolites) are: tannins, flavonoids, terpenoids, saponins, alkaloids, phenols and they have been investigated as an effective nutritional strategy to improve feed intake and general performance of animals (IDT, 2000; Agubosi *et al*., 2022). The chemicals concentrations of phytochemicals in plants varies from one plant species to another, other factors such as; storage conditions, location, climate as well as anti-nutrient could also play a key role (Liu *et al*., 2014; Agubosi *et al*., 2022). Among the potential plants with ethno-pharmacological or therapeutic properties is *Trichilia monadelpha*.

In the family Maliaceae, *Trichilia monadelpha* is one of the most important medicinal plant abundant in various phytochemicals of ethanopharmacological usefulness in livestock production. It is an evergreen semi-deciduous plant found mostly near river bank and widely distributed globally all over tropical and subtropical regions (Abbiw, 1990; Xie *et al*., 1994). The plant consists of over 90 speies and it can grow to about 12 – 20 meters high with 0.4 m in girth with many biomedical properties (Pupo *et al*., 2002). *Trichilia monadelpha* roots, leaves and stem bark are traditionally used for the treatment of rheumatism, malaria, gastrointestinal tract, cough, arthritis, inflammation, and asthma and skin diseases. Gas chromatography mass spectrometry (GC/MS) of ethanolic extract of *Trichilia monadelpha* stem bark revealed the presence of Azulene, α-cubebene, β-cayrophyllene, α-longipinene, γ-murollene, β-bisabolene, α-bergamotene, α-farnesene and β-cedrene having antimicrobial, antioxidant, cytotoxic, antianaphylatic, analgesic, antiplasmodial, anti-inflammatory and immunomodulatory properties (Geng *et al*., 2009; Agarwal *et al*., 2006).

Scientific studies have also proven that aqueous extract from *Trichilia monadelpha* stem bark, leaves and root are capable of inhibiting the activities of *E. coli*, *Salmonella spp*, *Staphyllococus spp* and other pathogenic microorganism making it a natural alternative to antibiotics (Ben *et al*., 2013; Tseng, 1991). Efficacy of *Trichilia monadelpha* stem bark extract (TMSBE) has been tested on adult albino male rats, the outcome of the experiment revealed that the test material is capable of scavenging free radicals thus preventing disease and mortality in animals. However, there is scanty information on the impact of TMSBE on the general performance of growing rabbits.

The present study aimed to examine the impact of feeding different levels of *Trichilia monadelpha* stem bark extract on the growth performance of rabbits as natural alternatives to synthetic antibiotics and to give a clue on its safety level.

Materials and methods

The research was carried out at Sumitra Research Institute, Gujarat, India located within with the coastline of 1,600 Km, 23° 13'N 72°41'E. The test material (*Trichilia monadelpha*) stem bark was collected at the Teaching and Research Farm, Sumitra Research Institute, India. The leaves were authenticated by a certified Taxonomist (Dr. Amit Shaka) at the Department of Taxonomy, Sumitra Research Institute where a voucher (MS/092/2021) sample of the plant was deposited.

Extraction of *Trichilia monadelpha* stem bark

Collected *Trichilia monadelpha* stem bark was washed with running tap water and then with distilled water to remove dust and other contaminants, chopped into smaller pieces using a kitchen knife to allow easy penetration of the solvent, air dried in a flat clean metallic tray for 16 days and pulverized into powder using a laboratory electric blender. 100 g of *Trichilia monadelpha* stem bark powder was transferred into Erlenmeyer flask and dissolve in 95 % ethyl alcohol (500 mL) for 24 hours, stirred frequently and filtered using Whatman No.1 filter paper into a beaker. The filtered liquid was set on a water bath at 40**ºC** to evaporate the solvent and recover the extract (TMSBE). It was later stored in the refrigerator at 4 **ºC** until use.

Animal feeding, health, housing and experimental design

40 growing New Zealand white × Chinchilla crossbred rabbits of 6-7 weeks age (472.6 ± 6.2 g) were used for the experiment. Animals were purchased from a renowned farm in Gujarat and transported to Sumitra Research Institute very early in the morning to reduce stress. The rabbits were subjected to a 2 weeks acclimatization period after balancing the weight and administered Ivomec® injection to treat each animal for parasites (endo- and ecto- parasites). The experimental animals were housed in wooden hutches with wire mesh raised from the floor about 150 cm. Rabbits were randomly assigned to 4 groups, each treatment was further divided into 5 replicates consisting of 2 rabbits each. Experimental diet was formulated to meet the nutrient requirement standards for growing rabbits according to Nutritional Research Council standards (1977). Animals were weighed at the beginning of the experiment to obtain their initial body weight (IBW) expressed in grams. The experimental diets were offered to the rabbits twice daily (7:00 am and 2:00 pm). Biosecurity measures were given top priority throughout the period of experiment (12 weeks) and the experimental design is a Completely Randomized Design.

Data collection with experimental set-up

Daily feed consumption was recorded and the feed leftover and/or wastage were weighed daily before supplying fresh feed. Fresh clean water was also provided always throughout the experiment. Records of average daily feed intake (calculated as total feed intake divided by the number of experimental days) and weekly body weight gain were taken. The daily feed intake was calculated by subtracting feed leftover and waste from the total daily feed offered. Mortality rate was monitored and Feed Conversion Ratio (FCR) was calculated as the ratio of feed intake to weight gain.

The experimental set-up was arranged as follows:

Group 1: Basal diet with no *Trichilia monadelpha* stem bark extract (TMSBE)

Group 2: Basal diet plus 3 mL TMSBE per rabbit/day

Group 3: Basal diet plus 6 mL TMSBE per rabbit/day

Group 4: Basal diet plus 9 mL TMSBE per rabbit/day

Laboratory analysis of *Trichilia monadelpha* stem bark extract using Gas Chromatography Mass Spectrometry (GC-MS)

Trichilia monadelpha stem bark extract (TMSBE) was subjected to laboratory analysis using SCION new generation Gas Chromatography Mass Spectrometry (GC-MS) system (GC-MS 436 Model) with an upper mass limit of 1200 m/z, programmable temperature vapourization injectors used in conjunction with "back flushing" to divert higher boiling point sample away from the column, high precision electronic pressure control, user created spectral libraries and multiple spectral databases. 3 mL of TMSBE was injected into the inlet of the machine after the power switch was put ON. The glass intervals of the machine prevent diffusion and the installed TekLinK ™ software was used for result validation.

Chemical analysis of experimental diet was carried out using Phoenix 5000 NIR feed analyzer, Argentina with the following specifications; wave length (1100 – 2500 nm), dimensions (14.0 in × 15.0 in × 20.75 in) (L×W×H) and temperature (35 – 105ºF). Dried sample of feed (200 g) was placed in the sample cap once the switch of the machine is powered. The machine automatically scans the entire feed surface and produced result in less than 30 seconds.

Statistical analysis

Data obtained were subjected to ANOVA using SPSS (22.0) and all the treatments were compared with Turkey's test and effects were considered significant where $P<0.05$.

The Statistical model used is:

$Y_i = \mu + T_{ij} + e_{ij}$

Where: Yi = effect on experimental observations, µ = general mean of the population Ti = the effect of the dietary treatment, eij = error in the experiment

Table 1: Gross composition of experimental diet fed to growing rabbits

Ingredient	Quantity (Kg)
Yellow Corn	24.00
Brewers dry grain	35.00
Palm kernel cake	14.00
Groundnut cake	6.60
Soy bean meal	15.00
Bone meal	2.00
Oyster shell	1.30
Salt	0.35
**Premix (Vitamins and minerals)	0.25
Toxin binder	0.20
Methionine	0.10
Lysine	0.20
Total	**100.0**
Determined analysis	
Dry matter (%)	91.22
Ash (%)	7.08
Crude protein (%)	16.10
Crude fibre (%)	15.40
Ether extract (%)	1.93
Digestible energy (Kcal/g)	280.18

**Each 1 kg contains: 7,500 IU vitamin A, 2621 IU vitamin D_3, 15.6 IU vitamin E, 2.10 mg vitamin K, 8.60 mg calcium pantothenate, 0.02 mg vitamin B_{12}, 0.55 mg folic acid, 300 mg choline chloride, 30.02 mg chlorotetracycline, manganese 150.30 mg, 62.75 mg iron, 44.04mg zinc, 2.7 mg copper, 1.50 mg iodine, 0.34 mg cobalt, 0.11 mg selenium

Table 2: Analysis of bioactive compounds in *Trichilea monadelpha* stem bark extract using GC-MS

Phytochemical components	Reaction time (min)	Area (%)	Molecular wgt (g/mol)
Copaene	2.81	25.40	204.3
α-santalene	4.52	0.02	203.8
Azulene	2.19	18.29	128.1
β-chamigrene	1.38	0.01	220.7
δ-cadinene	5.02	15.39	206.3
δ-selinene	9.27	0.44	205.2
β-elememe	13.10	0.02	194.3
α-cubebene	11.22	10.61	200.8

β-cayrophyllene	15.09	1.40	204.3
β-patcholene	10.30	0.49	204.4
α-longipinene	9.44	10.11	211.7
γ-murollene	10.29	2.50	154.3
α-pinene	15.25	1.67	188.5
γ-terpinene	19.01	0.77	180.6
γ-eudesmol	20.32	1.15	188.3
β-bisabolene	25.88	0.75	188.5
α-farnesene	24.30	1.04	171.4
β-lonupinene	27.01	0.71	200.9
α-bergamotene	27.02	0.22	196.3
Eicosane	29.14	1.93	192.7
β-cedrene	29.59	0.16	152.0
Aromadendrene	30.82	0.70	164.7
3-methoxy-p-cymene	30.52	1.23	154.2
α-Elemene	33.60	0.88	222.2
Sum total	-	95.89	-

Table 3: Impact of *Trichilea monadelpha* stem bark extract on the performance of growing rabbits

Variables	G1	G2	G3	G4	SEM
No of animals	10	10	10	10	-
Breed	NWZ ×CHIN	NWZ ×CHIN	NWZ ×CHIN	NWZ ×CHIN	-
IBW (g)	477.8	478.8	473.2	472.6	0.02
AWLW (g)					
5 – 6	531.6	522.9	530.4	529.4	10.91
6 – 7	728.1	743.8	750.7	755.2	12.33
7 – 8	891.2	900.8	923.6	940.4	13.04
8 – 9	1066.2[b]	1137.1[a]	1220.1[a]	1228.7[a]	18.70
9 -10	1102.2[c]	1334.1[b]	1502.5[a]	1511.4[a]	19.75
10 -11	1206.1[b]	1457.2[a]	1669.3[a]	1802.8[a]	15.03
11-12	1390.6[c]	1511.0[b]	1788.8[b]	1924.7[a]	12.48
AWWG (g)					
5 – 6	128.5c	120.4[b]	169.0[a]	170.6[a]	6.83
6 – 7	173.1[c]	195.8[b]	201.1[a]	218.7[a]	7.54
7 – 8	196.0[b]	211.8[a]	256.7[a]	266.2[a]	8.06
8 – 9	196.5[c]	298.3[b]	312.4[a]	318.4[a]	5.15
9 -10	163.1[c]	306.2[b]	408.7[a]	433.1[a]	3.86
10 -11	136.0[c]	356.3[b]	471.8[a]	480.2[a]	4.97
11-12	114.5[c]	381.1[b]	588.5[a]	597.1[a]	5.08
AWFI (g/w)					
5 – 6	614.8	618.7	620.6	622.4	9.12

6 – 7	770.0	764.2	766.8	767.2	6.17
7 – 8	805.4	811.7	810.4	809.1	8.32
8 – 9	847.1	852.9	860.1	859.6	6.51
9 -10	875.3	880.4	879.2	880.1	5.16
10 -11	896.5[b]	898.7[b]	900.1[a]	997.4[a]	7.47
11-12	712.1[c]	890.5[b]	918.7[a]	923.5[a]	6.02
FCR					
5 – 6	5.10[a]	4.93[b]	3.67[c]	3.64[c]	0.96
6 – 7	4.45[a]	3.90[b]	3.81[b]	3.51[c]	0.60
7 – 8	4.11[a]	3.83[b]	3.19[c]	3.04[d]	0.51
8 – 9	4.31[a]	3.01[b]	2.99[c]	2.91[c]	0.42
9 -10	5.36[a]	2.90[b]	2.88[b]	2.71[c]	0.21
10 -11	5.91[a]	2.87[b]	2.61[c]	2.60[c]	0.10
11-12	4.93[a]	3.00[b]	2.99[c]	2.90[c]	0.15
Overall AWWG (g)	912.8[c]	1032.2[b]	1315.6[a]	1452.1[a]	25.87
Overall AWFI (g/w)	865.20[c]	871.72[b]	900.34[a]	901.16[a]	21.04

Means with the same super scripts ([a-c]) are not significantly different where ($p>0.05$)

Results and discussion

Analysis of bioactive compounds in *Trichilea monadelpha* stem bark (TMSBE) extract using GC-MS

Phytochemicals also regarded as bioactive chemicals are natural chemicals of plant origin used by plants for defense against pathogens and growth. They also play a key role in sensory characteristics (colour, flavor and smell) of plants (Shittu and Alagbe, 2020). The result obtained in the GC-MS analysis of *Trichilea monadelpha* stem bark extract shows the presence of 24 compounds which exhibits different biological properties in livestock production and health for instance, copaene, azulene and δ-cadinene which contains 25.40 %, 18.29 % and 15.39 respectively are group of phenolic compounds possessing one or more aromatic rings with one or more hydroxyl groups. Phenolic compounds have antioxidant and anti-inflammatory properties as well as inhibiting the growth of pathogenic bacteria (Alagbe, 2021) while α-cubebene (10.61 %) and α-longipinene (10.11 %) are the second abundant compounds in TMSBE and they are categorized as group of flavonoids which possess antimicrobial, antiviral and antioxidant properties (Adewale *et al*., 2021). Flavonoids mostly occur in nature as conjugates in glycosylated or esterified form and can also turn out as aglycones during extraction processes (Agubosi *et al*., 2022). Other compounds [α-santalene (0.02%), β-chamigrene (0.01 %), δ-selinene (0.44 %), β-elememe (0.02 %), β-cayrophyllene (1.40 %), β-patcholene (0.49 %), γ-murollene (2.50 %), α-pinene (1.67 %), γ-terpinene (0.77 %), γ-eudesmol (1.15 %), β-bisabolene (0.75 %), α-farnesene (1.04 %), β-lonupinene (0.71 %), α-bergamotene (0.22 %), eicosane (1.93 %), β-cedrene (0.16 %), aromadendrene (0.70 %), 3-methoxy-p-cymene (1.23 %) and α-elemene (0.88 %)] obtained during the GC-MS analysis of TMSBE were less than 5 %. However, the have certain therapeutic properties such as: scavenging reactive or toxic compounds, co-factor of enzymatic activities, inhibitors of pathogenic intestinal bacteria and substrate for biochemical reactions (Alagbe *et al*., 2021). The outcome of this study agrees with the findings of Ravendra *et al*. (2011) but contrary to the findings of Purnima *et al*. (2006) on the analysis of bioactive compounds in *Trichilia connaroides* leaf extract. The differences in result can be ascribed to extraction method employed, age of plant, variations in geographical location and species (Akintayo and Alagbe, 2021; Musa *et al*., 2021).

Impact of *Trichilea monadelpha* stem bark extract on the performance of growing rabbits

Impact of *Trichilea monadelpha* stem bark extract on the performance of growing rabbits is exhibited in Table 3. The average weekly weight gain (g)/rabbit (AWWG) on weekly basis array from 128.5 – 170.6 g on the 5th – 6th week, 173.1 – 218.7 g, 196.0 – 266.2 g, 196.5 – 318.4 g, 163.1 – 433.1 g, 136.0 – 480.2 g and 114.5 – 597.1 g on 6th – 7th, 7th – 8th, 8th – 9th, 9th – 10th, 10th – 11th and 11th – 12th week respectively. The overall AWWG is highest in G3 and G4, intermediate in G2 and minimum in G1 (P<0.05). The higher weekly weight gain in G3 and G4 compared to other groups is a sign of increased enzymatic activity in the intestinal tract thereby increasing the absorption of nutrients from the feed supplied to the rabbits. Phytochemicals are known for its impact on stimulating internal secretions, improving animal's appetite and impeding the activities of harmful microorganisms aimed at enhancing the performance of animals (Ahmed, 2000). The result obtained shows that feeding growing rabbits TMSBE between 6-9 mL daily has a positive impact on animal performance compared to the other treatments (P<0.05). It could also imply that the animals feed TMSBE maintained a healthy gut and will benefit from optimal health and performance. Significant differences (P<0.05) were observed in the overall average weekly feed intake (AWFI) among the groups. Overall average weekly feed intake ranged from 865.20 g – 901.16 g. Rabbits in G3 and G4 consumed more and this translates to a better FCR compared to the other groups. The result in this experiment is in consonance with the findings of Dalle *et al*. (2016) and Attia *et al*. (2014) when phytogenics were fed to growing rabbits.

Conclusion

Plant extracts show a wider range of activities in animal nutrition than synthetic substances because they contain phytochemicals that have multiple therapeutic properties. They are less toxic and environmentally friendly. It was concluded that *Trichilea monadelpha* stem bark extract can promote performance in animals and can be feed to growing rabbits up to 9 mL/day without causing any deleterious effect on their growth.

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