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Research article

Solvents extraction of secondary plant metabolites from *Prosopis juliflora* (Sw.) DC. and annihilated fermentation on plant residues

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Abstract: The study evaluated cellular, cell wall, and secondary plant metabolites (SPM) composition in solvent extract residues of stem, leaves, fruits, and pods of *Prosopis juliflora* (Mesquite) and, their effect on the anaerobic fermentation *in vitro*. SPMs from different parts were extracted with 0.5 M HCl and 0.5 M NaOH, absolute acetone, and methanol. Dissolved solutes were more on methanol followed by acetone and 0.5 M HCl and least on 0.5 M NaOH. A significant difference in cell ($P< 0.01$) and, cell wall constituents ($P< 0.01$), nitrogen fractions ($P< 0.01$) and SPMs *viz*., total extractable tannins, alkaloids, glucosinolates, and hydrocyanic acid (HCN) was observed in the residues. Residues of leaves, fruits, and to the less extent the stem, were positive to cyanogenic glucosides. Despite better nutritional composition of solvent residues of leaves and fruits, anaerobic fermentation was annihilated due to the presence of SPM. Although anaerobic fermentation *in vitro* was absent on fresh fruits, oven drying resulted in comparable IVGP and reduced SPMs observed on naturally dried pods. The study concluded that SPMs recovery was better in methanol and Mesquite leaves were rich in SPM among all other plant parts and lethal to microbes thus, the leaf extracts from the weed could be explored to antimicrobial effect.

Keywords: Anti-nutritional - Fermentation - Mesquite - Plant parts - Residues - Weed.

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INTRODUCTION

Prosopis juliflora (Sw.) DC. is an ubiquitous weed in arid and semiarid regions worldwide and commonly known as Mesquite (Birhane *et al*. 2017). The thorny weed is rapidly invading new areas, roadsides, village common grazing lands, barren lands, etc. because small and hard seeds capable of surviving passage through different pH of the digestive system of grazing animals (Shiferaw *et al*. 2004). The dried pods of Mesquite have the nutritional potential for grazing ruminants (Srinivas & Chaturvedi 2019). Rural local governments are unpremeditated to eradicate its invasion in the farm and rangelands. Scientifically few solutions are suggested for using Mesquite as firewood, herbal medicine, and ruminant animal feed. Still, these alternatives are inadequate to prevent damage to local herbal and plant diversity because of the allelopathic effect from secondary plant metabolites (SPM) present in it (Khan *et al*. 2005). Eradication or management of Mesquite is necessary to protect rural communities from its wild spread and protecting biodiversity, and controlling through better utilization has been considered an attractive, purposive, and viable option (Shiferaw *et al*. 2004).

Mesquite is the repository of many SPM like tannins, alkaloids, glucosinolates, hydrogen cyanide (HCN), etc., and have used in pharmaceuticals, nutritional, bactericidal, disinfectant, and cosmetic application. Mesquite is widely used as a folk remedy for a large number of ailments (William & Jafri 2015). Accumulation of SPMs in different plant parts varies due to several factors relating to the plant and environment (Shitan 2016). Sheep and goat graze only dried pods of Mesquite but, not other plant parts (Srinivas & Chaturvedi 2019). Yahya *et al*. (2018) described conventional and contemporary techniques of SPMs extraction and separation which include organic and inorganic fluids or polar and non-polar solvents. The protocol followed for the extraction of SPMs

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from the weed included the right choice of plant parts, solvents, processing, recovery, etc. Exclusive processing protocols have been recommended to extract specific categories of SPMs (Jones & Kinghorn 2012). We attempted to identify the rich plant part of Mesquite for better recovery of SPMs using inorganic and organic solvents and evaluated the suitability of their residues as ruminant feed supplements *in vitro*.

Figure 1. The wild spreading of *Prosopis juliflora* (Sw.) DC. (Mesquite) in community lands and village roadside posing social threat for livestock and human.

MATERIALS AND METHODS

Solvent extraction of Mesquite plant parts

Stem, Leaves, stem, fruits, and pods of Mesquite plant each 25 g were taken in 500 ml sproutless beaker and soaked with 50 ml of organic solvents; absolute acetone and methanol, and, inorganic solvents; 0.5 molarity Hydrochloric acid (HCl) and Sodium hydroxide (NaOH) for 24 hrs. In the end, contents filtered through 500 ml Buchner funnel (pore size 15–40 µ), washed 5 to 6 times with lukewarm double distilled water (DDW) until free from the solvents and followed by drying in the oven at 60°C for 18 hrs. Dried residues were ground using a lab mill (M/s Jaico laboratory, India) using a 1 mm sieve.

Chemical analysis of cellular and cell walls

Residues were analyzed for total ash, nitrogen (CP = N × 6.25), EE, neutral (NDF) and acid (ADF) detergent fiber, and acid detergent lignin (ADL) according to (AOAC, 2012). Organic matter, total carbohydrates, non-fibrous carbohydrates (NFC), and hemicelluloses were calculated mathematically. Cellulose was estimated as a loss in weight in ADF by dissolving in 72 % H_2SO_4 (v/v). According to the Cornell net carbohydrate and protein system, N was fractionated to A (NH3, NO, amino acids, peptides), B1 (globulins and some albumins), B2 (most albumins and glutelins), B3 (prolamins and denatured proteins), and C (mailarad products and lignin bound N) fractions were estimated (Chalupa & Sniffen 1996).

Secondary plant metabolites

A qualitative test for cyanogenic glucosides (Method 936.11), HCN or prussic acid (Method 915.30), and total tannins (Method 955.35) were estimated according to AOAC (2012). Total glucosinolates were estimated by taking 5 g of dried sample in 100 ml of DDW, boiled for 5 minutes and, filtered through the Buchner funnel. Residue washed 3 to 4 times with hot DDW and final volume made to 250 ml. An aliquot of 25 ml was taken in a 100 ml beaker and added 10 ml of 0.1 N $AgNO_3$ and 25 ml of ethanol. Contents were refluxed for 45 min, cooled to room temperature, filtered using Whatman filter paper No. 42, volume made to 100 ml, and centrifuged at 1500 rpm. 25 ml of supernatant was taken in 125 ml of Erlenmeyer flask and added 2 ml of 6N HNO_3 (v/v) and 6 ml of 8% (w/v) ferric ammonium sulfate and titrated against 0.01 M potassium thiocyanate to pale salmon color. Total glucosinolates were calculated based on molecular weight (McGhee *et al*. 1964). Total alkaloids are estimated as for the procedure of Aasen *et al*. (1969). A sample of 5 g was macerated for 5 min in 100 ml of chloroform consisting of 0.05 N NH_3. The suspension filtered with suction to give a cloudy filtrate, of which 50 ml was shaken with 4 ml of 2 N dilute sulphuric acid and allowed to settle the contents. After settling, the chloroform layer and 2 ml of the aqueous layer were removed with a pipette fitted with a cotton bud in the tip and treated with 0.7 ml of concentrated aqueous NH_3. Total alkaloid extraction with 2 ml of chloroform was repeated in 3 lots. Chloroform extracts were dried over sodium sulfate, filtered, evaporated and the residue was titrated against 0.01 N of β – toluene sulphonic acid in chloroform. The number of milliequivalents of the base in the sample was calculated as titer × normality × 4.

Anaerobic fermentation of leaves, stem, fruits, and pods residues were tested using 100 ml polypropylene syringes according to Menke & Steingass (1988) using rumen liquor (RL) drawn from 3 mature sheep using

stomach tube and incubated at 39°C while removing any excess air in the syringes. Release of fermentative gases *in vitro* was measured at 0, $^{1}/_{2,}$ 1, 2, 4, 8, 12, 20, 24, 36, and 48 h. Buffer, and buffer plus inoculate blank were also run parallel.

Statistical analysis

Data were analysed for variance using complete randomized block design based on the model; $X_{IJ} = \mu + \alpha_i + ß_j + e_{ij}$. Where X_{ij} was the observation variable corresponding to observation from i^{th} replication and j^{th} treatment, μ was general mean and e_{ij} was the error component. Comparison between group means was tested by Duncan multiple range test using SPSS (V 17.0 M/s IBM India Pvt. Ltd).

RESULT AND DISCUSSION

Chemical composition of Prosopis juliflora plant residues

Table 1. Chemical composition of extraction residues of *Prosopis juliflora* (Sw.) DC. stem (on DM basis).

Parameter	HCl	NaOH	Acetone	Methanol	S.E.	P-value
Dissolved solutes, g kg^{-1}	8.30^{b}	3.83^{a}	24.25^{c}	50.25^{d}	1.215	0.001
Cellular and cell wall composition						
DM, g kg^{-1}	515^{d}	490^{c}	379^{a}	459^{b}	7.98	0.001
OM, g kg^{-1}	972^{d}	928^{a}	964^{b}	967^{c}	0.77	0.001
Total CP, g kg^{-1}	64^{a}	71^{b}	75^{c}	69^{b}	1.13	0.001
Total EE, g kg^{-1}	5.03^{a}	5.69^{b}	8.32^{b}	11.83^{d}	0.10	0.001
Total Carbohydrates, g kg^{-1}	903^{d}	851^{a}	881^{b}	887^{c}	1.39	0.001
Total Ash, g kg^{-1}	27.76	72.19	36.04	32.81	0.78	0.001
NDF, g kg^{-1}	685^{a}	743^{c}	708^{b}	708^{b}	2.46	0.001
NFC, g kg^{-1}	246^{c}	180^{a}	209^{b}	211^{b}	2.91	0.001
ADF, g kg^{-1}	543	534	533	533	3.21	0.133
Hemicelluloses, g kg^{-1}	143^{a}	210^{b}	175^{b}	175^{b}	4.55	0.001
Cellulose, g kg^{-1}	394^{b}	383^{a}	395^{b}	395^{b}	2.95	0.032
Lignin, g kg^{-1}	153^{c}	125^{a}	139^{b}	144^{b}	2.00	0.001
Nitrogen fractions						
Total-N, g kg^{-1}	10.29^{a}	11.32^{b}	11.93^{c}	10.95^{b}	0.182	0.001
A, g kg^{-1}	0.563^{a}	1.582^{b}	1.673^{b}	0.748^{a}	0.197	0.002
B1, g kg^{-1}	0.60^{a}	2.43^{c}	1.51^{b}	2.58^{c}	0.256	0.001
B2, g kg^{-1}	2.14^{b}	3.43^{c}	1.86^{b}	0.88^{a}	0.249	0.001
B3, g kg^{-1}	2.99^{b}	0.68^{a}	2.92^{b}	2.71^{b}	0.256	0.001
C, g kg^{-1}	4.00^{b}	3.20^{a}	3.96^{b}	4.03^{b}	0.188	0.019
Plant secondary metabolites						
Total phenols, g kg^{-1}	28.83^{c}	29.67^{c}	20.98^{a}	23.48^{b}	0.581	0.001
Total Tannins, g kg^{-1}	21.83^{b}	21.83^{b}	18.68^{a}	18.65^{a}	0.237	0.001
Total alkaloids, g kg^{-1}	8.67	7.50	7.30	8.68	1.033	0.598
Glucosinolates, g kg^{-1}	36.32^{b}	91.92^{c}	32.65^{b}	21.25^{a}	1.093	0.001
HCN, mg kg^{-1}	105.40	103.30	107.84	107.16	13.687	0.995

Note: Means in a row with different superscripts differ significantly

Mesquite is not only leguminous but also an evergreen plant. Dissolved solutes from stem, leaves, fruits, and dried pods were highest in methanol followed by acetone. Among inorganic solvents, 0.5 M HCl was better than 0.5 M NaOH. DM in the organic and inorganic solvent extraction residues of the stem, leaves, fruits, and pods was higher due to solubility in the organic and inorganic solvents and shrinking of the substrate. The chemical composition between residues of the stem (Table 1), leaves (Table 2), fruits (Table 3), and pods (table 4) was significant ($P< 0.01$). CP in the residues of leaves was 24.8% and least in the residues of stems. Stem contained 1.5, 2.0, and 2.4 times lesser CP than the residues of leaves, fruits, and dry pods which was contrary to total carbohydrates and cellulose content.

Excluding 3 to 4 % of the C fraction of N in the residues of plant parts of Mesquite, other N fractions were potentially digestible (Chalupa & Sniffen 1996). SPM like terpenes and resins are fat-soluble and high EE in leaves (2.5%). Oxalates are often bound with calcium in the total ash and it was 10% in the residues. Oxalates were of leaves that was two folds higher than stem or green fruit residues and least in dry pods (3.4%). The lignin increases with maturity hence, fruits had lesser lignin than pods. The residues of leaves and fruits although contained more nutrients, both were unpalatable (Pasiecznik 1999). Srinivas & Chaturvedi (2019) reported naturally dried pods are only palatable among different plant forts of Mesquite, but their CP and EE were 0.6 and 0.4% lesser in the extracts of pods than leaves.

Table 2. Chemical composition of extraction residues of *Prosopis juliflora* (Sw.) DC.leaves (on DM basis).

Parameter	HCl	NaOH	Acetone	Methanol	S.E.	P Value
Dissolved solutes, g kg^{-1}	40.62^{a}	37.67^{a}	111.57^{b}	169.27^{b}	2.969	0.001
Cellular and cell wall composition						
DM, g kg^{-1}	671^{c}	708^{d}	580^{a}	619^{b}	7.20	0.001
OM, g kg^{-1}	946^{b}	910^{a}	907^{a}	909^{a}	2.02	0.001
Total CP, g kg^{-1}	230^{c}	161^{a}	193^{b}	165^{a}	4.53	0.001
Total EE, g kg^{-1}	4.98^{b}	3.31^{a}	10.21^{d}	7.93^{c}	0.14	0.001
Total Carbohydrates, g kg^{-1}	711^{a}	745^{b}	704^{a}	736^{b}	5.44	0.001
Total Ash, g kg^{-1}	54.20^{a}	90.53^{b}	92.55^{b}	91.34^{b}	2.02	0.001
NDF, g kg^{-1}	443^{b}	393^{a}	454^{b}	505^{a}	6.14	0.001
NFC, g kg^{-1}	322^{a}	443^{b}	343^{a}	323^{a}	10.17	0.001
ADF, g kg^{-1}	311^{d}	285^{b}	266^{a}	301^{c}	2.29	0.001
Hemicelluloses, g kg^{-1}	131^{b}	107^{a}	188^{c}	204^{c}	6.69	0.001
Cellulose, g kg^{-1}	175^{d}	165^{c}	136^{a}	158^{b}	1.76	0.001
Lignin, g kg^{-1}	135^{c}	121^{a}	130^{b}	150^{d}	1.00	0.001
Nitrogen fractions						
Total-N, g kg^{-1}	36.87^{c}	25.72^{a}	30.91^{b}	26.32^{a}	0.727	0.001
A, g kg^{-1}	0.632	0.00	0.00	0.00	0.316	0.420
B1, g kg^{-1}	1.39^{b}	1.32^{b}	4.23^{c}	-4.57^{a}	0.577	0.001
B2, g kg^{-1}	26.79^{c}	4.92^{a}	5.00^{a}	14.72^{b}	0.131	0.001
B3, g kg^{-1}	4.14^{a}	14.13^{c}	17.34^{d}	9.54^{b}	0.182	0.001
C, g kg^{-1}	3.92^{a}	5.35^{b}	4.34^{b}	6.63^{c}	0.106	0.001
Plant secondary metabolites						
Total phenols, g kg^{-1}	23.58^{b}	23.52^{b}	22.83^{b}	18.80^{a}	0.445	0.001
Total Tannins, g kg^{-1}	18.70^{b}	18.68^{b}	21.80^{c}	15.63^{a}	0.213	0.001
Total alkaloids, g kg^{-1}	10.75	10.75	10.33	9.47	0.424	0.152
Glucosinolates, g kg^{-1}	122.56^{b}	154.57^{c}	96.80^{a}	97.57^{a}	1.340	0.001
HCN, mg kg^{-1}	993.20^{b}	1156.79^{c}	431.76^{a}	403.51^{a}	19.761	0.001

Note: Means in a row with different superscripts differ significantly.

Table 3. Chemical composition of extraction residues of *Prosopis juliflora* (Sw.) DC. fruits (on DM basis).

Parameter	HCl	NaOH	Acetone	Methanol	S.E.	P Value
Dissolved solutes, g kg^{-1}	25.93^{a}	20.17^{a}	64.20^{b}	95.80^{c}	2.275	0.001
Cellular and cell wall composition						
DM, g kg^{-1}	696^{b}	702^{b}	657^{a}	679^{ab}	8.56	0.008
OM, g kg^{-1}	962^{c}	883^{a}	940^{b}	947^{c}	1.75	0.001
Total CP, g kg^{-1}	222^{c}	175^{a}	204^{b}	206^{b}	2.38	0.001
Total EE, g kg^{-1}	12.89^{c}	18.24^{d}	7.86^{b}	6.57^{a}	0.15	0.001
Total Carbohydrates, g kg^{-1}	727^{b}	690^{a}	728^{b}	735^{b}	3.37	0.001
Total Ash, g kg^{-1}	38.41^{a}	117.03^{d}	59.76^{c}	53.00^{b}	1.75	0.001
NDF, g kg^{-1}	559^{b}	599^{e}	499^{a}	577^{c}	4.49	0.001
NFC, g kg^{-1}	206^{a}	208^{a}	289^{b}	210^{a}	5.63	0.001
ADF, g kg^{-1}	408^{c}	423^{c}	312^{a}	343^{b}	7.41	0.001
Hemicelluloses, g kg^{-1}	151^{a}	176^{ab}	187^{bc}	235^{c}	9.53	0.001
Cellulose, g kg^{-1}	379^{b}	384^{b}	285^{a}	302^{a}	6.06	0.001
Lignin, g kg^{-1}	42.22^{b}	51.12^{d}	35.03^{a}	47.34^{c}	0.881	0.001
Nitrogen fractions						
Total-N, g kg^{-1}	35.47^{c}	28.03^{a}	32.68^{b}	32.92^{b}	0.381	0.001
A, g kg^{-1}	13.10^{d}	2.12^{a}	10.32^{c}	7.85^{b}	0.375	0.001
B1, g kg^{-1}	1.62^{a}	4.12^{b}	1.65^{a}	3.75^{b}	0.229	0.001
B2, g kg^{-1}	3.07^{b}	1.31^{a}	3.75^{c}	3.23^{bc}	0.213	0.001
B3, g kg^{-1}	10.00^{b}	11.31^{c}	11.21^{c}	9.20^{a}	0.223	0.001
C, g kg^{-1}	7.68^{b}	9.17^{c}	5.75^{a}	8.88^{c}	0.143	0.001
Plant secondary metabolites						
Total phenols, g kg^{-1}	29.05^{d}	24.08^{c}	21.55^{b}	12.78^{a}	0.348	0.001
Total Tannins, g kg^{-1}	24.88^{c}	18.68^{b}	18.72^{b}	12.48^{a}	0.197	0.001
Total alkaloids, g kg^{-1}	5.60	4.08	6.05	4.33	0.633	0.120
Glucosinolates, g kg^{-1}	104.96^{b}	174.88^{d}	119.86^{c}	84.91^{a}	1.235	0.001
HCN, mg kg^{-1}	170.85^{a}	239.69^{b}	314.60^{c}	330.63^{c}	10.706	0.001

Note: Means in a row with different superscripts differ significantly.

Table 4. Chemical composition of extraction residues of *Prosopis juliflora* (Sw.) DC. pods (on DM basis).

Parameter	HCl	NaOH	Acetone	Methanol	S.E.	P Value
Dissolved solutes, g kg^{-1}	16.63^{a}	14.67^{a}	41.87^{b}	63.20^{c}	0.987	0.001
Cellular and cell wall composition						
DM, g kg^{-1}	945	951	940	943	4.76	0.430
OM, g kg^{-1}	960^{b}	917^{a}	940^{b}	952^{b}	7.20	0.004
Total CP, g kg^{-1}	186^{c}	159^{b}	132^{a}	161^{b}	1.85	0.001
Total EE, g kg^{-1}	4.00^{b}	5.34^{c}	3.61^{a}	6.66^{d}	0.13	0.001
Total Carbohydrates, g kg^{-1}	771	753	804	784	7.01	0.001
Total Ash, g kg^{-1}	39.72^{a}	82.82^{b}	59.59^{a}	47.59^{a}	7.20	0.004
NDF, g kg^{-1}	620^{c}	674^{d}	476^{a}	558^{b}	10.94	0.001
NFC, g kg^{-1}	190^{a}	161^{a}	388^{c}	274^{b}	10.45	0.001
ADF, g kg^{-1}	385^{b}	459^{c}	324^{a}	368^{b}	12.19	0.001
Hemicelluloses, g kg^{-1}	235^{b}	215^{b}	152^{a}	190^{ab}	18.83	0.039
Cellulose, g kg^{-1}	312^{bc}	378^{c}	264^{a}	293^{ab}	10.92	0.001
Lignin, g kg^{-1}	74.31^{ab}	87.36^{c}	63.35^{a}	80.43^{bc}	2.89	0.001
Nitrogen fractions						
Total-N, g kg^{-1}	29.71^{c}	25.48^{b}	21.19^{a}	25.82^{b}	0.296	0.001
A, g kg^{-1}	9.60^{b}	4.43^{a}	5.27^{a}	5.32^{a}	0.334	0.001
B1, g kg^{-1}	1.00^{ab}	1.63^{b}	0.60^{a}	2.74^{c}	0.274	0.001
B2, g kg^{-1}	10.03^{b}	6.23^{a}	6.18^{a}	6.02^{a}	0.273	0.001
B3, g kg^{-1}	3.15^{b}	7.28^{d}	1.57^{a}	5.63^{c}	0.254	0.001
C, g kg^{-1}	5.93^{a}	5.92^{a}	7.57^{b}	6.11^{a}	0.133	0.001
Plant secondary metabolites						
Total phenols, g kg^{-1}	23.88^{d}	18.93^{c}	14.02^{a}	15.20^{b}	0.335	0.001
Total Tannins, g kg^{-1}	18.72^{c}	15.60^{b}	12.45^{a}	12.48^{a}	0.154	0.001
Total alkaloids, g kg^{-1}	0.68^{bc}	0.92^{d}	0.52^{ab}	0.30^{a}	0.105	0.006
Glucosinolates, g kg^{-1}	120.44^{d}	90.23^{c}	57.80^{b}	29.07^{a}	1.219	0.001
HCN, mg kg^{-1}	98.53	98.27	95.07	93.22	3.715	0.701

Note: Means in a row with different superscripts differ significantly.

Solvents for extraction of secondary plant metabolites

In the absence of information on the solubility of various SPMs of Mesquite in different solvents, it is difficult to understand how strongly they are imbibed in the plant. Organic and inorganic solvents were selected to evaluate the potential extent of solutes solubility in typical solvents where, methanol is polar protic and acetone is apolar protic solvents involving the nucleophilic substitution of uni or bi molecule reactions, respectively. HCl and NaOH solvents were used for the separation of acidic, neutral and basic components using acid-base extraction of most organic carboxylic acids not soluble in the neutral water. Dissolved solutes of the stem, leaves, fruits, and pods were significantly ($P< 0.01$) more in the organic than inorganic solvents thus, indicated these are mostly bound to cellular macromolecules.

Secondary plant metabolites in Prosopis juliflora plant

Mesquite leaf residues contained more SPM than the other plant parts. Total tannins, glucosinolates, HCN, and alkaloids were recovered more in the organic than inorganic solvents. Among inorganic solvents where recovery in HCl was more but the difference was 80 to 93%. In organic solvents, solutes recovery was higher in methanol and 35% more than acetone. Except for HCN and total alkaloids, total tannins and glucosinolates in the stem were significantly ($P< 0.01$) different between the residues of different solvents. Glucosinolates recovery was better in 0.5 M NaOH. HCN recovery in the residues of pods was lesser in HCl extraction than other solvents and ranged from 170 to 330 mg kg^{-1} and fatal if an animal ingests more than 60 mg d^{-1}. Glucosinolates, HCN, and total alkaloids were many folds lesser in the residues of pods than fruits. Glucosinolates ($P< 0.01$) and total alkaloids ($P< 0.05$) in the residues of pods were lesser in organic than inorganic solvent extractions. SPMs were lesser in the pods than other plant parts of Mesquite because maturity and natural drying were environmental remediation to annihilate the SPMs.

Abdulrazak *et al*. (2001) reported that the total extractable tannin content of 13 and 25 g kg^{-1} DM in leaves and pods, respectively and mainly these were condensed tannins. Total tannins in the residues of inorganic and organic solvents were 16 to 22 g kg^{-1} and in the inorganic residues of the pod were 16 to 19 g kg^{-1} and recovery was only 13 g kg^{-1} in its organic solvent residues. Residues of leaves, fruits, and to the less extent the stem were positive to cyanogenic glucosides, and yield cyanide on hydrolysis. The cyanogenic glucosides test was negative in naturally dried pods. The presence of higher quantities of glucosinolates imparts bitterness to Mesquite leaves

thus, making them unpalatable (Srinivas & Chaturvedi 2019). Glucosinolates and its metabolites are goitrogenic and hepatotoxic however, ruminants are more resistant than monogastric animals (Tripathi & Mishra 2007). Hydrocyanic or prussic acid (HCN) in the solvent extracted residues of leaves and fruits of Mesquite was higher than the safer level of 60 mg d^{-1} (Mongo & Akhidue 2002). HCN was lesser in the residues of stem and pods. Hang & Preston (2005) reported reduced HCN content in the Cassava leaves from 863 to 80 mg kg^{-1} DM after sun drying. Although HCN content was reduced in the residues after inorganic and organic solvents, still they were beyond safer levels and may cause death to animals within few minutes to 3 h (Nkafamiya *et al*. 2007). Total alkaloids present even in the residues of leaves and fruits rendered this feed hazardous to livestock and affected fermentation due to fatal impact on rumen microflora. Silva *et al*. (2007) reported neuromuscular alterations due to gliosis damaging the neuronal cell health and integrity from piperidine alkaloids. Vimal & Tyagi (1986) identified 5 alkaloids from Mesquite. SPMs translocation was more from leaves to fruits and these reduced only when matured and pods were dried. Khan *et al*. (2005) reported the allelopathic effect of chemicals present in the leaves to inhibit the growth of other plants thus, affecting biodiversity. Leaves and, fruits were not palatable to grazing livestock due to the presence of different classes of SPMs (Srinivas & Chaturvedi 2019). Neither acid nor alkali soaking, not even organic solvents could annul the SPMs in the stem, leaves, and fruits and present in their residues. This indicated difficulty in removing SPMs from Mesquite plant parts.

Fermentation of Prosopis juliflora residues

Fermentation gas production was zero on the solvent residues of leaves and fruits of Mesquite until 48 h of incubation. No gas production on the solvent residues of leaves and fruits indicated an adverse impact on anaerobic fermentation. Fermentation gases as end products *in vitro* were observed only 11.8 ml g^{-1} on oven-dried leaves. This indicated oven drying of Mesquite leaves could only annul SPM moderately. Although no fermentation gases *in vitro* were observed on the solvent residues of fruits, oven-dried fruits had comparable fermentation gas production with naturally dried pods. SPMs in the residues of leaves and fruits were lethal to microorganisms on their solubility in rumen inoculum and annihilated the fermentation.

CONCLUSION

The tannins, HCN, glucosinolates, and alkaloids present in *Prosopis juliflora* (Mesquite) were firmly bound and their extraction was partial with typical inorganic and organic solvents. The leaves contained higher SPMs than the stem, fruits, or pods. Methanol was the best solvent followed by acetone, 0.5 M HCl, and least in 0.5 M NaOH. SPMs in leaves which may be soluble in anaerobic inoculum were lethal to microorganism, therefore; these extracts could be used as antimicrobial or pest control with caution to cost feasibility for SPMs extraction using different solvents. Mesquite pods are only suitable as livestock feed but not the stem, leaves or green fruits.

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