Phytochemicals, Antioxidant and Antimicrobial activities of *Ziziphus mauritiana* Lam.

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Abstract
Ziziphus mauritiana Lam. belongs to the family Rhamnaceae and is commonly known as "Indian jujube". The present study aimed at the screening, evaluation of phytochemicals and a comparison of antioxidant, antimicrobial activities of fruit extract of Z.mauritiana in raw and preserved form. Qualitative phytochemical analysis suggested the presence of different phytoconstituents. In DPPH assay the raw and preserved fruits exhibit highest percentage of inhibition at 2000µg/ml. It was suggested that the preserved fruit possess better antioxidant potential than raw fruit samples. In FRAP test, the methanolic extract of preserved fruit showed increased ferric reducing power than raw fruit sample for all the concentration. The raw fruit extract has no activity against E.coli at different concentrations. But it shows activity against S.aureus at the concentrations of 500 and 1000µg/ml.

The fungal strain A.niger also indicated sensitivity at concentrations 500 and 1000µg/ml and it was observed that there is no activity against C.albicans. The preserved fruit sample of Z.mauritiana do not have any activity against the two bacterial and fungal strains. Both the standards have zone of inhibition higher than the fruits samples. The methanolic extract of both raw and preserved fruit samples indicated mild antibacterial activity and moderate antioxidant potential.

Keywords: *Ziziphus mauritiana* Lam., phytochemicals, antioxidant, antimicrobial.

Introduction

Plants are considered to be one of the most important and significant sources of medicines. Medicines or drugs can be derived from any of the plant parts. Plants produce a number of chemical compounds in its parts in response of defence mechanism. Numbers of phytochemicals are produced in different parts of the plants. A single plant can have phytochemicals in the form of secondary metabolites which will provide the plants medicinal properties. Phytochemicals are deposited at different regions of plant parts such as fruit, flower, leaf, stem and root[6]. The concentration of the chemical deposition in plants varies from plant to plant. Different phytochemicals were identified with the help of preliminary phytochemical analysis. Antioxidants are compounds that help to prevent the oxidation process and are also called free radical scavengers. They lead to chain reactions and cause severe damages to the cells. Ascorbic acid is good antioxidant found in citrus fruits which can terminate the chain reactions produced by the free radicals. *Ziziphus mauritiana* leaves, fruits and seeds exhibit antioxidant activity. The ethanolic leaf extract of *Ziziphus mauritiana* exhibits significant antibacterial activity against tested pathogens like *S.pyogenes*, *S.aureus*, *E.coli* and *K.pnemoniae*[23]. *C.albicans* is the most common fungus which lives in our body as a normal flora. It forms a symbiotic relationship with the host body and does not cause any serious problems.

The methanol extract of *Ziziphus mauritiana* leaves has antifungal activity against *C.albicans*[11]. So, it can serve as the source for producing new oral health care products mainly for treating oral fungal infections. As per the report by Sivasankari et al[23], the leaves of *Ziziphus mauritiana* ethanolic and methanolic extract have moderate activity with *C. albicans*[23]. For the present study, DPPH and FRAP are used for the analysis of antioxidant activity and two bacterial and fungal strains were used for antimicrobial testing.

Material and Methods

Collection of Samples: The raw fruits of *Ziziphus mauritiana* (Fig. 1 and Fig. 2) were collected from P J J International Fruit Company Pvt. Ltd., Thiruvananthapuram, Kerala, India. Preserved fruit (Fig. 3) were procured from the sales outlet of a farm near Coimbatore manufactured by Agasthyia Organics (Brand Name: Mystique Hills-Organic Living). The raw fruit sample were used for phytochemical analysis and the extracts from raw and preserved fruits were compared for their bioactivities like antioxidant and antimicrobial.

Cold Extraction: The collected fruits samples were washed in running tap water. Unwanted parts along with seeds were removed using sterilized blade or knife, shade dried for three to four weeks and powdered into fine powder. 5gm of dried powder of both raw (Fig. 4) and preserved fruit (Fig. 5) were extracted with 20ml of methanol. These samples were placed in a gyratory shaker at 120rpm for 48 hours. The two extracts were filtered using Whatmann no.1 filter paper, dried and the yield and quality of extract were recorded. The final residues were stored in a refrigerator at 4^0C till further use.

Hot Extraction- Soxhlet Extraction: The collected fruits samples were washed well, dried and powdered. 50gm of powdered plant material was packed into a thimble and kept in Soxhlet apparatus. Methanol was used for the solvent

extraction. The whole apparatus was kept over a heating mantle and was heated continuously for eight hours at boiling point of methanol. The extract was concentrated to dryness and the residue was transferred to a sample bottle and was stored for further studies.

Fig. 1: Habit of *Ziziphus mauritiana* Lam.

Fig. 2: Raw Fruit of *Z. mauritiana* Lam.

Fig. 3: Preserved Fruit of *Z. mauritiana* Lam.

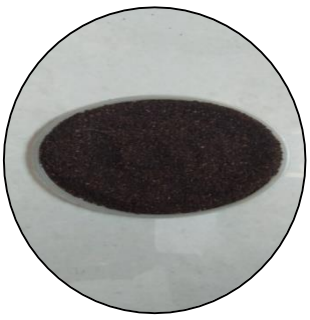


Fig. 4: Dry Powder of *Z.mauritiana* Raw Fruit

Preliminary Qualitative Phytochemical Analysis: A stock solution (mg/ml) of extract from methanolic solvent was prepared. These extracts along with blanks were analyzed qualitatively for the presence of various phytochemicals. Phytochemical investigations were carried out for raw fruit extract of *Ziziphus mauritiana* as per standard methods[24]. For the present study, alkaloids, flavonoids, terpenoids, tannin, cardiac glycosides, phenol, triterpenoids, anthraquinone glycosides, carbohydrates, proteins and saponins were tested.

Fig. 5: Dry Powder of *Z.mauritiana* Preserved Fruit

Quantitative Phytochemical Analysis: For the quantitative estimation, alkaloids[2], phenol[12], flavonoids[13] and terpenoids[15] were quantified.

Antioxidant Activity: Methanolic extracts of raw and preserved fruits were taken for antioxidant activity (DPPH and FRAP assay).

1.DPPH Radical Scavenging Assay: The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol[4]. IC_{50} values denote the concentration of the sample, required to scavenge 50% of DPPH free radicals.

2.Ferric Reducing Power Assay: The FRAP assay was performed according to the method of Nishaa et al[17].

Antimicrobial Assay by Agar Well Diffusion Method: Methanolic extract of raw and preserved fruits was taken for antimicrobial activity. The microorganisms used for the investigation of antibacterial activity were one Gram negative strain – *Escherichia coli* and one Gram positive strain- *Staphylococcus aureus*. The fungal strains selected were *Aspergillus niger* and *Candida albicans.* Streptomycin (standard antibacterial agent, concentration: 10mg / ml) and clotrimazole (standard antifungal agent, concentration: 10mg / ml) were used as the stanadard.

Culture of test organisms: Growth of culture was adjusted according to McFarland Standard, 0.5%.

- *Escherichia coli* (ATCC 25922)
- *Staphylococcus aureus* (ATCC 25923)
- *Aspergillus niger (ATCC 16404)*
- *Candida albicans(ATCC 10231)*

Statistical Analysis: The experimental data were recorded on basis of three independent trials of each parameter. Mean value and standard deviation (SD) were computed and the results were tabulated as mean $\pm$ SD for triplicate.

Results and Discussion

The phytochemical analysis of fruit extract of *Ziziphus mauritiana* Lam. was carried out using methanol as solvent. The present study aimed to detect the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, cardiac glycosides, phenolics, triterpenoids, anthraquinone glycosides, carbohydrates, proteins and saponins using the standard protocols and to assess the bioactivities of the extract from fruits in raw and preserved forms using hot extraction.

Yield of Extracts: The extraction yield in methanolic solvent was compared for the raw and preserved fruit samples of fruits of *Ziziphus mauritiana* Lam. The yield of crude extract was determined by measuring its dry weight and the yield per 100g of each sample was calculated and represented in table 1 using cold extraction.

$$\text{Extraction yield} = \frac{\text{Mass of extract}}{\text{Mass of dry matter}}$$

The yield of extract from preserved fruit was detected as 87.25% higher than that from raw fruit. Preservation of fruit with sodium chloride and sugar (jaggery) may bring about variations in physical and chemical properties of dried and preserved fruit samples and these alterations may facilitate better release of extractable components in comparison to raw sample.

Phytochemical Evaluation: Phytochemical evaluations on qualitative and quantitative attributes were carried out using standard protocols and the data were explained for the assessment of phytochemical profile of the fruit of *Ziziphus mauritiana*. The results of the qualitative analysis are illustrated in table 2. On the basis of the intensity of the reaction product of qualitative tests, the data were graded as very high (+++), high (++), moderate (+) and nil (-). In the present analysis, most of the major phytoconstituents were detected as positive except anthraquinone glycosides. The polar solvent (methanol) was detected to be successful in extracting the secondary metabolites effectively from the raw fruit sample of *Ziziphus mauritiana*. The major phytoconstituents detected were alkaloids, saponins and tannins. In addition to these phytoconstituents, flavonoids, terpenoids, triterpenoids, carbohydrates, cardiac glycosides, phenols and proteins were also detected in trace amounts.

Tests for alkaloids, saponins and tannins indicated more positivity with intense colour formation than other classes of phytochemicals. Phenol and carbohydrates also showed more intensity in the respective tests in the methanolic solvent.

Table 1
The yield and nature of extracts of fruits samples of *Ziziphus mauritiana* Lam. after extraction. (Duration of Extraction = 48 hours; Treatment Temperature=40^{0}C).

S.N.	Name of the plant part	Weight of powder (g)	Name of solvent	The yield of extract (g)	Yield per 100 g	Nature of the extract
1	Fruit (Raw)	5g	Methanol	1.02	20.4	Dark Brown
2	Fruit (Preserved)	5g	Methanol	1.91	38.2	Dark Brown

Table 2
Qualitative analysis of phytochemicals present in the methanolic extract of raw fruit of *Ziziphus mauritiana* Lam.

S.N.	Name of the phytoconstituents	Name of the test	Result
1	Alkaloids	Dragendroff's Test	++
		Mayer's Test	+++
2	Flavonoids	Ammonium Test	-
		Alkaline Reagent Test	+
3	Terpenoids	Liebermann-Burchard's Test	+
4	Tannin	Ferric Chloride Test	+++
5	Cardiac Glycosides	Keller-Killiani Test	+
6	Phenol	Ferric Chloride Test	++
7	Triterpenoids	Salkowski Test	+
8	Anthraquinone Glycosides	Hyhydroxyanthraquinone Test	-
9	Carbohydrates	Fehling's Test	++
10	Proteins	Biuret test	+
11	Saponin	Foam Test	+++

'+++' Very High; '++' High; '+' Moderate; '-' Nil.

Flavonoids detected by ammonium test resulted in negative and when tested by alkaline reagent test, showed positive result indicating the presence only in moderate level. Terpenoids, cardiac glycosides, proteins and triterpenoids were also present in moderate level. Anthraquinone glycosides were absent in the extract. In these, alkaloids, saponins and tannins showed more intensity than other phytochemicals. Methanolic solvent being highly polar can extract most of the phytochemicals in comparison to the non-polar solvent. The presence of –OH bond (alcoholic group) will result in the inner penetration of the membrane and cell wall causes the degradation and release of phytochemicals in the extract. Methanol is considered to be the best solvent for the extraction of bioactive compounds[7]. The earlier studies done by different authors supported the present phytochemical investigations[16,18].

The quantitative phytochemical analysis was carried out to estimate the quantity of phytoconstituents such as alkaloids, flavonoids, terpenoids and phenol. The results are illustrated in table 3. The present quantitative study revealed the amount of alkaloids content to be high in the fruit sample (99µg/mg). For the estimation of alkaloids, atropine (1mg/ml stock) was used as standard. The total flavonoid content was found to be 1.94µg/mg. Quercetin (1mg/ml stock) was used as standard. Concentration of phenol in gallic acid (standard: 1mg/ml stock) equivalent was measured as 5.21µg/mg. The total terpenoid content was found to be less in the methanolic extract and was estimated as 0.47µg/mg. On the comparison of the quantitative analysis of the phytoconstituents like alkaloids, flavonoids, terpenoids, phenol the total alkaloids content was found to be high and terpenoid content was detected to be less in methanolic extract.

The phytochemical study conducted by Rathore et al[20] reported that alkaloids were absent in the methanolic extract of fruit of *Ziziphus mauritiana* and other phytoconstituents such as flavonoids, phenol, sterols, lignin, tannins, saponins and glycosides were present[20]. In the present study, qualitative analysis of alkaloids by Dragendroff's and Mayer's test indicated positive response. The fruits also contain some terpenoids, phenolic acid, flavonoids[14], saponins and tannins[18] and were reported to possess antioxidant activity.

Antioxidant Assay: The antioxidant activity was carried out in the methanolic raw and preserved fruits extracts of *Ziziphus mauritiana*. DPPH assay and ferric reducing power assay were done to detect the antioxidant potential in both fruit samples. The percentage of scavenging and IC_{50} value were calculated and comparisons were made.

1. DPPH Radical Scavenging Assay: The DPPH radical scavenging activity of methanolic extracts of raw and preserved fruits of *Ziziphus mauritiana* was determined for the concentrations ranges 6.25 to 2000µg/ml and the results are illustrated in table 4 and figure 6. Ascorbic acid was used as standard (1mg/ml stock). The standard showed the highest percentage of inhibition (39.80%) at the lower concentration (6.25µg/ml). The extract from raw fruit exhibited moderate antioxidant activity when compared to preserved fruit extract. For the concentration ranges from 6.25 to 25µg/ml, both raw and preserved fruit had no inhibition and from 50µg/ml onwards, they showed antioxidant activity. The percentage of inhibition was highest at 2000µg/ml for raw (59.30%) and preserved fruit (71.67%) and lowest at 50 µg/ml (1.57% and 4.75% respectively). IC_{50} of ascorbic acid is 54.53µg/ml, for raw fruit, it is 1349.70 µg/ml and for preserved, it is 477.17 µg/ml.

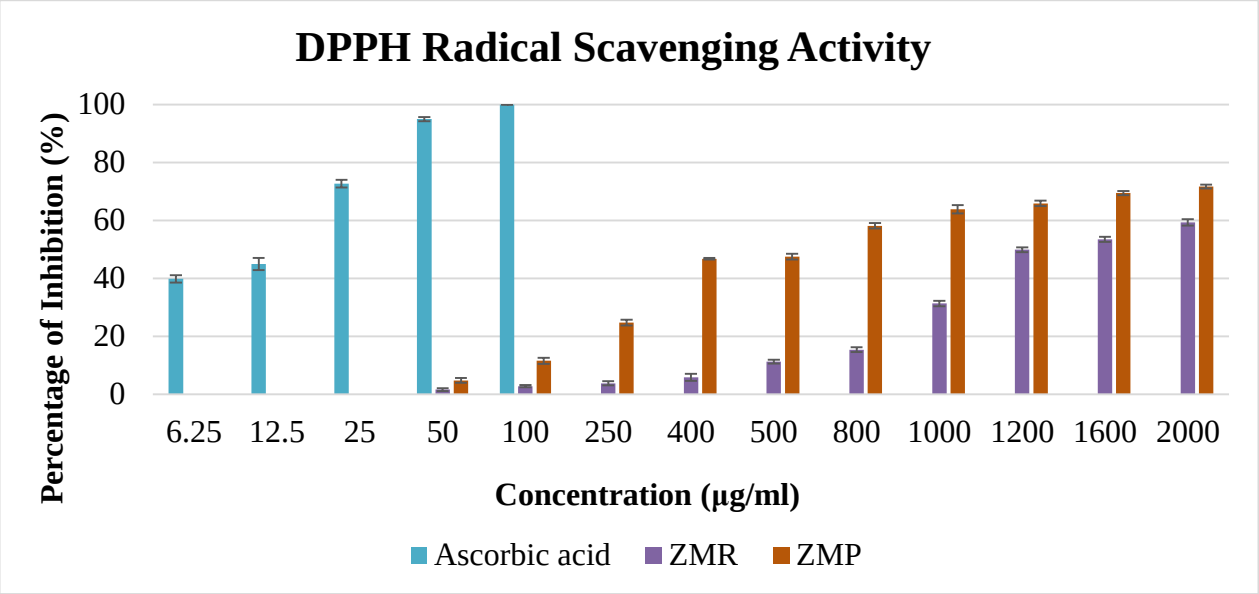
From the present investigation, it was observed that both raw and preserved fruit extracts had lower scavenging potential for DPPH radical in comparison to the standard ascorbic acid. The DPPH antioxidant assay is the best method for determining the antioxidant activity of the plant extract. It is very sensitive and easy method. The donor activity of the hydrogen atom present in the fruit extract causes the reduction in the absorbance as the concentration of the fruit samples increases, thereby increasing the percentage of inhibition. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical. It gives maximum absorption at the wavelength of 517nm. In the presence of antioxidant, the deep purple colour (DPPH) changes to colourless or pale yellow colour product, 1,1-diphenyl-2-picrylhydrazyl[21].

In the present study, the percentage of inhibition increases with increase in concentration of the fruit extract for both *Ziziphus mauritiana* raw (ZMR) and *Ziziphus mauritiana* preserved (ZMP) fruits. Both the fruits show antioxidant activity. Preserved fruits exhibit high antioxidant potential when compared to raw fruits. The standard ascorbic acid is recorded as highest percentage of inhibition than both the fruit extracts. The presence of fewer amounts of flavonoids (1.94µg/mg) and phenol (5.21µg/mg) may be responsible for the moderate antioxidant property of the fruits samples. The antioxidant activity of the fruits samples varies because of the method of extraction[19], different region[5,25] and so on. The phenolic and the flavonoid content were responsible for the free radical scavenging potential of the *Z.mauritiana* fruit[9].

Table 3
Quantitative analysis of phytochemicals present in the methanolic extract of raw fruit of *Ziziphus mauritiana* Lam.

S.N.	Phytoconstituents	Quantity (µg/mg)
1	Alkaloids	99.00± 1.06
2	Flavonoids	1.94± 0.58
3	Phenol	5.21±1.56
4	Terpenoids	0.47± 0.07

[Data shown as mean ± SD (n = 3)]



[ZMR – *Ziziphus mauritiana* Raw Fruit; ZMP – *Ziziphus mauritiana* Preserved Fruit]

Fig. 6: Determination of antioxidant activity of *Z.mauritiana* methanolic extract of raw and preserved fruits by DPPH assay ; Standard (Ascorbic acid)

Table 4

DPPH activity of methanolic extract of raw and preserved fruits of *Ziziphus mauritiana* Lam.

S.N.	Concentration (µg/ml)	Percentage of inhibition (%)		
		Standard (Ascorbic acid)	ZMR	ZMP
1	6.25	39.80± 1.27	-	-
2	12.5	44.93± 2.15	-	-
3	25	72.67± 1.34	-	-
4	50	94.98± 0.73	1.57± 0.49	4.75±0.86
5	100	100.00± 0.03	2.75± 0.39	11.50± 1.09
6	250		3.75± 0.73	24.70± 1.01
7	400		5.82± 1.25	46.75± 0.25
8	500		11.23± 0.62	47.48± 1.04
9	800		15.36± 0.82	58.11± 0.94
10	1000		31.30± 0.92	63.84± 1.43
11	1200		49.90± 0.83	65.84± 0.95
12	1600		53.44± 0.88	69.42± 0.72
13	2000		59.30± 1.06	71.67± 0.69
IC_{50}(µg/ml)		**54.53**	**1349.70**	**477.17**

[ZMR – *Ziziphus mauritiana* Raw Fruit; ZMP – *Ziziphus mauritiana* Preserved Fruit; Data shown as mean ± SD (n = 3)]

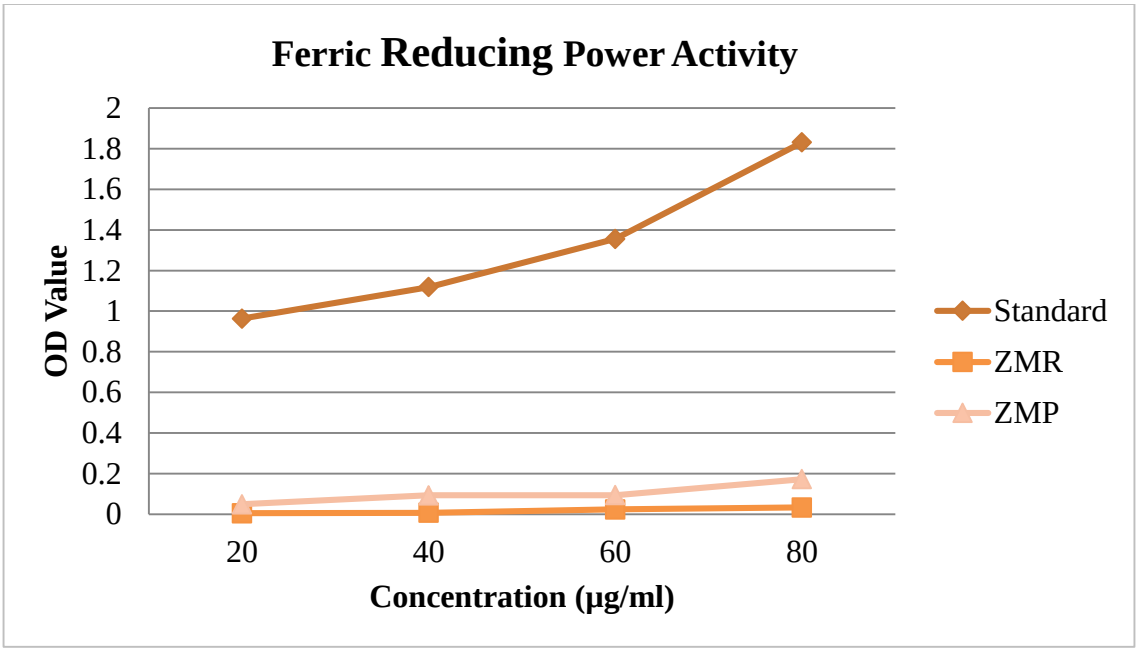
Table 5

FRAP assay of methanolic extract of raw and preserved fruits of *Ziziphus mauritiana* Lam.

S.N.	Concentration (µg/ml)	Absorbance (593nm)			FRAP Value (Concentration of $FeSO_4$ equivalent per µg of extract)	
		$FeSO_4$ (Standard)	ZMR	ZMP	ZMR	ZMP
1	20	0.963	0.005	0.049	50.61	54.00
2	40	1.119	0.007	0.093	47.23	53.84
3	60	1.356	0.024	0.094	47.15	52.53
4	80	1.831	0.033	0.173	41.07	51.84

Table 6
Antibacterial activity of methanolic extract of raw and preserved fruits of *Ziziphus mauritiana* Lam.

S.N.	Sample	*Escherichia coli*		*Staphylococcous aureus*	
		Concentration (µg/ml)	IZ (mm)	Concentration (µg/ml)	IZ (mm)
	Streptomycin	100	28	100	31
1	ZMR	250	Nil	250	Nil
		500	Nil	500	11
		1000	Nil	1000	11
	Streptomycin	100	28	100	27
2	ZMP	250	Nil	250	Nil
		500	Nil	500	Nil
		1000	Nil	1000	Nil



[ZMR – *Ziziphus mauritiana* Raw Fruit; ZMP – *Ziziphus mauritiana* Preserved Fruit]
Fig. 7: Determination of antioxidant activity of *Z.mauritiana* methanolic extract of raw and preserved fruits by FRAP assay ; Standard (Ferrous sulphate)

Table 7
Antifungal activity of methanolic extract of raw and preserved fruits of *Ziziphus mauritiana* Lam.

S.N.	Sample	*Aspergillus niger*		*Candida albicans*	
		Concentration (µg/ml)	IZ (mm)	Concentration (µg/ml)	IZ (mm)
	Clotrimazole	100	23	100	29
1	ZMR	250	Nil	250	Nil
		500	12	500	Nil
		1000	15	1000	Nil
	Clotrimazole	100	17	100	26
2	ZMP	250	Nil	250	Nil
		500	Nil	500	Nil
		1000	Nil	1000	Nil

2. Ferric Reducing Power Assay: Ferric reducing power assay was done to determine the antioxidant capacity of raw and preserved fruits samples of *Ziziphus mauritiana* and the results are illustrated in table 5 and figure 7. Ferrous sulphate ($FeSO_4$) (1mg/ml) was used as standard and absorbance was measured at 593 nm. The extract concentrations ranging from 20 to 80µg/ml were taken for the study. The reducing power capacity was detected as more for the preserved fruit than raw fruit extract. The antioxidant potential of the sample is measured through the reduction of Fe^{3+} TPTZ

complex (colourless) to Fe^{2+} TPTZ complex (blue coloration). The reduction of ferric ion to ferrous ion complex results in blue colouration and the absorbance was read at 593 nm. The absorbance value of standard ferrous sulphate peaked at 1.831 at 80µg/ml concentration. The raw and preserved fruits at 80µg/ml displayed the absorbance values 0.033 and 0.173 respectively. The blue colouration is because of the electron donating power of antioxidant present in the fruits samples.

In order to determine the antioxidant capacity of the raw and preserved fruit samples, the absorbance values were compared with those obtained from the linear standard curves of $FeSO_4$. The data in table 8 indicates that the absorbance of the samples – ZMR and ZMP proportionately increased due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration. The methanol extracts showed increased ferric reducing power with the increased concentration as standard antioxidant $FeSO_4$.

The extracts might be able to exert their action by breaking the free radical chain by donating electrons to free radicals. The sample ZMP showed increased ferric reducing power than ZMR for all the concentration levels in the range 20-80µg/ml. The concentration of $FeSO_4$ equivalent per µg of extract was determined to be higher for the extract of preserved fruit (ZMP sample) than the raw fruit extract (ZMR sample) for all the concentration levels (20 – 80µg/ml). In comparison to the standard $FeSO_4$, both extracts ZMR and ZMP indicated low reducing potential as indicated by the absorbance values. The reducing power activity of the fruits extracts increases with increasing concentration of the fruits extract[1].

The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and was expressed as $FeSO_4$ equivalents per micro gram of extract. Dorman et al[8] in their study concluded that ferric ion (Fe^{3+}) can be used as an electron donating indicator which is an important mechanism of phenolics[8].

Antibacterial Activity: The methanolic extracts of raw and preserved fruits of *Ziziphus mauritiana* were investigated for the antibacterial activity against one Gram negative bacteria (*Escherichia coli*) and one Gram positive bacteria (*Staphylococcus aureus*) and the observations are illustrated in table 6 and 7 and figures 8 and 9. The extract showed only moderate antibacterial activity when compared to the standard streptomycin. The zone of inhibition was measured in mm. The methanolic raw (Fig. 8a) and preserved fruit (Fig. 9a) extract showed no activity against *Escherchia coli* at the concentration of 250, 500 and 1000µg/ml compared with the standard streptomycin (28mm).

The methanolic extract of raw fruit has activity against *Staphylococcus aureus* (Fig. 8b) at the concentration ranges 500 (11mm) and 1000 µg/ml (11mm). But it shows no activity at the concentration of 250µg/ml. Standard streptomycin has 31mm zone of inhibition against *Staphylococcus aureus*. The preserved fruit (Fig. 9a and b) does not have any activity against both the bacterial strains. The standard has zone of inhibition 28 and 27mm respectively. Compared with the standard, the raw fruit extract has moderate antibacterial activity. The methanolic extracts of *Ziziphus mauritiana* fruit were reported to possess significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*[3].

Antifungal Activity: The methanolic extracts of raw and preserved fruits of *Ziziphus mauritiana* were evaluated for the antifungal activity. For the present investigation, two fungal strains *Aspergillus niger* and *Candida albicans* were used. The antifungal properties of both the extracts were identified by measuring the diameter of zone of inhibition in the culture plate. Clotrimazole (100µg) was used as standard. The concentrations taken for this present investigation are 250, 500 and 1000µg/ml. The methanolic raw fruit (Fig. 8c) extract of *Ziziphus mauritiana* shows activity against *Aspergillus niger* in 500 (12mm) and 1000µg/ml (15mm). At the range of 250µg/ml, no inhibition zone was observed.

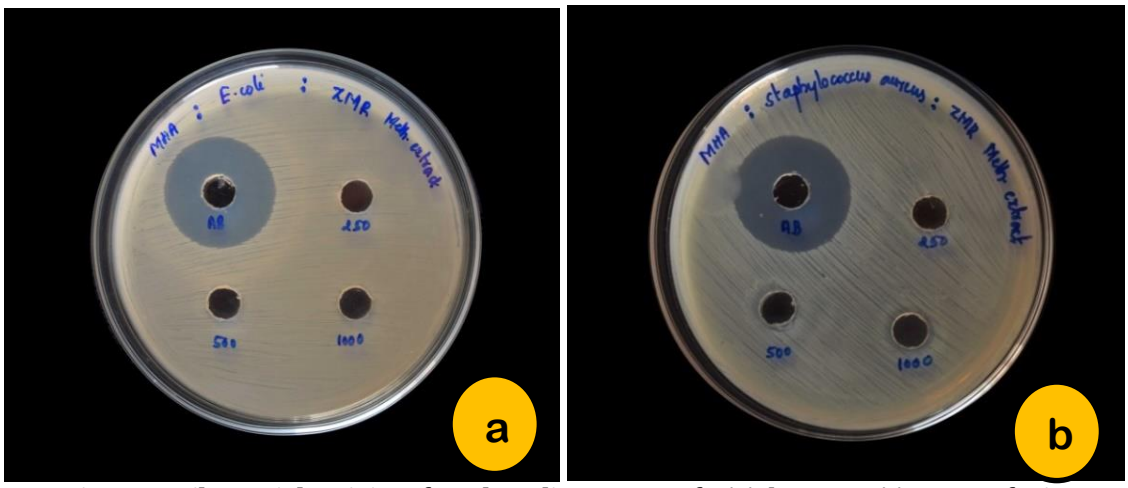


Fig. 8: Antibacterial activity of methanolic extracts of *Ziziphus mauritiana* raw fruit.
a) *Escherichia coli* b) *Staphylococcus aureus*

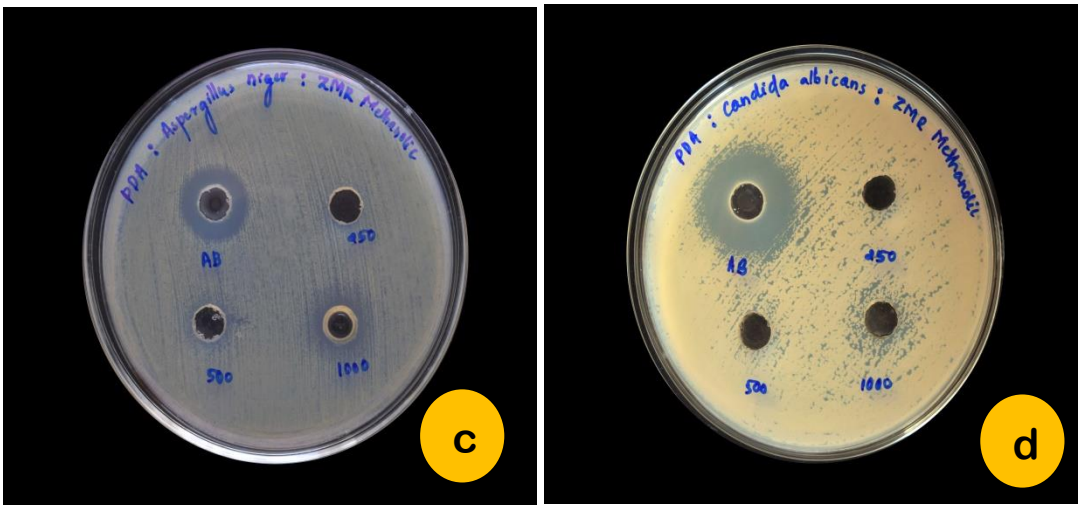


Fig. 8: Antifungal activity of methanolic extracts of *Ziziphus mauritiana* raw fruit.
c) *Aspergillus niger* d) *Candida albicans*

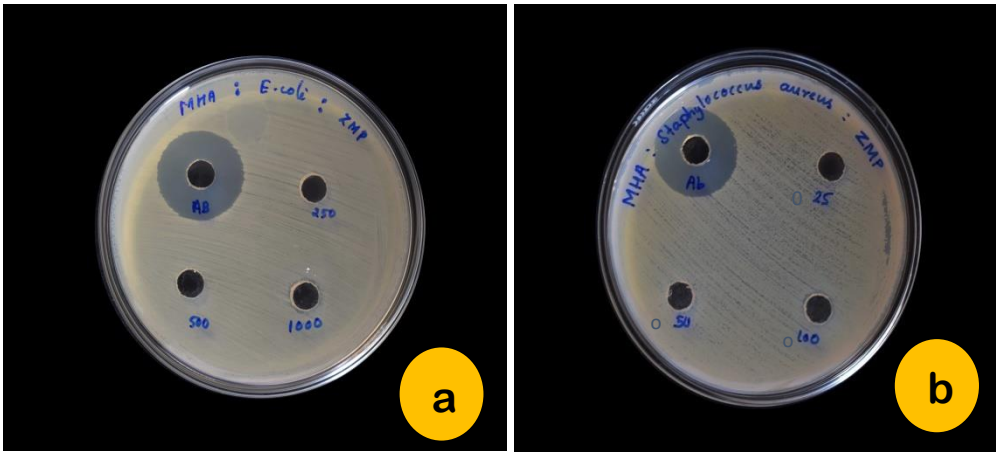


Fig. 9: Antibacterial activity of methanolic extracts of *Ziziphus mauritiana* preserved fruit.
a) *Escherichia coli* b) *Staphylococcus aureus*

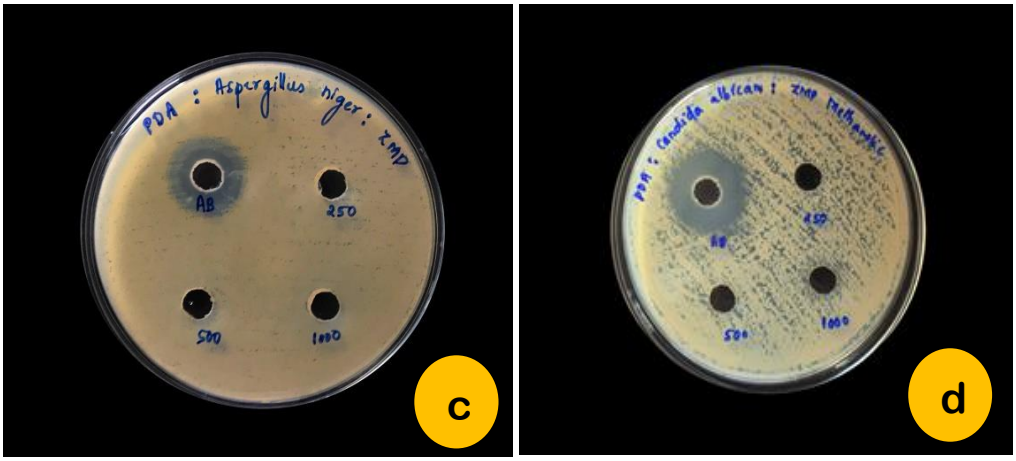


Fig. 9: Antifungal activity of methanolic extracts of *Ziziphus mauritiana* preserved fruit
c) *Aspergillus niger* d) *Candida albicans*

The antifungal activity of fruit extract was also investigated using *Candida albicans.* But both raw (Fig. 8d) and preserved fruit (Fig. 9d) extract have no activity against this fungal strain. The standard clotrimazole has 29 and 26mm zone of inhibition against *Candida albicans.* The preserved fruit (Fig. 9c and d) samples have no zone of inhibition against any fungal strains. Antimicrobial and antioxidant activity can be exhibited by most to the plant extracts due to the presence of high tannin content[21]. The differences in the zone of inhibition in antimicrobial activity are dependent on the nature of the chemicals, extraction methods[10,26] and several other factors.

The present study suggested that the fruit contains tannins only as a minor constituent and the major components detected were alkaloids. The relatively poor antimicrobial effect of the samples can be correlated with the results on phytoconstituent profile.

Conclusion

The major secondary metabolites detected from the qualitative phytochemical analysis of methanolic extract of raw fruit of *Ziziphus mauritiana* were alkaloids, flavonoids, tannin, phenol, carbohydrates, proteins, cardiac glycosides, triterpenoids, terpenoids and saponins. The highly polar solvent methanol gives almost all the phytoconstituents present in the raw fruit extract except anthraquinone glycosides, they were not detected from the fruit extract. The quantitative phytochemical analysis of the raw fruit extract of *Ziziphus mauritiana* suggested the presence of alkaloids (99µg/mg) as the major constituent and terpenoids (0.47µg/mg) as the least phytoconstituents present. The amount of saponin recorded was 56.46µg/mg. Alkaloids were the major constituents present in the methanolic fruit extract.

The DPPH and FRAP assays are conducted to determine the antioxidant potential of raw and preserved fruits of *Ziziphus mauritiana*. The present study revealed that the preserved fruit has high antioxidant potential than the raw fruit extract. Compared to standard ascorbic acid, it has high antioxidant potential. At 6.25 to 25µg/ml both the fruit extracts do not show any significant inhibition. From 50 to 2000µg/ml, the fruit extract shows antioxidant activity. The raw (59.30%) and preserved (71.67%) fruits exhibit highest percentage of inhibition at 2000µg/ml. The standard ascorbic acid has high antioxidant potential with IC_{50} 54.53µg/ml than the raw (IC_{50} 1349.70µg/ml) and preserved (IC_{50} 477.17µg/ml) fruit samples. The antioxidant potential of the samples (20 to 80µg/ml) was also determined by FRAP assay.

The results confirmed that the preserved fruit sample have high reducing power capacity than the raw fruit sample. The standard ferrous sulphate has high reducing power potential than the fruit samples. The antibacterial activity was determined with the help of *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) bacterial strains. The raw fruit extract indicated no zone of inhibition against *E.coli* and exhibited inhibition against *S.aureus* at the concentration ranges 500 (11mm) and 1000µg/ml (11mm). The preserved fruit sample did not indicate any zone of inhibition against *E.coli* and *S.aureus*. The standard streptomycin has high zone of inhibition than the fruits samples.

Antifungal potential of the fruit extract was also determined by two fungal strains *Aspergillus niger* and *Candida albicans*. The raw fruit extract showed zone of inhibition against *Aspergillus niger* at 500 (12mm) and 1000µg/ml (15mm). The standard clotrimazole has zone of inhibition 23mm against *Aspergillus niger*. The raw fruit has no inhibition against *Candida albicans* at 250, 500 and 1000µg/ml. The preserved fruit sample did not indicate any inhibition against *A.niger* and *C.albicans*. The standard clotrimazole has zone of inhibition higher than the fruit samples.

Acknowledgement

The authors are thankful to the Principal, University College, Thiruvananthapuram for providing Laboratory facilities for the study.

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(Received 22nd November 2023, accepted 05th February 2024)