Available online **www.ijpras.com**

International Journal of Pharmaceutical Research & Allied Sciences, 2017, 6(3):33-46

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Phytochemical Screening, Antioxidant and Antimicrobial Activities of Methanolic Extract of Ziziphus mauritiana Lam. Leaves Collected from Unaizah, Saudi Arabia

Ali Al Ghasham[1], Mohammed Al Muzaini[1], Kamal Ahmad Qureshi[1*], Gamal Osman Elhassan[1], Riyaz Ahmed Khan[1], Syeda Ayesha Farhana[1], Sana Hashmi[1], Elsayed El-Agamy[2], Wail E. Abdallah[3]

[1]Department of Pharmaceutics, Unaizah College of Pharmacy, Qassim University, Unaizah, K.S.A
[2]Department of Applied Medical Sciences, Unaizah Community College, Qassim University, Unaizah, K.S.A
[3]Department of Laboratory Sciences, College of Sciences and Arts, Qassim University, Al-Rass, K.S.A
**Correspondence Author: Kamal Ahmad Qureshi*
E-mail: Ka. afrah @ gmail. com

ABSTRACT

Ziziphus Mill. is the most important genera of the family Rhamnaceae, which is widely distributed all over the world and is used for centuries in locale medicine system. The main aim of this study was to investigate the phytochemical screening, antioxidant and antimicrobial activities of crude methanolic extract of Ziziphus mauritiana Lam. leaves against Staphylococcus aureus ATCC 25923, Streptococcus pneumoniae ATCC 49619, Enterococcus faecalis ATCC 29212, Bacillus cereus ATCC 10876, Escherichia coli ATCC 10536, Klebsiella pneumoniae ATCC 700603, Pseudomonas aerugenosa ATCC 27853, Proteus mirabilis ATCC 14153 and a pathogenic fungus; Candida albicans. The results of phytochemical screening have shown that, the plant extract has a detectable amount of bioactive constituents; cardiac glycosides, flavonoids, coumarins, tannins, phenols, phytosterols, quinones, resins, saponins and terpenoids. The results of antioxidant activity of plant extract have shown that, IC_{50} (half maximal inhibitory concentration) value of plant extract was 0.875 mg/mL, which suggests that, the plant extract has a significant level of free radicals scavenging activity. The plant extract has also shown a significant level of antibacterial activity against S.aureus ATCC 25923 (zone of inhibition diameter: 12 mm, MIC: 625 µg/mL, MBC: 1250 µg/mL), B.cereus ATCC 10876 (zone of inhibition diameter: 12 mm, MIC: 625 µg/mL, MBC: 1250 µg/mL), S.pneumoniae ATCC 49619 (zone of inhibition diameter: 10 mm, MIC: 156.25 µg/mL, MBC: 312.5 µg/mL) and antifungal activity against Candida albicans (zone of inhibition diameter: 10 mm, MIC: 125 µg/mL). Thus, the crude methanolic extract of Z.mauritiana Lam. leaves might be used for the curing of various human diseases.

Keywords: *Antibacterial, antifungal, antioxidant, phytochemical, plant extract*

INTRODUCTION

It is known that, plants are being used as main source of therapeutics for the human beings since centuries to till date. Medicinal plants have a great importance in the field of research because they are safe to use for the communities [1]. The medicinal plants have very complex chemical constituents called secondary metabolites,

which make them very important in the field of therapeutics [2, 3]. The secondary metabolites; alkaloids, glycosides, flavonoids, saponins, tannins and essential oils are known to have high therapeutic values.

Aromatic and medicinal plants are known as economically important plants because they are the major source of basic raw materials needed for locale pharma products, perfumery products, flavoring agents and cosmetic industries, thus they contribute into GDP of country via earning of foreign currency [4, 5]. Some medicinal plants have antibacterial, antifungal or antioxidant activities so they could be used as therapeutic agents for the curing of various human diseases like acne, boil, healing of wounds and various skin diseases, so they have great importance in locale medicine system [6].

The discovery of therapeutic agents for curing of various infectious diseases in human beings has started since long before the people were aware about the existence of microorganisms [7]. The emergence of antibiotic resistance in pathogenic microbes is the biggest challenge for the healthcare industry in both developing and developed countries. The emergence of multidrug-resistant (MDR) bacteria is the biggest challenge as well as burden in hospital and community settings [8].

Nowadays, the healthcare system is more advanced in antibiotic therapy, even though complications of infectious diseases are the most important causes of mortality in hospitalized patients. The synthetic antibiotics have many side effects which could remain lifelong in treated patients. To short out this problem, the actions must be taken for controlling and reducing of such cases and this can be done by controlling the use of antibiotics, by understanding the genetic role of antibiotic resistance, developing new antibiotics and changing the therapeutic strategies [9].

There are so many herbal plants which produce a variety of bioactive constituents of known therapeutic values. Antimicrobial activity is one of the most significant therapeutic values possessed by these medicinal plants [10]. The compounds, which could either, inhibit or kill the pathogens with no or minimum toxicity to host cells is regarded as the best candidates for developing the new antimicrobial agents [11, 12, 13].

Many reports have been published throughout the world during the last few years, which suggested that, the antimicrobial activities of medicinal plants have been increased [14, 15, 16].

Ziziphus Mill. is one of the most important genera of the family Rhamnaceae, which is widely distributed all over the world and is used for centuries in locale medicine systems. Till now, around 40 species of *Ziziphus* are in record and out of which *Z.mauritiana* Lam. is very common, especially in dry places. *Ziziphus* species are very rich source of carbohydrates, proteins, vitamins and fibers [17]. *Z.mauritiana* Lam. leaves are very rich in minerals, thus some animals like cattle, camels, goats feed on these leaves to get benefit for their health [18]. *Z.mauritiana* Lam. fruits are very rich in fibers and possess laxative activity and thus, very beneficial for human health [17]. *Z.mauritiana* Lam. leaves are utilized to treat some diseases like liver problem, asthma and fever, while the powder of root is used for wounds' healing [19].

The main aim of present study was to investigate the phytochemical screening, antioxidant activity and antimicrobial activities of methanolic extract (crude) of *Z.mauritiana* Lam. leaves against the bacteria; *S.aureus* ATCC 25923, *S.pneumoniae* ATCC 49619, *E.faecalis* ATCC 29212, *B.cereus* ATCC 10876, *E.coli* ATCC 10536, *K.pneumoniae* ATCC 700603, *P.aerugenosa* ATCC 27853, *P.mirabilis* ATCC 14153 and a pathogenic fungus; *Candida albicans.*

MATERIALS AND METHODS

Solvent and Materials

Absolute Methanol (Analytic grade), high speed centrifuge machine, Mueller Hinton agar, Mueller Hinton broth, sterile normal saline, sterile blank paper discs, standard antibiotic discs, Whatman filter paper grade No. 01 and 03, DPPH (1, 1-diphenyl-2-picrylhydrazyl), concentrated hydrochloric acid, Wagner's reagent, ferric chloride solution, glacial acidic acid, conc. sulphuric acid, chloroform, sodium hydroxide, Fehling solution, acetic anhydride, ammonia solution etc.

Collection of Plant Material and Identification

Fresh leaves of plant were collected from a farmhouse at Unaizah, Saudi Arabia and stored in a clean polythene bag, labeled with a temporary code; KA 05. The plant material (shown in figure 01) was identified via verification and authentication by Dr. Wail E. Abdallah; Plant taxonomist; College of Sciences and Arts at Al-Rass, Saudi Arabia, and a voucher herbarium specimen (Rass-KA 05/ 2017) was stored at the College Herbarium at Al-Rass, KSA.

Figure 01: Branch of *Z. mauritiana* Lam. showing leaves and immature fruits

Test organisms

The standard pathogenic bacteria; *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619, *Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 10536, *Klebsiella pneumoniae* ATCC 700603, *Proteus mirabilis* ATCC 14153, *Pseudomonas aerugenosa* ATCC 27853 and a pathogenic fungus; *Candida albicans* were collected from the department of Microbiology, King Saud Hospital, Unaizah, KSA.

Extraction [20, 21]

Z.mauritiana Lam. leaves were cleaned and rinsed thoroughly with distilled water and then leaves were dried in shade for 5-7 days at 30ºC temperature. The dried leaves were crushed to make fine powder using an electric blender and then powder was kept in a sterile airtight container until used. The maceration method was followed for the extraction.

10-15 grams powder of dried leaves was added into 100 mL of 80 % methanol in an Erlenmeyer flask (250 mL capacity) and resulting mixture was vortexed well. The maceration process was carried out in shaker incubator at 45ºC temperature with 50 rpm for 48-72 hrs. After incubation, the macerated sample was filtered by using Whatman filter paper grade 01 followed by high speed centrifugation at 5000 rpm for 15 minutes. Then, the centrifuged extract was re-filtered through 0.45 µm filter paper by using Millipore filter assembly. The supernatant was kept for evaporation in a glass petri dish at 45ºC for 24 hours. After evaporation of solvent, the dried methanolic extract was scratched by spatula. The dried methanolic extract was used for phytochemical screening, antioxidant and antimicrobial activities.

Phytochemical Screening [20-25]

The phytochemical screening was carried out to detect the following phytochemical constituents:

Alkaloids

50 mg plant extract was mixed well with 2 mL of 1% HCl solution, followed by slight heating till the steaming. After that, six drops of Wagner's reagent were added into 1 mL of acidified extract.

Cardiac glycosides

50 mg plant extract was dissolved into 2 mL of chloroform, followed by addition of few drops of H_2SO_4 solution at the side of test tube to form a layer.

Flavonoids

1 mL of plant extract (50 mg/mL) was added into 1 mL of 10 % lead acetate solution.

Phenols

1 mL of plant extract (50 mg/mL) was added into 0.5 mL of 10 % ethanolic ferric chloride solution.

Phlobatannins

2 mL of plant extract (50 mg/mL) was added into 2 mL of 1 % HCl solution, followed by boiling the mixture.

Steroids

1 mL of plant extract (50 mg/mL) was mixed with 1 mL of chloroform, followed by addition of 2 mL of acetic anhydride and then 1-2 drops of concentrated H_2SO_4 solution.

Tannins

Few drops of 1 % lead acetate were added into 2 mL of plant extract (50 mg/mL).

Terpenoids

2.5 mL of plant extract (50 mg/mL) was mixed with 1 mL of chloroform and then 1.5 mL of concentrated H_2SO_4 solution was added.

Volatile oil

An equal quantity (100 µL) of diluted sodium hydroxide and diluted hydrochloric acid were added into 2 mL of plant extract (50 mg/mL) and mixed well.

Saponins

5 mL of purified distilled water was taken into a test tube, followed by addition of 500 µL of plant extract (50 mg/mL) and then whole mixture was well stirred.

Leucoanthocyanins

2 mL of isoamyl alcohol was taken into a test tube, followed by slowly addition of 2 mL of plant extract (50 mg/mL).

Coumarins

1 mL of plant extract (50 mg/mL) was mixed with 1.5 mL of 10 % NaOH solution.

Emodins

1 mL of ammonia and 1.5 mL of benzene solutions were added into 1 mL of plant extract (50 mg/mL).

Phytosterols

50 mg of plant extract was treated with 2 mL of chloroform, followed by filtration through a Whatman filter paper grade 01. Few drops of acetic anhydride solution were added into filtrate, followed by boiling the filtrate. After cooling of filtrate, few drops of concentrated H_2SO_4 were added slowly at the side of test tube.

Quinones

1 mL of concentrated sulphuric acid was taken into a test tube, followed by addition of 1 mL of plant extract (50 mg/mL).

Resins

Few drops of acetic anhydride solution were added into 1 mL of plant extract (50 mg/mL), followed by addition of 1 mL of concentrated sulphuric acid.

Carboxylic acid

2 mL of sodium bicarbonate solution was added into 1 mL of plant extract (50 mg/mL).

Antioxidant (free radical scavenging) Activity [20, 21, 26, 27]

A modified DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay method was followed to detect antioxidant (free radical scavenging) activity of plant extract. The fresh working solution of DPPH was prepared by dissolving 3 milligrams (0.003 gram) powder of DPPH reagent into 100 mL of methanol (0.003 %). The working solution was kept in brown bottle at dark conditions till the use. Methanol was used as blank. The negative control was prepared by addition of 50 µL of methanol into 2 mL of freshly prepared DPPH solution. The positive control was prepared by addition of 50 µL of ascorbic acid into 2 mL of freshly prepared DPPH solution. Ascorbic acid was used as a standard antioxidant compound. The antioxidant assay was carried out by addition of 50 µL of plant extract into 2 mL of freshly prepared DPPH solution. Various concentrations of plant extract; 1, 2, 3 and 4 mg/mL were prepared, followed by determination of antioxidant activity of each concentration. All the test tubes (test, blank, negative control and positive control) were incubated in a dark room at room temperature for 30 minutes and then absorbance (O.D) of all the solutions were measured by using the UV-spectrophotometer at 517 nm. The whole protocol of determination of antioxidant activity has been summarized in table 01.

Table 01: Determination of antioxidant activity of *Z. mauritiana* Lam. leaves extract

Reagents	Blank	Control	Test
1. DPPH solution	-	2 mL	2 mL
2. Methanol	2 mL	50 µL	-
3. Plant extract	-	-	50 µL

The following formula was used to calculate the % inhibition of DPPH reagent:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of Negative Control} - \text{Absorbance of sample})}{(\text{Absorbance of Negative Control})} \times 100$$

The test values of antioxidant assay were used to plot an ascending graph (inhibition percentages vs. various concentrations of plant extract). The IC_{50} (half maximal inhibitory concentration) value of plant extract was obtained from this graph.

Antimicrobial Activities [11-12, 20-21]

Disc diffusion, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) assay methods were used to determine the antimicrobial activities of plant extract. 500 mg of plant extract was dissolved into 1 mL of 20 % DMSO (dimethyl sulphoxide) solvent and then 20 µL of resulting solution was pipette out on sterile Whatman filter paper grade 03 discs (6 mm). The suspensions of test organisms were prepared by mixing of around 5-6 colonies of each test organism in 1 mL of tryptic soy broth respectively. The turbidity of suspensions was adjusted equivalent to 0.5 McFarland standard.

100 µL quantity of each test organism suspension was inoculated on the surface of Mueller Hinton agar plates (for each test organism suspension, a separate MH agar plate was used). Sterile swabs were used to spread the suspensions on agar plates. The working plant extract discs (10 mg/disc) were dispensed on test agar plates and then test agar plates were incubated at 37ºC temperature for 24-48 hours. After incubation, the diameters of zone of inhibitions were measured. Vancomycin (30 µg) and Tobramycin (10 µg) antibiotic discs were used as standard antibiotics. The results of plant extract were compared with the results of standard antibiotics.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

MIC and MBC values of plant extract were determined by macro-broth dilution assay method. The stock solution of plant extract was prepared by dissolving 200 mg of methanolic extract into 2 mL of 20 % DMSO.

Minimum Inhibitory Concentration (MIC) for Bacteria

Around 5 colonies of each test bacteria (fresh culture) were suspended into 5 mL of sterile normal saline respectively and then, the turbidity of each bacterial suspensions were adjusted equivalent to 0.5 McFarland i.e. ~1-2 × 10^8 CFU/mL. Each bacterial suspension was diluted 1: 150 in sterile Mueller-Hinton broth. Now, the concentrations of resulting bacterial suspensions were approximately 1 × 10^6 CFU/mL. Before adjusting the concentration of bacterial inoculum, 1 mL quantity of various concentrations of plant extract were placed in 12 x 75 mm glass tubes. Within 15 minutes of inoculum standardization, 1 mL quantity of adjusted bacterial inoculum was added to each test tube in dilution series and vortexed well. The final concentrations of test bacteria were 5 × 10^5 CFU/mL respectively (1:1 plant extract: bacteria suspension). 1 mL suspension of test bacterium in Mueller-Hinton broth (without plant extract) was used as growth control tube. 1 mL of sterile Mueller-Hinton broth (without test organism) was used as Negative control.

Minimum Bactericidal Concentration (MBC) for Bacteria [20, 28]

100 µL of sample was taken from each MIC test tubes which showed no visible growth and then samples were inoculated on sterile tryptic soy agar plates. All inoculated plates were incubated at 37ºC temperature for 24-48 hrs.

Table 02: Dilution scheme of methanolic extract of *Z. mauritiana* Lam. Leaves

Sr. No.	Methanolic Extract: Stock Concentration (µg/mL)	Bacterial suspension + Methanolic Extract [1:1 Dilution] (mL)	Methanolic Extract: Final Concentration (µg/mL)	Fungal suspension + Methanolic Extract [1:10 Dilution] (mL)	Methanolic Extract: Final Concentration (µg/mL)
1.	100000	1 + 1	50000	0.9 + 0.1	10000
2.	50000	1 + 1	25000	0.9 + 0.1	5000
3.	40000	1 + 1	20000	0.9 + 0.1	4000
4.	20000	1 + 1	10000	0.9 + 0.1	2000
5.	10000	1 + 1	5000	0.9 + 0.1	1000
6.	5000	1 + 1	2500	0.9 + 0.1	500
7.	2500	1 + 1	1250	0.9 + 0.1	250

8.	1250	1 + 1	625	0.9 + 0.1	125
9.	625	1 + 1	312.5	0.9 + 0.1	6.25
10.	312.5	1 + 1	156.25	0.9 + 0.1	31.25

Minimum Inhibitory Concentration (MIC) for Fungi

Around 5 colonies of *C.albicans* (fresh culture) were suspended into 5 mL of sterile normal saline and then turbidity of suspension was adjusted equivalent to 0.5 McFarland standard i.e. ~1 x 10^6 to 5 x 10^6 cells/mL. The inoculum of *Candida albicans* was further diluted; 1:100, followed by 1:20 dilution by using sterile tryptic soy broth, which resulted in 5.0 x 10^2 to 2.5 x 10^3 cells/ mL.

Before adjusting the concentration of *Candida* cells in inoculum, 100 µL quantities of various concentrations of plant extract were placed in 12 x 75 mm test tubes. Within 15 minutes of inoculum standardization, 900 µL quantity of adjusted inoculum was placed into each test tube in the dilution series. All the test tubes of dilution series were vortexed well. This results in 1:10 dilution of each concentration of plant extract. The final concentration of *C.albicans* in suspensions was 50-25 cells/mL. 1 mL suspension of *C.albicans* in tryptic soy broth (without plant extract) was used as growth control tube. 1 mL of sterile tryptic soy broth (without *C. albicans*) was used as Negative control.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of Phytochemical screening of methanolic extract of *Z.mauritiana* Lam. leaves have showed that, the extract has significant level of bioactive compounds; coumarins, cardiac glycosides, flavonoids, phenols, phytosterols, quinones, resins, saponins, terpenoids and tannins. The results have been summarized in table 03.

Table 03: Phytochemical analysis of methanolic extract of *Z. mauritiana* Lam. leaves

Metabolites	Observation	Results	Metabolites	Observation	Results
Alkaloids	No Brownish-red precipitate formed	-	**Phenol/Polyphenols**	Blue green to dark blue coloration formed	+
Phlobatannins	No red precipitate Formed	-	**Phytosterols**	Brown colored ring formed at the junction	+
Carboxylic acid	No Effervescence formed	-	**Quinones**	Formation of red coloration observed	+
Cardiac glycosides	Brown ring formed at interphase	+	**Resins**	Formation of orange to yellow coloration observed	+
Coumarins	Yellow coloration formed	+	**Saponins**	Formation of continues effervescence observed	+
Emodins	No red coloration formed	-	**Steroids**	No dark green coloration observed	-
Flavonoids	Yellow colored precipitate formed	+	**Tannins**	Dark blue or greenish grey coloration observed	+

Terpenoids	Reddish brown color formed at interface	+	**Leucoanthocyanins**	No red coloration observed at upper layer	-
Volatile oil	No white precipitate formed	-			

- = Phytochemical Absent, + = Phytochemical Present

The results of Phytochemical screening suggest that, the methanolic extract of *Z.mauritiana* Lam. leaves is rich in various bioactive constituents such as coumarins, cardiac glycosides, flavonoids, phenols, phytosterols, quinones, resins, saponins, terpenoids and tannins. These phytochemicals are important for the use in health care.

The findings of the present study agreed with previous studies; Najafi in 2003 reported that, leaves of *Z.mauritiana* possess saponins, phenolic compounds, glycosides and tannins [29], Parmar *et al.* in 2012 reported that, leaves of *Z.mauritiana* possess phenolic compounds, glycosides, saponins, tannins and lignins [17]. Abdullah *et al.* in 2016 reported that, the methanolic extract of *Z.mauritiana* leaves contains saponins, tannins, alkaloids, phenolic compounds, terpenoids and flavonoids [20].

The effect of saponins on human health is anti-inflammatory, thus using those plants having saponins can strengthen the immune system. The tannins have antibacterial activity and can damage the bacterial cell wall [30].

Antioxidant (free radical-scavenging) Activity

The results of Antioxidant (free radical-scavenging) activity have shown that, IC_{50} (half maximal inhibitory concentration) value of methanolic extract of *Z.mauritiana* Lam. leaves was observed as 0.875 mg/mL. The results of free radical-scavenging Activity of plant extract were compared with the results of ascorbic acid; a standard antioxidant agent. The results have been summarized in Table 04 and Figure 02.

Table 04: Free radicals scavenging activity of methanolic extract of *Z. mauritiana* Lam. leaves

Compounds	**Concentration**	**Free radical-scavenging activity (% inhibition)**
Methanolic Extracts of *Z.mauritiana* Lam. leaves	1.0 mg/mL	56.69
	2.0 mg/mL	82.85
	3.0 mg/mL	84.45
	4.0 mg/mL	86.63
Ascorbic Acid	100 µg	72.50

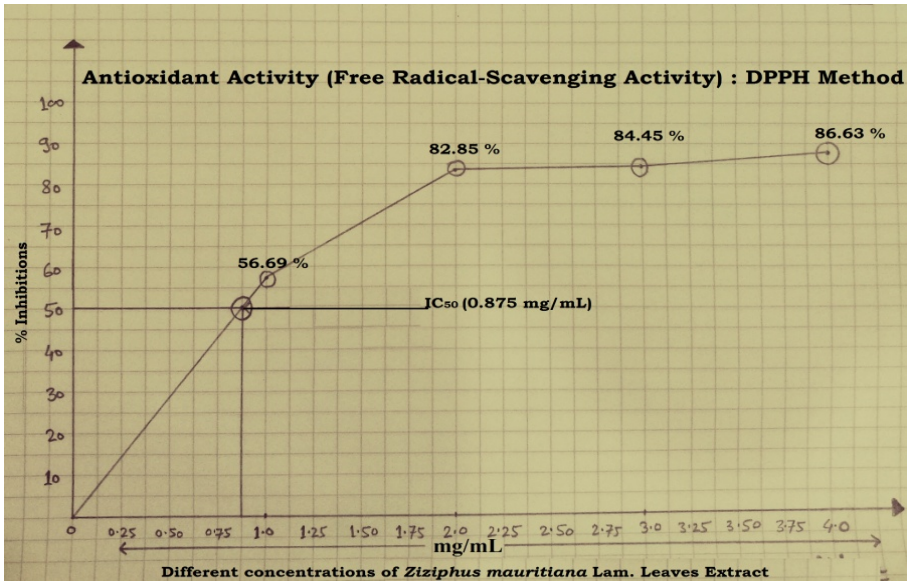


Figure 02: Free radical-scavenging activity (IC_{50}) of *Z. mauritiana* Lam. leaves

Alkaloids, phenolic compounds, glycosides, tannins, flavonoids and saponins are known as good antioxidant compounds which can help in controlling the oxidative stress related disorders [31]. During oxidation, free radicals generate, that is one of the major causes of degenerative diseases [33]. Therefore, in the present study, DPPH free radicals scavenging activity of methanolic extract of *Z.mauritiana* leaves were evaluated and in the present study, the results of DPPH free radicals scavenging activity of methanolic extract suggested that, *Z.mauritiana* leaves have a significant level of antioxidant activity. The results were compared to the results of ascorbic acid; a standard antioxidant agent.

Some studies supported this claim; Ashraf *et al*. in 2015 claimed that, IC_{50} of *Z.mauritiana* leaves was 0.11 mg/mL [34]. Sharma *et al*. in 2015 claimed that methanolic extract of *Z.mauritiana* has IC_{50} = 47.50 µg/mL [35]. Perumal *et al*. in 2011 claimed that, the extract of another species; *Z. jujube* leaves has IC_{50} = 20.62 µg/mL [36]. The antioxidant activity of methanolic extract of *Z.mauritiana* Lam. leaves might be due to the presence of antioxidant compounds such as flavonoids and ascorbic acid [37, 38].

Antimicrobial Activities

Disc Diffusion Method

The results of Antimicrobial activities of plant extract by disc diffusion method have shown that, the plant extract has a significant level of antibacterial activity against the gram-positive bacteria; *B.cereus* ATCC 10876 (12 mm), *S.aureus* ATCC 25923 (12 mm) and *S.pneumoniae* ATCC 49619 (10 mm) but has either very low or non-significant level of antibacterial activity against the gram-negative bacteria; *E.faecalis* ATCC 29212 (6.0±2.0 mm), *E.coli* ATCC 10536 (6.0±2.0 mm), *K.pneumoniae* ATCC 700603 (6.0±2.0 mm), *P.aerugenosa* ATCC 27853 (6.0±2.0 mm) and *P. mirabilis* ATCC 14153 (6.0 mm).

The result of antifungal activity has showed that, the plant extract has a significant level of antifungal activity against to *Candida albicans* (10 mm). The results of the disc diffusion method have been summarized in Table 05 and Figures 03-11.

Table 05: The Antimicrobial Activity of the methanolic extract of *Z. mauritiana* Lam. Leaves

Compounds	Mean diameter of zone of inhibition in mm (disc diameter 6 mm)		
	Gram-positive bacteria	Gram-negative bacteria	Fungi

	Bc	*Ef*	*Sa*	*Sp*	*Ec*	*Kp*	*Pm*	*Pa*	*Ca*
Extract (10 mg/disc)	12.0	6.0±2.0	12.0	10.0	6.0±2.0	6.0±2.0	6.0	6.0±2.0	10.0
Vancomycin (30 µg/disc)	16	14	12	16	-	-	-	-	N/A
Tobramycin (10 µg/disc)	-	-	-	-	20	10	17	15	N/A

Key: Disc diameter = 6.0 mm, 6.0 ± 2.0 = No activity or non significant activity, N/A = Not applicable, *Sa* = *Staphylococcus aureus* ATCC 25923, *Sp* = *Streptococcus pneumoniae* ATCC 49619, *Ef* = *Enterococcus faecalis* ATCC 29212, *Bc* = *Bacillus cereus* ATCC 10876, *Ec* = *Escherichia coli* ATCC 10536, *Kp* = *Klebsiella pneumoniae* ATCC 700603, *Pa* = *Pseudomonas aerugenosa* ATCC 27853, *Pm* = *Proteus mirabilis* ATCC 14153, *Ca* = *Candida albicans* (clinical isolate).

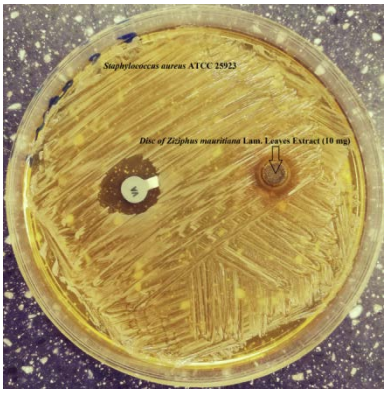


Fig. 03: *Staphylococcus aureus* ATCC 25923

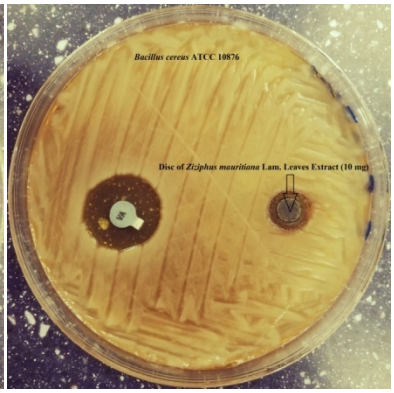


Fig. 04: *Bacillus cereus* ATCC 10876

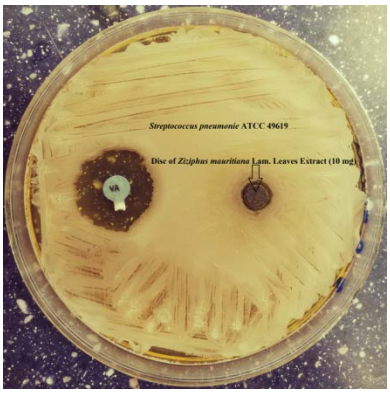


Fig. 05: *Streptococcus pneumoniae* ATCC 49619

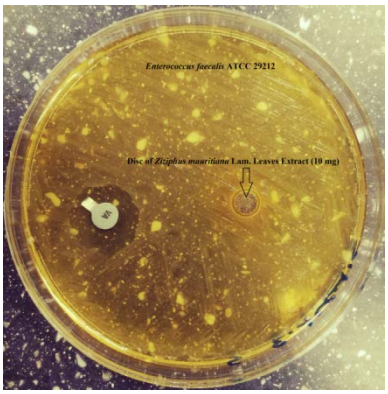


Fig.06: *Enterococcus faecalis* ATCC 29212

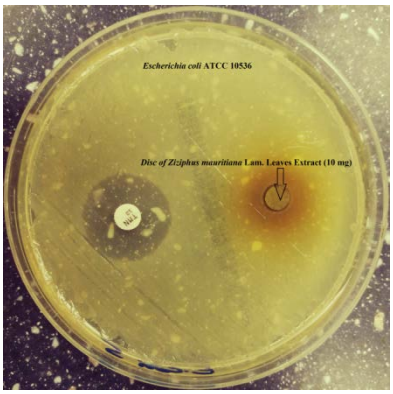


Fig.07: *Escherichia coli* ATCC 10536

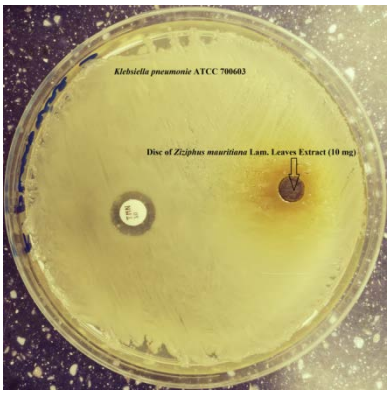
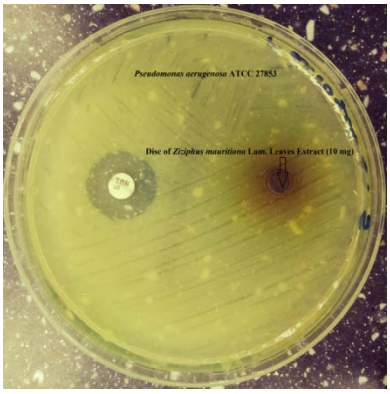
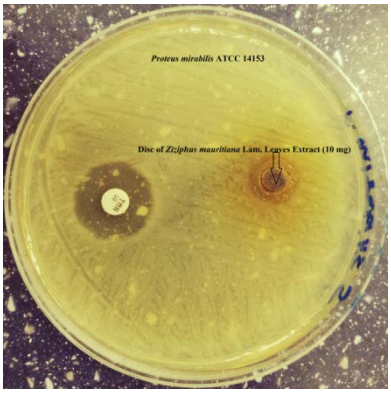


Fig.08: *Klebsiella pneumoniae* ATCC 700603





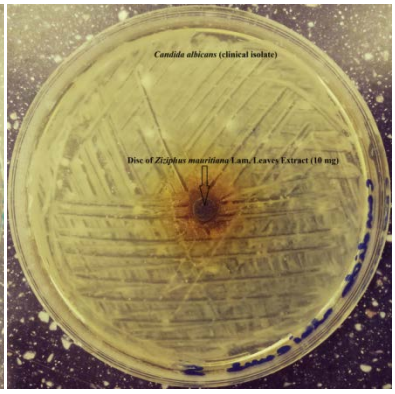


Fig.09: ***Pseudomonas aerugenosa*** **ATCC 27853** **Fig.10:** ***Proteus mirabilis*** **ATCC 14153** **Fig.11:** ***Candida albicans (clinical isolate)***

MIC (Minimum Inhibition Concentration) and MBC (Minimum Bactericidal Concentration)

The results of MIC and MBC have showed that, the MIC and MBC values of plant extract were observed as 625 µg/mL and 1250 µg/mL respectively for both *S.aureus* ATCC 25923 and *B.cereus* ATCC 10876 but for *S.pneumoniae* ATCC 49619; 156.25 µg/mL and 312.5 µg/mL respectively. The result of MIC for *C.albicans* was observed as 125 µg/mL.

The results of MIC and MBC have been summarized in table 06.

Table 06: Results of MIC and MBC of methanolic extract of *Z.mauritiana* Lam. leaves

Test organisms	MIC (µg/mL)	MBC (µg/mL)
1. ***Staphylococcus aureus*** **ATCC 25923**	625	1250
2. ***Bacillus cereus*** **ATCC 10876**	625	1250
3. ***Streptococcus pneumoniae*** **ATCC 49619**	156.25	312.5
4. ***Candida albicans*** **(clinical isolate)**	125	-

The results of antimicrobial activities of methanolic extract of *Z.mauritiana* Lam. leaves have revealed that, the plant extract has a significant level of antimicrobial activities against bacteria; *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619 and a pathogenic fungus; *Candida albicans*. These results further suggested that, the plant extract could be used in herbal preparations for the treatment of some skin diseases caused by these pathogens.

Some published studies have supported the claim of this study; Najafi in 2013 reported that, the methanolic extract of *Z.mauritiana* leaves exhibits a significant level of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [29]. Ashraf et al. in 2015 claimed that, the methanolic extract of *Z.mauritiana* leaves possesses a significant level of antibacterial effects against the *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* [32].

A recent study carried out by Abdullah et al. in 2016 reported that, the methanolic extract of *Z mauritiana* leaves has a significant level of antimicrobial activities against *Proteus vulgaris* and *Bacillus cereus* ATCC 10876 [20].

However, the current study suggested that, the plant extract has not possessed a significant level of antibacterial activity against gram-negative bacteria.

In addition to this, some published papers claimed that, leave extract of *Z.mauritiana* has none-significant level of antibacterial activity against to common bacteria; Mainasara *et al*. in 2012 claimed that, the ethanolic and methanolic extracts of *Z.mauritiana* leaves did not show a significant level of antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aerugenosa*, *Escherichia coli* and *Salmonella typhi* at 120 mg/mL concentration [30], which supports the claim of this study that, this plant extract has no significant level of antibacterial activity against gram negative bacteria.

CONCLUSION

The crude methanolic extract of *Ziziphus mauritiana* Lam. leaves is rich in phytochemical constituents which have significant level of antioxidant and antimicrobial activities. The isolation and purification of these bioactive phytochemical constituents may further yield more significant levels of antioxidant activity, antimicrobial activities or other curative properties against different health ailments.

ACKNOWLEDGEMENTS

This study was conducted for the partial fulfillment of the degree of PharmD. The authors are highly thankful of Dean; Unaizah College of Pharmacy: Dr. Alian Ali Alrasheedy for supporting throughout the research. The authors are also thankful of King Saud Hospital's staff members: Dr. Sulaiman Almeman, Dr. T. Padma Satya Kumari, Mr. Mamduh and Mr. Abdul Mohsen for providing technical help during this research.

REFERENCES

1. Najafi, S., Sanadgol, N., Sadeghinejad, B., Ashofteh Beira, S., Phytochemical screening and antibacterial activity of *Citrullus Colocynthis* (L.) Schrad. against *Staphylococcus aureus*, Journ. Oral Med. Path. Radio., 2010; 4(9): 2321-2325.

2. Karthikeyan, A., Shanthi, V., Nagasathaya, A., Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica* L., Int. J. Green Pharm., 2009; 3(1): 78-80.

3. Lozoya, M., Lozoya, X., Pharmacological properties *in vitro* of various extracts of Mimosa pudica Linn., Tepezcohuite Arch. Invest. Mex., 1989; 12; 87-93.

4. Aiyelaagbe, O., Antibacterial activity of *Jatropha multifida* roots, J. Fit., 2001; 72(5): 544-546.

5. Augusti, K.T., Therapeutic values of onion and garlic, Indian J. Exp. Biol.,1996; 34(3): 634-640.

6. Arora, D., Kaur, J., Antimicrobial activity of spices, Int. J. Antimicrob. Agents, 1999; 12 (3): 257-262.

7. Sofowora, A., Medicinal Plants and Traditional medicine in Africa. Published by John Wiley and Sons Ltd. 1st edition., 1982; 131: 168-171.

8. Cosgrove, S.E., Carmeli, Y., The impact of antimicrobial resistance on health and economic outcomes, Clin. Infect. Dis., 2003; 36(11): 1433-7.

9. Nascimento, G.G.F., Locatelli, J., Freitas, P.C., Silva, GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria, Braz. J. Microbiol., 2000; 31(4): 247-56.

10. Bolla, J.M., Alibert-Franco, S, Handzlik, J., Chevalier, J., Mahamoud, A., Boyer, G., Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria, FEBS. Lett., 2011; 585(11): 1682-90.

11. Qureshi, K.A., Bholay, A.D., Elhassan, G.O, Khan, R.A., El-Agamy. E.I., Isolation, purification and characterization of bacteriocin produced by *Bacillus pumilus* NJ-M2; A Future Biopreservative, Intern. Journ. Biol. Pharm. Allied Scien., 2016; 5(11): 2840-2862.

12. Alanazi, A.S., Qureshi, K.A., Elhassan, G.O., Elsayed El-Agamy E.I., Isolation, purification and characterization of antimicrobial agent antagonistic to *Escherichia coli* ATCC 10536 produced by *Bacillus pumilus* SAFR-032 isolated from the soil of Unaizah, Al Qassim province of Saudi Arabia, Pak. J. Bio. Sci. 2016; 19(5): 191-201.

13. Lee, C.K., Kim, H., Moon, K.H., Shin, K.H., Screening and isolation of antibiotic resistance inhibitors from herb materials-resistance inhibition of volatile components of Korean aromatic herbs, Arch. Pharm. Res., 1998; 21(1): 62-6.

14. Grosvenor, P.W., Supriono, A., Gray, D.O., Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2: antibacterial and antifungal activity, J. Ethnopharmacol., 1995; 45(2): 97-111.

15. Ahmad, I., Beg, A.Z., Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens, J. Ethnopharmacol, 2001; 74(2); 113-23.

16. Abu-Shanab, B.A., Adwan, G., Adwan, K.M., Abu-Shanab, F., Efficacy of aqueous and ethanol extracts of some Palestinian medicinal plants for potential antibacterial activity, Islam. Univ. J. Gaza, 2008; 16(2): 77-86.

17. Parmar, P., Bhatt, S., Dhyani, S., Jain, A., Phytochemical studies of the secondary metabolites of *Ziziphus mauritiana* Lam. Leaves, Int. J. Pharma. R., 2012; 4(3): 153-155.

18. Morton, J., Indian Jujube. In: Fruits of warm climates. Julia, F., Morton, J. (eds), Miami, FL., 1987; 272-275.

19. Dahiru, D., Sini, J.M., John-Africa, L., Antidiarrhoeal activity of *Ziziphus mauritiana* root extract in rodent, Afr. J. Biotech., 2006; 5(10): 941-945.

20. Abdallah, E.M., Elsharkawy, E.R., Ed-dra, A., Biological activities of methanolic leaf extract of *Ziziphus mauritiana*, Biosci. Biotech. Res. Comm., 2016; 9(4): 605-614.

21. Abdallah, E.M., Qureshi, K.A., Musa, K.H., Antimicrobial, antioxidant and phytochemical screening of Lupin seeds (*Lupinus termis* Forssk.) from Sudan, CIBTech J. Micro., 2017; 6 (1): 1-8.

22. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H., Phytochemical screening and Extraction: A Review, Int. Pharma., Sci., 2011; 1(1): 98-106.

23. Senguttuvan, J., Paulsamy, S.K., Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities, Asian Pac. J. Trop. Biomed., 2014; 4(1): 359-367.

24. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Yoga Latha, L., Extraction, isolation and characterization of bioactive compounds from plants' extracts, Afr. J. Tradit. Complement Altern. Med., 2011; 8(1): 1-10.

25. Yusuf, A.Z., Zakir, A., Shemau, Z., Abdullahi, M., Halima, S.A., Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* Linn., J. Pharma. Phyto., 2014; 6(2): 10-16.

26. Chandra Shekhar, T., Anju, G., Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves, A. J. Ethno., 2014; 1(4): 244-249.

27. Marinova, G., Batchvarov, V., Evaluation of the methods for determination of the free radical scavenging activity by DPPH, Bul. J. Agri. Sci., 2011; 17 (1): 11-24.

28. Doughari, J.H., Antimicrobial Activity of Tamarindus indica Linn., Trop. J. Pharm. Res., 2006; 5(2): 597-603.

29. Najafi, S., Phytochemical screening and antibacterial activity of leaf extract of *Ziziphus mauritiana* Lam., Int. Res. J. Appl. Basic Sci., 2013; 4 (11): 3274-3276.

30. Mainasara, M.M., Aliero, B.L., Aliero, A.A., Yakubu, M., Phytochemical and antibacterial properties of root and leaf extracts of *Calotropis procera*, Nig. J. Basic Appl. Sci., 2012; 20(1): 1-6.

31. Biapa, P.C., Agbor, G.A., Oben, J.E., Ngogang, J.Y., Phytochemical studies and antioxidant properties of four medicinal plants used in Cameroon, Afr. J. Trad. Complement Altern. Med., 2007; 4(4): 495-500.

32. Ashfaq, M., Shah, K.W., Ahmad, S., Singh, D., Preliminary phytochemical screening of alcoholic and aqueous extracts of Mentha longifolia Linn. Leaves, Int. J. Biol. Pharm. Res., 2012; 3(3): 384-386.

33. Pham-Huy, L.A., He, H., Pham-Huy, C., Free radicals, antioxidants in disease and health, Int. J. Biomed. Sci., 2008; 4(2): 89-96.

34. Ashraf, A., Sarfraz, R.A., Anwar, F., Shahid, S.A., Alkharfy, K.M., Chemical composition and biological activities of leaves of *Ziziphus mauritiana* Lam. native to Pakistan, Pak. J. Bot., 2015; 47(1): 367-376.

35. Sharma, K.R., Kalauni, S., Awale, S., Pokharel, Y.R., *In vitro* free radical scavenging activity of methanol extracts of some selected medicinal plants of Nepal, Austin J. Biotechnol. Bioeng., 2015; 2(1): 1-5.

36. Perumal, S., Mahmud, R., Piaru, S.P., Cai, L.W., Ramanathan, S., Potential antiradical activity and cytotoxicity assessment of *Ziziphus mauritiana* and *Syzygium polyanthum*, Int. J. Pharm., 2012; 8(6): 535-541.

37. Cheng, G., Bai, Y., Zhao, Y., Tao, J., Liu, Y., Tu, G., Ma, L., Liao, N., Xu, X., Flavonoids from *Ziziphus jujuba* Mill var. spinasa, Tetrahedron, 2000; 56: 8915-8920.

38. Preeti, K. and Tripathi, S., *Ziziphus jujuba*: a phytopharmacological review, Int. J. Res. Develop. Pharm. Life Sci., 2014; 3(3): 959-966.