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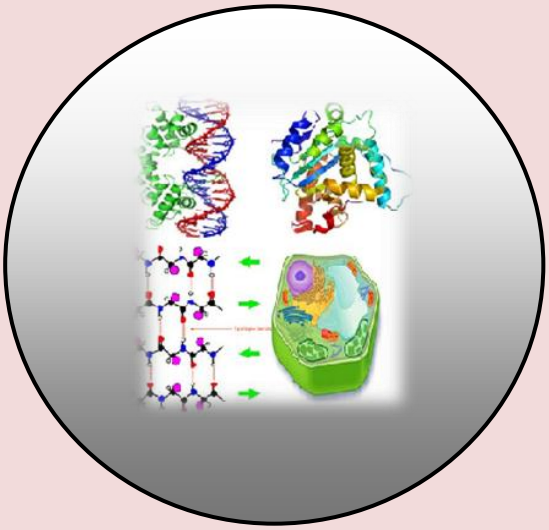

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Analysis of Antibacterial and Antioxidant Activities of *Melia azedarach* (L.)

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ABSTRACT

A number of Indian medicinal plants have been used for thousands of years in the traditional system of medicine (Ayurveda). The objective of this study was to evaluate the antibacterial and antioxidant activity of crude leaf and bark extracts (acetone and methanol) of Melia azedarach (L.). The evaluation of antibacterial activity for different extracts of plant was carried out by using the Agar-well diffusion method against pathogens such as Escherichia coli, Shigella dysenteriae, Listeria monocytogenes and Staphylococcus aureus at different concentrations (25%, 50%, 75% and 100%). Results showed low to significant antibacterial activity against the mentioned bacterial species. The maximum zone of inhibition was shown by methanol bark extract i.e. 16.0mm against S. dysenteriae followed by acetone bark extract (15.7mm) against same bacteria. The antioxidant capacity of the different extracts (methanol and acetone) of M. azedarach L. was evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) at different concentrations (20-100 µg/mL). The results showed that best antioxidant activity was shown by acetone bark extract of M. azedarach with lowest IC_{50} value i.e.18.76 µg/mL. The spectrum of activity observed in the present study justified the ethnic uses of M. azedarach against various infectious diseases.

Keywords: Medicinal plants, Melia azedarach L., antibacterial, antioxidant, Agar-well diffusion and DPPH.

INTRODUCTION

Medicinal plants are a source of great economic value in the Indian subcontinent. These plants contain the components of therapeutic value and have been used for centuries as remedies for human diseases (Nostro et al., 2000). In developing countries about 75-80 % of the whole population depends upon the herbal medicine for primary health care because it has fewer side effects on human body and has better culture acceptability (Parekh et al., 2006).

World Health Organization develops the great interest in the use and importance of Indian medicinal plants and this has strengthened the efforts on the documentation of ethno medicine data of medicinal plants (Dhar et al., 1968). India with respect to the traditional knowledge is one of the leading countries in Asia (Pant et al., 2009). There are more than 9000 plant species of medicinal plants in the Indian Himalayan Region (IHR), out of which 1748 species has various traditional and modern therapeutic uses (Samant et al., 1998). Himachal Pradesh, one of the pioneer states of the Himalaya, has great wealth of plants with medicinal value (Boktapa and Sharma, 2007). In recent years, it has been reported from all over the world that human pathogenic bacteria are exhibiting the multiple drug resistance (Piddock and Wise, 1989; Singh et al., 1992; Davis, 1994; Robin et al., 1998). Due to the present scenario of multiple drug resistance to various pathogenic bacteria, there is an emergence need of searching the new antimicrobial substances from the traditional medicinal plants (khan et al., 2017). The antibacterial and antioxidant properties of plants are due to the presence of many active phytochemicals also known as plant secondary metabolites including flavonoids, terpenoids, carotenoids, cumarins, alkaloids, lignins, saponins etc. (Bukhari et al., 2008). There is experimental evidence which shows that the large numbers of diseases are caused by the free radicals (FR) and reactive oxygen species (ROS) (Richards and Sharma, 1991; Niwa, 1991). Natural antioxidants possess the wide range of biochemical activities which include the alteration of intra cellular redox potential, inhibition of ROS generation and direct or indirect scavenging of free radicals (Abdollahi et al., 2005). *Melia azedarach* L. is a middle-sized deciduous tree and often planted in villages and along roadsides in the Himalaya. It is native of Indo-Malaya, Persia, China and Iran. In India it is found in North West Himalaya, Jhelum valley, commonly cultivated in India, wild in the sub-Himalayan tract. In Himachal Pradesh it is distributed in Bilaspur, Chamba, Kangra, Kinnaur, Kullu, Shimla, Sirmaur. Altitude range is up to 1500 m (Kumar, 2014)**.** Decoction of aerial plant parts is given for purifying blood. Poultice of bark is beneficial in joint pains. Juice of leaves prescribed for expelling intestinal worms and applied locally for killing lice. Powdered seeds and jaggery (1:1) in divided dose, is taken one hour before meals for 40 days, for curing piles. Wood is good for agricultural implements and building works (Sharma, 2004). It is in view of this, that the present research was set up to evaluate the antibacterial and antioxidant activity of *M. azedarach.*

MATERIALS AND METHODS

Collection of Plant Material

The whole plant *Melia azedarach* L. was collected from Malookpur village at an altitude of 393 m above sea level of Una, Himachal Pradesh, India. The collected plant material was brought to the laboratory for further analysis. The plant was identified at Department of Biosciences, HPU. Whole plant was first washed under tap water. After that the leaves and roots were cut into smaller pieces and allowed to shade dry for 15-20 days. After drying, plant material was crushed to form a fine powder with the help of pestle mortar. Prepared fine powders were stored at room temperature, in air tight containers.

Procurement of Bacteria

Different strains of bacteria (*Staphylococcus aureus, Escherichia coli, Shigella dysenteriae* and *Listeria monocytogenes*) have been procured from IGMC, Shimla and Department of Microbiology and Biotechnology, H.P. University Shimla for screening antibacterial properties of different plant extracts.

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C. Pure cultures of all the bacteria were maintained on nutrient medium broth and preserved in refrigerator. Sub-culturing was done at regular intervals in order to maintain the cultures. Each bacterial species was transferred from parent source to maintain and preserve the cultures.

Preparation of Acetone and Methanol Extracts

Acetone and methanol extracts of dried leaves and bark were prepared to screen antimicrobial activity. 3 g of material was taken in Erlenmeyer flask to which 30 mL of required solvents (i.e. methanol and acetone) were added. The flask was covered with aluminum foil and placed at dark place for 3-5 days for extraction. Material was filtered using Whatman filter paper no.1 and the extract was evaporated at 40ºC using rotary evaporator. The extract was collected and weighed. At last, a stock solution of 50 mg/ml conc. was prepared.

Screening for Antibacterial activity by using Agar-well diffusion method

Different extracts (acetone and methanol) of *Melia azedarach* L. were screened using agar-well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6ºC for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25, 50, 75 and 100% concentration of prepared plant extracts. The Petri plate kept as a control contained pure solvent only. The plates were incubated at 37±2ºC for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition zone diameter. Streptomycin was used as positive control.

Percentage of growth inhibition (%) = $\left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right) \text{x}100$

Where, Control = average diameter of bacterial colony in control. Test = average diameter of bacterial colony in treatment sets (Rana et al., 2016).

Screening for Antioxidant activity by using DPPH radical scavenging assay

The free radical scavenging activity of plant extracts was measured using 2, 2 Diphenyl-1-picrylhydrazyl (DPPH) as described by Blois (1958). Briefly, to 1mL of different concentrations (5, 10, 15, 20 and 25 µg/mL) of plant or test extract, 1 mL of DPPH (0.1 mM in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as reference standard. Mixture of 1mL methanol and 1 mL DPPH solution (without plant extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

DPPH scavenging effect (%) = $\left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample.
Graphs were plotted against percent inhibition v/s concentration of plant extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was evaluated using the following equation given below:

$$IC_{50} = \frac{50 - Y\text{-}Intercept}{Slope}$$

Statistical Analysis

Data are presented as mean± standard error of multiple time repeated experiments. To find out the IC_{50} value Regression value and value of Y-intercept were calculated using Microsoft Office Excel 2007.

RESULTS

Antibacterial Activity Screening

Streptomycin is a broad spectrum antibiotic which displayed great inhibition zone against all the tested bacteria.

Antibacterial screening of plant extracts (methanol and acetone) of *M. azedarach* against bacteria (*Escherichia coli, Listeria monocytogenes, Staphylococcus aureus* and *Shigella dysenteriae*).

The antibacterial activity of plant extracts of *M. azedarach* was determined by Agar-well diffusion method and the results of the above assay are shown in Tables 1-2 and in Figures 1-4. Results came out from the Table-1 concluded that at the minimum concentration (25%) of acetone extract of leaf, zones of inhibition for *S. aureus, E. coli, L. monocytogenes* and *S. dysenteriae* were 8.8 mm, 11.3 mm, 10.0 mm and 10.3 mm, respectively while at 100% concentration zones of inhibition were 12.8 mm, 13.7 mm, 13.3 mm and 13.3 mm respectively for all test bacteria. At 25% concentration of methanol extract of leaf, zones of inhibition were 8.7 mm, 9.2 mm, 9.0 mm and 8.8 mm and similarly at 100% concentration, zones of inhibition were 13.0 mm, 13.3 mm, 12.8 mm and 13.7 mm respectively(Table-1).

Table 1. Zones of inhibition produced by leaf extract of *M. azedarach* at different concentrations in acetone and methanol.

Extract	Conc. (%)	Inhibition zone diameter in mm (±S.E.)			
		S. aureus	***E. coli***	***L. monocytogenes***	***S. dysenteriae***
Acetone extract	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	8.8±0.17	11.3±0.33	10.0±0.00	10.3±0.33
	50	10.0±0.00	11.8±0.17	10.8±0.17	11.7±0.33
	75	11.3±0.33	13.0±0.00	12.3±0.33	13.0±0.00
	100	12.8±0.17	13.7±0.33	13.3±0.33	13.3±0.33
Methanol extract	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	8.7±0.33	9.2±0.17	9.0±0.00	8.8±0.17
	50	10.2±0.17	10.7±0.33	10.3±0.33	10.3±0.33
	75	11.3±0.33	11.7±0.33	11.7±0.33	11.7±0.33
	100	13.0±0.00	13.3±0.33	12.8±0.17	13.7±0.33

Each data point represents mean of three replicates ± S.E. (Standard Error)

In case of acetone bark extract of this plant, at 25% conc., zones of inhibition for *S. aureus, E. coli, L. monocytogenes* and *S. dysenteriae* were 9.6 mm, 11.7 mm, 10.3 mm and 11.3 mm and alike at 100% conc., zones of inhibition were 13.3 mm, 14.5 mm, 13.7 mm and 15.7 mm respectively. For methanol bark extract, zones of inhibition at 25% conc. were 10.7 mm, 10.7 mm, 10.2 mm and 10.7 mm. Similarly, for 100% conc. zone of inhibition were 14.7 mm, 14.3 mm, 14.2 mm and 16.0 mm respectively (Table-2).

Table 2. Zones of inhibition produced by bark extract of *M. azedarach* at different concentrations in acetone and methanol.

Extract	Conc. (%)	Inhibition zone diameter in mm (±S.E.)			
		S. aureus	***E. coli***	***L. monocytogenes***	***S. dysenteriae***
Acetone extract	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	9.6±0.33	11.7±0.33	10.3±0.33	11.3±0.33
	50	10.3±0.33	12.3±0.33	11.7±0.33	12.7±0.33
	75	11.7±0.33	13.3±0.33	12.3±0.33	14.3±0.33
	100	13.3±0.33	14.5±0.50	13.7±0.33	15.7±0.33
Methanol extract	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	10.7±0.33	10.7±0.33	10.2±0.17	10.7±0.33
	50	12.0±0.00	11.5±0.29	11.3±0.33	12.7±0.33
	75	13.7±0.33	13.3±0.33	12.7±0.33	14.7±0.33
	100	14.7±0.33	14.3±0.33	14.2±0.17	16.0±0.00

Each data point represents mean of three replicates ± S.E. (Standard Error)

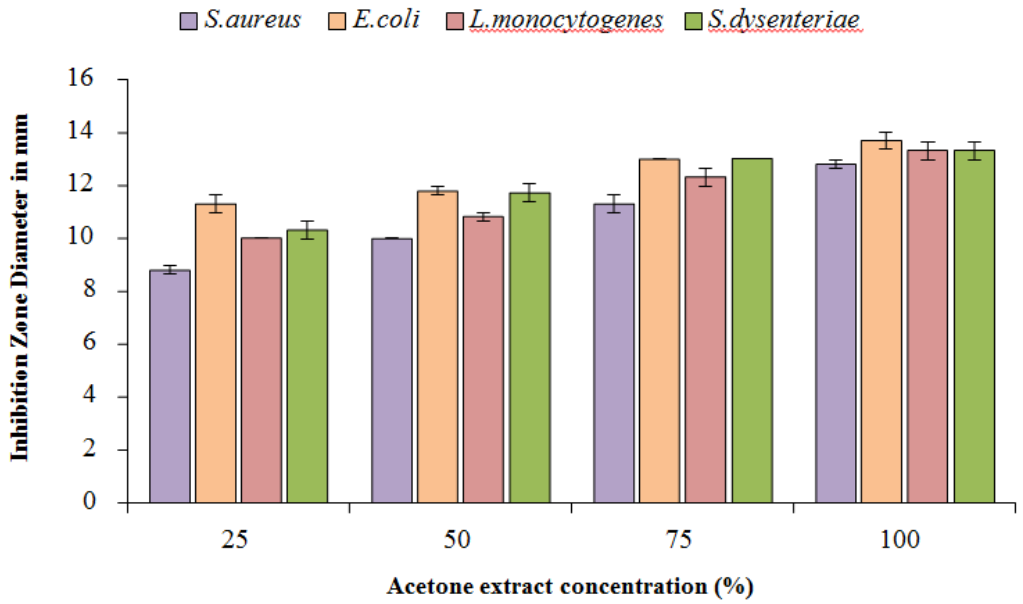

Figure 1. Antibacterial activity of Acetone leaf extract of *M. azedarach*.

Table 3. Free radical scavenging activity (%) of the control i.e. ascorbic acid at different concentrations.

	Concentration (µg/mL)	Free radical scavengingactivity (%)	IC_{50} Value (µg/mL)
Ascorbic acid (Control)	**5**	47.80±0.006	**5.84**
	10	53.65±0.004	
	15	59.08±0.001	
	20	61.37±0.002	
	25	63.13±0.001	

Table 4. Free radical scavenging activity (%) of the leaf extract of the *Melia azedarach* L. at different concentrations in methanol and acetone.

Name of the plant	Concentration (µg/mL)	Acetone extract	IC_{50} Value (µg/mL)	Methanol extract	IC_{50} Value (µg/mL)
***M. azedarach* (leaf)**	**5**	8.24±0.002	**33.68**	7.53±0.002	**42.03**
	10	16.83±0.002		11.89±0.008	
	15	23.40±0.001		16.24±0.005	
	20	29.96±0.001		23.95±0.001	
	25	37.54±0.001		30.98±0.004	

Table 5. Free radical scavenging activity (%) of the bark extract of the *Melia azedarach* L. at different concentrations in methanol and acetone.

Name of the plant	Concentration (µg/mL)	Acetone extract	IC_{50} Value (µg/mL)	Methanol extract	IC_{50} Value (µg/mL)
***M. azedarach* (bark)**	**5**	27.51±0.002	**18.76**	16.66±0.004	**27.08**
	10	36.55±0.001		23.28±0.003	
	15	43.94±0.002		30.68±0.006	
	20	54.20±0.001		41.79±0.002	
	25	58.11±0.002		45.50±0.003	

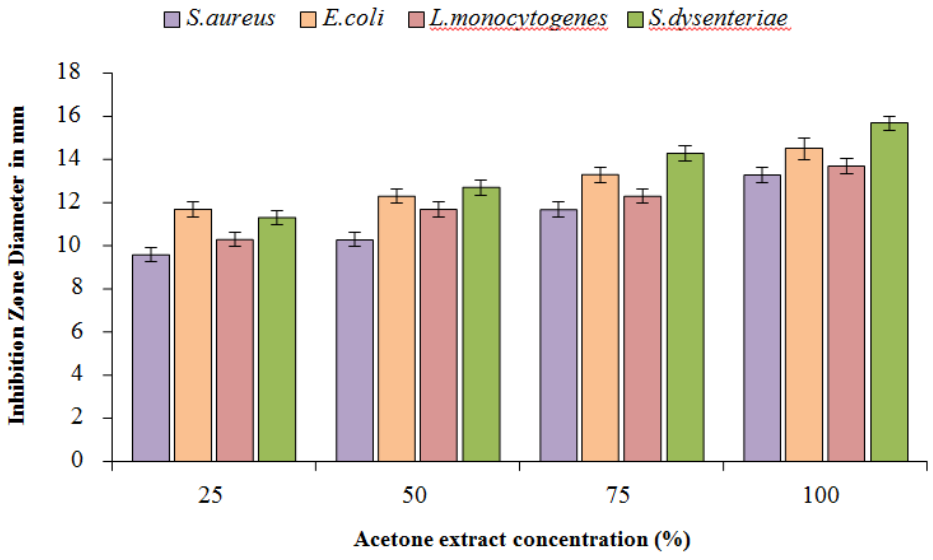

Figure 2. Antibacterial activity of Methanol leaf extract of *M. azedarach*.

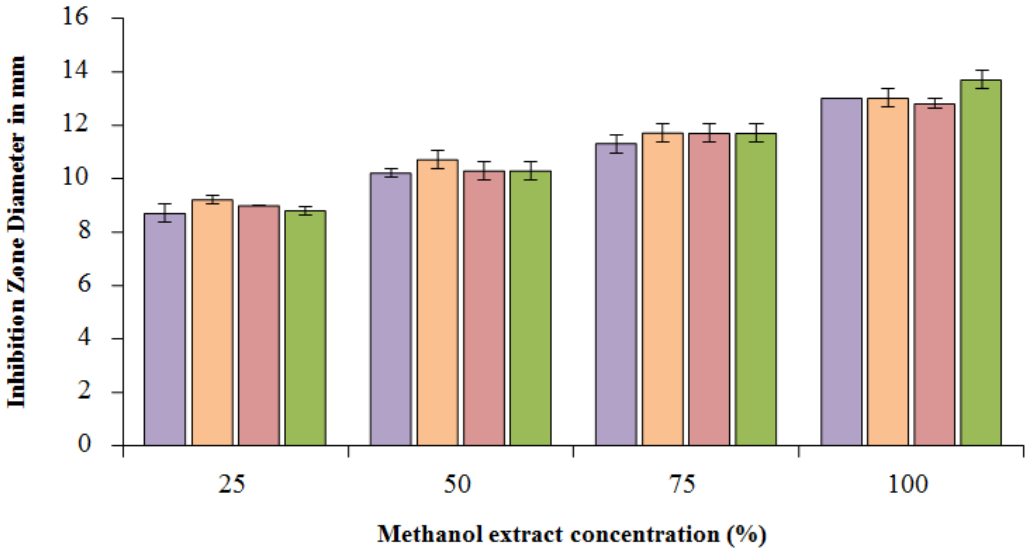

Figure 3. Antibacterial activity of Acetone bark extract of *M. azedarach*.

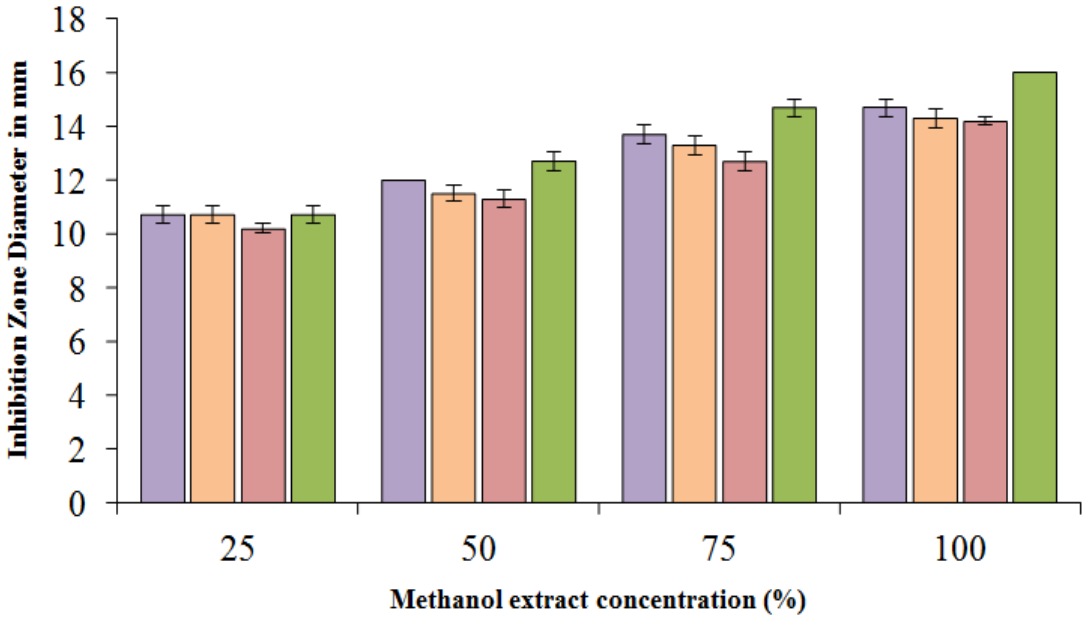

Figure 4. Antibacterial activity of Methanol bark extract of *M. azedarach.*

Antioxidant Activity

DPPH radical scavenging activity of methanol and acetone extracts of *M. azedarach* leaves and barks were calculated and the results were expressed in percentage inhibition corresponding to the various concentrations of *M. azedarach* extracts. The graph was plotted between the concentrations of the extract used and scavenging activity (%) of DPPH free radical. The results are represented in Tables 3-5 and Figure 5. Ascorbic acid was taken as the standard which exhibited IC_{50} value of 5.84µg/mL (Table-3).

In case of *M. azedarach* IC_{50} value for acetone leaf and bark extracts were 33.68 µg/mL and 18.76 µg/mL, respectively and for methanol leaf and bark extracts were 42.03 µg/mL and 27.08 µg/mL, respectively.

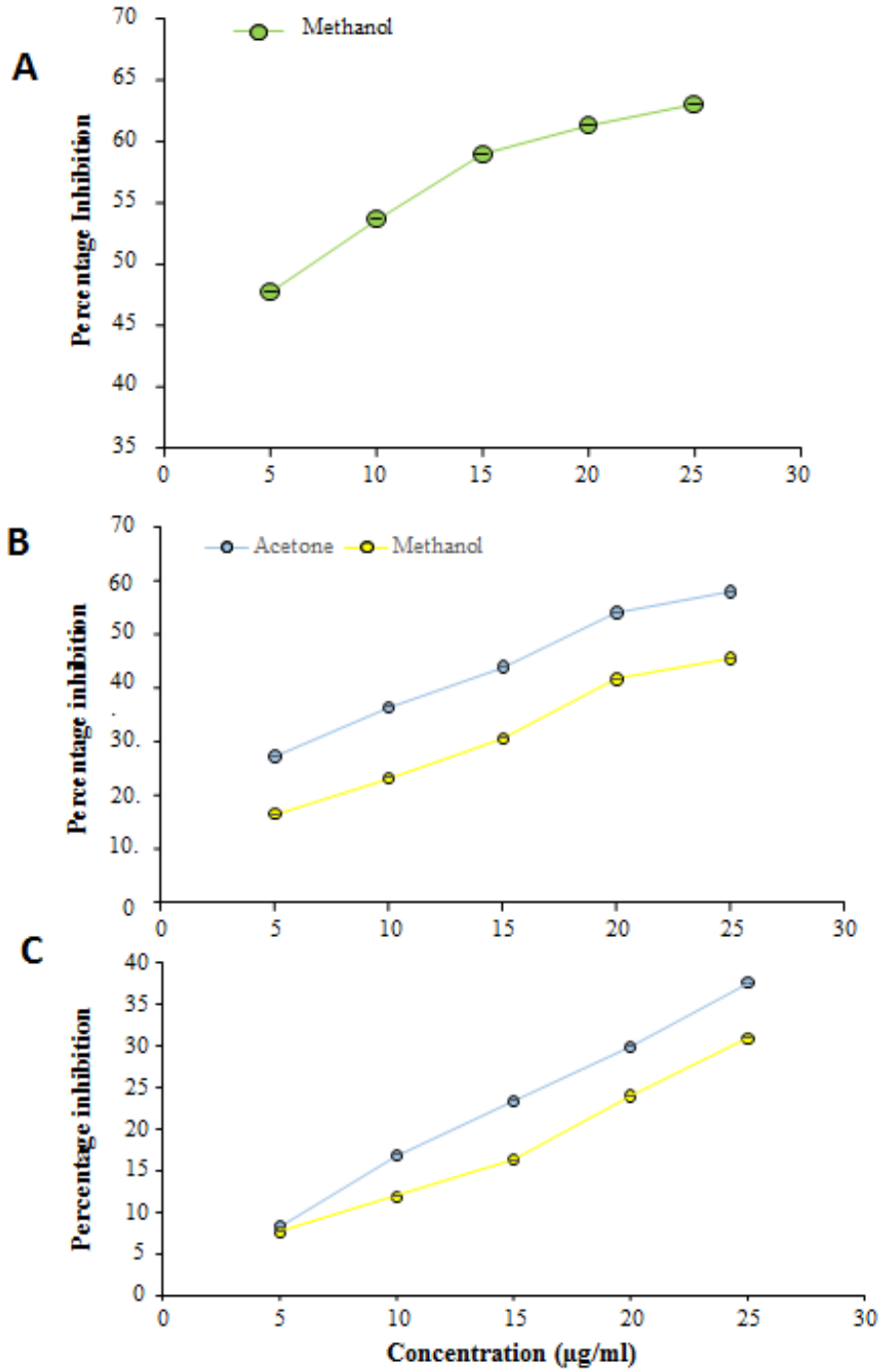

Figure 5. (A)Percentage inhibition of DPPH shown by Ascorbic acid in Methanol (B) Percentage inhibition of DPPH shown by Acetone and Methanol extracts of *Melia azedarach* L. Leaves; (C) *Melia azedarach* L. Bark.

DISCUSSION

Antibacterial Activity Screening

It has been observed that with increase in concentration of leaf and root extracts, the zones of inhibition also increased. In addition to antibacterial activity of *M. azedarach*, this plant showed that methanol leaf and bark extracts had the greater zones of inhibition for the four concerned bacteria followed by acetone extract. The maximum zone of inhibition was found against *E. coli* in leaf acetone extract (13.7 mm) and in *S. dysenteriae* in leaf methanol extract (13.7 mm). And in bark, maximum zone of inhibition was found in *S. dysenteriae i.e.* 16.0 mm in methanol extract.

Khan *et al*. (2001) studied the antimicrobial activity of the methanol extracts of leaves, root, stem and bark of *M. azedarach.* The activity was increased on fractionation particularly in the dichloromethane fraction of the stem bark of *M. azedarach.*

Suresh *et al*. (2008) evaluated the antibacterial activity of the leaf extracts of *M. azedarach* against pathogenic bacteria like Gram-positive and Gram-negative by *in vitro* Agar-well diffusion method. The aqueous leaf extract of plant showed pronounced inhibition than chloroform leaf extract. Leaf extracts showed more inhibitory action on *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia*.

Antioxidant Activity

Table-4 shows free radical scavenging activity (%) of the leaf extract of *M. azedarach* in acetone and methanol solvents at different concentrations with 33.68 µg/mL IC_{50} value of acetone extract and 42.03 µg/mL IC_{50} value of methanol extract. Free radical scavenging activity (%) of the bark extract of *M. azedarach* in acetone and methanol solvents at different concentrations shown in Table-5, where acetone bark extract shows lesser IC_{50} value (18.76 µg/mL) than methanol bark extract IC_{50} value (27.08 µg/mL).

Khatoon et al. (2014) evaluated the antioxidant potential of n-hexane and methanolic extracts of *Melia azedarach* L. bark. Studies on the antioxidant activity by DPPH scavenging method revealed significant antioxidant potential of n-hexane and methanol extracts with IC_{50} value 84.37 and 66.79µg/mL, respectively.

CONCLUSION

From the performed investigation it was concluded that the selected plant showed considerable activity against all the tested pathogenic bacteria. Maximum diameter of zone of inhibition (16.0 mm) was reported for the methanol bark extract against *S. dysenteriae.* In case of antioxidant activity, acetone bark extract showed strongest activity than the other extracts. This study suggests that the plant extracts possess potent antibacterial and antioxidant activity, which might be helpful in preventing or slowing the progress of various bacterial and oxidative stress-related diseases.

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