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Evaluation of *in vitro* antibacterial and antioxidant activities of *Melia azedarach* L. bark

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Abstract: *The objective of the present investigation was to evaluate the antibacterial and antioxidant potential of n-hexane and methanolic extracts of Melia azedarach* L. *bark. The antibacterial potential of M. azedarach* L. *bark was tested against human pathogens causing diarrhoea and dysentery such as Shigella flexneri (MTCC-9543), Salmonella enterica ser typhi (MTCC-733), Bacillus subtilis (MTCC-1305), Streptococcus mitis (MTCC-2897), Klebsiella pneumoniae (MTCC-109) and Staphylococcus aureus (MTCC-1430) using agar well diffusion method. The results of the study revealed that n-hexane extract of bark sample was highly effective against Shigella flexneri whereas Streptococcus mitis, Staphylococcus aureus and Bacillus subtilis showed no response and other test pathogens under study responded moderately. It was observed that methanolic extract had high inhibition potential against Salmonella enterica ser typhi and Streptococcus mitis while moderate effect against other test bacteria. Studies on the antioxidant activity by DPPH scavenging method revealed significant antioxidant potential of n-hexane and methanolic extracts with IC_{50} value 84.37 and 66.79 respectively.*

Key Words*: Agar well diffusion, Antibacterial activity, Antioxidant activities, Melia azedarach*

I. INTRODUCTION

Plants have been a source of food, fibre and medicine since the begining of the human civilization. The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine system [1]. Since antiquity, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, triterpenes which are therefore, should be utilized to combat the disease causing pathogens [2][3][4]. Application of plant-derived biocides in agriculture have been more popular during the past for their low health risk and feasibility. In recent years much attention has been given to non-chemical systems for seed treatment to protect them against many plant pathogens [5]. With the advancement in science and technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs [6]. Antibiotics are undeniably one of the most important therapeutic discoveries of the twentieth century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products [7]. This is because of the emergence of resistant pathogens as a consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics [8] [9]. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistant inhibitors from plants [10] [11]. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics might be active against drug resistant pathogens [12].

Melia azedarach L. is a deciduous tree native to southern Asia and Australia [13]. It is an old tree in Egypt [14] with reputed value for its antifungal properties[15]. The plant has traditionally been used as anthelmintic, diuretic, astringent and stomachic [16]. Various scientific studies reported the anticancer [17], antimalarial activity, analgesic and anti-inflammatory activity [18]. In the earlier studies, ethanolic extract of *M. azedarach* leaves showed activity against fever, nausea, thirst and skin diseases [19][20]. It has also showed antioxidant activity [21] and analgesic activity [22]. Leaves and fruits showed antifeedant activity [23][17]. Antibacterial, antifungal, antimalarial, and cytotoxic activities of *M. azedarach* has been reported by earlier workers [24][25][26][27]. Leaf extract of *M. azedarach* was a good inhibitor of *Bipolaris micropus* [29] but it was partial inhibitor to *Alternaria solani* with little or no effect on *Fusarium oxysporum* isolated from tomato [28].

In the present investigation, n-hexane and methanolic extracts of *Melia azedarach* L. bark have been evaluated for their antimicrobial and antioxidant activities.

II. MATERIALS AND METHODS

2.1 Collection and identification of plant material

The plant *Melia azedarach* was collected from the Chandaka reserve forest area near Bhubaneswar, Odisha in the month of March 2012. Identification of the voucher specimen was done by following available literatures. The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar. The bark samples were collected in bulk amount, washed in running tap water, dried under shade and made to coarse powder form.

2.2 *Processing of plant material and preparation of extract*

The collected bark which was shade dried and ground to form course powder and had been successively extracted with the solvent n-hexane and methanol by Soxhlet apparatus [30] and the extract was recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.

2.3 *Evaluation of the extracts for antibacterial activity*

The *in vitro* antibacterial screening was carried out against selected bacterial pathogens causing diarrhoea and dysentery in human. The bacterial pathogens were *Shigella flexneri* (MTCC-9543), *Salmonella enterica ser typhi* (MTCC733), *Bacillus subtilis* (MTCC1305), *Streptococcus mitis* (MTCC2897), *Klebsiella pneumoniae* (MTCC-109), *Staphylococcus aureus* (MTCC-1430). These species were procured from microbial type culture collection centre (MTCC) and Gene Bank, Chandigarh, India. The remaining test bacteria were procured from P.G. Department of Microbiology, OUAT, Bhubaneswar, Odisha. These organisms were identified by standard microbial methods[31]. The antibacterial screening of the extracts were carried out by determining the zone of inhibition using agar well diffusion method [32] . Ciprofloxacin was taken as reference antibiotic.

2.4 *Agar well diffusion assay*

Agar well diffusion method[30] was followed to determine the zone of inhibition of microbes in Nutrient Agar (NA, HiMedia Laboratories Ltd., Mumbai) plates which were swabbed (sterile cotton swabs) with 8 hr old broth culture of bacteria. Wells (8 mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. Stock solution of plant extracts were prepared at a concentration of 3 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 hours. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37 °C for 24 hours for bacterial pathogens. Triplicates were maintained and the diameter of the zone of inhibition (mm) was measured and the data were statistically analysed.

2.5 *Antioxidant Test*

Antioxidant potential of the extract was estimated on the basis of the extract's scavenging activity of stable DPPH radical. Initially 25mg of extract was dissolved in 50 ml of methanol to prepare stock solution. Then 250, 200, 150, 100 and 50 µg / ml solution was prepared by diluting the stock solution. Then 1.5 ml of solution from the above was added to 1.5 ml of DPPH. This was kept in dark for 20 minutes for allowing reaction. Absorbance of the samples were measured by using UV Spectrophotometer at 517 nm against blank. A blank was prepared without adding the extract. 10 mg of dry ascorbic acid was dissolved in 10 ml of methanol to prepare 5 different concentrations viz. 10, 20, 30, 40 and 50 µg/ml of ascorbic acid. This was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavengered (5) = A Control – A Text × 100

(Absorbance of the central reaction - Absorbance in the present of sample of the extracts × 100)

The antioxidant activities of the extracts were expressed in IC_{50}.

III. RESULT AND DISCUSSION

3.1 Antibacterial Screening

The bark extract of this plant subjected to antibacterial screening against three gram-positive (*Bacillus subtilis, Streptococcus mitis* and, *Staphylococcus aureus*) and three gram-negative (*Shigella flexneri*, *Salmonella typhi* and *Klebsiella pneumoniae*) bacteria causing diarrhoea and dysentery. The results indicated that n-hexane extract of the bark sample exhibited highest zone of inhibition against *S. flexneri* (21.2 ± 0.75), least against *K. pneumoniae* (11.03 ± 0.75) and resistant to *S. mitis, S. aureus, B. subtilis.* This extract exhibited moderate activity against *S. typhi* (13.06 ± 0.30). While methanolic extract exhibited highest zone of inhibition on *S. mitis* (17 ± 0.2), least against *K. pneumoniae* (11.16 ± 0.3) and moderate against *S. typhi* (15.5 ± 0.40) followed by *Shigella flexneri* (13.3 ± 0.3), *S. aureus* (11.86 ± 0.3) and *B. subtilis* (11.86 ± 0.32).

3.2 Antioxidant Study

n-Hexane extract of *M. azedarach* showed comparatively high antioxidant activity than methanolic extract and ascorbic acid. The IC_{50} value of n-hexane extracts, methanol extract and ascorbic acid was found 84.27, 66.79and 25.63 respectively.

Table-1 *In vitro* antibacterial activity (zone of inhibition in mm) of various plant extracts of *Melia azedarach*

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Microorganisms	n-hexane extract (2mg/ml)	Methanol extract (2mg/ml)	Ciprofloxacin 0.5mg/ml (RA)
Bacteria	Zone of Inhibition in mm	Zone of Inhibition in mm	Zone of Inhibition in mm
Streptococcus mitis	---	17.02 ± 0.2	28±0.816
Staphylococcus aureus	---	11.86 ± 0.23	20±0.816
Shigella flexneri	21.2 ± 0.72	13.3 ± 0.3	29±1.69
Bacillus subtilis	---	11.86 ± 0.32	21±0.816
Klebsiella pneumoniae	11.03 ± 0.75	11.16 ± 0.37	27±1.69
Salmonella enteric ser. typhi	13.06 ± 0.30	15.56 ± 0.40	32±1.69

(zone of inhibition with S.D); (---) No inhibition ; RA-reference antibiotic.

Table-2 Antioxidant activity of *Melia azedarach*

Conc. (µg/ml)	% of scavenging			IC_{50} value		
	n-hexane extract.	Methanolic extract.	Standard Ascorbic acid solution	n-hexane extract	Methanolic extract	Standard ascorbic acid solution
50	42.54	48.45	34.02			
100	54.51	53.14	47.13	84.27	66.79	25.63
150	62.24	61.54	53.12			
200	71.45	68.56	64.36			
250	81.47	79.28	71.45			

IV. CONCLUSION

It was found that *Melia azedarach* L had more inhibitory effect against bacterial strains studied in the present investigation. The methanolic extracts of this plant showed more antimicrobial activity than n-hexane extract. The findings of the present study to control the pathogenic bacterial strains can be further explored in order to discover new drug molecules to combat human diseases. The peculiar event which was evident during the study that the n-hexane extract of the plant was having more inhibitory activity against *Shigella flexneri* whereas its methanolic extract had still more effect on *S. typhi*. Since the methanol extract of *M. azedarach* registered lowest IC_{50} value, thereby showing highest antioxidant potential. The plant can be further studied for the isolation of important chemical constituents for the exploration of specific antibiotic and antioxidants.

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