

***In Vitro* Antioxidant Activity and Antibacterial Activity of Ethyl Acetate Extract of Medicinal Tree Species, *Anacardium occidentale* L. and *Mangifera indica* L.**

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ABSTRACT

This study was undertaken to evaluate the antioxidant property and antibacterial activity of ethyl acetate extract of mature leaves and flower of the medicinal tree species, *Anacardium occidentale* and *Mangifera indica*. 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) free radical scavenging method and agar well diffusion method was used to detect antioxidant activity and antibacterial activity respectively. Various plant extracts of both the tree species studied were found to have potent antioxidant activity against DPPH and antibacterial activity against five human pathogens such as *Pseudomonas aeruginosa*; *Salmonella typhi*; *Bacillus subtilis*; *Escherichia coli* and *Staphylococcus aureus*. Therefore ethyl acetate extracts of mature leaf and flower extract of both *A.occidentale* and *M.indica* can be considered as a new potential source of natural antioxidants and antibacterial agent for pharmaceutical industries.

Highlights

- The present study involves the comparison of antioxidant activity and antibacterial activity in ethyl acetate extract of mature leaves and flower of *A.occidentale* and *M.indica*.
- Various plant extracts of both the tree species showed potent antioxidant activity against DPPH and antibacterial activity against five human pathogens such as *Pseudomonas aeruginosa*; *Salmonella typhi*; *Bacillus subtilis*; *Escherichia coli* and *Staphylococcus aureus*.

Keywords: *Anacardium occidentale*, *Mangifera indica*, Antioxidant activity, Antibacterial activity, DPPH

During the last few decades there has been an increasing demand in the study of medicinally important traditional plants in different parts of the world. *A.occidentale* is a tropical tree indigenous to Brazil, a member of the family Anacardiaceae, which is now widely grown in other tropical countries like India and is a multi-purpose plant (Togun 1977). Many parts of this tree are used in traditional medicine. Commercially important two parts are cashew nut for diet and the liquid from nut shell (CNSL) for various industrial and medical applications (Joseph 1975; Pillai *et al.* 1990). Anacardic acid is having very much demand in the international market (Rodrigues *et al.* 2004). Apart from this, a large number of other phenolic

compounds are founds in very small quantities in the shell (Miraliakbari and Shahidi 2008). Phenol is seen throughout the plant system though it is mainly concentrated in the nut shell. The mango (*Mangifera indica* L.) is one of the choicest fruit crops of tropical and sub-tropical regions of the world, especially in Asia. Its popularity and importance can easily be realized by the fact that it is often referred as 'King of fruits' in the tropical world (Singh *et al.* 1991). Mangiferin is the most important phenolic compound in mango leaves, bark, peels and kernels and is present in particularly high quantities in young leaves (Barreto *et al.* 2008). Mangiferin has many biological activities, including anticancer, antimicrobial, anti-allergenic, anti-inflammatory,

analgesic, immunomodulatory and hypolipidemia, as well as antioxidant activity (Masibo and He 2008).

Antioxidants act as a defense mechanism that protect against oxidative damage and include compounds to remove repair damaged molecules. It can prevent the oxidation caused by free radicals and sufficient intake of antioxidants is supposed to protect against diseases. Plants are the source of medication for preventive, curative, protective purposes. Many of these herbal medicines are finding this way into the world market as alternatives to prescribed allopathic drugs currently available to treat various disorders and ailments.

The development of new microbial agents against resistant pathogens is increasing interest. Therefore, the ethyl acetate extract from different parts of medicinal plants used locally in folk medicine was evaluated for antimicrobial activity. Antimicrobial agents are used to inhibit the activity of microorganisms. The use of plant extracts and phytochemicals, with established antimicrobial properties, could be of great significance in preventive and/or therapeutic approaches. In view of the above, the present study has been made to investigate the antioxidant potential as well as the antimicrobial activities of a traditionally used medicinal tree plants such as *A. occidentale* L. and *M. indica* L.

MATERIALS AND METHODS

Plant material

The plant materials (mature leaves and flower) of *A. occidentale* and *M. indica* were collected from mother stock trees. The shade dried mature leaves and flower of the study species were made into fine powder.

Preparation of plant extracts

The shade dried plant materials were ground and used for preparing extracts. Powdered samples were extracted with ethyl acetate by maceration and kept it for a period of 24 hrs at room temperature at a ratio of 1:100 (g:ml). Homogenized samples were centrifuged at 10,000 rpm for 15 minutes and supernatants were pooled. Then the extract was filtered to remove all undissolved matter, including cellular material and other constituents that are insoluble in the extraction solvent and each

extract was concentrated in a rotary evaporator to remove ethyl acetate. The residue thus obtained was dissolved in ethyl acetate and stored at 4-8°C in a refrigerator for further analysis (Muthusamy *et al.* 2013; Harborne 1998)

DPPH radical scavenging activity

The 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) scavenging activity was measured (Kumarasamy *et al.* 2007). Ethyl acetate extract of the samples at various concentrations (20, 40, 60, 80 and 100 µg/ml) was added separately to each 1ml of 0.1mM ethyl acetate solution of DPPH and allowed to stand for 20min. Absorbance at 517nm using spectrophotometer was measured. Ascorbic acid was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

$$\% \text{ Inhibition} = (A_0 - A_1/A_0) \times 100$$

Where A_0 and A_1 stand for absorption of the control sample and absorption of tested extract solution respectively. The control samples contained all the reagents except the extract. IC_{50} value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis.

Determination of antibacterial activity

The antibacterial activity of the ethyl acetate extracts was screened against *Pseudomonas aeruginosa* (MTCC 1034); *Salmonella typhi* (MTCC 1168); *Bacillus subtilis* (MTCC 2340); *Escherichia coli* (MTCC 56) and *Staphylococcus aureus* (MTCC 9760). Antibacterial activities of the different extracts were investigated by the agar well diffusion method (Alzoreky and Nakahara 2003) using Mueller-Hinton agar plates previously inoculated with 18 hour old nutrient broth culture for the bacteria. Bacterial plates were incubated at 37°C for 24 hour. The zone of inhibitions produced by inhibitory action of different plant extracts and control were taken as the antibacterial activity.

Statistical analysis

All analyses were carried out in triplicate and the



data were reported as means \pm SD. The data were subjected to one way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's Multiple Range Test ($P < 0.05$) using the statistical software package (SPSS for Windows, ver.17, 2008).

RESULTS AND DISCUSSION

Antioxidant activity

The free radical scavenging activity of ethyl acetate extracts of *A. occidentale* and *M. indica* were determined using ethyl acetate solution of DPPH reagent. It has been observed that the percentage of radical scavenging effect on the DPPH radical was increased with the increase in the concentration of all the extracts from 20 to 100 $\mu\text{g/ml}$. The results of the present study confirmed that all the plant extracts showed antioxidant activity. Among various plant extracts of *M. indica* and *A. occidentale* tested for antioxidant activity, the various plant extracts of *A. occidentale* showed the highest percent of inhibition compared to various plant extracts of *M. indica*. In both the tree plants, antioxidant activity of various plant parts can be ranked as: mature leaves > flower (Table 1).

Among the four extracts and standard tested for antioxidant activity, the ethyl acetate extract of mature leaves of *A. occidentale* showed the highest percent of inhibition from $26.33 \pm 1.96\%$ at 20 $\mu\text{g/ml}$ to $34.72 \pm 1.53\%$ at 100 $\mu\text{g/ml}$ while the ethyl acetate extracts of flower of *M. indica* showed the least ($11.6 \pm 0.54\%$ at 20 $\mu\text{g/ml}$ and $18.17 \pm 1.35\%$ at 100 $\mu\text{g/ml}$), which is comparable to the standard antioxidant activity of ascorbic acid ($45.25 \pm 0.36\%$ at 20 $\mu\text{g/ml}$ and $49.89 \pm 0.99\%$ at 100 $\mu\text{g/ml}$) (Table 1). Studies also clarified the relationship between phenolic compounds and antioxidant activity (Zhao *et al.* 2014) and many scientists have confirmed that phenolic compounds are most appropriate antioxidants in *A. occidentale* (Jaiswal *et al.* 2012, Tan and Chiang 2014). Antioxidant activity of ethanol extract of leaves and flower of *A. occidentale* were explored (Silva *et al.* 2016). The free radical scavenging activity of young leaves, barks, roots and kernels of *M. indica* were also studied (Samba *et al.* 2018). Mangiferin is the major phenolic compound present in mango leaves (Barreto *et al.* 2008).

The antioxidant activity of different plant part extracts of *A. occidentale* and *M. indica* were also expressed in terms of IC_{50} ($\mu\text{g/ml}$) values (Table 2) and it ranged from $97.34 \pm 1.33 \mu\text{g/ml}$ to

Table 1: Free radical scavenging activities of various extracts of *A. occidentale* and *M. indica* measured using the DPPH assay

Test compound (Ethyl acetate extract)	DPPH radical scavenging activity (%)					
	Concentration ($\mu\text{g/ml}$)					
	20	40	60	80	100	
<i>A. occidentale</i>	Mature leaves	26.33 \pm 1.96	27.96 \pm 1.56	30.13 \pm 1.32	32.71 \pm 0.97	34.72 \pm 1.53
	Flower	14.03 \pm 0.81	16.31 \pm 1.71	18.19 \pm 1.63	20.4 \pm 1.47	22.31 \pm 1.57
<i>M. indica</i>	Mature leaves	23.17 \pm 1.14	25.17 \pm 1.31	28.03 \pm 1.11	31.07 \pm 1.31	33.19 \pm 1.41
	Flower	11.6 \pm 0.54	12.9 \pm 1.21	14.1 \pm 0.96	16.3 \pm 1.33	18.17 \pm 1.35
Control	Ascorbic acid	45.25 \pm 0.36	46.43 \pm 1.97	48.15 \pm 0.98	49.1 \pm 0.86	49.89 \pm 0.99

Results are expressed as means \pm SD for triplicates.

Table 2: Antioxidant activity of investigated plant extracts of *A. occidentale* and *M. indica*

Test compound (ethyl acetate extract)		IC_{50} ($\mu\text{g/ml}$)
<i>A. occidentale</i>	Mature leaves	242.23 \pm 1.54b
	Flower	367.34 \pm 0.67d
<i>M. indica</i>	Mature leaves	288.65 \pm 1.34c
	Flower	487.88 \pm 1.66e
Control	Ascorbic acid	97.34 \pm 1.33a

The mean (\pm SD) values within a column followed by different letters are significantly different by Duncan's multiple range test ($p < 0.05$)

487.88±1.66µg/ml. The result revealed that flower of *M. indica* showed weak antioxidant activity, with IC₅₀ value of 487.88±1.66µg/ml. While the ethyl acetate extract of mature leaves of *A. occidentale* showed the highest antioxidant activity, with IC₅₀ value of 242.23±1.54µg /ml. The IC₅₀ value for standard ascorbic acid was 97.34±1.33 µg/ml. The IC₅₀ value decreased with the increase of antioxidant activity of each explant type and vice versa. The result of the present study highlighted that the IC₅₀ value differ significantly (p < 0.05) among the various extracts (Table 2).

Antimicrobial activity

In the present study, ethyl acetate extracts of different plant parts of *A.occidentale* and *M.indica* were tested for its antibacterial activity against five typical bacterial strains of *Staphylococcus aureus* (MTCC 9760), *Pseudomonas aeruginosa* (MTCC 1034), *Salmonella typhi* (MTCC 1168), *Bacillus subtilis* (MTCC 2340) and *Escherichia coli* (MTCC 56).

All the plant extract of *A. occidentale* showed inhibitory action against *P. aeruginosa*, *S. typhi*, *B. subtilis* and *E. coli* whereas the ethyl acetate extract of flower of *A. occidentale* and *M. indica* showed no inhibitory action against *S. aureus* as given in the Table 3. The control (ethyl acetate) showed no zone of inhibition against five typical bacterial strains (Fig. 1).

Table 3: Zones of inhibition produced by ethyl acetate extracts of *A. occidentale* and *M. indica*

Test compound (Ethyl acetate extract)		Zone of inhibition (mm)				
		Bacteria				
		<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>
<i>A. occidentale</i>	Mature leaves	19	12	20	19	21
	Flower	10	24	19	19	—
<i>M. indica</i>	Mature leaves	24	9	17	23	23
	Flower	11	11	—	20	—

Mature leaves (C₂), Flower (C₃) - *A. occidentale*; Mature leaves (M₂), Flower (M₃) - *M. indica*.

Among the two extracts of *A. occidentale* tested, flower showed maximum zone of inhibition (24mm)

against *S. typhi*. Flower showed same range of inhibitory action against *B. subtilis* and *E. coli* with a zone of inhibition of 19mm (Table 3 & Fig. 1).

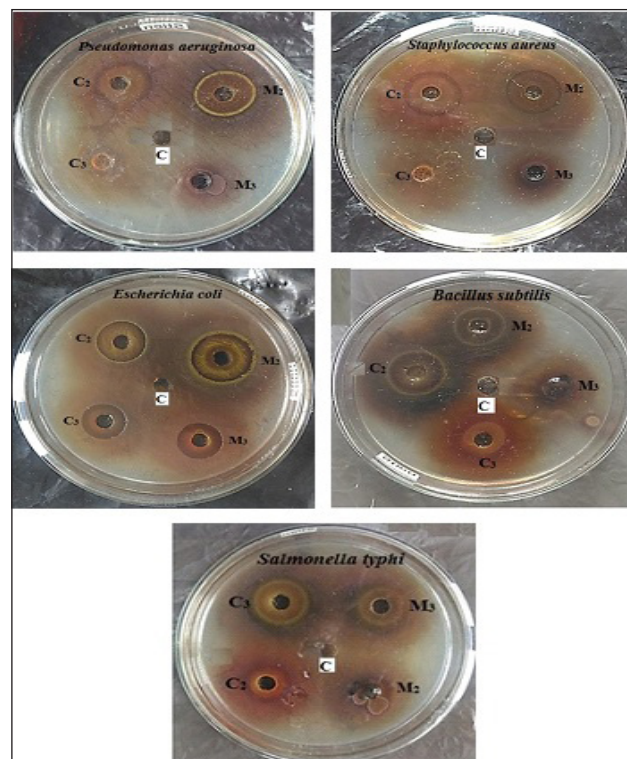


Fig. 1: Zones of inhibitions revealed by various plant parts of *A. occidentale* and *M. indica* against *P.aeruginosa*, *S. aureus*, *E. coli*, *B. subtilis* and *S. typhi*

C₂ (Mature leaves), C₃ (Flower) - *A. occidentale*
M₂ (Mature leaves), M₃ (Flower) - *M. indica*
C-control

It was observed that ethanol and ethyl acetate leaf extract also showed zone of inhibition against *S. aureus* and *E. coli* (Chbisika *et al.* 2014). Thus the present study is in conformity with early reports. The antibacterial activities of different plant parts of *A. occidentale* are mainly due to the presence of a phenolic lipid known as anacardic acid (Sujatha *et al.* 2011).

Result of the present study also revealed that ethyl acetate extract of mature leaves of *M. indica* showed zone of inhibition against *P. aeruginosa*, *B. subtilis*, *E. coli*, *S. typhi* and *S. aureus*. It was observed that among the two extract of *M. indica*, ethyl acetate extract of mature leaves showed comparatively high antimicrobial activity against *P. aeruginosa* with a zone of inhibition 24mm. It also showed 23mm zone of inhibition against *S. aureus*, 17mm zone of inhibition against *B. subtilis* (Table 3 & Fig. 1).



It was also observed that mature leaf extract of *M. indica* showed maximum zone of inhibition (24mm) against *P. aeruginosa* compared to the mature leaf extract of *A. occidentale* (19mm) (Table 3 & Fig. 1). This study was also supported by early study, there the antibacterial activity of flower of *M. indica* against *E. coli* was investigated (Poongothai and Rajan 2013). It was also confirmed that ethanolic and methanolic extract of mango leaf showed relatively high zones of inhibition against *S. typhi*. (Zakaria *et al.* 2006). Plant extracts with antibacterial activities have therapeutic potential to heal several infectious diseases and are related with lesser side effects compared to the synthetic drugs.

CONCLUSION

This research provides information about the antioxidant property and antibacterial activity of various plant parts of *A. occidentale* and *M. indica*. Hence, it is identified that these two species can be used as a source for the manufacturing of drugs of scavenging property and antibacterial activity. However, large scale *in vivo* studies are required to confirm the scavenging property and antimicrobial activity before going for commercialization.

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