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Antioxidant and antimicrobial activity of different plant parts of *Anacardium occidentale* L. and *Mangifera indica* L.: a comparative study

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ABSTRACT

Anacardium occidentale L. and *Mangifera indica* L. has been used worldwide both for pharmaceutical, food and cosmetic industries due to the presence of biological activities of some of its metabolites. The present study comprises the correlation of antioxidant activity and antimicrobial activity in ethyl acetate extract of young leaves and bark of *A. occidentale* and *M. indica*. The activity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) on radical scavenging effect of the extracts was carried out by spectrophotometrically. All the plant extracts showed DPPH radical scavenging activity and among the extracts, *A. occidentale* young leaves indicated higher antioxidant potential in comparison with those of the other extracts. The antibacterial activity of various extracts was also screened against some human pathogens of clinical importance; *Pseudomonas aeruginosa*; *Salmonella typhi*; *Bacillus subtilis*; *Escherichia coli* and *Staphylococcus aureus.*

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

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INTRODUCTION

Medicinal plants have become an element of international importance both in the case of medicinal and economical. The use of plant biochemical for therapeutic use is known since ancient time. Antioxidants have the ability to scavenge the free radicals found in a variety of food stuffs. Now a day, many antioxidants were extracted from plants to replace synthetic ones and also play a major role in protecting plants against harsh sunlight and live under severe oxygen stress. [1-4] Vitamins, carotenoids and phenolic compounds are the major compounds that exhibit antioxidant activity. [5-6] Phenolics and flavonoids play a major role in contributing the antioxidant activity to the members of Anacardiaceae. [7-8] Considering this fact, the medicinally important tree species such as *A. occidentale* and *M. indica* were chosen for the evaluation of antioxidant property. It will help to find out new sources of natural antioxidants to use them in food industries and pharmaceutical industries to replace synthetic ones.

The potent plant components can contest human and plant pathogenic bacteria, fungi and virus without any side effects and environmental hazards. Due to this favourable reason, search for plant products with antimicrobial properties intensified in recent years. [9-10] Microbial infections are one of the major reasons for physical disabilities, health problems and mortalities around the world. In medical field, phytochemicals have been used as antimicrobial agents. Compared to commercial antibiotics, natural phytochemicals are found to be more effective with fewer side effects that is why they are used as an alternate remedy for the treatment of various diseases. Bioactive compounds (alkaloids, tannins, flavonoids, phenolics etc.) also showed antimicrobial activity in the area of food preservation, pharmaceutics etc. Many pathogenic microbial species showed resistance to antibiotics has led to the screening of medicinal plants for their potential antimicrobial activities. [11-12] Based on the role of plant extracts in curing many infectious diseases, the present study was carried at comparison of the antibacterial activity of various extracts of *A. occidentale* and *M. indica*. These plant extracts were investigated for antibacterial activity against selected pathogenic microbes (*S. aureus, E. coli P. aeruginosa*, *S. typhi* and *B. cereus*), that cause human skin disorders.

MATERIALS AND METHODS

Plant materials

Plant materials (young leaves and bark) of *A. occidentale* and *M. indica* were collected from the mother trees grown at the Sree Narayana College Campus, Kollam. The plant parts collected were cleaned and air-dried and later ground to fine powder.

Preparation of plant 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). The samples were centrifuged at 10,000 rpm for 15 minutes and supernatants were collected. The filtered extract was concentrated in a rotary evaporator to remove ethyl acetate. The residue thus obtained was dissolved in ethyl acetate and kept at 4-8^{o}C in a refrigerator. [11-12]

DPPH free radical scavenging activity

The DPPH free radical scavenging activity was assessed by the modified method. [13] DPPH (20 mg) was dissolved in ethyl acetate (250 ml) in order to get the concentration of 80µg/ml. The plant extract was prepared in ethyl acetate (1 mg/ml). Serial dilutions were done to obtain concentration of 20, 40, 60, 80 and 100µg/ml. Ascorbic acid was used as standard (1-125µg/ml). Spectrophotometric reading was taken at 517 nm after 30 minutes dark incubation at room temperature. Freshly prepared DPPH solution in ethyl acetate was used for absorbance measurements. Percentage of inhibition was calculated using the following expression.

$$\% \text{ Inhibition} = (A_{control} - A_{sample} / A_{control}) \times 100$$

Non-linear regression analysis was used to calculate the concentration exhibiting 50% of DPPH free radical scavenging activity (IC_{50}).

Antibacterial activities

The antibacterial activity of the ethyl acetate extracts of various plant parts were carried out against some human pathogenic bacteria; *Pseudomonas aeruginosa* (MTCC 1034); *Salmonella typhi* (MTCC 1168); *Bacillus subtilis* (MTCC 2340); *Escherichia coli* (MTCC 56) and *Staphylococcus aureus* (MTCC 9760) obtained from the Microbiology Laboratory of the Department of Biotechnology, Sree Narayana College, Kollam, Kerala. Agar well diffusion method [14-15] was employed to assess the antibacterial activities of the different plant extracts using Mueller- Hinton agar plates. The bacteria (1 ml bacterial broth of approximately 10^5 cells) were swabbed with a sterile cotton swab into the Mueller-Hinton agar plates. The plant extract (250 mg/ml) was transferred to the well of 6 mm diameter. Then the microbial plates were incubated at 37^{o}C for 24 hours. The zone of inhibitions produced by the inhibitory action of different plant extracts and control were taken as the antibacterial activity.

Statistical analysis

All the determinations were conducted at least 3 times (n = 3); The statistical analysis was carried out using a SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver.17, 2008). One way analysis (ANOVA) and the Duncan's New Multiple range test were applied to the result at 0.05 level of significance (p<0.05).

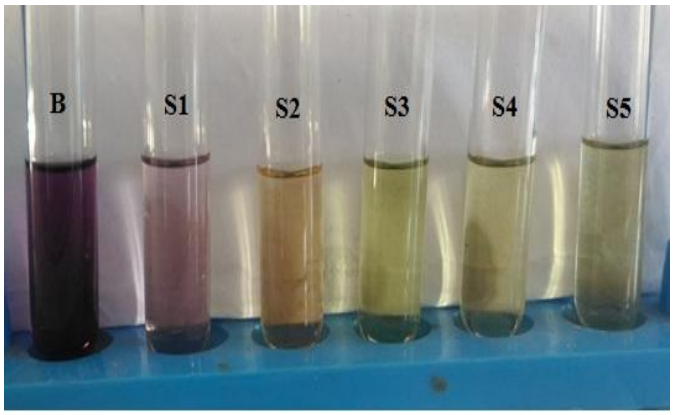

Fig. 1: Discoloration of DPPH under the influence of ascorbic acid at different concentrations (25-125µg/ml)

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant property of ethyl acetate extracts of various plant parts of *A. occidentale* and *M. indica* were determined using ethyl acetate solution of DPPH reagent along with ascorbic acid as standard. Antioxidant present in the given samples reacted with DPPH and its colour changed to yellow. The antioxidant activity of standard ascorbic acid at various concentrations is represented in Figure 1. The results of the present investigation showed that all the plant extracts showed antioxidant activity. Among various

plant extracts of *A. occidentale* and *M. indica* checked for antioxidant activity, the various plant extracts of *A. occidentale* showed the maximal 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: young leaves > bark (Table 1). It was noticed that the DPPH radical scavenging activity was increased with the increase in the concentration of all the extracts from 20 to 100µg/ml. Among the four extracts and standard tested for antioxidant activity, the ethyl acetate extract of young leaves of *A. occidentale* revealed the topmost percent of inhibition from 36.44 ± 0.58% at 20µg/ml to 44.31 ± 1.71% at 100µg/ml while the ethyl acetate extracts of bark of *M. indica* showed the least (1.74 ± 0.77% at 20µg/ml and 9.47 ± 1.55% at 100µg/ml), which is corresponding to the antioxidant activity of standard ascorbic acid (45.25 ± 0.36% at 20µg/ml and 49.89 ± 0.99% at 100µg/ml) (Table 1). Radical scavenging activities of plant phenolic compounds and the relationship between phenolic compounds and antioxidant activity was also studied [16] and many researchers have affirmed that phenolic compounds are most adequate antioxidants in *A. occidentale*. [17-18] Radical scavenging activity of ethanol extract of flower, leaves and stem bark of *A. occidentale* were explored. [19] The antioxidant activity of young leaves, barks, roots and kernels of *M. indica* were also studied. [20] Mangiferin is the most important phenolic compound present in the various parts of *M. indica*. [21]

The free radical scavenging activity of different plant part extracts of *A. occidentale* and *M. indica* were also expressed in terms of IC_{50} (µg/ml) values (Table 2) and it ranged from 160.39 ± 1.74µg/ml to 526.29 ± 1.71µg/ml. The result revealed that bark of *M. indica* showed weak antioxidant activity, with IC_{50} value of 526.29 ± 1.71µg/ml; while the ethyl acetate extract of young leaves of *A. occidentale* revealed the greatest antioxidant activity, with IC_{50} value of 160.39 ± 1.74µg /ml. The IC_{50} value for ascorbic acid was 97.34 ± 1.33µg/ml. In each explant type, the IC_{50} value decreased with the increase of antioxidant activity and vice versa. The result of the current study highlighted that the IC_{50} value differ significantly ($p < 0.05$) among the various extracts (Table 2).

Antimicrobial activity

In this 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 *Pseudomonas aeruginosa* (MTCC 1034); *Salmonella typhi* (MTCC 1168); *Bacillus subtilis* (MTCC 2340); *Escherichia coli* (MTCC 56) and *Staphylococcus aureus* (MTCC 9760) (Fig. 2).

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

Among the two extracts of *A. occidentale* tested, young leaves showed maximum zone of inhibition (24 mm) against *P. aeruginosa*. Young leaves showed same range of inhibitory action against *E. coli* and *S. aureus* with a zone of inhibition of 23 mm (Table 3 & Fig. 3).

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 (µg/ml)				
		20	40	60	80	100
A. occidentale	Young leaves	36.44 ± 0.58	38.11 ± 1.34	39.97 ± 1.23	41.96 ± 0.91	44.31 ± 1.71
	Bark	10.34 ± 0.94	11.86 ± 0.91	15.17 ± 1.48	17.90 ± 1.22	19.1 ± 1.33
M. indica	Young leaves	31.33 ± 0.63	32.1 ± 1.79	34.3 ± 0.87	36.31 ± 1.74	37.91 ± 1.23
	Bark	1.74 ± 0.77	3.51 ± 0.64	5.73 ± 1.12	7.14 ± 1.68	9.47 ± 1.55
Control	Ascorbic acid	45.25 ± 0.36	46.43 ± 1.97	48.15 ± 0.98	49.1 ± 0.86	49.89 ± 0.99

Results are expressed as means ± SD for triplicates

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

Test compound (ethyl acetate extract)		IC_{50} (µg/ml)
A. occidentale	Young leaves	160.39 ± 1.74b
	Bark	358.18 ± 0.97d
M. indica	Young leaves	239.59 ± 1.28c
	Bark	526.29 ± 1.71e
Control	Ascorbic acid	97.34 ± 1.33a

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

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				
		P. aeruginosa	*S. typhi*	*B. subtilis*	*E. coli*	*S. aureus*
A. occidentale	Young leaves	24	19	20	23	23
	Bark	9	-	9	13	-
M. indica	Young leaves	25	14	24	19	19
	Bark	9	-	12	9	13

Young leaves (C_1), Bark (C_2) - *A. occidentale*; Young leaves (M_1), Bark (M_2) - *M. indica*

It was recognized that ethanol extract of stem bark showed the zone of inhibition of 12.5 mm and cashew leaves showed the zone of inhibition of 13 mm against *S. aureus*. [22] Thus the present study is in conformity with early reports. The antimicrobial properties of various plant parts of *A. occidentale* are mainly due to the presence of a phenolic lipid known as anacardic acid and other chemical compounds such as tannins, flavonoids, phenols, alkaloids, saponins, steroids or triterpenes. [23-24]

Result of the current study also detected that ethyl acetate extract of young leaves and bark of *M. indica* showed zone of clearance against *P. aeruginosa, B. subtilis, E. coli* and *S. aureus*. It was observed that among the two extract of *M. indica,* ethyl acetate extract of young leaves demonstrated relatively great antimicrobial activity against *P. aeruginosa* with a zone of inhibition 25 mm. It also showed 19mm zone of inhibition against *S. aureus*, 24mm zone of inhibition against *B. subtilis* (Table 3 & Fig. 3).

It was also observed that young leaf extract of *M. indica* showed maximum zone of inhibition (25 mm) against *P. aeruginosa* compared to the young leaf extract of *A. occidentale* (24 mm) (Table 3 & Fig. 3).

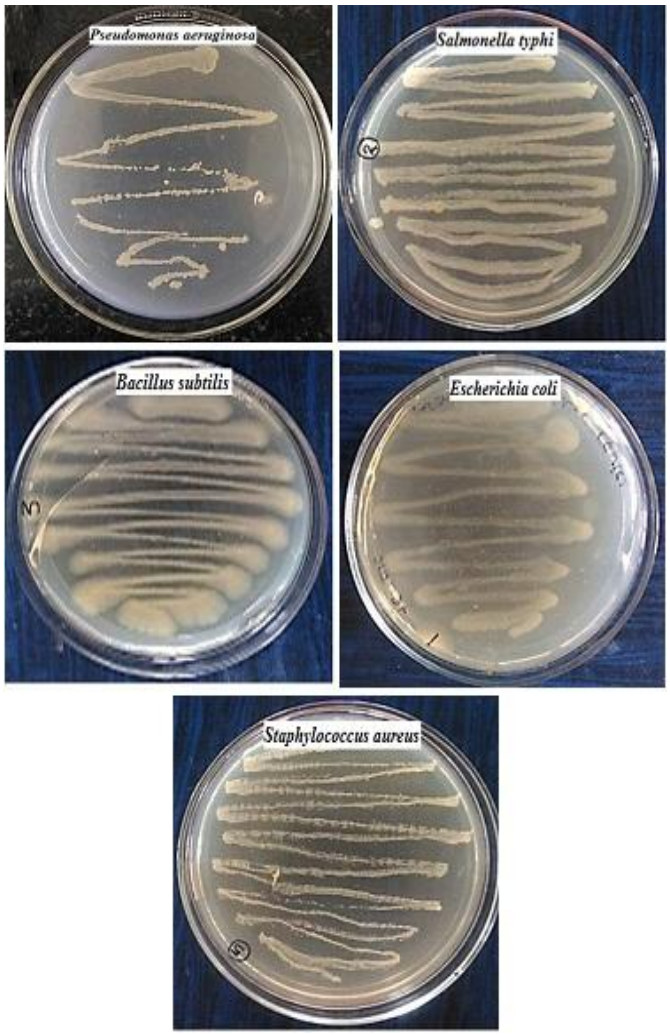

Fig. 2: Pure cultures of *P. aeruginosa; S. typhi; B. subtilis; E. coli* and *S. aureus* on nutrient agar plate

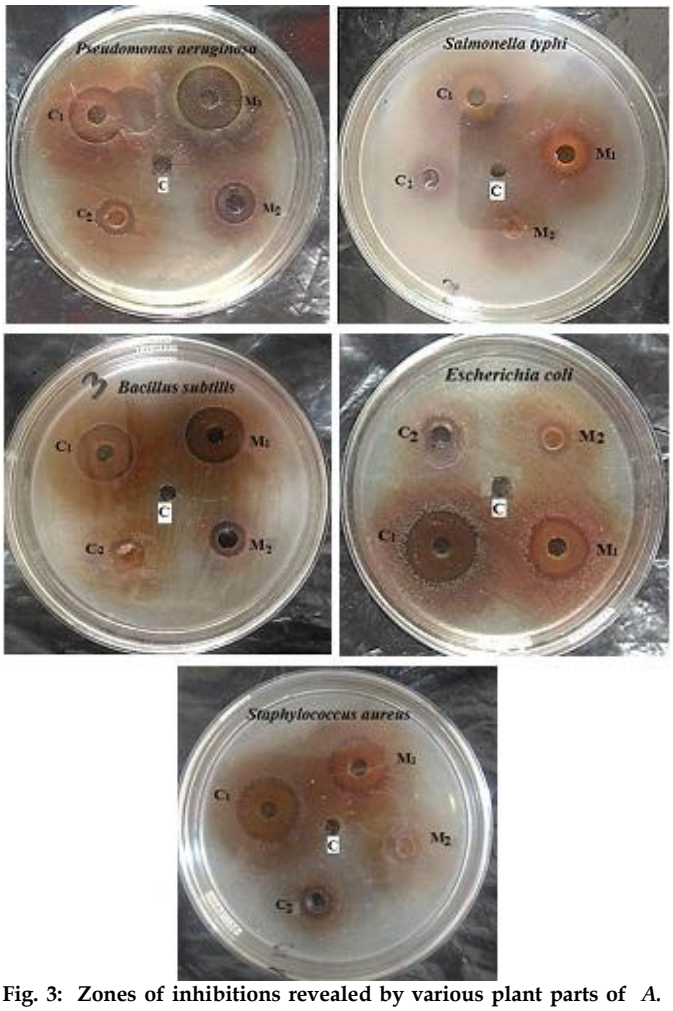

Fig. 3: Zones of inhibitions revealed by various plant parts of *A. occidentale* and *M. indica* against *P. aeruginosa, S. typhi, B. subtilis, E. coli* and *S. aureus*
C_1 (Young leaves), C_2 (Bark) - *A. occidentale*, M_1 (Young leaves), M_2 (Bark) - *M. indica*, C-control

In the case of *B. subtilis*, young leaf extract of *M. indica* showed maximum zone of inhibition of 24 mm compared to other plant extracts of *A. occidentale*. Young leaf extract of *A. occidentale* showed zone of inhibition (23 mm) against *E. coli* and *S. aureus* (Table 2 & Fig. 3).

This study was also supported by early study; there the antibacterial activity of young leaves of *M. indica* against *S. typhi* was investigated. [25] This study also helped to find out new sources of natural antioxidants to use them in food industries and pharmaceutical industries to replace synthetic ones. Antibacterial activities of plant extracts have lot of therapeutic application to treat many infectious diseases and they also have fewer side effects related to synthetic drugs.

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