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Research Article

ANTIMICROBIAL PROPERTY OF POTENT MEDICINAL PLANT *ACACIA NILOTICA* (L.) WILD. EX. DELILE SUBSP. *INDICA* (BENTH.) BRENAN

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ABSTRACT

Objectives: In the present study, the antimicrobial property of extracts obtained from leaves of *A. nilotica* was evaluated as the leaves of *A. nilotica* are traditionally useful for treating infectious diseases.

Methods: The air-dried *A. nilotica* leaves were packed into a Soxhlet apparatus and were extracted sequentially with petroleum ether (PE), benzene (BZ), dichloromethane (DCM), chloroform (CF), ethanol (EA) and water (AQ). Three gram negative (*Escherichia coli, Klebsiella pneumonia, Proteus mirabilis*) and three gram positive *(Staphylococcus aureus* , *Enterococcus faecalis, Streptococcus pneumoniae*) organisms were selected for determining the antimicrobial potency of extracts by using agar well diffusion method and minimum inhibitory concentration(MIC) was also determined for all the extracts.

Results: The results indicated that the various extracts of this plant exhibited different extent of antimicrobial activities but the ethanol extract of *A. nilotica* showed a higher potency than the positive control kamamycin at higher concentration tested in agar well diffusion method. MIC values also have indicated that the ethanol extract was considered as good antimicrobial agents whereas benzene, dichloromethane and aqueous extracts as moderately active agents and petroleum ether as weakly active. The potency of ethanol could be due to the presence of enormous amount of flavonoids and phenolic compounds.

Conclusion: Thus the crude ethanol extract of leaves of *A. nilotica* can be used as a potential source of natural antibiotics.

Keywords: *Acacia nilotica,* Antimicrobial property, Minimum inhibitory concentration, Agar well diffusion method.

INTRODUCTION

Plants served mankind as a source of medicine from time immemorial. According to World Health Organization, the best way of obtaining drugs could be from medicinal plants. About 80% of populations from developed countries use traditional medicine as a cure for ailments. The phytochemicals from safe traditional medicinal plants serve as lead compounds for discovering chemotherapeutic drugs [1-3]. Therefore, such plants should be investigated to evaluate their properties, safety and efficacy.

Plants are considered as chemical storehouses as they contain a variety of multidisciplinary bioactive compounds. Both plant extracts and phytochemicals with known pharmacological properties can be of great significance in medicinal field [4-5]. The phytochemicals responsible for pharmacological property could be secondary metabolites such as phenolic compounds, tannins, essential oils etc [6-9]. There is large number of studies conducted in different countries to evaluate the pharmacological efficiency of medicinal plants.

Dietary phytochemicals with antioxidant activity are associated with a lower risk of mortality from many of the diseases like diabetes, acute hypertension, cancer, infectious diseases and cardiovascular diseases [10-11] Plant-derived natural products such as flavonoids, terpenes, alkaloids, α-tocopherol, and carotenoids have received considerable attention in recent years due to their diverse pharmacological properties, including cytotoxic and chemo preventive effects [12-13].

The plant *A. nilotica* is a tree 5-20m high with a dense spheric crown, stems and branches usually dark to black colored, fissured bark, grey-pinkish slash, exuding a reddish low quality gum. The tree is used for cold, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma [14]. It was reported that the plant is rich in phenolics consisting of condensed tannin and phlobatannin, gallic acid, protocatechuic acid pyrocatechol, (+) -catechin, (_) epigallocatechin- 7-gallate, and (_) epigallocatechin-5, 7-digallate [15-16].

In our previous study, we showed that the ethanol extract prepared from leaves of *Acacia nilotica* had potent antioxidant and anti cancer activities and we found that the phenolic compound ethyl gallate was responsible for these properties [17-19].

However, to the best of our knowledge, no study has been carried out on the possible antimicrobial action of leaves of this plant. Therefore in the present study, the possible antimicrobial property of extracts obtained from leaves of *A. nilotica* (L.) Wild. Ex. Delile Subsp. *indica* (Benth.) Brenan was evaluated because the leaves of *A. nilotica* are traditionally useful for treating infectious diseases like throat infection, cold, pneumonia, meningitis and urinary problems.

MATERIALS AND METHODS

Plant preparation and extraction

The *Acacia nilotica* was collected from vicinity of VIT University, Vellore during the month of August 2007. *A. nilotica* was identified as *Acacia nilotica* (L.) Wild. Ex. Delile subsp. *indica* (Benth.) Brenan and voucher specimen deposited at Botanical Survey of India, South circle, Coimbatore.

For sequential extraction, air-dried *A. nilotica* leaves were packed into a Soxhlet apparatus and were extracted sequentially with petroleum ether (PE), benzene (BZ), dichloromethane (DCM), chloroform (CF), ethanol (EA) and water (AQ). The extracts were subjected to further analysis and all the assays were done in triplicates.

Determination of antimicrobial property

Culture media

Mueller Hinton agar (MHA) is used to test bacteria. MHA was prepared and sterilized in autoclave at 121ºC for 15 minutes and poured in sterilized petriplates and allowed for solidification.

Test organisms used

The test organisms were selected on the basis that they cause a lot of infections in humans. Both Gram-positive and Gram-negative bacterial species were selected as test organisms.

Gram positive bacteria

Staphylococcus aureus (ATCC 9144); *Enterococcus faecalis* (ATCC 35550); *Streptococcus pneumoniae* (ATCC 33400).

Gram negative bacteria

Escherichia coli (ATCC 13534); *Klebsiella pneumonia* (ATCC 15380); *Proteus mirabilis* (ATCC 7002).

Preparation of inoculum and standardization of culture

The inoculum was prepared by inoculating a loop of each organism from a 24 h old culture into a sterile nutrient broth aseptically and kept in incubator shaker at 37ºC for 24 h.

The overnight culture is taken and checked until the visible turbidity is equal or greater than the 0.5 McFarland standard at 625 nm using spectrophotometer. Sterilized nutrient broth is used as blank [20]. If the absorbance is higher, then the culture is diluted with sterilized nutrient broth and absorbance is noted. The culture is stored for the further analysis.

Determination of antimicrobial assay

The Plant extracts were assessed for its antimicrobial activity by agar well diffusion method [21]. MHA plates were seeded with standardized 24 hr broth culture of test bacteria. In each of these plates five wells of 6mm were made using a sterile cork borer. The plant extracts of different concentrations (10, 50,100, 250 and 500 µg) were added to respective wells in the volume of 100 µl. Separate plates were kept for both positive and negative control. Solvent was used as negative control and kanamycin antibiotic as positive control. The plates were kept at room temperature for 20 min for diffusion of extracts into the medium and incubated at 37ºC for 24 h. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in triplicate and the diameter of the inhibition zones in mm was calculated.

Determination of minimum inhibitory concentration

Plant extracts at different concentrations were added in the test tubes and incubated at 37ºC for 30 minutes. After incubation 1 ml of purple broth and 1 ml of 1% dextrose were added in all the test tubes. Control was also maintained with nutrient broth along with purple broth and dextrose. The tubes were incubated at 37ºC for 24 h.

The retention of purple color in the test tubes at the minimum concentration indicated the minimum inhibitory concentration of an antimicrobial that will inhibit the visible growth of an organism after overnight incubation [22].

RESULTS AND DISCUSSION

Antimicrobial activity

Medicinal plants have become the subject of global attention due to the evolution of resistance genes to antibiotics of microbial origin and synthetic compounds [23]. A great deal of interest has developed in the fields of ethno botany and complementary medicine to identify bioactive compounds for their chemo preventive and chemotherapeutic potentials. [24]. The antibacterial activity of the plant extracts against six bacterial strains was quantitatively assessed by the presence or absence of inhibition zone by measuring the diameter of area around the well (Table 1).

Table 1: Antimicrobial activity of different extracts obtained from *Acacia nilotica*

Extracts	Conc. (µg/ml)	Zone of inhibition (mm)					
		1	2	3	4	5	6
PE	10	-	-	-	-	-	-
	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
	250	-	8.2 ± 0.17	-	-	-	-
	500	-	9.5 ± 0.29	-	-	-	-
BZ	10	-	-	-	-	7.3 ± 0.333	-
	50	-	-	-	-	8.3 ± 0.33	-
	100	-	-	-	-	9.3 ± 0.33	-
	250	-	-	-	6.5 ± 0.29	10.3 ± 0.33	-
	500	-	-	-	7.3 ± 0.33	11.3 ± 0.33	-
DCM	10	-	6.5 ± 0.29	-	6.3 ± 0.33	-	-
	50	-	7.16 ± 0.17	-	7.3 ± 0.33	-	-
	100	-	8.16 ± 0.16	-	8.3 ± 0.33	-	-
	250	-	9.16 ± 0.16	-	10.3 ± 0.33	-	-
	500	-	10.5 ± 0.28	-	11.3 ± 0.33	-	-
CF	10	-	6.16 ± 0.16	-	8.5 ± 0.28	-	-
	50	-	8.5 ± 0.28	-	9.16 ± 0.16	-	-
	100	-	10.3 ± 0.3	-	10.3 ± 0.33	-	-
	250	-	11.3 ± 0.3	-	11.3 ± 0.33	-	-
	500	-	12.5 ± 0.28	-	12.16 ± 0.16	-	-
EA	10	-	7.5 ± 0.29	-	-	9.3 ± 0.33	7.16 ± 0.16
	50	10.5 ± 0.29	8.5 ± 0.28	9.33 ± 0.33	8.3 ± 0.33	11.5 ± 0.28	8.5 ± 0.28
	100	12.2 ± 0.16	9.2 ± 0.16	11.33 ± 0.33	9.2 ± 0.16	12.3 ± 0.33	9.3 ± 0.33
	250	13.2 ± 0.16	10.33 ± 0.33	12.33 ± 0.33	10.5 ± 0.28	13.3 ± 0.33	10.3 ± 0.33
	500	17.5 ± 0.28	11.3 ± 0.33	14.33 ± 0.33	11.5 ± 0.29	14.5 ± 0.28	11.3 ± 0.33
AQ	10	-	-	-	7.5 ± 0.28	-	-
	50	-	-	-	8.3 ± 0.33	-	-
	100	-	-	-	10.3 ± 0.33	-	-
	250	-	8.3 ± 0.33	-	-	-	-
	500	-	9.3 ± 0.33	-	-	-	-
Kanamycin	30 µg/disc	16.3 ± 0.33	18.3 ± 0.33	15.0 ± 0.57	12.3 ± 0.33	18.3 ± 0.33	11.33 ± 0.33

1. Staphylococcus aureus 2. Streptococcus pneumonia 3. Enterococcus faecalis 4. Escherichia coli 5 Proteus mirabilis 6. Klebsiella pneumoniae

PE: Petroleum ether; BZ: Benzene; DCM: Dichloromethane; CF: Chloroform; EA: Ethanol; AQ: Water

Note: mm - millimeter; µg - microgram; ml - milliliter

The majority of the clinically useful antibiotics are active against the test strains at least at 10µg /ml concentration. A compound is not considered as an antibiotic if it is not active at least at 100µg /ml. On this basis, tests on plant extracts were carried out at five different concentrations (10, 50,100, 250 and 500 µg). All the extracts behaved differently in the present investigation and the distinct

microbial activities of different extracts can be due to the diverse chemical nature of various phytochemicals [25]. Gradual increase in the diameter of inhibition zone was noted with corresponding increase in concentration of extracts used. In the present work, the ethanol extract of *Acacia nilotica* was found to be effective against both gram-positive and gram-negative bacteria at all the concentrations tested. Our results are consistent with the previous reports indicating that ethanolic leaf extracts of *Aegel marmelos*, showed maximum inhibition against Gram-positive and Gram-negative bacteria. [26]. Dichloromethane, chloroform and aqueous extracts were effective against *Streptococcus pneumonia* and *Escherichia coli* but benzene extract was effective against *Proteus mirabilis* only. Petroleum ether extract was not effective against all the organisms tested but effective against *Streptococcus pneumonia* only at higher concentrations. When compared to ethanol extract, all the extracts showed less antibacterial activity. Similar results were also reported in the past study in bark extract of *A. nilotica* [27] where the ethanol stem bark extract was effective against *Streptococcus viridans, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Shigella sonnei.*

When compared to the antibiotic kanamycin, the ethanol extract exhibit higher zone of inhibition against the tested organism at higher concentrations. This reveals that the plant contains compounds that may have potent antimicrobial activity against the tested microorganisms than the ethanol extract and antibiotics tested. The positive control exhibited the zone of inhibition at varying ranges, *S. aureus* at 16mm, *S. pneumoniae* at 18mm, *E. faecalis* at 15mm, *E. coli* at 12mm, *P. mirabilis* at 18mm, *K. pneumoniae* at 11mm.

Minimum inhibitory concentration

Minimum inhibitory concentrations are considered as the 'gold standard' for determining the susceptibility of organism to antimicrobial activity and therefore used to judge the performance of all other methods of susceptibility testing. It is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation [20].

Minimum inhibitory concentration (MIC) was assessed for all the extracts of *A. nilotica*. The results indicated that the ethanol extract was effective than other extracts against the tested organism. Ethanol extracts inhibited the growth of *S. aureus, S. pneumoniae, E. faecalis, E. coli, P. mirabilis and K. pneumoniae* at concentrations of 10 & 5 0µg/ml (Table 2). Extracts with MIC values less than 100 µg/ml were considered with good antimicrobial activity; whereas MIC values of 100- 500 µg/ml were moderately active and 500-1000 µg/ml were weakly active [8]. Thus ethanol extract was considered as good antimicrobial agents whereas benzene, dichloromethane and aqueous extracts as moderately active agents and petroleum ether as weakly active. Similar results were observed in the previous study assessed for antimicrobial activity of ethanol extracts of five plants including *Acacia nilotica* against 11 clinical pathogens including *S. aureus*, *E. faecalis*, *E. coli, K. pneumoniae.* This study revealed that, the most potent antimicrobial plant was *A. nilotica* (MIC range 9.75-313µg/ml) [28].

Table 2: Minimum inhibitory concentration of different extracts obtained from *Acacia nilotica*

Extracts	Minimum inhibitory concentration (µg/ml)					
	1	**2**	**3**	**4**	**5**	**6**
PE	-	-	-	-	-	-
BZ	-	-	-	500	500	-
DCM	-	500	-	500	-	-
CF	-	250	-	500	-	-
EA	50	10	50	50	10	10
AQ	-	500	-	500	-	-

1. Staphylococcus aureus 2. Streptococcus pneumoniae 3. Enterococcus faecalis 4. Escherichia coli 5. Proteus mirabilis 6. Klebsiella pneumonia

PE – Petroleum ether; BZ – Benzene; DCM – Dichloromethane; CF – Chloroform; EA – Ethanol; AQ – Water extracts

Previous reports about *Acacia* species demonstrate that they are rich in polyphenolic compounds [29-30]. It was also reported that the plant was found to be rich in gallic acid, methyl gallate and catechin. The highest antimicrobial activity of ethanol extract could be due to the presence of high amount of phenolics and flavonoids.

CONCLUSION

The various extracts of leaves of *A. nilotica* in this study exhibited different extent of antimicrobial activities but the ethanol extract of *A. nilotica* showed a higher potency than the positive control kamamycin at higher concentration tested. This could be due to the presence of enormous amount of flavonoids and phenolic compounds, which are responsible for the immense antimicrobial property. The results of the present study would certainly help to ascertain the potency of the crude ethanol extract of leaves of *A. nilotica* as a potential source of natural antibiotics.

Since, *A. nilotica* is a commonly available plant; it may represent a potential, economical therapeutic agent for infectious diseases, due to its antimicrobial activities.

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REFERENCES

1. Bouhlel I, Mansour H, Limem I, Sghaier M, Mahmoud A, Chibani J et al.. Screening of antimutagenicity via antioxidant activity in different extracts from the leaves of *Acacia salicina* from the center of Tunisia. Environ Toxicol Pharmacol 2007; 23: 56-63.
2. Ramya S, Kalaivani T, Rajasekaran C, Jepachanderamohan P, Alaguchamy N, Kalayansundaram M, Jayakumararaj R Antimicrobial activity of aqueous extracts of bark, root, leaves and fruits of *Terminalia arjuna* Wight & Arn. Ethnobotanical Leaflets 2008; 12: 1192-1197.
3. Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar N, Siva R, Jayakumararaj R Investigations on antibacterial activity of leaf extracts of *Azardirachta indica* A. Juss (Meliaceae): A traditional medicinal plant of India. Ethnobotanical Leaflets 2008a; 12:1213-1217.
4. Kagithoju S, Godishala V, Pabba SK, Kurra HB, Swamy R Anti bacterial activity of flower extract of *Pongamia pinnata* linn – An elite medicinal plant. Int J Pharmacy and Pharmaceutical Sci 2012; 4(3): 130-132.
5. Neelavathi P, Venkatalakshmi P, Brindha P Antibacterial activities of aqueous and ethanolic extracts of *Terminalia catappa* leaves and bark against some pathogenic bacteria. Int J Pharmacy and Pharmaceutical Sci 2013; 5(1): 114-120.
6. Basile A, Sorbo S, Giordano S, Ricciardi L, Ferrara S, Montesano D et al Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. Fitoterapia 2000; 71:.110-116.
7. Paiva S, Figueiredo M, Aragão T, Kaplan M Antimicrobial activity *in vitro* of plumbagin isolated from Plumbago species. Memórias do Instituto Oswaldo Cruz 2003; 98: 959-961.
8. Pretto J, Cechinel-Filho V, Noldin V, Sartori M, Isaias D, Cruz A Antimicrobial activity of fractions and compounds from Calophyllum brasiliense (Clusiaceae/Guttiferae). Zeitschrift Fur Naturforschung C 2004; 59: 657-662.
9. Kalaivani T, Mathew L Phytochemistry and free radical scavenging activities of *Oroxylum indicum*. Environ We Int J Sci Tech 2009; 4: 45-52.

10. Rice-Evans C, Flavonoids and isoflavones: absorption, metabolism, and bioactivity. Free Radical Biology and Medicine 2004; 36: 827-828.
11. Dixon R, Xie D, Sharma S Proanthocyanidins: a final frontier in flavonoid research? New Phytologist 2005; 165: 9-28.
12. Jayaprakasha G, Patil B *In vitro* evaluation of the antioxidant activities in fruit extracts from citron and blood orange. Food Chem 2007; 101: 410-418.
13. Kviecinski M, Felipe K, Schoenfelder T, de Lemos Wiese L, Rossi M, et al Study of the antitumor potential of *Bidens pilosa* (Asteraceae) used in Brazilian folk medicine. J Ethnopharmacol 2008; 117: 69-75.
14. Ambasta S The useful plants of India. Condensed and updated version of 'The Wealth of India, Raw Materials series C, 5000. CSIR, New Delhi. 918. 1986
15. Kirtikar K, Basu B.. Liliaceae: Chlorophytum. Indian medicinal plants. Allahabad: LM Basu. 2508–9. 1975
16. Raghavendra MP, Satish S, Raveesha KA *In vitro* evaluation of anti-bacterial spectrum and phytochemical analysis of *Acacia nilotica*. J Agri Tech 2006; 2: 77–88.
17. Kalaivani T, Mathew L Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. ex Delile, an Indian medicinal tree. Food and Chemical Toxicology 2010; 48: 298-305.
18. Kalaivani T, Rajasekaran, C, Suthindharan K, Mathew L "Free radical scavenging, cytotoxic and hemolytic activities from leaves of *Acacia nilotica* (L.) Wild. ex. Delile subsp. *indica* (Benth.) Brenan". eCAM 2010; doi:10.1093/ecam/neq060
19. Kalaivani T, Rajasekaran C, Mathew L Free Radical Scavenging, Cytotoxic, and Hemolytic Activities of an Active Antioxidant Compound Ethyl Gallate from Leaves of *Acacia nilotica* (L.) Wild. Ex. Delile Subsp. *indica* (Benth.) Brenan. J Food Sci 2011; DOI: 10.1111/j.1750-3841.2011.02243.x
20. Andrews J Determination of minimum inhibitory concentrations. J Antimicrobial Chemotherapy 2001; 48: 5-16.
21. Collins CH, Lyne PM, Grange JM Microbiological Methods 7th Edn. Butterwort-Heinemann Ltd. Britain, 175-190 1995.
22. Ushimaru PI, Da Silva MTN, Di Stasi LC, Barbosa L, Fernandes A Antimicrobial activity of medicinal plant extracts. Brazilian J Microbiol 2007; 38: 717-719.
23. Lee E, Chen J, Huda M, Kuroda T, Mizushima T, Tsuchiya T Functional cloning and expression of emeA, and characterization of EmeA, a multidrug efflux pump from *Enterococcus faecalis*. Biological and Pharmaceut Bull 2003; 26: 266-270.
24. Kalaivani T, Rajasekaran C, Mathew L *In vitro* free radical scavenging potential of *Picrorhiza kurroa*. J Pharmacy Res 2010a; 3: 849-854.
25. Kalaivani T, Sasirekha M, Arunraj D, Palanichamy V, Rajasekaran C *In Vitro* Evaluation of Antibacterial Activity of Phytochemical Extracts from aerial parts of *Bacopa monnieri* (L.) Wettest (Scorphulariaceae). J Pharmacy Res 2012; 5(3): 1636-1639
26. Rajasekaran C, Meignanam E, Premkumar N, Kalaivani T, Siva R, Vijayakumar V, Ramya S, Jayakumararaj R *In vitro* evaluation of antibacterial activity of phytochemical extracts from leaves of *Aegle marmelos* (L.) Corr. (Rutaceae). Ethnobotanical Leaflets 2008b; 12: 1124-1128.
27. Banso A Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. J Med Plant Res 2009; 3: 82-85.
28. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali S, et al Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. Molecules 2009; 14(2): 586-597.
29. Kaur K, Michael H, Arora S, Härkönen P, Kumar S *In vitro* bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from *Acacia nilotica* (L.) Willd. ex Del. J Ethnopharmacol 2005; 99: 353-360.
30. Sultana B, Anwar F, Przybylski R Antioxidant activity of phenolic components present in barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica*, and *Eugenia jambolana* Lam. trees. Food Chem 2007; 104:1106-1114.