



ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *ADENANTHERA PAVONINA* AND *MUSSAENDA PHILIPPICA* AGAINST ISOLATED BACTERIA AND FUNGI.

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ABSTRACT

Long before mankind discovered the existence of microbes, the idea of using certain plants for medicinal purpose because of their healing potential due to antimicrobial principles, was well accepted. In the past few decades, the search for new anti-infection agents has occupied many research groups in the field of ethnopharmacology. In the present paper, we analyze two medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds that can act as new anti-infection agents. The antibacterial and antifungal activity of three solvent extracts of *Adenanthera pavonina* and *Mussaenda philippica* on microbes isolated from dairy cattle rearing unit was studied. The results revealed that these extracts have antibacterial activity against *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and hence these crude extracts can be used as anti-infection agents in dairy cattle rearing unit to avoid infections especially to calves which are prone to infections at birth.

KEYWORDS: *Antibacterial, antifungal, isolated strains, diffusion assay, phytochemicals, disinfectant.*

INTRODUCTION

Nature has gifted us with a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the world. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities¹. There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation of them to yield active principles^{2, 3,4,5}. These antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms⁶. From an estimated 250,000 higher plants in the world only 5–15% have been studied for a potential therapeutic value^{7,8}. Hence the rest of the plants remain to be investigated. Herbal medicines have received much attention as a

source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and for environment⁹. But, resistance to antimicrobial agents is emerging in a wide variety of pathogens and multiple drug resistance is becoming common in diverse organisms^{10, 11,12}. Most of the farmers express desire to learn more about the proper use and application of ethnoveterinary practices as these were economically, socially and culturally more acceptable for marginalized communities¹³. This has necessitated a search for new antimicrobial substances from plants. *Adenanthera pavonina* belongs to the family Mimosaceae, commonly known as Anai kundumani or Manjadi in Tamil, is an important medicinal plant from the 'Indian subcontinent'. This species is endemic to Southern China and India. Various parts of this plant have

also been used in traditional medicine for the treatment of asthma, boil, diarrhoea, gout, inflammations, rheumatism, tumour and ulcers, and as a tonic^{14,15,16}. Traditionally, the ground seed is widely used for the treatment of various human ailments such as treatment of boils, inflammation, blood disorders, arthritis, rheumatism, cholera, paralysis, epilepsy, convulsion, spasm and indigestion¹⁷. *Mussaenda philippica* of the family Rubiaceae, commonly known as Flag Bush is found throughout South East Asia. Due to the colourful nature of the plant it is usually used for landscaping. It is used as a medicinal plant in India and other South Asian countries. It is reported to have analgesic¹⁸, anticonvulsant¹⁹ and antitumor activity²⁰. The antibacterial activity of the leaves of *M. roxburghii* has been studied²¹. Antimicrobial activity of the extracts of these two plants against very few microbial species have been studied^{22,23,24}. In this study, the antimicrobial and antifungal activity of chloroform, ethyl acetate and ethanol extracts of the leaves of *M. philippica* and *A. pavonina* were assessed against bacteria and fungi isolated from dairy units.

MATERIALS AND METHODS

Collection

Fresh leaves of *Adenanthera pavonina* and *Mussaenda philippica* were collected from the suburbs of Chennai, Tamil Nadu, India. The taxonomic authentication was done by Dr. P. Jayaraman, Plant Anatomy Research Centre, Tambaram, Chennai. The voucher specimen number for *A. pavonina* is PARC/2014/2022 and *M. philippica* is PARC/2015/3131. The collected plants were washed with running tap water, again washed with distilled water, air dried, homogenized to a fine powder and stored in air-tight bottles.

Preparation of crude extracts.

Dried plant material of both *A. pavonina* and *M. philippica* were extracted with chloroform, ethyl acetate and ethanol separately. They were kept on a rotary shaker for 9 days, changing the solvents once in three days to elute maximum amount of extracts from the plants. The supernatant was collected by filtration using Whatman no.1 filter paper and the filtrate was evaporated at room temperature to retain thermo labile compounds in the extract. The extract was stored at 4°C in airtight sterile vials for further studies.

Bacterial Isolates

Bacterial isolates used in this study were isolated

from dairy units using standard isolation techniques and identified based on Bergys manual on Determinative Bacteriology²⁵. The bacterial strains include *Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Clostridium perfringens*. All the strains were confirmed by cultural and biochemical characteristics and maintained in slants for further use.

Fungal Isolates

Fungal isolates used in this study were *Candida albicans* and *Aspergillus niger*. They were isolated from dairy cattle rearing unit and both were identified by staining and cultural characteristics in sabouraud dextrose agar.

Agar disc diffusion method

Agar disc-diffusion method was carried to determine the antimicrobial and antifungal activity. Brain Heart Infusion agar (BHI) plates for bacteria and Potato Dextrose agar plates for fungi were swabbed with 8 hour old - broth culture of the isolates. Stock solution of chloroform, ethyl acetate, and ethanol extracts of *A. pavonina* and *M. philippica* were prepared at a concentration of 100mg/ml in DMSO. About 20 µl of different concentrations (2 mg, 1mg and 0.5 mg) of chloroform, ethyl acetate, and ethanol extracts of *A. pavonina* and *M. philippica* and DMSO (20ul) were added to the disc and allowed to dry at room temperature. Then the discs were placed over the swabbed plates. DMSO was used as negative control and Enrofloxacin (30mcg/disc) for bacterial strains and Amphotericin B (20mcg/disc) for fungal strains were used as positive control. The plates were incubated overnight at 37°C/28°C for bacterial / fungal isolates. The diameter of the inhibition zone (mm) was measured.

RESULTS

Chloroform, ethyl acetate, and ethanol extracts of both *M. philippica* and *A. pavonina* showed antibacterial activity against few isolates but did not show any activity against fungal isolates. The details of the microbial activity of *A. pavonina* are shown in Table1 and *M. philippica* is shown in Table 2. In the present study, the extracts of both the plants exhibited promising anti-bacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* was resistant to all the three extracts of *A. pavonina*

and ethyl acetate extract of *M.philippica*. Both the plant extracts showed no activity against *Proteus vulgaris*, *Enterobacter aerogenes*, and *Clostridium perfringens*. Ethanol extract of *M.philippica* showed antibacterial activity against all the bacterial species studied except *Proteus vulgaris*

and *Enterobacter aerogenes*. The zones of inhibition of all the extracts were comparatively lesser than the positive control Enrofloxacin (Table 1). DMSO which was used as a carrier (negative control) did not show any growth inhibition.

Table 1
Antimicrobial activity of A.pavonina.

Test organism	Diameter of inhibition zone(mm)									
	CL			EA			ET			EX
Bacteria										
Concentration per disc	2mg	1mg	0.5mg	2mg	1mg	0.5mg	2mg	1mg	0.5mg	120mcg
<i>Staphylococcus aureus</i>	12	12	12	18	14	10	19	18	12	40
<i>Escherichia coli</i>	14	13	11	10	10	10	8	-	-	24
<i>Klebsiella pneumoniae</i>	8	8	8	10	8	8	12	10	8	13
<i>Salmonella enteritidis</i>	-	-	-	10	8	8	8	8	8	35
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	24
<i>Bacillus subtilis</i>	12	12	8	8	8	8	8	8	-	23
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	-	26
<i>Enterobacter aerogenes</i>	-	-	-	-	-	-	-	-	-	23
<i>Clostridium perfringens</i>	-	-	-	-	-	-	-	-	-	40
Fungi	AB									
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	10
<i>Aspergillus niger</i>	-	-	-	-	-	-	-	-	-	10

CL-Chloroform, EA-Ethyl Acetate, ET-Ethanol, EX-Enrofloxacin, AB-Amphotericin B.

Both the fungal strains *Candida albicans* and *Aspergillus niger* were resistant against extracts of *A.pavonina* and *M.philippica*. Amphotericin B used as positive control showed an inhibition zone of 10

mm diameter. DMSO showed no activity against the two fungal strains. The results indicated that the plant extracts has effect on few Gram positive and Gram negative bacteria.

Table 2
Antimicrobial activity of M.philippica

Test organism	Diameter of inhibition zone(mm)									
	CL			EA			ET			EX
Bacteria										
Concentration per disc	2mg	1mg	0.5mg	2mg	1mg	0.5mg	2mg	1mg	0.5mg	120mcg
<i>Staphylococcus aureus</i>	12	6	6	10	9	8	8	8	8	40
<i>Escherichia coli</i>	-	-	-	12	9	-	14	12	10	24
<i>Klebsiella pneumoniae</i>	8	8	8	10	8	8	12	10	8	13
<i>Salmonella enteritidis</i>	-	-	-	-	-	-	8	8	-	35
<i>Pseudomonas aeruginosa</i>	12	12	11	-	-	-	11	8	-	24
<i>Bacillus subtilis</i>	-	-	-	9	8	8	13	11	10	23
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	-	26
<i>Enterobacter aerogenes</i>	-	-	-	-	-	-	-	-	-	23
<i>Clostridium perfringens</i>	-	-	-	-	-	-	-	-	-	40
Fungi	AB									
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	10
<i>Aspergillus niger</i>	-	-	-	-	-	-	-	-	-	10

CL-Chloroform, EA-Ethyl Acetate, ET-Ethanol, EX-Enrofloxacin, AB-Amphotericin B

DISCUSSION

The traditional ethno-veterinary medicinal practices are being followed by the rural folk through which a number of veterinary diseases are managed in the developing countries. There is an increased concern over the past decade due to the indiscriminate use of antibiotics and chemical products in management and control of diseases in food animals. Indiscriminate use of antibiotics and chemicals has made way into the food chain has led to development of drug resistance and serious health hazards in human well being. The use of antibiotics and other chemical products are banned for animal healthcare in a number of countries because of human healthcare. The World Health Organization (WHO) states that 74% of the plants derived medicines have a modern indication that correlates with their traditional, cultural (and sometimes ancient) uses²⁶. Hence ethno-veterinary practices can be tried as an alternative for the control of diseases. Screened plant extracts have shown antibacterial activity against some bacterial isolates. All the three extracts of *A. pavonina* displayed profound activity against *S. aureus*. Similar results were obtained against *S. aureus* with methanol extract of the same plant²³. Ethyl acetate and ethanol extracts of *A. pavonina* showed moderate activity against *S. paratyphi*. Similar results were obtained in a previous study with methanol extract²³. In the present study *P. aeruginosa* was resistant against all the three extracts of *A. pavonina* but in an earlier study the methanol extract showed activity²⁴. Both *C. albicans* and *A. niger* showed resistance against all the three extracts of both the plants studied. In a previous study hexane extract of *A. pavonina* showed activity against *A. niger* while no activity against *C. albicans*. Also *A. niger* was resistant to methanol extract of *A. pavonina*, but hexane extract showed antifungal activity²⁴. *M. philippica* showed profound activity against *K. pneumoniae* and no activity against *P. vulgaris*. *Cl. perfringens* and *E. aerogenes*. In an earlier study, methanol extract of the leaves of *M. philippica* showed activity against *B. subtilis* and the bacteria was resistant against ethyl acetate and hexane extract²². In the present study ethyl acetate and ethanol extracts showed activity against *B. subtilis*. Considering the large number of different groups of chemical compounds present in plants, it is most likely that their antibacterial activity is not attributed to one specific mechanism but that there are several targets in the cell. Various publications have

documented that polyphenols and phenolic compounds which are widely seen in plants are responsible for their antimicrobial activity. A preliminary screening exhibited good antioxidant properties of the sepals of *M. 'dona aurora'* a cultivar of *M. philippica*²⁷. A new iridoid glycoside, sanshiside-D, has also been identified. The abundance of the iridoids may be related to the antioxidant and hepatoprotective activities of this plant²⁸. Phytochemical analysis of leaf of *A. pavonina* contains phenols, flavanoids, and tannins²⁹. Phenolics, alkaloids, and terpenoids are present in the leaves of *M. philippica*³⁰. Phenolic compounds have their effects on cellular membranes. They have been seen to attack not only cell walls and cell membranes, thereby affecting their permeability and the release of intracellular constituents, but also to interfere with membrane functions such as electron transport, enzyme activity or nutrient uptake. Thus, active phenolic compounds might have several targets which could lead to the inhibition of bacteria³¹. Since flavonoids are known to be synthesized by plants in response to microbial infection, it can be counted to be in vitro effective antibacterial substances against a wide array of infectious agents³². Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic and antiparasitic effects³². The presence of the secondary metabolites like alkaloids, flavanoids and tannins in the extracts of these two plants may be responsible for the antibacterial activity.

CONCLUSION

With this knowledge in the background the extracts of these potential plants can be prospected to reach the active fraction or molecule(s), which can be further formulated, also the dried plant material itself could be utilized as a disinfectant by premixing it with water while cleaning dairy cattle rearing units. This will be helpful to the farmers of the developing countries who lack access to modern medicine and it will also help them economically and more over it will be environmental friendly

ACKNOWLEDGEMENT

The authors sincerely thank Times of India (ILCTOI14AZB001) for the partial funding of this work.

REFERENCES

- Hamburger M, Hostettmann K, Bioactivity in plants: the link between phytochemistry and medicine, *Phytochemistry* 30:3864–387, (1991).
- Rabe T, Van Staden J, Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*, *Journal of Ethnopharmacology*, 73:171–174, (2000).
- Palombo EA, Semple SJ, Antibacterial activity of traditional Australian medicinal plants, *Journal of Ethnopharmacology*, 77:151–157, (2001).
- Portillo A, Vila R, Freixa B, Adzet T, and Cañigueral S, Antifungal activity of Paraguayan plants used in traditional medicine, *Journal of Ethnopharmacology* 76: 93–98, (2001).
- Zgoda-Pols JR, Freyer AJ, Killmer LB, Porter JB, Antimicrobial resveratrol tetramers from the stem bark of *Vatica oblongifolia* ssp. *Oblongifolia*, *Journal of Natural Products* 65:1554–1559, (2002).
- Mitscher LA, Drake S, Gollapudi SR, Okwute SK, A modern look at folkloric use of anti-infective agents. *Journal of Natural Products* 5:1025–1040, (1987).
- Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH, Natural plant chemicals: sources of industrial and medicinal materials, *Science* 228: 1154–1160, (1985).
- Kinghorn AD. Plants as sources of medicinally and pharmaceutically important compounds. In: Nigg, H.N., Seigler, D. (Eds.), *Phytochemical Resources for Medicine and Agriculture*. Plenum Press, New York, pp. 75–95, (1992).
- Abdul MM, Sarker AA, Saiful IM, Muniruddin A, Cytotoxic and antimicrobial activity of the crude extract of *Abutilon Indicum*, *Int J Pharmacogn Phytochem Res* 2: 1 – 4, (2010).
- Ahmad I and Beg AZ, Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens, *J Ethnopharmacol*, 74:113-123, (2001).
- Threlfall EJ, Frost JA and Ward LR, Increasing spectrum of resistance in multi-resistant *Salmonella typhimurium*, *Lancet*, 343:1052 – 1054, (1996).
- Gowan MM, Plant products as antimicrobial agents, *Clin Microbiol Rev*, 12: 564-582.
- Doss H, Muhamed Mubarak, M.Vijayasanthi and R.Venkataswamy in-vitro antibacterial activity of certain wild medicinal plants against bovine mastitis isolated contagious pathogens, *Asian Journal of Pharmaceutical and Clinical Research*, 5(2), (2012)
- Watt JM, Breyer-Brandwijk MG, The medicinal and poisonous plants of southern and eastern Africa. London: E and S Livingstone Ltd., (1962)
- Kirtikar KR, Basu BD, Indian medicinal plants. India: International book distributors, (1981).
- Burkill IH, A dictionary of the economic products of the Malay Peninsula Edited by: Ministry of Agriculture (Malaysia). Crown Agents for the colonies London, 839, (1966).
- Balogun AM, Fetuga BM, Fatty acid composition of seed oils of some members of the leguminosae Family. *Food Chem* 17:175–82, (2004).
- Siddique AB, Sikder MAA, Rashid RB, Islam F, Hossain AKMN. Screening of Analgesic activity for *Mussaenda philippica* on Swissalbino mice. *Medscape Gen Med*. 9, 60, (2007).
- Mates MJ, Jimenez S, Fransisca. Evaluation of anticonvulsant activity of leaves and sepals of *Mussaenda philippica*. *Asian Pac J Trop Biomed*. C576,32, 157,170, (2000).
- Lakshmi BVS, Sudhakar M, Anubindu E, Antioxidant and antitumor activity of *Mussaenda philippica* sepal extract against in vivo colon cancer and breast cancer in mice. *Int. J. Advances in Pharmacy Medicine and Bioallied Sciences*, (2014).
- F Islam, MR Kuddus, F Latif, MK Hossain, Preliminary antimicrobial activity and cytotoxicity of leaf extracts of *Mussaenda roxburghii* Hook. f. *Bol Latinoam Caribe Plant Med Aromat* 12(6): 612 – 617, (2013).
- Erlina Abdullah, Raha Ahmad Raus and Parveen Jamal, Extraction and Evaluation of Antibacterial Activity from Selected Flowering Plants, *American Medical Journal*, 3 (1): 27-32, (2012).
- Yodesh Mahida and JSS Mohan, Screening of plants for their potential antibacterial activity against *Staphylococcus* and *Salmonella* spp., *Natural Product Radiance*, 6(4):301-305, (2007).
- MS Ali, I Azhar, F Ahmad, VU Ahmad and K Usmanghani, Antimicrobial Screening of

- Mimaceae Plants, Pharmaceutical Biology, 39(1):43–46, (2001).
25. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST, Ed. Bergey's manual of determinative bacteriology. 9th Edn. Baltimore (MD): Williams & Wilkins. (1994).
26. Wynn GS, Herbs in Veterinary Medicine. Alternative Veterinary Medicine, 21(47), 38, (2001).
27. Vidyalakshmi KS, Dorni C and Vasanthi HR, Antimitotic and cytotoxic activity of *Mussaenda queensirikit*. J. Pharm. Toxic. 2, 660 – 665, (2007).
28. Vidyalakshmi KS, Sulochana N, and Rajamanickam GV, An iridoid with anticancer activity from *Mussaenda 'dona aurora'* (sepals). Ind. J. Chem, 329-334, (2009).
29. Kothale KV and Rothe SP, Phytochemical screening of *Adenanthera pavonina* Linn, World Journal of Science and Technology, 2(6):19-22, (2012).
30. Melfei Estrada Bungihan and Claribel Asuncion Matias , Determination of the Antioxidant, Phytochemical and Antibacterial Profiles of Flowers from Selected Ornamental Plants in Nueva Vizcaya, Philippines , Journal of Agricultural Science and Technology B 3 (2013) 833-841, (2013).
31. Amensour M, Bouhdid S, Fernández-López J, Idaomar M, Senhaji NS, Abrini J. Antibacterial activity of extracts of *Myrtus communis* against food-borne pathogenic and spoilage bacteria, International Journal of Food Properties, 13(6):1215-24, (2010).
32. Jamine R, DaisyJamine R, Daisy P and Selvekumar BN, *In vitro* Efficacy of Flavonoids from *Eugenia jambolana* Seeds Against ESL-Producing Multidrug-Resistant Enteric Bacteria, Research Journal of Microbiology 2 (4): 369–374, (2007).
33. Naveen Prasad R, Viswanathan S, Renuka Devi , Vijayashree Nayak., Sweth VC, Archana Parathasarathy N and Johanna Rajkumar,. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. Journal of Medicinal Plants Research 2(10): 268-270, (2008).