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Screening of Phytochemical and Antibacterial Potential of Four Indian Medicinal Plants

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Abstract: A study was undertaken to investigate the qualitative phytochemicals and antimicrobial properties of different solvent crude extracts of four Indian medicinal plants namely, *Nerium oleander*, *Lippia nodiflora*, *Wattakaka volubilis* and *Wrightia tinctoria* against various bacterial pathogens. The presence of steroids, triterpenoids, phenolic compounds, tannins, alkaloids, saponins, flavanoids and reducing sugars was indicated by the tests conducted. The plant extracts showed different diameters of inhibition zone ranging between 4 to 28 mm. Among themselves, methanol, ethyl acetate, hexane and petroleum ether extracts of *N. oleander* exhibited the highest inhibition zone against *S. typhi*. The *in vitro* antibacterial assay and preliminary phytochemical analysis may open way for complementary future investigations in identifying potentially useful properties of chemical and pharmacological importance.

Key words: Medicinal plant % *Wattakaka volubilis* % Inhibition zone % *In vitro*

INTRODUCTION

India is rich in medicinal plant diversity. All known types of agro-climatic, ecologic and edaphic conditions are met within India. India is rich in all three levels of biodiversity, as species diversity, genetic diversity and habitat diversity [1]. Plants have been the traditional source of raw materials for medicine. A rich heritage of knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharva veda, Charaka, Sushruta, etc. An estimate suggests that about 13,000 plant species worldwide are known to have use as drugs.

Plants have proved to be signifigant natural resources for medicines; documentation of their use in medicine originates from ancient times. Ethnobotanical and ubiguitous plants provide a rich resource for natural drug research and development [2]. Medicinal plant based drugs have the added advantage of being simple, effective and offering a broad spectrm of activity with greater emphasis on preventive action [3]. Higher plants play an important role in providing new remedies and in some cases, they are important sources for old remedies. According to Van *et al*. [4], 50% of the drugs used in the clinical treatment are derived from the plant sources. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens [5,6]. The trend of using natural products has increased and the active plant extracts are frequently for new drug discoveries and for the presence of antimicrobials [7]. The objective of this paper study was to evaluate the qualitative analysis of phytochemicals and antibacterial efficacy of some selected four Indian medicinal plants.

MATERIALS AND METHODS

Plant Sample Collection: The leaves of the four medicinal plants viz., *N. oleander*, *L. nodiflora*, *W. volubilis* and *W. tinctoria* which are free from disease and were collected in Tiruchirappalli district, Tamil Nadu. The leaf

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samples were washed thoroughly for 3 times in running tap water and finally with sterile distilled water. After drying and crushing, the powdered plant materials have been subjected to various extractions.

Preparation of Solvent Extracts: 20 g of each powdered leaf plant material was extracted separately in the ratio of 1:6 at room temperature using various solvents namely hexane, petroleum ether, ethyl acetate, chloroform, acetone and methanol with gentle stirring for seven days (three times within this period) till colorless extract were obtained on the top of the extractor. Each of the solvent extract was concentrated separately under reduced pressure and preserved at 4° C in airtight bottle for further investigations.

Growth Condition of Microorganisms: The bacterial cultures of *Escherichia coli* (MTCC 1195), *Klebsiella pneumoniae* (MTCC 2405), *Enterobacter aerogens* (MTCC 2823), *Salmonella typhi* (MTCC 733), *Proteus vulgaris* (MTCC 1771), *Pseudomonas aeruginosa* (MTCC 2642), *Staphylococcus aureus* (MTCC 1430), *Bacillus cereus* (MTCC 1272) were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The cultures of *Serratia marcescens* and *Proteus mirabilis* were supplied by the Department of Microbiology, Institute of Basic Medical Science, Chennai, India and the bacterial cultures were maintained on nutrient broth at 37° C.

Preparation of Inoculum: The bacterial strains preserved in the nutrient agar at 4°C were revived in nutrient broth (liquid medium) and incubated at 37±1°C for overnight and the suspensions were checked to provide approximately 10^5cfu mL^{-1}.

Qualitative Phytochemical Analysis: The Preliminary phytochemical constituents were qualitatively analyzed for four medicinal plants by using Brindha *et al.* [8].

Antibacterial Activity: The disc diffusion assay methods of Iennette [9] as described by Rosoanaivo and Ratsimanaga- Urverge [10], Rabe and Van Staden [11] were used with modification to determine the growth inhibition of bacteria by plant extracts. The diluted bacterial culture (200 ìL) was spread over nutrient agar plates using sterile glass L-rod. Fifty microliter of the each extracts was applied per filter paper disc (Whatman No. 1, 6 mm diameter) and was allowed to dry before being placed on the layer of the agar plate. Each extracts was tested in triplicate (3 discs plate^{-1}) and the plates were incubated at 37±1°C for 24 h. After incubation, the diameter of the inhibition zones and the sensitivity were measured in mm. Standard antibiotic of Chloramphenical (30 mg disc^{-1}) was used as reference of positive control.

RESULTS

Preliminary Phytochemical Screening: Table 1 reveals that the qualitative phytochemical screening of the leaf extracts of *N. oleander*, *L. nodiflora*, *W. volubilis* and *W. tinctoria*, which demonstrated the presence of steroids, triterpenoids, sugars, saponins, phenolic compounds and alkaloids, while flavonoids and tannins were absent in some extracts. The anthraquinones and amino acids were absent in almost all the extracts of four medicinal plants.

Antibacterial Activity: The anti-bacterial activities of four selected medicinal plant extracts against ten bacterial strains were summarized in Table 2, 3, 4 and 5. The results revealed that the plant extracts showed significant antibacterial activity with varying magnitudes. The naked eye judgment method was used to determine the end point of inhibition at the edge where the growth starts. Generally most of the tested organisms were sensitive to number of the selected medicinal plant extracts. Out of four plants with 24 extracts showed antibacterial activity against one or more bacteria. The diameters of inhibition zone ranging between 4 to 28 mm. The highest antibacterial activity was obtained from methanol extracts than hexane, ethyl acetate and petroleum ether extracts of *N. oleander L.* which exhibited inhibition zone ranging between 23 to 28 mm. According to Eloff [12], the methanol was the most effective solvent for plant extraction than ethanol, n-hexane and water. This also proved in our present study that the methanol extracts of *N. oleander* exhibited the highest antibacterial activity followed by ethyl acetate and n-hexane extracts against *S. typhi*. The moderate activity was obtained from the chloroform and acetone extracts of *N. oleander* against *P.vulgaris*. But the least antibacterial activity was recorded by methanol extract of *N.oleander* against *S. aureus* only. *S. marcescens, B. cereus, E. aerogens, E. coli, P. mirabilis and P. aeruginosa* showed resistant to all the extracts of *N. oleander*.

Mostly, all the extracts of *L. nodiflora* showed well inhibition zone against the maximum number of antibacterial strains and represented in Table 3. Among them, methanol and ethyl acetate extracts were showed

Table 1: Preliminary phytochemical screening of leaf extracts of some selected medicinal plants

Chemical	Petroleum ether				Ethyl acetate				Chloroform				Methanol				Acetone				Hexane			
Compounds	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Steroids	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	-	-	+
Triterpe-noids	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Reducing	+	+	-	-	-	-	-	+	+	-	+	-	-	-	+	-	-	+	+	+	-	-	+	-
Sugars	-	-	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-	+	-	-	-	+	-	-
Alkaloids	-	+	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	+
Phenolic compounds	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-
Catachins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	-	-	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	-	+	-	+	-
Saponins	-	-	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+
Tannins	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
Anthro-quinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amino- acids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A= *Nerium oleander*, B= *Lippia nodiflora*, C= *Wattakaka volubilis*, D= *Wrightia tinctoria* (+) Presence; (-) Absence

Table 2: Inhibition zone (mm) of leaf extracts of *Nerium oleander L.* by disc diffusion method

Bacterial strains	Different solvent extracts						Chloramphenical (30 mg discG^{1})
	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Acetone	Hexane	
Escherichia coli	-	-	-	-	-	-	24±0.88
Klebsiella pneumoniae	-	-	-	19±0.44	-	-	32±1.45
Enterobacter aerogens	-	-	-	-	-	-	40±1.45
Salmonella typhi	23±0.33	25±0.57	-	28±0.57	-	23±0.88	22±1.76
Proteus vulgaris	-	-	12±0.88	-	14±1.20	19±1.45	26±0.88
Proteus mirabilis	-	-	-	-	-	-	27±1.45
Pseudomonas aeruginosa	-	-	-	-	-	-	32±1.45
Staphylococcus aureus	-	-	-	10±0.88	-	-	24±1.20
Serratia marcescens	-	-	-	-	-	-	33±0.88
Bacillus cereus	-	-	-	-	-	-	29±1.73

Values are mean inhibition zone (mm) ± S. E of three replicates

Table 3: Inhibition zone of leaf extracts of *Lippia nodiflora Rich*. by disc diffusion method

Bacterial strains	Different solvent extracts						Chloramphenical (30 mg discG^{1})
	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Acetone	Hexane	
Escherichia coli	-	14±0.88	11±0.88	12±1.45	9±0.88	-	24±0.88
Klebsiella pneumoniae	-	11±0.28	-	-	-	-	32±1.45
Enterobacter aerogens	-	15±0.70	-	-	-	-	40±1.45
Salmonella typhi	-	-	-	17±0.35	-	-	22±1.76
Proteus vulgaris	-	-	-	10±0. 33	-	-	26±0.88
Proteus mirabilis	10±0.88	-	11±1.45	-	-	-	27±1.45
Pseudomonas aeruginosa	-	-	-	-	-	-	32±1.45
Staphylococcus aureus	13±0.55	-	-	-	-	-	24±1.20
Serratia marcescens	-	-	-	-	12±0.35	-	33±0.88
Bacillus cereus	-	-	-	-	-	15±0.0	29±1.73

Values are mean inhibition zone (mm) ± S. E of three replicates

Table 4: Inhibition zone of leaf extracts of *Wattakaka volubilis L.* by disc diffusion method

	Different solvent extracts						
Bacterial strains	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Acetone	Hexane	Chloramphenical (30 mg discG^{1})
Escherichia coli	12±0.57	-	-	15±1.73	-	-	24±0.88
Klebsiella pneumoniae	-	11±2.02	-	-	-	-	32±1.45
Enterobacter aerogens	-	-	-	-	-	-	40±1.45
Salmonella typhi	-	-	-	-	12±1.73	13±1.45	22±1.76
Proteus vulgaris	-	-	-	-	14±1.85	-	26±0.88
Proteus mirabilis	-	15±2.18	-	-	-	-	27±1.45
Pseudomonas aeruginosa	10±0.88	-	-	-	-	-	32±1.45
Staphylococcus aureus	-	-	-	-	-	-	24±1.20
Serratia marcescens	-	-	6±0.88	-	-	-	33±0.88
Bacillus cereus	-	-	-	-	-	7±0.88	29±1.73

Values are mean inhibition zone (mm) ± S. E of three replicates

Table 5: Inhibition zone of leaf extracts of *Wrightia tinctoria* (Roxb) R.Br. by disc diffusion method

	Different solvent extracts						
Bacterial strains	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Acetone	Hexane	Chloramphenical (30 mg discG^{1})
Escherichia coli	-	-	22±1.45	-	-	19±2.60	24±0.88
Klebsiella pneumoniae	-	-	-	-	22±1.52	-	32±1.45
Enterobacter aerogens	-	-	-	-	-	5±0.88	40±1.45
Salmonella typhi	-	-	-	-	-	14±1.45	22±1.76
Proteus vulgaris	-	6±1.76	-	-	-	-	26±0.88
Proteus mirabilis	21±1.76	-	-	-	-	-	27±1.45
Pseudomonas aeruginosa	-	-	9±1.76	-	-	-	32±1.45
Staphylococcus aureus	-	-	-	-	10±0.88	-	24±1.20
Serratia marcescens	4±0.88	-	-	-	-	-	33±0.88
Bacillus cereus	-	-	-	8±0.57	-	-	29±1.73

Values are mean inhibition zone (mm) ± S. E of three replicates

the highest antibacterial activity against *S. typhi* (17 mm) and *E. aerogens* (15 mm), respectively. Table 4 showed the antibacterial activity of various solvent extracts of *W. volubilis*. In which, the highest antimicrobial activity was obtained in both ethyl acetate and methanol extracts against *P. mirabilis* and *E. coli.* The chloroform and hexane extracts showed only the minimum inhibition zone against *S. marcescens* and *B. cereus*, respectively. Table 5 represented that the antimicrobial activity of leaf extracts of *W. tinctoria* .The plant extracts such as petroleum ether, chloroform, acetone and hexane extracts showed the highest inhibition zone between 19 to 22 mm against *E. coli*, *P. mirabilis* and *K. pneumonia*, respectively. The moderate antibacterial activity was obtained from hexane, acetone and chloroform extracts. The lowest inhibition zone was ranging from 4 to 8 mm. Among four selected medicinal plants namely, *N. oleander*, *L. nodiflora*, *W. volubilis* and *W. tinctoria*, the methanol extract of *N.oleander* showed maximum inhibition zone (28 mm) compared to other extracts of all the tested plants. This gives an indication of the presence of promising antibacterial compounds. Most of the identified components with antimicrobial activity extracted from plants are aromatic or saturated organic compounds and they are more soluble in methanol and ethanol [13].

DISCUSSION

Drug resistant human pathogenic microorganism has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This condition has forced scientists

to search for new antimicrobial substances from various sources [14]. In India, large section of people especially, in villages using the herbal medicine to combat the infectious diseases and disorders. Gradually people move towards the traditional medicine. The reason for that is, trusts on herbal medicine, which improve the diseases conditions, after the herbal medicine treatment. No side effect or fewer side effects is observed due to herbal medicine. Another reason is the cost of the drugs and cost of the treatment is low. People in developing countries now prefer the herbal medicine [15].

Higher plants are much more important in the production of economically important organic compounds, pharmaceuticals and pesticides [16]. Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides [17]. In our present investigation, the leaves extracts of *N. oleander*, *L. nodiflora*, *W. volubilis* and *W. tinctoria* were possess the highest inhibition zone against *E.coli*, *K. pneumoniae*, *S. typhi*, *P. vulgaris* and *P. mirabilis*. There are reports in the literature that methanol is a better solvent for consistent extraction of antimicrobial substances from medicinal plants [18,19]. The earlier report has also been proved in our present study that methanol extract of *N. oleander* exhibited the highest activity (28 mm) against *S. typhi* than standard antibiotics (Chloramphenical) around 22 mm. This may be attributed to two reasons; firstly, the nature and potentiality of biological active components (alkaloids, flavonoids, essential oil, biterpenoids etc), which could be enhanced in the presence of methanol. Secondly, the stronger extraction capacity of methanol could have produced greater number/amount of active constituents responsible for antibacterial activity [20].

The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant; such as phenols [21], terpenoids [22,23], alkaloids [24] and flavonoids [25]. Based on our results, it is concluded that the plant extracts have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms.

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REFERENCES

1. Zafar, M., A. Iqbal and M. Faiz, 1999. Indian medicinal plants: a potential source for anticandidal drugs. J. Ethnopharmacol, 37: 237-242.
2. Kong, J.M., N.K. Goh, L.S. Chia and T.F. Chia, 2003. Recent Advances in traditional plant drugs and orchids. Acta pharmacol Sin., 24: 7-21.
3. Chin, Y., M.J. Balunas, H.B. Chai and A.D. Kinghorn, 2006. Drug discovery from natural sources. AAPSJ; 8: E 239-253.
4. Van Wyk, B., B.Van Qudtshoorn and N. Gerika, 1997. Medicinal Plants of South-Africa. Briza Publication, Pretoria.
5. Cragg, G.M., D.J. Newman and K.M. Snader, 1997. Natural products in drug discovery and development. J. Nat. Prod., 60: 52-60.
6. Recio, M.C., 1989. A review of some antimicrobial compounds isolated from the medicinal plants reported in the literature 1978-1988. Phytother. Res., 3: 117-125.
7. Das, S., S. Das, S. Pal, A. Mujib and S. Dey, 1999. Biotechnology of medicinal plants- Recent advances and potential. 1st Ed. Vol 2. UK 992 Publications, Hyderabad, pp: 126-139.
8. Brindha, P., B. Sasikala and K.K. Purushothaman, 1981. Pharmacological Studies on Merugan kizhangu. Bulletin of Medico-Ethno-Botanical Research (BMEBR), 3(1): 84-96.
9. Iennette, E.H., 1985. Manual of Clinical Microbiology. 4th Edn. American Association for Microbiology, Washington, D.C., pp: 978-987.
10. Rosoanaivo and Ratsimanaga-Urverge, 1993. Biological evaluation of plants with reference to the Malagasy flora. Monograph for the IFs. NAPRECA Workshop on Bioassays. Antananavivo, Madagascar, pp: 72-79.
11. Rabe, T. and Van Staden, 1997. Isolation of an antibacterial sesqui-terpenoid from *Warbugia salutaris*. J. Ethnopharmacol, 73: 171-174.
12. Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants. J. Ethnopharmacol, 60: 1-8.

13. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.
14. Subramanian, S., D. Sathish Kumar, P. Arul Selvan and G.P. Senthil Kumar, 2006. *In vitro* antibacterial and antifungal activities of ethanolic extract of *Aloe vera* leaf gel. J. Plant Sci., 1: 348-355.
15. Kandhasamy, M. and K.D. Arunachalam, 2008. *In vitro* Antimicrobial Activity of Tuber Extracts of *Zhenaria scabra*. J. plant Sciences, 3(2): 224-229.
16. Hostettmann, K. and J.L. Wolfender, 1997. The search for biologically active secondary metabolites. Pesticide Science, 51: 471-482.
17. Varma, J. and N.K. Dubey, 1999. Prospectives of botanical and microbial products as pesticides of Tomorrow. Current Sci., 76: 172-179.
18. Sengul, M., H. Ogutcu and A. Adiguzel, 2005. Antimicrobial effects of *Verbascum georgicum* Bentham extract. Turk. J. Biol., 29: 105-110.
19. Ozturk, S. and S. Ercisli, 2006. The chemical composition of essential oil and *in vitro* antibacterial activities of essential oil and methanol extract of *Ziziphora persica* Bunge. J. Ethnopharmacol, 106: 372-376.
20. Ghosh, A., B.K.Das, S.K. Chatterjee and G. Chandra, 2008. Antibacterial potentiality and phytochemical analysis of mature leaves of *Polyalthia longifolia* (Magnoliales: Annonaceae). The South Pacific. J. Natural Sci., 26: 68-72.
21. Kazmi, M.H., A. Malik, S. Hameed, N. Akhtar and S.N. Ali, 1994. An anthraquinone derivative from *Cassia italica.* Phytochemistry, 36: 761-763.
22. Habtemariam, S., A.I. Gray and P.G. Waterman, 1993. A new antibacterial sesquiterpene from *Premna oligotricha*. J. Natural Product, 56: 140-143.
23. Taylor, R.S.L., F. Edel, N.P. Manandhar and G.H.N. Towers, 1996. Antimicrobial activities of southern Nepalese medicinal plants. J. Ethnopharmacol, 50: 97-102.
24. Omulokoli, E., B. Khan and S.C. Chhabra, 1997. Antiplasmodial activity of four Kenyan medicinal plants. J. Ethnopharmacology, 56: 133-137.
25. Batista, O., A. Duarte, J. Nascimento and M.F. Simones, 1994. Structure and antimicrobial activity of diterpenes from the roots of *Plectranthus hereroensis.* Journal of Natural Produc., 57: 858-861.