

In vitro Antimicrobial Activity and Phytochemical Analysis of few Indian Medicinal Plants

Gomathi Rajashyamala L¹, Elango V²

Department of Siddha Medicine, Tamil University, Thanjavur, Tamil Nadu, India

Abstract: The antibacterial effect of some selected Indian medicinal plants was evaluated on bacterial species like *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and the fungal species *Candida albicans*. The solvent used for the extraction of plants was methanol. The in vitro antimicrobial activity was performed by agar disc diffusion method. The extracts of *Crataeva nurvala* could not inhibit any of the microbial strains investigated. The most active antibacterial and antifungal plant was *Evolvulus alsinoides*. The significant antimicrobial activity of active extracts was compared with the standard antimicrobics, chloramphenicol for bacteria (30 µl/disc) and fluconazole for fungi (30 µl/disc). The results obtained in the present study suggest that *Evolvulus alsinoides* can be used in treating diseases caused by the test organisms.

Keywords: anti microbial activity, *Evolvulus alsinoides*, Phytochemistry, microorganism, *binencasa hispida*.

1. Introduction

Currently 80% of the world population depends on plant-derived medicine as the first line of primary health care for human beings since it has no side effects⁽¹⁾. Hence, alternative medicines are available for those who do not want conventional medicine or who cannot be helped by conventional medicine. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as a tool in discovering new biologically active molecules has been most productive in the area of antibiotics⁽²⁾. Antibiotic resistance has become a global concern⁽³⁾. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens⁽⁴⁾. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases⁽⁵⁾. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections⁽⁶⁾. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity^(7,8). India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties⁽⁹⁾. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents⁽¹⁰⁾. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections

⁽¹⁰⁾. Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments⁽¹¹⁾. The aim of this study was to evaluate the activity of extracts from 4 plants against several pathogenic microorganisms in vitro.

2. Materials and Methods

2.1 Collections of Plant materials:

The Fresh plant/plant parts were collected from Thanjavur, Tamil Nadu, India from a single herb. The plants and the parts screened, together with their families and vernacular names, are given in Table 1.

2.2 Preparation of alcoholic extract

The collected Fresh plant materials were washed several times with distilled water to remove the traces of impurities from the plant material. They were dried at room temperature and coarsely powdered. The powder was extracted with methanol (70%) for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extracts were stored in refrigerator until used.

2.3 Preliminary phytochemicals screening:

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993), Trease and Evans (1989) and Harborne (1973)^(13,14,15).

2.3.1 Test for carbohydrates

Benedict's test: To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 min. A characteristic red colored precipitate indicates the presence of sugar.

2.3.2 Test for Aminoacid:

One ml of the extract was treated with few drops of Ninhydrin reagent. Appearance purple colour shows the

presence of amino acids

2.3.3 Test for Polyphenols

Ethanol (10.0 ml) was added to each extracts and the resulting solution (3.0 ml) was transferred in test tubes and warmed in a water bath (15 minutes). Three drops of freshly prepared ferric cyanide solution were added to the extract solution. Formation of a blue green colour indicated the presence of polyphenols.

2.3.4 Test for Tannins:

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.3.5 Test for anthraquinones:

Five ml of the extract solution was hydrolysed with diluted Conc. H₂SO₄ extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

2.3.6 Test for Flavonoids:

- 1) Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.
- 2) Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.
- 3) A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

2.3.7 Test for alkaloids:

1. Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

2.3.8 Test for Phlobatannins:

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

2.3.9 Test for Saponin:

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.3.10 Test for Steroids:

Two ml of acetic anhydride was added to 0.5 g methanolic extract of each sample with 2 ml H₂SO₄. The colour changed

from violet to blue or green in some samples indicating the presence of steroids.

2.3.11 Test for Terpenoids (Salkowski test):

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.3.12 Test for triterpenoids:

Ten mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc. H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenoids.

2.3.13 Test for Cardiac glycosides (Keller-Killani test):

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.4. Determination of antimicrobial activity

The antimicrobial activity was performed by agar disc diffusion method.

Microorganisms

Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, pseudomonas aeruginosa and fungi as Candida albicans were the microorganisms used and they were obtained from the Microbiology Laboratory of the Thanjavur Medical College Hospital, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37°C for 24 hours except for Candida albicans which was incubated at 25°C for 24-48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8°C until use.

Sample solutions for the experiment

The sample solutions as 50µl, 100µl and 150µl were used for the experiment. Standard antibiotic solution as chloromphenical for bacteria (30µl/disc) and fluconazole for fungi (30 µl/disc) used to compare the test solution.

Preparation of dried filter paper discs

Whatman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization After sterilization, the discs were loaded with different concentrations of prepared sample solutions again kept under refrigeration for 24 hrs.

Application of discs to inoculated agar plates

Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5°C for 1 hr to permit good diffusion and then transferred to incubator at 37°C for 24 hrs. After completion of 24hrs, the plates were inverted and placed in an incubator set to respective temperature for 24 hrs.

Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

Results and Discussion

The antimicrobial activity of 4 plant species extract was assayed in vitro by agar disc diffusion method against 4 bacterial species and 1 fungal species. Table 3 summarizes the microbial growth inhibition of methanol extracts of the screened plant species. The methanol extract of only three plants showed antimicrobial activity (*Binencasa hispida*, *Evolvulus alsinoides* and *Celastrus peniculatus*); the other did not show any antimicrobial activity. The maximum antibacterial activity was shown by *Evolvulus alsinoides*, followed by *Binencasa hispida* and *Celastrus peniculatus*, respectively. The methanol extracts of the investigated plants showed maximum antimicrobial activity against *K. pneumonia*, *Staphylococcus aureus*, *pseudomonas aeruginosa* and *Candida albicans*. Similar results were also reported by Venkatesan et al. ⁽¹⁶⁾, Prescott et al. ⁽¹⁷⁾, and Stainer et al. ⁽¹⁸⁾, who reported diseases such as pneumonia, urinary and respiratory tract infection, nosocomial pathogens and opportunistic infections caused by those bacterial species. The extracts of *crataeva nurvala* could not inhibit any of the bacterial species and fungal species. But very

slight zone(1mm) formed in *K. pneumonia* were studied. The significant antibacterial activity of the active plant extracts was comparable to the standard chloromphenical (30 µl/disc) and fluconazole (30 µl/disc)

Preliminary phytochemical analysis revealed the presence of tannin, alkaloids, steroids, anthroquinone and saponins. The other secondary metabolites like flavonoids, phlobatanin, amino acids, carbohydrate, cardiac glycosides, etc. were present in trace amounts in some of the plants (Table 2). It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It is quite possible that some of the plants that were ineffective in this study do not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in methanol or water ⁽¹⁸⁾. The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials⁽⁸⁾. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial phytochemical evaluation of few plants forms a primary platform for further pharmacological studies.

In conclusion, *Evolvulus alsinoides* extracts possess a broad spectrum of activity against a panel of pathogens responsible for the most common microbial diseases. These promissory extracts open the possibility of finding new clinically effective antimicrobial compounds.

Table 1: Ethnobotanical information of some traditionally used Indian medicinal plant species selected for phytochemical studies and antimicrobial activity.

Plant species	Family	Common name	Part used	Therapeutical uses
<i>Crataeva nurvala</i>	Capparidaceae	Maavilangam	Bark	antiinflammatory, urolithiatic, antidiabetic, antibacterial, analgesic, antiinfertility, antidiarrhoeal, antinociceptive and cardioprotective activity
<i>Binencasa hispida</i>	Cucurbitaceae	Venpoosani	Seed	central nervous effects (anxiolytic, muscle relaxant, antidepressant, in the treatment of Alzheimer's disease and to minimize opiates withdrawal signs), antioxidant, anti-inflammatory, analgesic, antiasthmatic, diuretic, nephroprotective, antidiabetic, hypolipidemic and antimicrobial effects
<i>Evolvulus Alsinoides</i>	Convolvulaceae	Vishnu grandi	leaves	anti-amnesic, anti stress (adaptogenic), antimicrobial and gastro protective activity, Antiulcer and anticatonic activity, Antioxidant activity, anti inflammatory activity, Antipyretic, Antidiarrheal, Cardioprotective Effects, Antidyskinesial Activity, Gastroprotective Activity, Anti-Stress Constituents, Anticonvulsant Activity.
<i>Celastrus peniculatus</i>	Celastraceae	Valuluvai	Seed	antiinflammatory, urolithiatic, antibacterial, analgesic, antioxidant activity.

Table 2: Preliminary phytochemical analysis of screened medicinal plant species

Test	<i>Creativa nurvala</i> (maavilangam)	<i>Binencasa hispida</i> (ven poosani)	<i>Evolvulus Alsinoides</i> (Vishnu grandii)	<i>Celastrus peniculatus</i> (valuluvai)
Tannin	++	+	+++	+
Phlobatannins	-	-	-	-
Saponin	+	++	+++	++
Flavonoids	++	-	+++	+
Steroids	++	++	+++	+++
Terpenoids	+	+	+++	+
Triterpenoids	-	++	+++	+
Alkaloids	++	++	+++	+++
Carbohydrate	+	-	++	+
Amino acid	-	+	+	+
Anthroquinone	++	+	+++	+
Polyphenol	-	-	+++	-
Glycoside	++	++	+++	+

(-) indicates absence; (+) indicates presence; (+++) indicates high concentrations

Table 3: Antibacterial activity of methanol extracts of screened medicinal plants.

Micro organism	Concentration (µl/disc)	Zone of inhibition (diameter in mm)				Standard (30µl/disc)
		<i>Creativa nurvala</i> (maavilangam)	<i>Binencasa hispida</i> (ven poosani)	<i>Evolvulus Alsinoides</i> (Vishnu grandii)	<i>Celastrus peniculatus</i> (valuluvai)	
<i>E.coli</i>	50	0	2	7	0	13
	100	0	3	9	0	
	150	0	5	10	0	
<i>Klebsiella pneumoniae</i>	50	0	0	4	0	13
	100	0	0	6	1	
	150	1	0	8	3	
<i>Staphylococcus aureus</i>	50	0	6	5	2	12
	100	0	7	7	4	
	150	0	9	9	5	
<i>Pseudomonas aeruginosa</i>	50	0	0	5	1	10
	100	0	1	7	2	
	150	0	3	9	4	
<i>Candida albicans</i>	50	0	4	5	4	12
	100	0	6	7	6	
	150	0	8	8	7	

References

- [1] Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. In glossary of Indian medicinal plants. *Council Sci. Indus. Res.*, New Delhi. 1: 197.
- [2] Gerhartz, W., Yamamoto, Y.S., Campbell, F.T., Pfefferkorn, R. and Rounsaville, J.F. 1985. *Ullmann's Encyclopedia of Industrial Chemistry*, Vol. A 5, (VCH-Verlag, Weinheim 1986) pp. 490-516.
- [3] Westh H, Zinn CS, Rosdahl VT et al. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microb Drug Resist* 10: 169-176, 2004.
- [4] Bandow JE, Brotz H, Leichert LIO et al. Proteomic approach to understanding antibiotic action. *Antimicrob Agents Chemother* 47: 948-955, 2003.
- [5] Rojas R, Bustamante B, Bauer J et al. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol* 88: 199- 204, 2003.
- [6] Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensm-Wiss u-Technol* 37: 263-268, 2004.
- [7] Colombo ML, Bosisio E. Pharmacological activities of *Chelidonium majus* L (Papaveraceae). *Pharmacol Res* 33: 127-134, 1996.
- [8] Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J. ed. *Perspectives on New Crops and New Uses*. Alexandria, VA: ASHS Press; 1999: pp. 457-462.
- [9] Martins AP, Salgueiro L, Goncalves MJ et al. Essential oil composition and antimicrobial activity of three Zingiberaceae from S. Tome e Principe. *Planta Med* 67: 580-584, 2001.
- [10] Krishnaraju AV, Rao TVN, Sundararaju D et al. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci Eng* 2: 125-134, 2005.
- [11] Balandrin MF, Kjocke AJ, Wurtele E et al. Natural plant chemicals: sources of industrial and mechanical materials. *Science* 228: 1154-1160, 1985.
- [12] Tanaka H, Sato M, Fujiwara S. Antibacterial activity of isoflavonoids isolated from *Erythrina variegata* against methicillinresistant *Staphylococcus aureus*. *Lett Appl Microbiol* 35: 494- 498, 2002.
- [13] Sofowara A (1993). *Medicinal plants and Traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.

- [14] Trease GE, Evans WC (1989). Pharmacognosy. 11th edn. Brailliar Tiridel Can. Macmillan publishers.
- [15] Harborne JB. (1973). Phytochemical methods, London. Chapman and Hall, Ltd. pp. 49-188.
- [16] Venkatesan M, Vishwanathan MB, Ramesh N et al. Antibacterial potential from Indian Suregada angustifolia. J Ethnopharmacol 99: 349-352, 2005.
- [17] Prescott LM, Harley JP, Klein DA. Microbiology 4th ed. Boston: The McGraw-Hill Companies Inc.; 1999: pp. 685.
- [18] Stainer RY, Ingraham JL, Wheelis ML et al. General Microbiology, 5th ed. London: The MacMillan Press Ltd.; 1986.