

SCREENING OF SELECTED PLANT EXTRACTS FOR THEIR LOCALIZATION OF PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY

Jancy Mathew^{1*}, Jampala Siva Satya Mohan² and Vinay M. Raole¹

¹Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara (390002),
Gujarat, India.

²Department of Biosciences, Sardar Patel University, Anand (388120), Gujarat, India.

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*Corresponding Author

Jancy Mathew

Department of Botany, The
Maharaja Sayajirao
University of Baroda,
Vadodara (390002), Gujarat,
India.

ABSTRACT

Traditional medicine particularly herbal medicine considered as a major healthcare provider around the globe, particularly in rural and remote areas. Herbal medicines are gaining much importance nowadays as it is cheap, easily available and have lesser side effects. The current investigation deals with efficacy of methanolic leaf extracts by carrying out the phytochemical screening and qualitative analysis as well as to check their efficacy against selected antibiotic susceptible and resistant microorganisms. Phytochemicals obtained from selected leaf extract of seven taxa were alkaloids, tannins, flavonoids, saponins, steroids and phenol which were traditionally used to treat cough, low B.P, respiratory infections and gonorrhea. Over and

above, the results confirmed the ethnomedicinal uses of four plant taxa by showing the inhibitory action against microorganisms; among which the most significant one is *Polyalthia longifolia*, against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Serratia marcescens*.

KEYWORDS: Ethnomedicinal, Microorganism, Phytochemical, Screening.

INTRODUCTION

Medicinal plants are not only used for the treatment of diseases but also as a potential material for maintaining good health. Many countries in the world, depends upon herbal medicines because of their availability locally, cultural acceptability, better compatibility and adaptability with the human body physiology and has lesser side effects.^[1] Infectious and non

infectious diseases represent an important cause of morbidity and mortality among the human population, particularly in developing and underdeveloped nations. Therefore, a search to develop new antimicrobial drugs in recent years, especially due to the constant emergence of many microorganisms resistant to conventional antimicrobials. Scientific validation of active constituents extracted from the plants can be used in drug discovery in the future.

According to the World Health Organization (2002) medicinal plants would be the best source to obtain an assortment of drugs and drug plants. Many plants as a whole are loaded with number of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids which have been found to have antimicrobial properties during in vitro testing.^[2]

In India, about 65% of total^[3] and 85% of the rural^[4] population depend on such traditional knowledge for healthcare. India has a tremendous wealth of medicinal plants due to its unique geography, climate and environmental conditions.^[5] The country has an ancient cultural background and about 300 tribal communities with 53 million population.^[6] These people are using 7000-7500 plants to overcome different kind of health problems.^[7-9] The aim of the study is to validate the plant species scientifically based on the antimicrobial screening and qualitative analysis of phytochemicals and comparing it with the available literature of ethnobotanical uses of the selected plants. Though medicinal uses of plants were reported by many researchers but scientific validation of ethno-botanically significant flora is not reported much in Gujarat, India. By and large, there is no detail systematic study conducted till date.

MATERIALS AND METHODS

Collection of different plant materials

Seven plant species named *Aegle marmelos* (L.) Correa, *Catharanthus roseus* (L.) G. Don, *Melia azedarach* L., *Neolamarckia cadamba* (Roxb.) Bosser, *Nerium indicum* L., *Polyalthia longifolia* (Sonn.) Thwaites, *Tamarindus indica* L. belonging to 6 different families- Rutaceae, Apocynaceae, Meliaceae, Rubiaceae, Annonaceae and Fabaceae were collected in the form of leaves from Vallabh Vidhyanagar, Anand. Identity of selected plant taxa was also confirmed with Flora of Gujarat state.^[10]

Preparation of leaf extract

Healthy leaves of all the selected plants were washed thoroughly under running tap water to remove the dust particles and were dried by keeping it in oven for 4-5 days at 45°C and

powdered using mortar and pestle. Extract was prepared by infusion extract method given by Houghton and Raman.^[11] For extraction 10g of dry powder of each sample was soaked in 100 ml of methanol at room temperature for 24 hours. Extracts were filtered through Whatman filter paper no.1 and the filtrates were centrifuged at 3000 rpm for 10 minutes to remove solid debris. The supernatant was collected and stored in refrigerator until further use.

Qualitative analysis of crude plant extracts

Qualitative analysis was carried out by standard procedures described by Houghton and Raman^[11] to identify the various constituents present in crude leaf extracts of selected plants prepared in organic solvents.

Dragendroff's test for presence of alkaloids

The extract is treated with few drops of Dragendroff's reagent. The orange brown precipitate coloration if observed indicates the presence of alkaloids.

Braymer's test for presence of tannins

2ml extract is treated with 2 ml water and 2-3 drops of 5% FeCl₃. The blue black precipitate if observed indicates the presence of tannins.

Sodium hydroxide test for presence of flavonoids

1ml extract is treated with 1ml of 10% NaOH. The yellow color indicates the presence of flavonoids.

Acetic anhydride test for presence of terpenoids

2 ml extract is treated with 2ml acetic acid and 2-3 drops of concentrated H₂SO₄. The deep red color indicates the presence of terpenoids.

Foam test for presence of saponins

5 ml extract is treated with 5 ml distilled water and both were mixed by shaking. The foam production indicates the presence of saponins.

Test for steroids

2 ml extract is treated with 2 ml chloroform and 2 ml concentrated H₂SO₄. The brown colored ring between the sample and H₂SO₄ indicates the presence of steroids.

Ferric chloride test for phenols

Few drops of extract are treated with few drops of 5 % FeCl_3 . The dark green color indicates the presence of phenol.

Selected microorganisms for Bioassay

The microorganisms selected for the experiment are one gram-positive bacteria- *Staphylococcus aureus* and four gram-negative bacteria- *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Serratia marcescens*. The bacterial cultures were grown on nutrient broth medium (Hi Media pH 7.4) at 37°C and maintained at 4°C .

Inoculum preparation

A fresh microbial suspension was prepared by subculturing the bacterial colonies into the nutrient broth medium (Hi Media pH 7.4) and incubated at 37°C in order to maintain approximately uniform growth rate of each organism. The bacterial cultures from the fresh media was compared with 0.5 Mc Farland turbidity standard, which is equivalent to approximately 1×10^8 bacterial cell density^[12] was used throughout the experimentation.

Bioassay

The present study, the antimicrobial activity of different plant methanolic extracts were screened by agar well diffusion method.^[13] A stock solution (100mg/ml) of each plant extract was prepared in DMSO for antibacterial activity. Approximately 20 ml sterilized nutrient agar medium was poured into the sterile petriplates in aseptic conditions using laminar air flow cabinet and then allowed to solidify at room temperature. The prepared agar plates were then marked and labeled with organism and extract name. Fresh bacterial culture inoculum of 100 μl having 1×10^8 CFU/ml was spread on agar plates with sterile glass spreader. A well of 8mm diameter punched off at previously marked petriplates into agar medium with sterile cork borer and then was filled with 100 μl of each plant extract. Plates were placed in refrigerator for 30 minutes for pre diffusion of plant extract and then incubated at 37°C for 24 hours until the appearance of inhibition zone (excluding well diameter) was measured as a property of antibacterial activity. The assays were conducted in duplicates.

RESULTS AND DISCUSSION

Methanolic plant extracts prepared by infusion extract method from leaves of seven different plant species i.e., *A. marmelos*, *C. roseus*, *M. azedarach*, *N. cadamba*, *N. indicum*, *P. longifolia*, *T. indica* were selected as experimental materials to compare its efficacy with the

ethnobotanical uses recorded on the basis of phytochemical analysis and antimicrobial screening.

Phytochemical analysis

Many phytochemical constituents such as alkaloids, tannins, flavonoids, terpenoids, saponins, steroids, phenol, glycosides and several other aromatic compounds are secondary metabolites produced by plants for their defense mechanism against many microorganisms, insects and herbivores.^[14] In the present study leaf extracts of *Aegle marmelos*, *Catharanthus roseus*, *Melia azedarach*, *Neolamarckia cadamba*, *Nerium indicum*, *Polyalthia longifolia*, *Tamarindus indica* prepared in methanol were used for presence of phytochemical constituents such as alkaloids, tannins, flavonoids, terpenoids, saponins, steroids and phenols.

All the leaf extracts of selected plants ie: *Aegle marmelos*, *Catharanthus roseus*, *Melia azedarach*, *Nerium indicum*, *Polyalthia longifolia*, *Tamarindus indica* prepared in methanol posses tannins, terpenoids, saponins, steroids and phenol. Alkaloids were present in all the selected plant extracts except *Neolamarckia cadamba*.

In *Aegle marmelos*, the phytochemicals obtained from methanol leaf extracts are alkaloids, tannins, flavonoids, terpenoids, saponins, steroids, phenol. The obtained results were agreeing with study conducted by Mujeeb et al.^[15] In *Catharanthus roseus*, as reported by Aziz et al,^[16] Kabesh et al,^[17] phytochemicals obtained were alkaloids, tannins, terpenoids, saponins, steroids and phenols. In *Melia azedarach*, alkaloids, tannins, flavonoids, saponins, steroids were present and the results were same as mentioned by Muhammad et al^[18] and Farook et al.^[19] As reported by Ganjewala et al^[20] and Kumar et al,^[21] the results obtained were tannins, flavonoids, saponins, steroids and phenol in *Neolamarckia cadamba*. In *Polyalthia longifolia*, the results obtained were alkaloids, tannins, flavonoids, saponins, steroids, phenol as mentioned by Arun and Chandrashekhar^[22] and Mudhafar et al,^[23] and in *Tamarindus indica* the phytochemicals obtained were alkaloids, tannins, flavonoids, terpenoids, steroids, phenols and these results were similar to the one reported by Arora et al,^[24] Abdallah and Muhammad.^[25]

The phytochemical analysis of different plant methanolic extracts from the experiment confirmed the presence of phytochemicals extracted in previous screening by different researchers, mentioned above.

Antimicrobial activity of plant extracts

The data pertaining to the antibacterial activity of plant extracts and inhibition zone formed by them are listed in the Table I. It was revealed from the results that each medicinal plant shows different degree of inhibition zone against selected microorganism in the study. The diameter of inhibition zone depends upon extrinsic and intrinsic factors. The extrinsic factors like pH of the medium, period and temperature of incubation, size of the well, volume of the inoculums, concentration of plant extracts can be fixed and standardized during the experiment and hence no error due to extrinsic factors. The intrinsic factors such as nature of medicinal plants including its components, solubility, and diffusing property are predetermined.

Methanolic extract of *P. longifolia* inhibited significantly against all the tested microorganisms with the highest inhibition zone of 22mm against *E. coli*, *P. aeruginosa* and *S. typhi* followed by *S. aureus* (20mm) and *S. marcescens* (18 mm).

The maximum activity was shown by *Polyalthia longifolia*, followed by *Nerium indicum*, *Tamarindus indica*, *Neolamarckia cadamba*, *Catharanthus roseus*, *Aegle marmelos* and *Melia azedarach* against the tested microorganisms.

Table I: Antibacterial activity of leaf extracts in methanol.

Sr no.	Tested microorganisms	Diameter of the zone of inhibition in mm shown by methanol plant extracts against tested microorganisms						
		AM	CR	MA	NC	NI	PL	TI
1	<i>E. coli</i>	10	10	8	8	16	22	18
2	<i>P. aeruginosa</i>	8	8	3	10	12	22	12
3	<i>S. aureus</i>	4	16	8	10	12	20	10
4	<i>S. typhi</i>	10	6	8	10	10	22	6
5	<i>S. marcescens</i>	0	8	6	12	16	18	16

AM- *Aegle marmelos*, CR- *Catharanthus roseus*, MA-*Melia azedarach*, NC- *Neolamarckia cadamba*, NI-*Nerium indicum*, PL-*Polyalthia longifolia*, TI- *Tamarindus indica*.

The earlier study has reported the moderate antimicrobial activity of methanol leaf extract against *Escherichia coli* and *Staphylococcus aureus* in all the selected plants; *Aegle marmelos*,^[26] *Catharanthus roseus*,^[27] *Neolamarckia cadamba*,^[28] *Nerium indicum*,^[29] *Polyalthia longifolia*,^[30-31] and better activity against *Salmonella typhi* in *Polyalthia longifolia*.^[31]

Plant extracts prepared in methanol showed a broad range of activity against the tested microorganisms. *E. coli*, *P. aeruginosa*, *S. aureus* were most susceptible organisms and *S. marcescens* was the most resistant one among all.

Comparision of ethnobotanical uses with its efficacy towards microorganisms

***Aegle marmelos*:** The ethnomedicinal uses reported by Dutta et al^[32], reveals that the leaf extract is used to treat diseases like diarrhea (EC, ST), respiratory infections (PA, SM), abscess (SA) high fever (ST) caused by the tested organisms *E.coli*, *S. typhi*, *P. aeruginosa*, *S. marcescens*, and *S.aureus*.

***Neolamarckia cadamba*:** The ethnomedicinal uses reported by Negi^[33] indicates that the leaf extract is used to treat diseases like diarrhea (EC, ST), skin diseases (PA, SA) conjunctivitis and eye infections (SM) caused by the tested organisms *E.coli*, *S. typhi*, *P. aeruginosa*, *S. marcescens*, and *S.aureus*.

***Polyalthia longifolia*:** The ethnomedicinal uses reported by Subramanion et al^[34] reveals that the leaf extract is used to treat fever (ST), skin diseases (PA) diarrhea (EC, ST) respiratory infections (PA, SM) caused by the tested organisms *E.coli*, *S. typhi*, *P. aeruginosa*, *S. marcescens*, and *S.aureus*.

***Tamarindus indica*:** The ethnomedicinal uses reported by Bhadoriya et al^[35] discloses that the leaf extract is used to treat respiratory problems (PA,SM), fever (ST), diarrhea(EC, ST), eye infections (SM) caused by the tested organisms *E.coli*, *S. typhi*, *P. aeruginosa*, *S. marcescens*, and *S.aureus*.

From the results after comparing with the available literature of ethnobotanical uses, it revealed that the methanolic leaf extract of *Aegle marmelos*, *Neolamarckia cadamba*, *Polyalthia longifolia*, and *Tamarindus indica* showed the antimicrobial efficacy against the tested microorganisms which caused the problems like abscess formation, vomiting, cold, cough, bronchitis, diarrhea, fever, skin diseases, respiratory infections, eye diseases etc.

CONCLUSION

Although, some plant part extracts show evidence of good antibacterial potency, in contrast to our expectation. Over and above, limited antibacterial potency of some plants suggests that there is no concurrence between the traditional uses of medicinal plants in the crude form for the remedy of infectious diseases. Another possibility for the limited antibacterial potency of

some plants may be due to the extraction method and the use of crude extracts. Even though we showed potent *in vitro* activity of a few traditional plant extracts for certain bacteria, it may not be translated *in vivo*. However, further detail study is necessary to explore their effectiveness in inhibiting the growth of different pathogens. From the current investigation, it can be concluded after comparison of ethnomedicinal uses with results of antimicrobial screening against selected microorganisms and qualitative analysis four plants namely- *Aegle marmelos*, *Neolamarckia cadamba*, *Polyalthia longifolia*, *Tamarindus indica* among the seven selected plants can be validated scientifically based on the information available in the literature.

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