Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants

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Abstract

The success of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains. Methanol extracts of six plant species traditionally used in Indian folklore medicine for the treatment of various bacterial and fungal infections were investigated for *in vitro* antimicrobial activity against pathogens namely *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* by disc diffusion method. Methanol extracts of *Eugenia jambolana* and *Cassia auriculata* showed the highest toxicity against all the bacteria. The plant extracts showed antibacterial activity but not antifungal activity against any of the fungi used. Minimum inhibitory concentration (MIC) assay were determined for these two extracts against bacteria. *E. jambolana* revealed the highest antimicrobial activity at a minimum concentration (0.75 mg/ml) against *S. aureus.* The phytochemical analysis carried out revealed the presence of coumarins, flavanoids, glycosides, phenols, tannins, saponins and steroids**.** Alkaloids were not detected from any of the plant extracts under study. The results provide justification for the use of the plants in folk medicine to treat various infectious diseases.

Keywords: antibacterial activity; folk medicinal plants; methanol extract; antibiotics

1. Introduction

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds[1].

Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem[2]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action[3,4]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[5]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents. Systematic screening of them may result in the discovery of novel active compounds[6].

In this study, methanol extracts of six plants, which had been described in herbal books and folklore medicine of India, were screened for their antimicrobial activity. The species tested were: *Andrographis paniculata* (leaves), *Eugenia jambolana* (kernel), *Cassia auriculata* (flowers), *Murraya koenigii* (leaves), *Salvadora persica* (stem) and *Ipomoea batatas* (leaves) against five bacteria; two gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and three gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and against two fungi (*Candida albicans* and *Aspergillus niger*).

2. Materials and Methods

Plant Materials

Six folk medicinal plants viz., *Eugenia jambolana* (kernel), *Cassia auriculata* (flowers) *Murraya koenigii* (leaves), *Salvadora persica* (stem) and *Ipomoea batatas* (leaves) and *Andrographis paniculata* (leaves)

were screened. The shade dried coarsely powdered material of the above mentioned plants were purchased from an ayurvedic shop at Gudiyatham.

Extract Preparation

Air-dried and coarsely powdered plant was extracted for 8 hours with Methanol in Soxhlet apparatus and then the extract was filtered and allowed to evaporate in open air. The dried extract is dissolved in 10% DMSO and stored in refrigerator until used.

Phytochemical Analysis

Freshly prepared extracts were subjected to standard phytochemical analyses to find the presence of the following phyto constituents phenols, flavanoids, alkaloids, coumarins, glycosides, tannins, saponins and steroids.[1,7].

Test Microorganisms

The test organisms were supplied by Department of Microbiology, School of Bio-Sciences and Technology, VIT University. Two gram-positive bacteria: *Staphylococcus aureus* and *Staphylococcus epidermidis*; three gram-negative bacteria: *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and two fungi: *Candida albicans* and *Aspergillus niger* were used in the study.

Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi that were incubated without agitation for 24 h at 37°C and 25°C respectively. To 5ml of MHB and SDB, 0.2 ml of culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 McFarland solution[8] at 600nm which is equivalent to 10^6–10^8 CFU/ml.

Antimicrobial Assay

Disc diffusion method

Kirby-Bauer method was followed for disc diffusion assay[9]. *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The same procedure has been followed for the fungi using Sabouraud dextrose agar. The different concentrations of extracts (1, 2 and 4 mg/disc) were loaded on 5 mm sterile individual discs. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h. Negative control was prepared using respective solvent. Gentamycin (10 µg/disc) was used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Minimum Inhibitory Concentration (MIC) Asssay

The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disk diffusion (Kirby–Bauer) method. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of a microorganism is known as MIC[10]. Selected plant extracts were subjected to a serial dilution (25 mg/ml to 0.37 mg/ml) using sterile nutrient broth medium as a diluent. In a 96-well titre plate 20 µl of an individual microorganism and 20 µl of selected plant extract were loaded and inoculated at 37° C for 24 h. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract.

A control experiment was run in parallel to study the impact of the solvent alone (without plant extracts) on growth of the five test organisms. Methanol was diluted in a similar pattern with sterile nutrient broth followed by inoculation and incubation.

3. Results & Discussion

The methanol extracts of six medicinal plants were tested against the pathogenic microbes viz., *E.coli* , a most common bacteria of which virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis; *Klebsiella pneumonia* which is the causative organism of pneumonia, *Staphylococcus aureus*, a wound infecting pathogen which can cause septicemia, endocarditis and toxic shock syndrome; *Staphylococcus epidermidis* which causes septicemia and endocarditis in immunocompromised patients; *Pseudomonas aeruginosa* which infects the pulmonary tract, urinary tract, burns and wounds; *Candida albicans* a causal agent

of opportunistic oral and genital infections in humans and *Aspergillus niger* which causes Aspergillosis, a serious lung infection, if large amounts of spores are inhaled.

Out of 6 plants tested for antimicrobial activity, 5 plant species showed antibacterial activity by inhibiting one or more microorganisms. The results of the antimicrobial activity of plant extracts tested against microorganisms by disk diffusion method are shown in Table -1 & 2. Among the plants screened, the methanolic extract of *E. jambolana* and *C. auriculata,* showed significant inhibition of all tested bacteria followed by *M. koenigii* and *I. batatas* against few pathogens. The plant extracts showed antibacterial activity but not antifungal activity.

The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone against the test microorganisms. *E. jambolana* (4 mg/disc) exhibited the prominent antibacterial activity all the five bacteria but was more susceptible against *E.coli* and *S. aureus* whereas *C. auriculata* (4 mg/disc) was more susceptible against *S. aureus* and *S. epidermidis. M. koenigii* (4 mg/disc) showed antibacterial activity against all bacteria used in this study except *K. pnemoniae*. It was highly efficient against *S. aureus* and *S. epidermidis*; *A. paniculata* (4 mg/disc) showed activity against *S. epidermidis*, *K. pneumoniae* and *E. coli*; *I. batatas* (4 mg/disc) showed mild activity against *E. coli* but *S. persica* did not show any antimicrobial activity against the pathogens studied.

Methanol extracts of *E. jambolana* and *C. auriculata* that showed maximum antimicrobial activity was taken for MIC assay. The result of MIC assay is shown in Table-3. *E. jambolana* exhibited the highest antibacterial efficacy against *S. aureus* at 0.75 mg/ml concentration followed by *E. coli* and *P. aeruginosa* at 1.5 mg/ml concentration. Least efficacy was shown against *S. epidermidis* which was inhibited at 12 mg/ml concentration. Oliveira *et al.*[11] reported the antimicrobial activity of *Syzygium cumini* extract from leaves. The antimicrobial activity of *E. jambolana* may be due to tannins and other phenolic compounds. It is known to be very rich in gallic and ellagic acid polyphenol derivatives[12,13]. *C. auriculata* showed inhibition at 3.0 mg/ml concentration against *P. aeruginosa*, *S. aureus*, and *S. epidermidis* followed by *E. coli* and *K. pneumonia* at a concentration of 6.0 mg/ml. Samy and Ignachimuthu[14] reported that *C. auriculata* exhibited significant activity against *E. coli* and *S. aureus*. The antimicrobial activity of *C. auriculata* extract may be due to the presence of phenolic constituents.

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores[15]. The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical constituents of the selected plants investigated are summarized in Table-4. Analysis of plant extracts revealed the presence of coumarins, flavonoids, glycosides, phenols, saponins, steroids and tannins in most of the selected plants which could be responsible for the observed antimicrobial property. Alkaloids were absent in the selected plant extracts studied. These bioactive compounds are known to act by different mechanism and exert antimicrobial action.

Tannins bind to proline rich proteins and interfere with the protein synthesis[16.] Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls[17]. Coumarins are also known act against gram positive bacteria and it is produced in carrots in response to fungal infection which could be attributed to its antimicrobial activity[18]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell[19]. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes[20].

In this study, *S. aureus* was found to be sensitive to five plant extracts. The highest sensitivity of *S. aureus* may be due to its cell wall structure and outer membrane[21]. Our results suggest that gram-positive bacteria are generally more sensitive to the spice and herb extracts. This was consistent with the previous studies on other spices and herbs[22].

4. Conclusion

E. jambolana and *C.auriculata* exhibited the highest antimicrobial activity at a minimum concentration against *S. aureus.* The results provide justification for the use of these plants in folk medicine to treat various infectious diseases.

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6. References

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Table-1. Antimicrobial activity of *Andrographis paniculata*, *Eugenia jambolana* and *Cassia auriculata* tested against microorganisms by disk diffusion method

		Mean diameter of growth inhibition zone (mm)					
	Conc. (mg/ml)	*E.c*	*K.p*	*P.s*	*S.a*	*C.a*	*A.n*
Andrographis paniculata	1	–	–	–	–	–	–
	2	–	8	–	–	–	–
	4	7	12	18	–	–	–
Eugenia jambolana	1	–	–	–	15	–	–
	2	12	7	11	17	–	–
	4	23	12	18	23	–	–
Cassia auriculata	1	–	–	–	15	–	–
	2	–	–	7	17	–	–
	4	8	9	10	21	–	–

E.c, Escherichia coli; K.p, Klebsiella pneumonia; P.s, Pseudomonas aeruginosa; S.a, Staphylococcus aureus; S.e, Streptococcus epidermidis; C.a, Candida albicans; A.n, Aspergillus niger. [a] Diameter of inhibition zone less than 6.mm is represented as "–".

Table-2. Antimicrobial activity of *Murraya koenigii*, *Salvodara persica* and *Ipoema batatas* tested against microorganisms by disk diffusion method

		Mean diameter of growth inhibition zone (mm*)					
	Conc. (mg/ml)	*E.c*	*K.p*	*P.s*	*S.a*	*C.a*	*A.n*
Murraya koenigii	1	–	–	–	–	–	–
	2	–	–	–	–	–	–
	4	7	–	–	12	–	–
Salvodara persica	1	–	–	–	–	–	–
	2	–	–	–	–	–	–
	4	–	–	–	–	–	–
Ipoema batatas	1	–	–	–	–	–	–
	2	–	–	–	–	–	–
	4	7	–	–	–	–	–

E.c, Escherichia coli; K.p, Klebsiella pneumonia; P.s, Pseudomonas aeruginosa; S.a, Staphylococcus aureus; S.e, Streptococcus epidermidis; C.a, Candida albicans; A.n, Aspergillus niger. [a] Diameter of inhibition zone less than 6.mm is represented as "–".

Table-3. Minimum inhibitory concentration (MIC) of the most efficacious herbal extracts[b] on five test organisms

	Efficacious herbal extracts		Positive control- Antibiotic
	E.jambolana (mg/ml)	*C.auriculata* (mg/ml)	Gentamicin (µg/ml)
E. coli	1.5	6	12.5
K. pneumonia	3	6	100
P. aeruginosa	1.5	3	1.5
S. aureus	0.75	3	1.5
S. epidermidis	12.5	3	12

[b] Extracts exhibited ≥ 20mm diameter of inhibition zone in disk diffusion test.

Table-4. Phytochemical constituents of Plant extracts

Species	**Alkaloid**	**Coumarin**	**Flavanoids**	**Glycosides**	**Phenols**	**Tannins**	**Saponins**	**Steroids**
A. paniculata	–	+	–	+	–	+	–	+
C. auriculata	–	+	+	+	+	+	+	+
E. jambolana	–	+	+	+	+	+	–	+
I. batatas	–	+	+	+	+	+	–	+
M. koenigii	–	+	+	+	+	+	+	+
S. persica	–	–	–	–	–	+	+	–

"+" indicates presence and "–" indicates absence.