RJC
http://www.rasayanjournal.com

RASĀYAN *J. Chem.*
Vol.3, No.4 (2010), 615-620
ISSN: 0974-1496
CODEN: RJCABP

ANTIMICROBIAL ACTIVITY OF PLANTS TRADITIONALLY USED AS MEDICINES AGAINST SOME PATHOGENS

Abhishek Mathur*[1], Rakshanda Bhat[2], G.B.K.S. Prasad[3], V.K. Dua[4], Satish K. Verma [5]and Pavan K. Agarwal[5]

[1]Department of Biochemistry, [2]Department of Microbiology ,[5]Department of Biotechnology, Sai Institute of Paramedical and Allied Sciences, Dehradun (U.K.) India.
[3]Department of Biochemistry, Jiwaji University, Gwalior
[4]National Institute of Malaria Research, BHEL, Haridwar (U.K.), India.
*E-mail: abhishekmthr@gmail.com

ABSTRACT

The antimicrobial activity and minimum inhibitory concentration (MIC) of various plant extracts in different solvents such as ethanol (98%), hexane (99%) and distilled water of plants traditionally used as medicines as Bidens pilosa L., Bixa orellana L., Cecropia peltata L., Cinchona officinalis L., Gliricidia sepium, Jacaranda mimosifolia, Justica secunda Vahl., Piper pulchrum, P. paniculata L. and Spilanthes americana were evaluated against five bacteria Staphylococcus aureus, Streptococcus β hemolytic, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli and fungus Candidia albicans. These plants are used in Indian folk medicine to treat infections of microbial origin.

Keywords: Antimicrobial activity, infections, folk medicine, % Relative Inhibition zone diameter (RIZD), microorganisms.

INTRODUCTION

In developing countries and particularly in India low income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infections[1]. These plants are ingested as decoctions, teas and juice preparations to treat respiratory infection. They are also made into a poultice and applied directly on the infected wounds or burns. When people from these remote communities get an infectious disease, they are usually treated by traditional healers and shamans because of their expertise in such procedures as making diagnoses, treating wounds, setting bones and making herbal medicines. Traditional healers claim that their medicine is cheaper and more effective than modem medicine. Patients of these communities have a reduced risk to get infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics. However, if they are treated in a hospital the chance of contracting a nosocomial infection is increased. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases[2]. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. We chose ten species used in folk medicine to determine their antimicrobial activity: *B. pilosa L.* (Asteraceae), *B. orellana L.* (Bixaceae), *C. peltata L.* (Moraceae), *C. officinalis L.* (Rubiaceae), *G. sepium HB & K* (Fabaceae), *J. mimosifolia D. Don* (Bignoniaceae), *J. secunda Vahl.* (Acanthaceae), *P. pulchurum C.DC* (Piperaceae), *P. paniculata L.* (Polygonaceae), and *S. americana* (Asteraceae). In general, these plants are used in folk medicine in the treatment of pharyngitis, gingivitis, bronchitis, infected wounds, topical ulcers, and as antiparasitic agents[3].

The extract of *B. pilosa* is used in folk medicine as an anti-helminthic and protozoacide agent; it also has antiseptic properties. It contains flavanoids[4]. The ethanol extract of the leaves of *B. orellana* possesses antimicrobial activity against Gram (+) microorganisms and *C. albicans*[5]. Also, its leaves have been employed to treat malaria and leishmaniasis. Its seeds contain carotenoid[6, 7]. The ethanol extract of *C. peltata* has been used as an antibilious, cardiotonic and diuretic agent[8]. In addition, its leaves have been employed against blennorrhea and warts[9]. The decoction of the leaves of *C. officinalis* is used to treat ameobiasis[10, 11]. Its dry bark is active against *P. falciparum*, and herpes. It contains quinoline alkaloid[12, 13]. Branches and leaves of *G. sepium* are used to reduce fever in children and adults. It has also been used as insecticide and to treat infections produced by *Microsponum canis*, *Trichophyton mentagrophytes*, and *Neisseria gonorrohae*. Its leaves have iridoids, triterpenes, saponins (I and II) [14, 15]. The water extract of *J. mimosifolia* is active against *P. aeruginosa.* Its flowers contain flavones and flavanoids. Its leaves have terpinods, triterpenes, flavones, and steroids[16-17].
J. secunda has been used to disinfect scorpion wound. *P. pulchrum* is used to disinfect snakebites[18-19]. Other species exhibit antimicrobial activity against *P. aeruginosa* and *C. albicans. Polygala* spp. possesses trypanocidal activity[20]. It contains coumarins[21,22]. Flowers of *S. americana* are used to treat mouth infections and some varieties of herpes. It contains spilantol[23]. Evidently, there are not sufficient scientific studies that confirm the antimicrobial properties of these plants. This study looks into the *in vitro* antimicrobial activity of these plants against six pathogenic microorganisms that cause the most common cases of infectious diseases of poverished communities in India.

EXPERIMENTAL

Plant material

All the plants were collected by farmers and traditional healers from the villages of different parts. All the species were identified by Taxonomist.

Preparation of plant extracts

The plant extracts were prepared using the modified method[24]. Briefly, three 100 g portions of the dried powdered plants material were soaked separately in 500 ml of distilled water, ethanol (98 %) and n-hexane (99%), for 72 h. Then, each mixture was refluxed followed by agitation at 200 rpm for 1h. The filtrates obtained were concentrated under vacuum at 40°C to obtain the dry extracts.

Determination of antimicrobial activity

Microorganisms used

The pure cultures of test organisms (*S. aureus* ATCC 29737, *S. B hemolytic* ATCC 10389, *B. cereus* ATCC 14603, *P. aeruginosa* ATCC 25619, *E. coli* ATACC 10536, and *C. albicans* ATCC 53324) were obtained from the Microbiology laboratory Sai Institute of Paramedical and Allied Sciences, Dehradun (U.K.), India.

Culture media

The medium used for the activation of the microorganisms was Soybean casein broth (SCB). The following selective agar media used for the antimicrobial test. Baird-Parker *(S. aureus*), Cetrimide (*P. aeruginosa*), Mc-Conkey (*E.coli*), Blood (*S. β hemolytic*), Nutritive(*B. cereus*), and Sabouraud's Dextrose(*C. albicans*).

Inoculum

The microorganisms were inoculated into SCB and incubated at 35 ± 2°C for 4 h. The turbidity of the resulting suspensions was diluted with SCB to obtain a transmittance of 25.0 % at 580 nm. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/ml.

Agar diffusion assay

The modified agar well diffusion method[25] was employed. Each selective medium was inoculated with the microorganism suspended in SCB. On the agar was solidified, it was punched with a six millimeters diameter wells and filled 25 µL of the plants extracts and blanks (ethanol, distilled water, and hexane). The concentration of the extracts employed was 25 µg/ml. Simultaneously, gentamycin sulfate (*S. aureus*, *P. aeruginosa*, *E. coli*, and *B. cereus*), clindamycin *(S. β hemolytic*) and nystatin (*C. albicans*) were used

as positive controls at a concentration of 1.0, 0.3 and 1.0 µg/ml respectively. The dilution medium for the positive controls was sterile distilled water. The test was carried out by triplicate. The plaques were incubated at 35 ± 2°C for 24h, except for *C. albicans* which was incubated at 29 ± 2°C. The antimicrobial activity was calculated by applying the expression.

$$\% \text{ RIZD} = [(\text{IZD Sample- IZD negative control}) / \text{IZD antibiotic standard}] \text{ X } 100$$

Where RIZD is the percentage of relative inhibition zone diameter and IZD is the inhibition zone diameter (mm). Equation compensates the possible effect of the solvent (blank) other than water on the IZD. The resulting IZD of the samples were either higher than or equal to the IZD of the blanks. Therefore, the obtained percentages were positives (Table 1). The test was considered negative (-) when the IZD of the sample was equal to the IZD of the blank.

Phytochemical screening

The method[10] was implemented to identify the general phytochemicals groups of compounds in the extracts (Table 1). The test for amino acids was conducted by dissolving 10 mg of dry extracts in 1 ml of ethanol and adding 1 droplet of ninhydrin reagent. For flavanoids, Shimoda's test was adopted (15 mg of dry extract was dissolved in 1 ml of ethanol, concentrated HCl, and magnesium turnings were added). Anthocyanins were identified by adding 1 ml of boiling water, 0.5 ml of 37 % HCl to 10 mg of dry extract. The solution was heated at 100^0C, cooled and added, 0.4 ml of amylic alcohol. The test for phenolic compounds was carried out by dissolving 10 mg of dry extract in 1ml of 1 % ferric chloride solution. For tannins, 1 ml of the gelatin reagent was added to 1 ml of the filtered aqueous extract Quinones were identified by extracting 10 ml of the aqueous extract with dichloromethane, evaporating the organic phase, and adding 5 ml of ethanol, 1 ml of hydrogen peroxide 5 % and 1 ml of sulfuric acid 50 % respectively. The solution was heated, cooled, extracted with benzene and 1 ml of ammonia solution added. Cardiac glycosides were identified by evaporating 1 ml of the organic phase, dissolving the residue in I ml of ethanol and adding 0.5 ml of Kedde's reagent. For detection of triterpenoids and steroids, 0.5 ml of acetic anhydride and 1 droplet of 37 % sulfuric acid solution were added to 0.5 ml of the organic phase. The test for alkaloids was carried out by adding 0.5 ml of the aqueous extract into four test tubes; boiled, filtered and one droplet of the reagents of Mayer, Valser, Dragendroff's and ammonium Reineckate was added respectively.

RESULTS AND DISCUSSION

The ethanol extracts of *B. orellana* (seeds), *G. sepium*, *J. mimosifolia* and *P. pulchrum* were the most active against the microorganisms studied. In some cases, the three extracts of the same plant had antimicrobial activity against the same microorganism. Highest yield of extractable substances in the water extract (15.0%). Ethanol extracts exhibited a higher degree of antimicrobial activity as compared with water and hexane extracts fraction. *E. coli, B. cereus and S. aureus* were the most susceptible bacteria to all plant extracts. On the contrary, *S. β hemolytic, P. aeruginosa* and *C. albicans* were the most resistant microorganisms. None of the extracts was more active against *S. β hemolytic* than the positive control (clindamycin). Only three plants (*J. secunda, P. pulchrum and P. paniculata*) were most active against *C. albicans*. Steroids and anthocyanins of *B. orellana* (seeds) could be responsible for their antimicrobial activity against *S aureus*, *B. cereus* and *E. coli.* Similarly, the presence of steroids and amino acids in *C. peltata* could correspond to its high antimicrobial activity exhibited against *E. coli*. *B. pilosa* showed a low activity against *S. aureus* and *B. cereus.* However, some studies established that the methanol extract of this plant is highly active against *S. aureus, S. epidermidis* and *B. subtilis. C. officinalis* was the species that exhibited the greatest variety of secondary metabolites. It also showed antimicrobial activity against all the pathogens studied. Similarly, *S. americana* presented antimicrobial activity against all the microorganisms studied except for *C albicans.* Alkaloids and steroids found in this plant might account for this. Former studies associated the alkaloid spilantol with its biological activity. This plant also showed the lowest yield of extractable solids among all the plants (0.02%).

CONCLUSION

All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these plants were more effective than traditional antibiotics to combat the pathogenic microorganisms studied. The chance to find antimicrobial activity was more apparent in ethanol than water extracts of the same plants. Three species (*B. orellana, J. secunda* and *P. pulchurum* presented the lowest MIC compared to the antibiotic standard. These plants could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity. This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. However, none of the plants are recommended the treatment of infections produced by *S. β hemolytic* and *P. aeruginosa.*

Table-1a: Effect of Ethanolic extracts

S.No.	Plant	Microorganisms (% RIZD)					
		SA	SβH	BC	PA	ECO	CA
1	*B. pilosa. L.*	77.8	68.7	64.2	68.8	87.6	54.3
2	*B. orellana L.*	80	75	71	85	92	62
3	*C. peltala L.*	76.4	50.7	61.7	68.6	91.3	51.8
4	*C. officinalis*	78.5	54.3	68.4	64.3	85.6	47.8
5	*G. sepium*	85	72	76	75	80	61
6	*J. mimosifolia*	78	62.4	81.4	74.8	82.3	66.5
7	*J. secunda Vahl.*	76.3	56.4	63.2	61.8	82.4	79.8
8	*P. pulchrum*	79.5	66.5	72.1	72.9	87	82.4
9	*P. paniculata*	72.4	54.2	76.5	75.7	81.0	81.14
10	*S. americana*	78.4	73.1	74.3	71.8	86.5	NA

Table-1b: Effect of aqueous extracts

S.No.	Plant	Microorganisms (% RIZD)					
		SA	SβH	BC	PA	ECO	CA
1	*B. pilosa L*	NA	NA	NA	NA	NA	NA
2	*B. orellana L.*	NA	NA	NA	NA	NA	NA
3	*C. peltata L.*	NA	NA	NA	NA	NA	NA
4	*C. officinalis*	NA	NA	NA	NA	NA	NA
5	*G. sepium*	NA	NA	NA	NA	NA	NA
6	*J. mimosifolia*	NA	NA	60.2	NA	72.4	NA
7	*J. secunda Vahl*	NA	NA	NA	NA	NA	NA
8	*P. pulchrum*	62.2	NA	60.1	NA	71.8	NA
9	*P. paniculata*	NA	NA	NA	NA	NA	NA
10	*S. americana*	NA	NA	NA	NA	NA	NA

ACKNOWLEDGEMENTS

The authors wish to thank to the Chairman, Mr. Harish Arora and Vice Chairperson, Mrs. Rani Arora of Sai Institute of Paramedical and Allied science, Dehradun (U.K.), India. Thanks are due to the faculties of Dept. of Microbiology, Biochemistry & Biotechnology for their support and cooperation. We acknowledge the research staff of NIMR, Hardwar (U.K.) and Sai Institute, Dehradun (U.K.) for providing us the facilities in Research .

Table-1c: Effect of hexane extracts

S.No.	Plant	Microorganisms (% RIZD)					
		SA	SβH	BC	PA	ECO	CA
1	*B. pilosa L*	76.5	65.4	61.5	64.3	71.2	53.4
2	*B. orellana L.*	72.2	51.5	66.5	66.5	81.4	56.7
3	*C. peltata L.*	73.4	48.6	58.6	63.4	80.5	50.8
4	*C. officinalis*	75.4	52.5	56.5	61.5	82.3	46.9
5	*G. sepium*	68.5	49.5	74.3	65.6	75.6	51.5
6	*J. mimosifolia*	75.6	43.5	76.2	74.3	74.3	62.8
7	*J. secundaVahl*	74.2	52.5	61.5	71.1	68.5	74.3
8	*P. pulchrum*	75.7	62.3	68.7	72.4	83.2	71.2
9	*P. paniculata*	70.5	51.5	73.3	73.5	84.6	75.7
10	*S. americana*	56.4	56.8	51.2	48.7	44.2	NA

SA = *Staphylococcus aureus*
SβH = *S β hemolytic*
BC = *Bacillus cereus*
PA = *Pseudomonas aeruginosa*
ECO = *E. coli*
CA = *Candida albicans*
NA = No activity

REFERENCES

1. D.S. Fabricant, N.R. Farnsworth, *Environmental Health Perspectives Supplements*, **109**, 69 (2001).
2. J. Gonzalez. *J Ethnopharmacology*, **2**, 43 (1980).
3. T. Rabe, I. Van. , *J. Ethnopharmacology*, **56**, 81 (1997).
4. P.M. Shah. *Clinical Microbiology and Infection*, **11**, 36 (2005).
5. T.C. Fleischer, E.P.K. Ameade, M.L.K. Mensah, I.K. Sawer.,*Fitoterapia*, **7**,136 (2003).
6. O.N. Irobi, M. Moo-Young, W.A. Anderson.,*Pharm Bio.*, **34**, 87 (1996).
7. O.A. Binutu, B. Lajubutu, *Afr. J. Med. Sci.*, **23**, 269 (1994).
8. A. Caceres, B.R. Lopez, M.A. Giron, H. Logemann, *J. Ethanopharmacol.*, **31**, 263 (1991).
9. J. Hikawczuk, V. Saad, T. Guardia, A.O. Juarez, O.S. Giordano, Anales *de la Asociacion Quimica Argentina*., **86**, 167 (1998).
10. A. Martinez, G. Valencia. Universidad de Antioquia., Marcha fitoquimica, pp. 59-65 (2003).
11. S. Kiokias, M.H. Gordon, *Food Chem.*, **83**, 523 (2003).
12. D.C. Warhurst, *Cinchona alkaloids and malaria, Lancet*, **2**, 1346(1981).
13. O.B. Wijesekera, L.S. Rajapakse, D.W. Chelvarajan, *J Chromatogr.*, **A21**, 388(1976).
14. M.P.Gupta, 270 Plantas Afedicinales Iberoamericanas, **1**. Bogota: Presencia Ltd: pp. 378-379 (1995).
15. L. Rastrelli, A. Caceres, F. De Simone, R. Aquino, *J. Agric Food Chem.*, **47**, 1537(1999).
16. V. Gambaro, J.A.Garbarino Galeffi C, M. Nicoletti, G.B. Messana L Marini-Bettolo.,**19**, 17-19 (1988).
17. M.L. Vasquez,. Plantas y frutas medicinales de Colombia y America.. 1. Bogota: Climent., Cecropia, pp. 134-135 (1982).
18. R. Otero, V.Nunez, J. Barona, R. Fonnegra, S.L. Jimenez, R.G Osorio, M.D.A. Saldarriaga, *J Ethanopharmacol*., **73**, 233 (2000).
19. C. Lans, T. Harper, K. BMC complementary and alternative medicine, **1**, 1-10 (2001).
20. M.G. Pizzolatti, A.H. Koga, E.C. Grisard, M. Steindel, *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, **10**, 422(2003)
21. G. Bader, Y. Kulhanek, H. Ziegler-Boehme, *Pharmazie*, **45**, 618(1990).

22. M. Hamburger, M. Gupta, K. Hostettmann, *Planta Medica*, **3**, 215(1985).
23. L.S. Ospina, J. Olarte, E. Nunez, *Phytochemical studies. Rev. Colomb Cienc Quim Pharm*., **15**, 37 (1986).
24. P.I. Alade, O.N. Irobi, *J Ethanopharmacol*, **39**, 171(1993).
25. C. Perez, M. Pauli, P. Bazevque, *Acta Biologiae et Medicine Experimentalis*, **15**, 113(1990).

[RJC- 574/2010]
