International Journal of Drug Development & Research
| January-March 2012 | Vol. 4 | Issue 1 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.03,& H index 2

Antimicrobial screening of Medicinal plants against human Pathogens- A Comparative account of two different methods of Extraction

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Abstract

The present study is an attempt to find out whether medicinal plants maintain same kind of effectiveness or otherwise when extracted by different methods. Five medicinal plants were selected to screen their antimicrobial efficacy against four bacterial strains *viz Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris* and two fungal strains *Candida albicans* and *Aspergillus niger*. Extraction was carried out by soxhlet and cold percolation method. There was much variation in the results. Compared to aqueous extract lipophilic extracts showed good result with both the methods.

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Key words:
Antimicrobial tests, soxhlet extracts, cold percolation extracts.

How to Cite this Paper:
Ranjita R. Kurdekar, G. R. Hegde*, Gurumurthi Hegde and S. S. Hebbar "Antimicrobial screening of Medicinal plants against human Pathogens- A Comparative account of two different methods of Extraction", Int. J. Drug Dev. & Res., Jan-March 2012, 4(1): 82-89

Article History:----------
Date of Submission: 17-09-2011
Date of Acceptance: 18-11-2011
Conflict of Interest: NIL
Source of Support: NONE

Introduction

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. The use of plants as medicine is widespread throughout the world. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. [1] However, the blind dependence on synthetics is over and people are returning to the naturals with the hope of

safety and security. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. [2] Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. Medicinal plants and their derivatives are thus looked upon not only as a source of affordable healthcare but also as an important commodity item of international trade and commerce.

The present study ascertained whether the plant extracts could effect growth of the test organism *in-vitro*. Further it was to find out whether the inhibition of the growth was dependent on the method of extraction of the plant material. Five plants *viz. Persicaria piripu* (Polygonaceae), *Lobelia nicotianaefolia* (Lobeliaceae), *Costus speciosus* (Costaceae), *Lagenandra toxicaria* (Araceae) and *Remusatia vivipara* (Araceae) were selected, mainly based on their ethnomedicinal properties which have been enlisted in the Table 1. All the five plants were tested for their antimicrobial activity against both bacterial and fungal strains by extracting the plant material using two different methods *viz.* soxhlet and cold percolation method.

Table 1. Details of medicinal plants and their properties

SI. No	Name of the plants	Family	Part used	Medicinal properties
1	*Persicaria piripu* (DC.) M.R.Almeida	Polygonaceae	Leaves	Antidiarrhic, antiscorbutic, vulnerary and herb tonic. [7] Stem juice used as febrifuge, jaundice and leucorrhoea. [8] warts and also to cure amoebiasis. [9]
2	*Lobelia nicotianaefolia* Roth ex R. & S	Lobeliaceae	Leaves and roots	Bronchitis, asthma, insect and scorpion-bite, induce nausea and vomiting. [10] Anti-asthmatic [11] and also as an antiseptic.[7]
3	*Costus speciosus* (L.) Spreng.	Costaceae	Rhizome	Fever, cough, [8] gynecological problems. It is used to make sexual hormones and contraceptives. [12] It is bitter, astringent, cooling, digestive, stimulant, good for heart, dyspepsia, diabetes, oedema, blood diseases, leprosy and other skin ailments. [13] Root is used for snake bite; juice of boiled plant is used in earache. [14]
4	*Lagenandra toxicaria* Dalz.	Araceae	Rhizome	Insecticidal properties, remedy for itch. [8]
5	*Remusatia vivipara* (Roxb.) Schott & Endl.	Araceae	Corm	Antioxidant, wound healing, [15] remedy for itch. [8]

Materials and methods

All the five plants were collected from Kumta taluk of Uttar Kannada district, Karnataka State, India. The collected plants were authentically identified with the help of floras, such as *Flora of Presidency of Bombay* [3] *Flora of British India* [4] *Flora of Presidency of Madras* [5] and *Flora of Karnataka*. [6] All the plant materials were powdered using electric grinder.

a) Extraction of the plant material

In order to see the differences in the quality of extracts two methods were employed as follows:

i) *Soxhlet method* -The powdered plant material was subjected to extraction in four different solvents *viz.* chloroform, acetone, ethanol and water in the order of increasing dielectric constants of the solvents. [16]

ii) *Cold percolation method* -This is similar to the traditional method of extraction used by herbalists throughout the world. A known amount of dried material (5gm/50ml) was soaked in the desired solvent and kept for continuous shaking for nearly 48 hrs. This was followed by filtration and evaporation of excess solvent without applying heat. Each time before extracting with the next solvent, the material

was air dried at room temperature [16] and the same method was repeated for the next solvent. [17]

b) Preparation of extracts- In both the methods, extracts were weighed and re-dissolved in Dimethyl formamide (DMF) to obtain the extract solution. Three different concentrations- 25, 50 and 100 mg/ml were used for screening purpose.

c) Microbial Cultures-The bacterial strains *Staphylococcus aureus* (MTCC 737), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1688) and *Proteus vulgaris* (MTCC 1771) and a fungal strain *Candida albicans* (MTCC 183) were procured from the MTCC (Microbial Type Culture and Collection) Chandigarh, India. Another fungal strain *Aspergillus niger* was obtained from the stock culture maintained in the Mycology Laboratory, Department of Botany, Karnatak University, Dharwad.

d) Antimicrobial activity: The antimicrobial activity was studied by disc diffusion method. [18] The inoculum suspensions of all the strains were prepared. For all the bacterial strains and the fungal strain, *C. albicans* peptone water was selected as the growth medium and sterilized distilled water was taken as the growth medium for the fungus *A. niger*. Nutrient agar (Hi-media) was selected as the bacterial medium and Potato Dextrose Agar (Hi-media) as fungal medium. Twenty milliliter of the sterilized medium was poured in the pre-autoclaved petriplates and allowed to solidify. The 12 hrs culture broth was swabbed on the agar surface. Sterile discs impregnated with 10 µl of the extract were placed on the media and gently pressed down to ensure contact with the medium. Then the plates with bacterial strains were incubated at 37° C for 24 hrs and 48 hrs for fungi. The zone of inhibition was noted. Streptomycin was used as standard for bacteria and Nystatin was used as standard for fungi.

e) Determination of Minimum Inhibitory Concentration (MIC) - Serial tube dilution method [19]

The MIC values were determined by serial tube dilution method. The concentrations of the extracts range in the decreasing order from 100 mg/ml to 5.55 mg /ml in DMF against the inhibitory microorganism.

f) Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) [20]

The tubes that showed no visible growth were streaked on fresh nutrient agar plates (for bacteria) and on PDA plates (for fungi). The plates were incubated at 37° C for 24 hrs and examined for growth. MBC or MFC was regarded as the lowest concentrations of the extracts that prevented the growth of the bacterial or fungal colony on solid medium.

g) Statistical analysis- In order to ascertain significant difference in the results obtained from antimicrobial effect of the tested plants, variance analyses were carried out using SPSS 16.0 software package. Values of $p<0.05$ were considered as significantly different. The results were subjected to analysis of variance (ANOVA) and mean values were separated according to Duncan's multiple range test at p=0.05 level. [21]

Results and Discussion

The antimicrobial activities of the plants selected are shown in Table 2 and 3 for both the methods. The soxhlet extract of leaves of *Persicaria piripu* showed good result against *Staphylococcus aureus* and *Candida albicans* [22] where as the cold extract did not show inhibition against the strains tested except for *C. albicans* for which it showed an inhibition zone of 9.0mm.

Table 2: Antimicrobial activity for the extracts obtained by soxhlet method

Name of the plants	Extracts	Zone of inhibition (mm)					
		K. pneumoniae	*S. aureus*	*P.vulgaris*	*P. aeruginosa*	*C. albicans*	*A.. niger*
L. nicotianaefolia (leaves)	Chloroform	- - 8.0±0.00[a]	- - -	- - -	- - -	8.3±0.33[c] 9.5±0.28[b] 11.16±0.16[a]	- - -
	Acetone	- - -	- - -	- - -	- - -	- - -	- - -
	Ethanol	- - -	- - -	- - -	- - -	- 9.16±0.16[b] 10.0±0.00[a]	- - -
	Water	- 7.33±0.33[b] 10.33±0.33[a]	- - -	- - -	- - -	- - -	- - -
L. nicotianaefolia (Roots)	Chloroform	7.00±0.00[c] 9.10±0.16[b] 10.1±0.16[a]	13.0±0.00[c] 15.2±0.16[b] 17.5±0.50[a]	- 7.16±0.16[b] 8.33±0.33[a]	10.00±0.00[c] 16.16±0.16[b] 23.33±0.33[a]	- - -	- - -
	Acetone	7.00±0.00[c] 8.30±0.33[b] 9.83±0.44[a]	- 10.16±0.16[b] 12.33±0.33[a]	- - -	7.16±0.16[c] 8.50±0.50[b] 10.50±0.28[a]	- - -	- - -
	Ethanol	7.00±0.00[c] 7.83±0.16[b] 8.83±0.16[a]	- - -	7.00±0.00[c] 8.16±0.16[b] 9.00±0.00[a]	7.16±0.16[c] 8.46±0.26[b] 10.16±0.16[a]	- - -	- - -
	Water	- - -	- - -	- - 8.3±0.33[a]	- - -	- - -	- - -
Costus speciosus (Rhizome)	Chloroform	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-
	Water	- - 10.1±0.16[a]	- - -	- - -	- - -	- - -	- - -
Lagenandra toxicaria (Rhizome)	Chloroform	7.16±0.16[c] 7.33±0.33[b] 8.16±0.16[a]	- - -	- - -	- - -	- - -	- - -
	Acetone	- 7.16±0.16[b] 8.33±0.33[a]	- - -	- - -	- - 8.16±0.16[a]	7.16±0.16[c] 10.16±0.16[b] 11.16±0.16[a]	- - -
	Ethanol	- - 7	- - -	- - -	- - -	- 7.16±0.16[b] 9.16±0.16[a]	- - -
	Water	-	-	-	-	-	-
Remusatia vivipara (Corm)	Chloroform	7.0±0.00[c] 8.00±0.00[b] 9.16±0.16[a]	- - -	- - -	- - -	- - -	- - -
	Acetone	- - 6.16±0.16[a]	- - -	- - 7.0±0.00[a]	7.00±0.00[c] 7.83±0.16[b] 8.83±0.16[a]	- - 7.16±0.16[a]	- - -
	Ethanol	- - -	- - -	- - -	- - -	- - 7.00±0.00[a]	- - -
	Water	-	-	-	-	-	-
Standard value*		25mm	18mm	28mm	22mm	31mm	21mm

*Streptomycin (10 µl/disc) for bacteria and Nystatin (100 units/disc) for fungi

The soxhlet extracts of leaves of *Lobelia nicotianaefolia* did not show any promising result but the extracts obtained by cold percolation method showed very good activity against all the bacterial and fugal strains except for *Proteus vulgaris* and *Aspergillus niger* respectively. The chloroform extract of leaves of this plant showed very good activity against *S. aureus* with a highest inhibition zone of 15.16mm and 18.3mm for acetone extract (which is equal to the standard value).

Table 3: Antimicrobial activity for the extracts obtained by cold percolation method

Name of the plants	Extracts	Zone of inhibition (mm)					
		K. pneumoniae	*S. aureus*	*P.vulgaris*	*P. aeruginosa*	*C. albicans*	*A.. niger*
Persicaria piripu (leaves)	Chloroform	- - -	- - -	- - -	- - -	- - -	- - -
	Acetone	- - -	- - -	- - -	- - -	- - 9.0±0.00 [a]	- - -
	Ethanol	- - -	- - -	- - -	- - -	- - -	- - -
	Water	- - -	- - -	- - -	- - -	- - -	- - -
L. nicotianaefolia (Leaves)	Chloroform	7.0±0.00 [c] 8.16±0.16 [b] 9.0±0.00 [a]	11.5±0.00 [c] 13.5±0.00 [b] 15.16±0.16 [a]	- - -	7.16±0.16 [c] 8.16±0.16 [b] 9.33±0.33 [a]	7.33±0.33 [b] 8.16±0.16 [b] 11.3±0.33 [a]	- - -
	Acetone	- 7.16±0.16 [b] 8.16±0.16 [a]	12.1±0.16 [c] 15.5±0.28 [b] 18.3±0.33 [a]	- - -	10.0±0.00 [c] 11.3±0.33 [b] 13.5±0.28 [a]	8.0±0.00 [c] 9.5±0.28 [b] 10.6±0.33 [a]	- - -
	Ethanol	7.00±0.00 [c] 9.16±0.16 [b] 10.33±0.33 [a]	8.00±0.00 [c] 11.16±0.16 [b] 14.33±0.33 [a]	- - -	7.0±0.00 [c] 9.8±0.44 [b] 11.5±0.28 [a]	7.0±0.00 [c] 8.16±0.16 [b] 10.33±0.33 [a]	- - -
	Water	7.00±0.00 [b] 7.00±0.00 [b] 9.33±0.33 [a]	7.00±0.00 [b] 8.33±0.33 [b] 10.66±0.66 [a]	- - -	- - -	7.00±0.00 [c] 8.16±0.16 [b] 9.00±0.00 [a]	- - -
L. nicotianaefolia (Roots)	Chloroform	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-
	Ethanol	- - -	- - -	- - -	- - -	- - -	- - -
	Water	- - -	- - -	- - -	- - -	- - -	- - -
Costus speciosus (Rhizome)	Chloroform	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-
	Water	- - -	- - -	- - -	- - -	- - -	- - -
Lagenandra toxicaria (Rhizome)	Chloroform	- - -	- - 7.16±0.16 [a]	- - 6.16±0.16 [a]	- - -	- - 10.00±0.00 [a]	- - -
	Acetone	- - -	- - 7. 00±0.00 [a]	- - -	- - 9. .00±0.00 [a]	- - 9.5±0.50 [a]	- - -
	Ethanol	- - -	- - -	- - 7.00±0.00 [a]	- - -	- - -	- - -
Remusatia vivipara (Corm)	Chloroform	- - -	- - -	- - -	- - -	7.00±0.00 [b] 7.50±0.28 [b] 9.00±0.00 [a]	- - -
	Acetone	- - -	- - -	7.3±0.16 [b] 8.3±0.33 [b] 9.3±0.33 [a]	- - -	- - -	- - -
	Ethanol	- - -	- - -	- - -	- - -	- - -	- - -
Standard value*		25mm	18mm	28mm	22mm	31mm	21mm

*Streptomycin (10 µl/disc) for bacteria and Nystatin (100 units/disc) for fungi.

The root extract of *L. nicotianaefolia* obtained by both the methods showed much bioactive difference. The soxhlet chloroform extract showed highest inhibition of 23.33mm for *Pseudomonas aeruginosa* which is more than the standard value (22mm) and 17. 5mm for *S. aureus*

which is slightly less than the standard value. None of the cold extracts showed activity against the strains tested. Rhizome extract of *Costus speciosus* for both cold and soxhlet method did not show activity against any of the strains tested. The soxhlet and cold extracts of *Lagenandra toxicaria* rhizome showed weak to moderate activity against most of the strains tested. No significant result was obtained for both cold and hot extracts of *Remusatia vivipara* corm.

The MIC was performed to determine the minimum concentration of all the extracts, which inhibited the growth of bacterial and fungal strains. The extracts showed MIC values ranging from 50 to 12.5 mg/ml (Table 4 & 6.) where as none of the extracts showed minimum bactericidal concentration between 100 to 200 mg/ml except for chloroform extract of roots of *L. nicotianaefolia* (Table 7) which showed MBC value at 50mg/ml (Table 5).

Table 4. Minimum Inhibitory Concentration of *Lobelia nicotianaefolia* roots (Soxhlet method)

Extracts	Value in mg/ml				
	K. p	S. a	P. v	P. a	C. a
Chloroform	-	25	-	12.5	-
Acetone	-	50	-	-	-
Ethanol	-	-	-	-	-

K. p- *Klebsiella pneumoniae*, S. a- *Staphylococcus aureus*, P. v- *Proteus vulgaris*, C. a- *Candida albicans*

The extracts showing more than 12mm of zone of inhibition were selected to continue further to find out the MIC values.

Table 5. Minimum Bactericidal Concentration of *Lobelia nicotianaefolia* roots (Soxhlet method)

Extracts	Value in mg/ml				
	K. p	S. a	P. v	P. a	C. a
Chloroform	-	50	-	100	-
Acetone	-	>200	-	-	-
Ethanol	-	-	-	-	-

K. p- *Klebsiella pneumoniae*, S. a- *Staphylococcus aureus*, P. v- *Proteus vulgaris*, C. a- *Candida albicans*

Table 6: Minimum Inhibitory Concentration of *Lobelia nicotianaefolia* leaves (Cold method)

Extracts	Value in mg/ml				
	K. p	S. a	P. v	P. a	C. a
Chloroform	-	25	-	-	-
Acetone	-	12.5	-	25	-
Ethanol	-	25	-	-	-

K. p- *Klebsiella pneumoniae*, S. a- *Staphylococcus aureus*, P. v- *Proteus vulgaris*, C. a- *Candida albicans*

Table 7: Minimum Bactericidal Concentration of *Lobelia nicotianaefolia* leaves (Cold method)

Extracts	Value in mg/ml				
	K. p	S. a	P. v	P. a	C. a
Chloroform	-	>100	-	-	-
Acetone	-	>100	-	>100	-
Ethanol	-	>100	-	-	-

K. p- *Klebsiella pneumoniae*, S. a- *Staphylococcus aureus*, P. v- *Proteus vulgaris*, C. a- *Candida albicans*

The extraction of all the five plants was done by using four different solvents. None of the extracts showed activity against the fungus *Aspergillus niger*. This may be due to the concentration quotient used is too minimal to inhibit the growth. However all the test organisms including the most resistant Gram negative bacteria like *P. aeruginosa* and *K. pneumoniae* were susceptible against the tested extracts. While screening for the antimicrobial activity of the 13 medicinal plants Swarnakar and Katewa [23] have ended up with the result, *Costus speciosus* exhibited no inhibition against *Escherichia coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and fungus *C. albicans*. And also Aparna Saraf [24] tested the phytochemical and antimicrobial studies of *C. speciosus* (Koen.) and reported the presence of secondary metabolites like alkaloids, flavanoids, cardiac glycosides, saponins, sterols, tannins and anthroquinone glycosides. Further the methanolic extract failed to inhibit the tested strains *viz S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* but the aqueous extract appeared to have antibacterial activity only against *S. aureus*. Shale *et al* [25] stated that some of the extracts did not show any activity against the tested strains, they may be active against other microbial species which were not tested. The same kind of comparative work was

done by Duru and Nkechi [26] where they have evaluated the antimicrobial and phytochemical analysis of seeds of *Voacanga africana* by cold and hot percolation method. The result was some what similar with respect to both the methods but in our experiments, the plants *Persicaria piripu* and *Lobelia nicotianaefolia* showed wide variation in the results.

Conclusion

From our work it is clear that method of extraction of the plant material affects the bioactive nature of the plant extracts. Data from the literature as well as from our work it is clear that the structure of the secondary metabolites would change with the heat treatment and possibly the cold percolation is more nearer to the traditional system of preparation of medicines. To support the above investigations further isolation and characterization of the plant extracts having promising result is needed.

Acknowledgements

The authors are thankful to the UGC-SAP-DRS-III, New Delhi, India for their financial assistance and Karnataka University, Dharwad for the facilities provided.

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