INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF COMPOUNDS FROM SELECTED MEDICINAL AND AROMATIC PLANTS

Lavanya, G. & Brahmaprakash, G. P.
Department of Agricultural Microbiology, Gandhi Krishi Vignana Kendra,
University of Agricultural Sciences, Bengaluru- 560 065, Karnataka, India.

ABSTRACT
The present study focused on developing a protocol, separation of compounds by chromatographic technique and screening antimicrobial compounds from selected medicinal and aromatic plants. Antimicrobial compounds from these plants were screened for their antimicrobial activity against four bacteria and two test fungi. Plants were extracted with different polar and non-polar solvents. TLC profiling of all the plant extracts in different solvent system indicated the presence of different groups of phytochemicals in these plants. Terpenes, phenolic acids and flavonoid compounds were effective in inhibiting the growth of microorganisms. Compounds from plant extracts showed highest activity against plant pathogens tested. Ethanol was found to be better for purifying the compounds followed by chloroform.

KEYWORDS: Thin layer chromatography, retention factor, polar and non-polar solvents.

INTRODUCTION

Plants have been used for a long time for their medicinal properties. Plant derived products *viz*., gums, resins, oils and extracts have been used for therapeutic purpose since ages. Systematic screening of folk medicinal plants has resulted in the discovery of novel effective compounds against harmful organisms (Tomoko *et al*., 2002).

Antimicrobial compounds are a group of chemical compounds which are biosynthetically or synthetically produced which either destroy or usefully suppress the growth and metabolism of variety of microorganisms. These compounds have various functional groups to be active. Some antimicrobial agents are effective in controlling infectious diseases in plants, animals and humans.

Aromatic and medicinal plants are a group of unique plants containing certain chemicals having antimicrobial properties. Plant protection measures have been developed by the use of safer biocides which are environmentally and ecologically safe and could be exploited for commercialization. During recent years, these crops have been reported to possess potent antifungal and antibacterial activity (Mishra *et al.,* 1993). Among aromatic and medicinal crops *Pogostemon patchouli* (Patchouli), *Rosmarinus officinalis* (Rosemary), *Lantana camara* (Lantana) and *Chromolaena odorata* (Eupatorium) are important crops possessing antimicrobial compounds in them.

The investigations regarding the separation of few class of compounds; terpenes, phenolic acids and flavonoids by chromatographic technique is scanty from the medicinal and aromatic plants used in the experiment, a study was conducted for the isolation of terpenes, phenolic acid and flavonoid group of compounds from four different medicinal and aromatic plants using thin layer chromatography and also to establish the best procedure to obtain extracts containing active constituents and tested for antimicrobial activity.

MATERIALS AND METHODS

A laboratory experiment was conducted to test the antimicrobial activity of compounds from two aromatic plants *Pogostemon patchouli* (Patchouli) and *Rosmarinus officinalis* (Rosemary) and two medicinal plants *Lantana camara* (Lantana) and *Chromolaena odorata* (Eupatorium). Biomass extracts from all these four plants were extracted with different polar (ethanol and ether) and non polar (hexane and chloroform) solvents.

Procurement of plant materials: The fresh plant materials such as whole biomass (leaves, stem, branches, roots) of *Pogostemon patchouli* (Patchouli) and *Rosmarinus officinalis* (Rosemary) were collected from Aromatic crop section, Division of Horticulture, latitude of 12^0 58' N and longitude of 77^0 38' E, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru- 560 065.

The plant materials of *Lantana camara* (Lantana) and *Chromolaena odorata* (Eupatorium) were collected around Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru- 560 065. (latitude of 12^0 58' N and longitude of 77^0 38' E)

Preparation of extract: Powdered plant material (15 grams) was soaked with 450 ml of ethanol for five days and then filtered. The filtrate was evaporated to a thick residue at 40°C using a rotary evaporator. The residue was dissolved in 5 ml of ethanol solvent and the extract was stored in the refrigerator for further studies. Similarly different plant material was extracted with different polar and non-polar solvents by following the above procedure (Solomen *et al*., 2005). Patchouli, Rosemary, Lantana and Eupatorium extracts were similarly prepared with different polar and non-polar solvents.

Thin layer chromatographic technique (TLC) was adopted for separating the bioactive compounds (Alicekurian and

Ashashankar, 2007). Retention factor values was calculated using the formula,

$$Rf = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

The solvent systems used as mobile phase were Hexane: ethyl acetate (4:1), Benzene: acetic acid: water (6:7:3) and Conc HCl: acetic acid: water (3:30:10). Preliminary phytochemical tests were conducted to confirm the presence of major bio active compounds in Pacthouli, Rosemary, Lantana and Eupatorium biomass extracts (Nayak and Lexley, 2006). Based on the bioactive compounds found in the herbal plant samples, solvent system was used.

Pathogens used: Antimicrobial compounds were tested against four bacterial pathogens, *Escherichia coli, Staphylococcus aureus, Erwinia* sp., *Xanthomonas campestris* pv. campestris, and two fungal pathogens, *Candida albicans* and *Fusarium oxysporum.*

Method of screening: Paper disc method **(**Gangrade *et al*., 1989**)** was followed for testing the antimicrobial activity.

Statistical analysis: Bioactive compounds from the selected medicinal plants for antimicrobial activity were analyzed using two- way analysis with interaction (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

TLC profiling of all plant extracts have resulted in directing towards the presence of number of phytochemicals. Various phytochemicals have different Rf values in different solvent system. Different Rf values of the compound also reflects about their polarity. Compound showing high Rf value in the solvent system have low polarity and with less Rf value have high polarity. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. In the present experiment, TLC profiling of all the plant extracts in different solvent system indicated the presence of different groups of phytochemicals in the plants.

The retention factor values for the plants extracted with different solvents (ethanol, chloroform, ether) ranged between 0.3 to 0.9 in terpenes, phenolic acids and in an optimum range from 0.05-0.85 and 0.5 to 0.9 of flavonoids which indicated the presence of terpene, flavonoid and phenolic group of compounds in the plant extracts. This range of retention factors was also reported by other researchers (Fecka and Cisowski, 1999; Muchuweti *et al.*, 2005; Talukdar *et al*., 2010). Rf values for these group of compounds varies with the different mobile phases.

Terpene**,** phenolic acids and flavonoid group of compounds were screened for activity against six test organisms. The separated group of compounds actively inhibited the test bacteria and fungi. Terpene group of compounds from ethanol extract of two aromatic plants *Pogostemon patchouli* (Patchouli) and *Rosmarinus officinalis* (Rosemary) and two medicinal plants *Lantana camara* (Lantana) and *Chromolaena odorata* (Eupatorium) inhibited the growth of all pathogens tested. *Erwinia* sp. was highly inhibited by the phenolic acid compounds from ethanol extract of *Pogostemon patchouli* (Patchouli) (Table 1).

Xanthomonas campestris was highly inhibited by the phenolic acid compounds from chloroform extract of Rosemary (Table 2 and plate 1). Phenolic acid compounds separated from chloroform extract inhibited all the organisms tested. Hence, Phenolic acid group of compounds were found to be potential antimicrobial compounds which was similar with the results reported by Chung *et al* in 1993.

Terpene compound(s) from ethanol extract of *Lantana camara* had a high inhibition against *Erwinia* sp. (Table 3). Similarly with methanolic extracts of *Lantana camara* inhibited the growth of 12 microorganisms which was reported by Kumar and his co-workers in 2006.

TABLE 1: Antimicrobial activity of phenolic acids from chloroform extract of *Pogostemon patchouli* (Patchouli)

Test organisms	Diameter of inhibition (mm) Phenolic acids	
	Band 1	Band 2
Escherichia coli	12.58	12.23
Staphylococcus aureus	14.40	12.83
Erwinia sp.	19.50	15.10
Xanthomonas campestris	15.00	13.50
Fusarium oxysporum	14.27	13.17
Candida albicans	12.37	12.43
F test	A*	B* AB*
SE m	0.18	0.32 0.45
CD	0.54	0.94 1.32

Zone of inhibition includes diameter of sterile/susceptibility discs= 8mm

A: difference between compounds, B: difference between organisms, AB: interaction between compound and test organisms

*: significant at 5%.

Note: Band 1: represents the first group of compounds separated from thin layer chromatographic plate.

Band 2: represents the second group of compounds separated from thin layer chromatographic plate.

TABLE 2. Antimicrobial activity of phenolic acids from chloroform extract of *Rosmarinus officinalis* (Rosemary)

Test organisms	Diameter of inhibition (mm) Phenolic acids		
	Band 1	Band 2	
Escherichia coli	13.33	13.06	
Staphylococcus aureus	13.87	12.26	
Erwinia sp.	17.17	14.37	
Xanthomonas campestris	18.93	14.40	
Fusarium oxysporum	13.27	11.20	
Candida albicans	14.20	13.90	
F test	A*	B*	AB*
SE m	0.157	0.273	0.386
CD	0.460	0.797	1.127

Zone of inhibition includes diameter of sterile/susceptibility discs= 8mm
A: difference between compounds, B: difference between organisms, AB: interaction between compound and test organisms
*: significant at 5%.
Note: Band 1: represents the first group of compounds separated from thin layer chromatographic plate.
Band 2: represents the second group of compounds separated from thin layer chromatographic plate.

TABLE 3. Antimicrobial activity of terpenes from ethanol extract of *Lantana camara* (Lantana)

Test organisms	Diameter of inhibition (mm) Terpenes		
	Band 1	Band 2	Band 3
Escherichia coli	12.00 (1.113)	11.07 (1.081)	- (0.00)
Staphylococcus aureus	11.80 (1.107)	11.11 (1.083)	11.00 (1.078)
Erwinia sp.	15.89 (1.227)	14.33 (1.185)	15.88 (1.227)
Xanthomonas campestris	10.55 (1.062)	10.55 (1.062)	11.67 (1.102)
Fusarium oxysporum	11.89 (1.110)	10.89 (1.075)	- (0.00)
Candida albicans	13.11 (1.149)	14.11 (1.179)	14.79 (1.198)
F test	A *	B*	AB*
SE m	0.02	0.028	0.049
CD	0.057	0.080	0.14

Zone of inhibition includes diameter of sterile/susceptibility discs= 8mm
A: difference between compounds, B: difference between organisms, AB: interaction between compound and test organisms
*: significant at 5%. Values in the parenthesis correspond to log transformed value. (log X+1)
Note: Band 1: represents the first group of compounds separated from thin layer chromatographic plate.
Band 2: represents the second group of compounds separated from thin layer chromatographic plate.
Band 3: represents the third group of compounds separated from thin layer chromatographic plate.

Flavonoid group of compounds from chloroform extract of *Lantana camara* has highly inhibited the plant pathogens. The results were previously reported with the extracts of *Croton bonplandianum* made from chloroform solvent were found to be effective against most of the organisms tested as reported by Shekar *et al.*, 2004. Compounds extracted with ether as a solvent showed a little activity against the tested organisms (Table 4) which was similar with the results of Singh and Karnwal, 2006.

Ether is found to be slightly polar in nature and has limited solubility. Hexane solvent found ineffective in dissolving and separating the compounds from all the plant species. Similarly *Croton bonplandianum* was found ineffective against the organisms tested (Shekar *et al.*, 2004). Compounds that exhibit antifungal and antibacterial activity have been isolated in recent years from different plant species and from many microorganisms, invertebrate and vertebrate species. Hence the priority is to be given for the development of alternative drugs to fight against animal and plant bacterial and fungal pathogens.

TABLE 4: Antimicrobial activity of flavonoids from ether extract of *Chromolaena odorata* (Eupatorium)

Test organisms	Diameter of inhibition (mm) Flavonoids	
	Band 1	Band 2
Escherichia coli	15.00 (1.204)	- (0.00)
Staphylococcus aureus	11.44 (1.094)	- (0.00)
Erwinia sp.	11.50 (1.096)	13.89 (1.172)
Xanthomonas campestris	- (0.00)	- (0.00)
Fusarium oxysporum	22.77 (1.376)	20.67 (1.335)
Candida albicans	11.00 (1.079)	11.67 (1.102)
F test	A*	B* AB *
SE m	0.005	0.009 0.013
CD	0.015	0.027 0.038

Zone of inhibition includes diameter of sterile/susceptibility discs= 8mm
A: difference between compounds, B: difference between organisms, AB: interaction between compound and test organisms
*: significant at 5%. Values in the parenthesis correspond to log transformed value. (log X+1)
Note: Band 1: represents the first group of compounds separated from thin layer chromatographic plate.
Band 2: represents the second group of compounds separated from thin layer chromatographic plate.

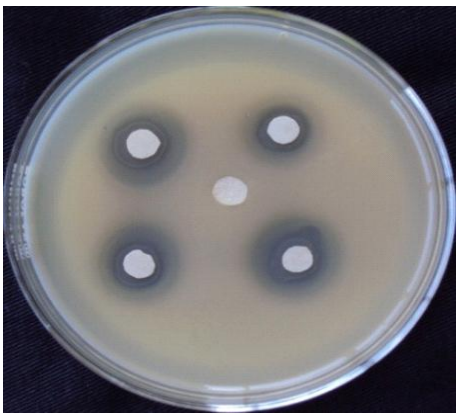

Plate 1: Antimicrobial assay of chloroform extract of Rosemary (*Rosmarinus officinalis*)

In the present experiment, qualitative tests for plant extracts showed significant indication about the presence of metabolites. Terpenes, phenolic acids and flavonoids were found to be present in the biomass extracts of the plants used. Our results confirmed that these antimicrobial compounds had a better inhibition of test organisms even at smaller quantities. Based on the polarity, compounds separated from ethanol extract showed better separation followed by chloroform extract. The results indicated the rich diversity of bioactive compounds of therapeutic use, separation and screening of antimicrobial compounds is found advantageous for future investigation into the field of pharmacology, phytochemistry and other biological actions for drug discovery.

REFERENCES

Alicekurian. and Ashashankar, M. (2007) Medicinal plants, *Horticulture Science Series*-2, P.28.

Chung, K.T. Stevans, JR. S. E., W.F. and C. I. Wei (1993) Growth inhibition of selected food borne bacteria by tannic acid, propyl gallate and related compounds, *Lett. Appl. Microbiol*. **17**, 29-32.

Fecka, I. and Cisowski. W. (1999) Multiple Gradient Development TLC in Analysis of Complex Phenolic Acids from *Lycopus europaeus. Chromatographia*. **49**, 256-260.

Gangrade, S. K., Shrivastava, R. D., Sharma, O. P., Jain, N. K. and Trivedi, K. C. (1989) Evaluation of antifungal properties of essential oils of *Ocimum* species. *Indian Perfumer*. **33**, 97-101.

Kumar, P. V., Chauhan, N. S., Padh, H. M. and Rajani, M. (2006) Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J. Ethnopharmacol*. **107**, 182–188.

Mishra, D. C. O., Samuel. and Tripathi, S. C. (1993) Synergistic antifungal efficacy of essential oils of *Apium graveolens* and *Cuminum cymium*. *Indian Perfumer*, **37**, 134-140.

Muchuweti, M., Zenda, G., Ndhlala, A. R. and Kasiyamhuru, A. (2005) Sugars, organic acid and phenolic compounds of *Ziziphus mauritiana* Fruit. *Eur. Food Res. Technol*. **221**, 570–574.

Nayak, B. S. and Lexley, M., (2006) *Catharantus roseus* flower extract has wound-healing activity in Sprague Dawley rats. BMC Complementary and Alternative Medicine. **6**, 41-45.

Shekar, D. R., Prasad, S. H. K. R., Rao, K. V. and Lakshmi, M. V. (2004) *In vitro* evaluation of antibacterial spectrum of leaf extracts of *Croton bonplandianum*. *Indian J. Microbiol.* **44**, 145-146.

Singh, P. and Karnwal, P. (2006) Antifungal activity of *Cassia fistula* leaf extract against *Candida albicans. Indian J. Microbiol.* **46**, 169-170.

Solomon, J**.,** Kallidass, S. and Vimalan., J. (2005) Isolation, Identification and study of antimicrobial property of bioactive compound in an Indian medicinal plant *Acalypha indica* (Indian nettle). *World J.Microbiol. Biotechnol.* **21**, 1231-1236.

Steel, R. G. D. and Torrie, J. H. (1980) Principles and procedure of statistics, second edition. *New York: McGraw-Hill Book Co.*

Talukdar, A. D., Choudhury, M. D., Chakraborty, M. and Dutta, B. K. (2010) Phytochemical screening and TLC profiling of plant extracts of *Cyathea gigantea* (Wall. Ex. Hook.) Haltt. and *Cyathea brunoniana*. Wall. ex. Hook. (Cl. & Bak.) *Assam University J. Sci. & Tech.: Biological and Environmental Sciences*. **5**, 70-74.

Tomoko, N., Takashi, A., Hiromu, T., Yuka, I., Hiroko, M., Munekazu, I., Totshiyuki, T., Tetsuro, I., Fujio, A., Iriya, I. and Tsutomu, N., Kazuhito, W. (2002) Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *J. Health Sci.* **48**, 273–276.