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Antifungal Activity and Phytochemical Analysis of *Cympogon citratus, Sauropus androgynus* and *Spillanthes acmella* Plants

V. Senthamarai Selvi, G. Govindaraju and Anusha Basker

[1]Department of Biochemistry, PRIST University, Vallam, Thanjavur, India

Abstract: The present paper deals with the antifungal screening and phytochemical screening for the therapeutic importance from three rare medicinal plants such as *Cympogon citratus, Sauropus androgynus* and *Spillanthes acmella.* The compounds such as carbohydrates, proteins, alkaloids, flavonoids, phenols etc, mostly found in all plants and plant related foods that play in most important characteristics. The present work shows positive result in some fungal organisms. In the study of phytochemical analysis the experimental results were compared with reported available literature. Nutritious indigenous plants, fruits, vegetables have the potential to promote wide usage.

Key words: Antifungal · Phytochemical · *Cympogon citratus* · *Sauropus androgynus* and *Spillanthes acmella*

INTRODUCTION

Natural products such as herbs, fruits and vegetables become popular in recent years due to public awareness and increasing interest among consumers and scientific community [1]. Epidemiological evidence has been provided that constituents in natural products show many biological and pharmacological activities including antioxidative, antinflammatory and antiviral effects [2]. Phytochemicals are the chemicals extracted from plants. These chemicals play important role in plant metabolism, such as Carbohydrates, proteins, alkaloids derived from aminoacids, terpenes are a group of lipids and phenolics that are derived from Carbohydrates. Plant produe a very impressive array of antioxidant found naturally occurring [3, 4].

Cymbopogon citratus (DC.) Stapf, popularly known as citronella grass or lemongrass is widely used as a herb in Asian cuisine. It has a citrus flavor and can be dried and powdered or used fresh. *Cymbopogon citratus* is commonly used in teas, soups, and curries. Lemongrass oil is used as a pesticide and a preservative. Research shows that lemongrass oil has anti-fungal properties [5]. *Cymbopogon citratus* leaves are employed as an antihypertensive and anti-inflammatory folk medicine [6]. Phytochemical components like alkaloids, tannins and cardiac glycosides found in the powder are believed to be associated with the preservative and antimicrobial effects of lemon grass [7]. The tea from its leaves has been widely used as an antiseptic, antifever, antidyspeptic, carminative, tranquilizer and stomachic. Several investigations have demonstrated the sedative, Central Nervous System depressor, analgesic, anti-microbial, and fungistatic activities [8, 9].

Sauropus androgynus L. Merr. (Euphorbiaceae, Thai name: Pak-Wanban) is a traditional vegetable in Thailand. It is native to South Asia and Southeast Asia including India, Sri Lanka, Thailand, Laos, Malaysia and Indonesia etc [10]. From the proximate composition of Sauropus androgynus leaf it was observed that its nutritive value is superior to other commonly consumed leafy vegetables in India. Sauropus androgynus leaf was previously reported to contain considerable amounts of the alkaloid papavarine 580mg per 100gm fresh leaf [11]. Vegetables and fruits commonly consist of some nutrients and phytochemicals, which can exhibit antioxidant capacity. The major antioxidants of vegetables and fruits are vitamin C, vitamin E, carotenoids and phenolic compounds, especially flavonoids. These antioxidants

Corresponding Author: V. Senthamarai Selvi, Department of Biochemistry, Research Scholar, PRIST University, Vallam, Thanjavur, India. E-mail: lotus10selvi@yahoo.co.in.

act as scavenging radicals and inhibit the chain initiation or break the chain propagation [12].

Spilanthes acmella Murr. is an indigenous herb growing as an annual throughout the tropics. The whole plant is claimed to possess medicinal properties. The flowers are chewed to relieve tooth ache and the crushed plant is used in rheumatism [13, 14]. The plant is generally known as tooth ache plant. It is also evaluated the anti-inflammatory and analgesic activity of the aqueous extract of *Spilanthes acmella* [15]. Preliminary phytochemical studies showed the presence of flavonoids.. There are reports that some flavonoids are predominant inhibitors of either cyclo-oxygenase or lipo-oxygenase [16, 17]. Flavonoids are hydrolyzed by saliva to deliver aglycones that have protective effect in the oral cavity [18].

MATERIALS AND METHODS

Anti Fungal Activity Disc Diffusion Method [19]: The potato dextrose agar plates were inoculated with each fungal culture for 10 days inoculation. The filter papers discs 5mm diameter impregnated with ethanolic extracts and water extract were placed on test organism-seeded plates. Blank disc impregnated without plant extract was used as control. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

Phytochemical Analysis

Test for Carbohydrates:

Benedicts Test: One ml of a sample solution is placed in a test tube. Two ml of Benedict's reagent (a solution of sodium citrate and sodium carbonate mixed with a solution of copper sulfate) is added. The solution is then heated in a boiling water bath for three minutes. The formation of a reddish precipitate within three minutes.

Molisch's Test: Two ml of a sample solution is placed in a test tube. Two drops of the Molisch reagent (a solution of α-napthol in 95% ethanol) is added. The solution is then poured slowly into a tube containing two ml of concentrated sulfuric acid so that two layers form. The formation of a purple product at the interface of the two layers.

Test for Protein

Ninhydrin Test: Add 0.1 mL of 0.1% ninhydrin solution (in 95% methanol) to 2 mL of the sample. Incubate the mixture for 10 min at 100°C. Blue color indicates, free amino acids or the presence of amino groups.

Test for Tannins: 0.5 g of the crude powder was stirred with 10 ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as evidence for the presence of tannin [20].

Test for Saponins: 0.5 g of crude powder was shaken with water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of saponins [21, 22].

Test for Flavonoids: A portion of crude powder was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration [23].

Test for Terpenoids: 0.5 g of crude powder was dissolved in 5 ml of methanol. 2 ml of the extract was treated with 1 ml of 2, 4-dinitrophenyl hydrazine dissolved in 100 ml of 2M HCl. A yellow-orange coloration was observed as an indication of terpenoids [24].

Test for Glycosides: 0.5 g of crude powder was dissolved in 5 ml of methanol. 10 ml of 50% HCl was added to 2 ml of methanolic extract in a test tube. The mixture was heated in a boiling water bath for 30 min. 5 ml of Fehling's solution was added and the mixture was boiled for 5 min to observe a brick red precipitate as an indication for the presence of glycosides [20].

Test for Phenols: To 2ml of test solution, added alcohol and then few drops of neutral ferric chloride solution was added. The test result was observed.

Test for Steroids: 0.5 g of crude powder was dissolved in 5 ml of methanol. 1 ml of the extract was treated with 0.5 ml of acetic acid anhydride and cooled in ice. This was mixed with 0.5 ml of chloroform and 1 ml of concentrated sulphuric acid was then added carefully by means of a pipette. At the separations level of the two liquids, a reddish-brown ring was formed, as indication of the presence of steroids [24].

Test for Alkaloids: 0.5 g of crude powder was defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 min with 5 ml of aqueous HCl on a boiling water bath. The resulting mixture was centrifuged for 10 min at 3000 rpm. 1 ml of the filtrate was treated with few

drops of Mayer's reagent and a second 1 ml with Dragendroff's reagent and turbidity was observed [16, 20].

RESULTS

The antibacterial activity of three plant extract was assayed in vitro by disc diffusion method against five fungal species and the microbial growth inhibition of extract of the screened plant species is mentioned in Table: 1. Among the three plants the maximum antibacterial activity was shown by *Cympogon citrates* and *Sauropus androgynus,* where as *Spillanthes acmella* showed very minimum activity. The leaves of *Cympogon citrate, Sauropus androgynus* effective inhibition when compared to flowers and root. The antibacterial results indicated among these *Cympogon citrates, Sauropus androgynus* and *Spillanthes acmella* were found to be effective against *Aspergillus flavus, Candida albicans* and moderate effectiveness in *Mucor species* tested. The leaves, flowers of *Cymbopogon citrates, Sauropus Androgynus, Spillanthes acmella* showed less inhibition against *Penicillium species and in Rhizopus species* also. The roots of *Sauropus Androgynus, Spillanthes acmella* and flowers of *Cymbopogon citrates* show no inhibition property.

The screening of secondary metabolites were done and presented in Table: 2. The leaves of *Cymbopogon citrates,* the leaves of *Sauropus Androgynus* showed the strongest presence of Carbohydrates, Proteins, Tannins, Saponins Flavonoids, Terpenoids, Glycosides, Phenolic, Sterioids and Alkaloids. The roots of *Cymbopogon citrates,* the roots of *Sauropus Androgynus* and the flowers of *Spillanthes acmella* showed moderate presence of Carbohydrates, saponins, glycosides, Phenolics and alkaloids. The flowers, of *Sauropus Androgynus* and the flowers of *Spillanthes acmella* showed the presence of Protein. Where as the flowers of *Cymbopogon citrates,* the leaves, roots of *Spillanthes acmella* showed absence result. The phytochemical

Table: 1 Antifungal Activity of *Cympogon citratus, Sauropus androgynus* and *Spillanthes acmella* Plant Extracts

		Zone of inhibition (mm)								
		Cymbopogon citrates			*Sauropus Androgynus*			*Spilanthes Acmella*		
S. No	ASSAY Organisms	Leaves	Flower	Root	Leaves	Flower	Root	Leaves	Flower	Root
1	*Aspergillus flavus*	08	03	05	09	02	02	02	06	-
2	*Candida albicans*	07	03	06	15	04	03	04	04	-
3	*Mucor species*	06	03	04	01	02	02	02	04	-
4	*Penicillium species*	06	-	04	05	01	-	02	04	-
5	*Rhizopus species*	06	-	06	01	01	-	02	04	-

Table 2: Phytochemical Screening of the Leaves, Flower, Root Ethanolic Extract *Cymbopogon citrates, Sauropus Androgynus and Spillanthes Acmella*

		Phytochemical analysed in selected Plants								
		Cymbopogon citrates			*Sauropus Androgynus*			*Spilanthes Acmella*		
S. No	Name of the Test	Leaves	Flower	Root	Leaves	Flower	Root	Leaves	Flower	Root
1	Carbohydrates I)Benedicts Test ii) Molisch's test iii)Fehling test	+	-	+	++	+	+	+	+	-
2	Protein I) Bradford Test	++	+	+	++	+	+	+	+	-
3	Tannins	+	-	+	++	+	+	-	+	-
4	Saponins	++	-	+	++	+	+	-	+	-
5	Flavonoids	++	-	+	++	+	+	-	+	-
6	Terpenoids I) Molisch's test ii)Fehling test iii)Benedict's test	++	-	+	++	+	+	-	+	-
7	Glycosides	+	+	+	++	+	+	-	+	-
8	Phenolic	++	+	+	++	+	+	-	+	-
9	Steroids	+	-	+	++	+	-	-	-	-
10	Alkaloids I) Mayer's test ii) Wagner's test	++	+	+	++	+	+	-	+	-

analysis result indicated that the presence of alkaloids, flavonoids, phenolic compounds and glycosides etc., in the plant extracts, these compounds might be responsible for antibacterial activity against micro-organisms and may also have pharmacological activities.

DISCUSSION

The present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization [25]. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the words population. There are about 45,000 plant species in India with capacity to produce a large number or organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity [26, 27]. In the present study several biochemical constituents and antifungal activities of three medicinal plants were evaluated. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants [28]. In the present work ethanolic extract of *Cymbopogon citrates, Sauropus Androgynus* showed higher activity to the majority of the organism tested when compared to *Spillanthes acmella*. Some of the plants were ineffective in this study do not possess any inhibition.

The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful [29]. The result of phytochemicals in the present investigation showed the presence of biochemical constituents such as alkaloids, phenolic compounds, flavanoids etc,. This study also shows the presence of strong different phytochemicals that can be of valuable therapeutic activity.

CONCLUSION

The extracts from *Cymbopogon citrates, Sauropus Androgynus* and *Spillanthes acmella* were good antifungal in this model. The *Cymbopogon citrates* and *Sauropus Androgynus* may act as antioxidant property. It was proposed that the compounds display the important role as antioxidant might be flavonoids, alkaloids, phenolics, ect., and other phytochemicals also. Continued further exploration of plant-derived antimicrobials is needed today. However, the present study of in vitro antimicrobial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies. In the present study, we have found that the biologically active phytochemicals were present in the ethnaolic extracts of few parts of medicinal plants. Further research is necessary to determine the identity the compounds from these plants and also to determine their full spectrum of efficacy.

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REFERENCES

1. Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D.H. Byrne, 2006. Comparision of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruits extracts. J. Food Compos. Anal., 19: 669-675.
2. Pawlowska, A.M., W. Oleszek and A. Braca, 2008. Quali-Quantitative analyses of flavonoids of Morus nigra L. and Morus alba L. (Moraceae) fruits. J. Agri. Food Chem., 56: 3377-3380.
3. Duduku Krishnaiah, Rosalam Sarbatly and Awang Bono, 2007. Phytochemical antioxidants for health and medicine – A move towards nature. Biotechnology and Molecular Biology Review, 1(4): 097-104.
4. Hollman P.C.H., 2001. Evience for health effects? Journal of Science Food Agri., 81: 842-852.
5. Shadab, Q., M. Hanif and F.M. Chaudhary, 1992. Antifungal activity by lemongrass essential oils. Pak. J. Sci. Ind. Res., 35: 246-249.
6. Carbajal, D., A. Casaco, L. Arruzazabala, R.Gonzalez. and Z. Tolon, 1989. Pharmacological study of *Cymbopogon citratus* leaves. J. Ethnopharmacol., 25(1): 103-107.
7. Adegoke, G.O.K. and B.A. Odesola, 1996. Storage of maize and cowpea and inhibition of microbial agents of biodeterioration using the powder and essential oil of lemon grass (*Cymbopogon citratus*). International Biodeterioration & Biodegradation, 37(1-2): 81-84.
8. Negrelle, R.R.B. and E.C. Gomes. 2007. *Cymbopogon citratus* (DC.) Stapf: chemical composition and biological activities. Rev. Bras. Pl. Med., 9: 80-92.

9. Mishra, A.K. and N.K. Dubey, 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. Appl. Environ. Microbiol., 60: 1101-1105.
10. Frits Stoepman, G.V.N., 1996. Plant Resources of South-East Asia 8: Vegetables. (2nd ed). Prosea Foundation. Bogor: Indonesia.
11. Padmavathi, P. and M. Prabhakara Rao, 1990. Nutritive value of Sauropus androgynus leaves. Plant Foods for Human Nutrition. 40(2): 107-113.
12. Shi, H., N. Noguchi and E. Niki, 2001. Natural antioxidants. In: Antioxidants in food: Practical application. J. Pokorny, N. Yanishlieva and M. Gordon (Eds.), (1st ed). Cambridge : CRC Press Woodhead Publishing Ltd.
13. Balian S., S. Ahmad and R. Zafar, 2006. J. Pharmacol., 38: 213.
14. Kirtikar, K.R. and B.D. Basu, 1935. Indian Medicinal Plants, International Book Distributors, 9/3, Dehradun, India.
15. Chakraborty, A., B.R.K. Devi, Rita, S. Sharatchandra and I. Singh, 2004. Indian J. Pharmacol., 36: 148.
16. Trease, G.E. and W.C. Evans, 1972. Pharmacognosy. 4th ed. London: Bailliere Trindall; Phenolic compounds and tannins, pp: 146-8.
17. Rajnarayana, K., M.C. Reddy, M.R. Chaluvadi and D.R. Krishna, 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J. Pharmacol., 33: 2-16.
18. Chakraborty, A., B.R.K. Devi, R. Sanjebam, S. Khumbong and I.S. Thokchom, 2010. Preliminary studies on local anesthetic and antipyretic activities of Spilanthes acmella Murr. in experimental animal models. Indian J. Pharmacol., 42(5): 277-279.
19. Taylor, R.S.L., N.P. Manandhar and G.H.N. Towers, 1995. Screening of selected Medicinal Plants of Nepal for antimicrobial activities. J. Ethnopharmacol., 46: 153-159.
20. Harborne, J.B., 1973. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall, New York, pp: 279.
21. Smolenski, S.J., H. Silinis and N.R. Farnswoth, 1974. Alkaloids screening. V. Lloydia, 37: 506-536.
22. Kapoor, L.D., A. Singh, S.L. Kapoor and S.N. Shrivastava, 1969. Survey of Indian medicinal plants for saponins, alkaloids and flavonoids. Lloydia, 32: 297-302.
23. Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian Medicinal plants. African J. Biotechnol., 4: 685-688.
24. Kolawole, O.M., S.O. Oguntoye, O. Agbede and A.B. Olayemi, 2006. Studies on the efficacy of *Bridelia ferruginea* Benth. bark extract in reducing the coliform load and BOD of domestic waste water. Ethnobotanical Leaflets, 10: 228-238.
25. Vogel, H.G., 1991. Similarities between various systems of traditional medicine. Considerations for the future of ethnopharmacology. J. Ethnopharmacol., 35: 179-90.
26. Yiming, L.I., L.E. Seans and Zhang, 2004. The antimicrobial potential of 14 natural herbal plants. J. Am. Dent. Assoc., 135: 1133-1141.
27. Mahato R.B. and R.P. Chaudhary, 2005. Ethano medicinal study and antibacterial activities of selected plants of palpa district, Nepal. Scientific World, 3(3): 26-31.
28. Veermuthu, D., A. Muniappan and I. Savarimuthu, 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamilnadu, India. BMC Complementary and Alternate Medicine, 6(35).
29. Kanatt S.R, R, Chander and A, Sharma. 2007. Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chem., 100: 451-458.