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Antifungal Activity of Verbenaceae

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Traditional curatives based on herbal medicinal rationales are time tested and widely accepted across various cultural and socioeconomic strata all around the world. The emergence of drug resistant germs makes the circumstances more assailable for emerging pathogens, especially fungi. Thus an attempt to assess the spectrum of antifungal activity among selected Verbenaceae members (*P. serratifolia, L. nodiflora, V. trifolia, V. negundo* var. *negundo, V. negundo* var. *pubescens, V. altissima, V. leucoxylon, V. pinnata, S. jamaicensis, C. inermis* and *C. serratum*) was conducted against a panel of 8 fungi (*Aspergillus flavus, A. fumigatus, A. niger, Penicillium* sp., *Verticillium* sp., *Curvularia* sp., *Mucor* sp. and *Fusarium* sp.). All of the tested plants were inhibitory to many of the tested fungi and both of the extracts (petroleum ether and methanolic) showed appreciable antifungal effects, though the phytochemical composition was dissimilar.

Key words: Verbenaceae, Antifungal, Fungi, Antimicrobial.

Traditional therapeutics based on herbal medicinal principles is time tested and widely accepted across various cultural and socioeconomic strata all around the world. A number of therapeutic drugs which are currently available in market have plants as origin. In short, the plant kingdom is a treasure house of potential drugs and in recent years there has been a burgeoning interest among the scientific community. Drugs from the plants are easily available, less expensive, safe, and efficient and seldom have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most axiomatic choice for examining the current search for therapeutically effective new drugs such as anticancer drugs, (Haldar *et al.,* 2010), antimicrobial drugs (Namita and Mukesh, 2012), antidiabetic drugs (Rao *et al.,* 2001) and anti hepatotoxic compounds (Manokaran *et al.,* 2008).

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Family Verbenaceae comprises a multitude of species with medicinal and ornamental uses, which occurs in virtually all terrestrial ecosystems. Several members of the family Verbenaceae are considered as medicinal and are used from time immemorial. Rahmatullah (2011) critically reviewed the folk medicinal uses of Verbenaceae family plants in Bangladesh.

The antimicrobial activities of members of Verbenaceae are well reported like *Lippia palmeri* (Ortega-Nieblas *et al*., 2011); *Clerodendrum viscosum* (Oly *et al*., 2011); *Vitex negundo* (Mahmud *et al.,* 2009); *Lippia triplinervis* (Leitão *et al*., 2011). However, there exists a lacuna of knowledge with regard to antifungal activity. However, traditional ayurvedic healers are using a wide ambit of plants in the family to treat various ailments. Thus scientific validation of existing knowledge is indispensible for developing new drug combinations and treatment modalities to combat fungal diseases, as fungal diseases are overwhelmingly emerging. The present study is mooted in this background.

MATERIALS AND METHODS

Extract preparation

Leaves of 11 plants of Verbenaceae were collected from different regions in the State of Kerala. The taxonomic identification was performed by using standard taxonomic keys and expert consultations. Herbarium was also prepared and voucher numbers and the codes are assigned. The collected plants are dried under shade, crushed and subject to soxhlet extraction with petroleum ether and methanol. The extract was filtered and concentrated.

Antifungal susceptibility testing

Fungal isolates

The test species used for this investigation are *Aspergillus flavus, A. fumigatus, A. niger, Penicillium* sp., *Verticillium* sp., *Curvularia* sp., *Mucor* sp. and *Fusarium* sp. The fungi were maintained on Sabouraud Dextrose Agar (SDA) slants at 10°C and subcultured for the study.

Agar well diffusion bioassay

The antifungal activity of the extracts was determined by using the agar well diffusion assay as depicted by Patel and Trivedi (1962) and Holder and Boyce (1994) with some modifications. SDA plates were prepared and a well of 5mm diameter was bored at the middle of the plate aseptically. Then spore suspension of the fungi to be tested was prepared (0.2-2.5 x 105 CFU/mL) and swabbed on to the agar surface. 100 µl of extract was added to the well and incubated. The respective solvents used for extraction was (petroleum ether or methanol) used as a negative control. Four plates were prepared for each test and were incubated at room temperature. The antifungal activity was taken on the basis of diameter of zone of inhibition around the well, which was measured after 4-8 days of incubation and the mean value along with standard deviation of the readings are presented.

RESULTS AND DISCUSSION

Since antiquity, botanicals have provided a wide spectrum of compounds of healing properties, for several ailments. In recent years, antimicrobial properties of plant extracts are reporting incessantly throughout the world. The quantum of medicinal plants and herbal medicinal products used worldwide has climbed up

Table 1. Sensitivity pattern of petroleum ether extracts (mm)

S.No.	Plants tested	*A. flavus*	*A. niger*	*A. fumigatus*	*Verticillium sp.*	*Curvularia sp.*	*Penicillium sp.*	*Mucor sp.*	*Fusarium sp.*
1.	*P. serratifolia*	15±0.1	17.21±2.10	-	17.25±0.2	20.11±0.11	13±2.89	-	-
2.	*L. nodiflora*	-	20.11±1.11	-	22.11±3.12	-	17.02±0.87	-	26.72±0.71
3.	*V. trifolia*	33.01±0.89	28.67±3.18	24.00±1.81	14.68±0.19	18.85±0.88	27.77±0.17	36.76±1.01	29.91±2.09
4.	*V. negundo* var. *negundo*	24.26±3.21	18.00±1.09	19.71±2.01	-	14.11±0.09	14.75±0.11	16.67±0.19	17.19±0.11
5.	*V. negundo* var. *pubescens*	30.67±4.16	31.00±1.61	19.29±0.99	12.18±0.05	12.80±0.18	16.80±0.88	17.79±0.04	12.82±0.13
6.	*V. altissima*	26.92±2.13	25.71±1.16	24.49±2.77	-	16.00±0.10	14.28±0.81	20.18±0.37	27.62±0.83
7.	*V. leucoxylon*	32.11±1.18	29.00±1.69	19.98±1.31	14.11±0.17	15.00±0.02	19.81±0.09	23.38±0.11	32.00±1.39
8.	*V. pinnata*	-	-	-	-	12.95±0.02	-	13.18±0.11	15.48±0.91
9.	*S. jamaicensis*	-	-	-	-	13.71±0.71	12.11±0.88	-	-
10.	*C. inermis*	23.18±0.39	20.71±1.99	17.11±1.37	17.78±0.33	24.66±0.49	12.68±0.11	-	-
11	*C. serratum*	-	-	-	-	-	14.91±0.18	24.73±1.37	24.50±2.17

dramatically in the last decades (Reich and Schibli, 2006). The Family Verbenaceae comprises several species with a myriad of characteristics and applications which occurs in virtually all terrestrial ecosystems. In folkloric medicine, various parts of the plants are used to rectify several disease conditions (Cowan, 1999; Burkill, 2000; Das *et al*., 2003 and Edeoga *et al*., 2005).

The antifungal effects of *P. serratifolia, L. nodiflora, V. trifolia, V. negundo* var. *negundo, V. negundo* var. *pubescens, V. altissima, V. leucoxylon, V. pinnata, S. jamaicensis, C. inermis* and *C. serratum* against fungi such as *Aspergillus flavus, A. fumigatus, A. niger, Penicillium* sp., *Verticillium* sp., *Curvularia* sp., *Mucor* sp. and *Fusarium* sp. were evaluated. In an overall analysis, all of the tested plants were inhibitory to many of the tested fungi. Interestingly, both of the extracts (petroleum ether and methanolic) showed appreciable antifungal effects, though the phytochemical composition was dissimilar.

The petroleum ether extract of *P. serratifolia* was more effective against *Curvularia* sp. (20.11±0.11) followed by *Verticillium* sp. (17.25±0.2). On the other hand, the methanolic extract of *P. serratifolia* exhibited more effect against *Penicillium* sp. (16.11±0.13) (Table 1 and 2). The obtained results are in tune with the available results. The antimicrobial activity of *P. serratifolia* was well established (Rajendran and Basha, 2010; Singh, 2011). Several phytochemicals from both leaves and roots, are reported from *P. serratifolia* like 1HCycloprop [e]azulen -7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]- (2.98 %), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (2.44 %), 2-Hydroxy-3-methylbenzaldehyde (6.39 %), :2s,6s-2,6,8,8-Tetramethyltricyclo [5.2.2.0(1,6)] undecan -2-ol (6.35 %), Benzofuran, 2,3-dihydro- (29.94 %), Glycerin (1.14 %), n-Hexadecanoic acid (13.94%), 2-Propenoic acid and 3-(4-methoxyphenyl)- (13.84 %) (Singh *et al*., 2011). The obtained antifungal activity is the result of synergestic action of all these active components.

L. nodiflora petroleum ether extract was also promising in controlling the tested fungi, *Fusarium* sp. (26.72±0.71), *Verticillium* sp. (22.11±3.12) while the methanolic extract was effective against the genus *Aspergillus* only. Durairaj *et al*. (2007) critically studied the antimicrobial and lipid peroxide scavenging activity

Table 2. Sensitivity pattern of methanolic extracts (mm)

S.No.	Plants tested	*A. flavus*	*A. niger*	*A. fumigatus*	*Verticillium sp.*	*Curvularia sp.*	*Penicillium sp.*	*Mucor sp.*	*Fusarium sp.*
1.	*P. serratifolia*	-	-	14.11±0.18	12.00±0.08	-	16.11±0.13	-	-
2.	*L. nodiflora*	16.11±1.87	18.01±2.07	14.00±0.17	-	-	-	-	-
3.	*V. trifolia*	26.71±0.59	31.07±3.18	25.77±0.19	17.79±1.34	15.09±2.18	25.79±0.19	35± 0.18	29.06±0.11
4.	*V. negundo* var. *negundo*	25.43±1.20	19.11±0.14	20.16±1.11	-	15.08±0.15	17.77±1.61	15.13±1.29	18.06±0.89
5.	*V. negundo* var. *pubescens*	25.43±1.32	33.01±2.90	-	-	14.91±1.93	22.18±2.17	18.01±1.16	14± 0.03
6.	*V. altissima*	26± 2.27	23.14±0.19	25.17±3.29	13.73±0.43	16.72±0.78	-	-	-
7.	*V. leucoxylon*	31± 3.11	18.12±1.09	23.89±2.08	-	-	33.72±1.75	-	-
8.	*V. pinnata*	-	-	-	14.76±0.59	18.77±1.04	19.18±0.86	13.18±2.87	-
9.	*S. jamaicensis*	-	-	-	-	18.70±1.08	13.09±1.04	-	13.01±0.08
10.	*C. inermis*	19.80±1.05	16± 0.07	16.44±0.34	14± 0.24	-	13.89	-	19.81±2.05
11	*C. serratum*	-	-	-	-	13.17±0.10	16.91±0.15	14± 0.01	16.84±2.13

of *Lippia nodiflora* with significant outputs. The present results also supported the findings. Moreover, other potentials of the plant such as hepatoprotective activity were also well accomplished (Naik *et al*., 2012).

The genus *Vitex* showed copious antifungal activity irrespective of petroleum ether and methanol extracts. The petroleum ether extract of the *Vitex* sp. *V. trifolia, V. negundo* var. *pubescens* and *V. leucoxylon* demoed notable antifungal effect against all the tested fungi. *V. trifolia* demonstrated significant antifungal effects towards *Mucor* sp. (36.76±1.01) followed by *A. flavus* (33.01+0.89) and *Fusarium* sp. (29.91±2.09). *V. negundo* var. *pubescens* showed commendable activity against *A. flavus* (30.67±4.16) and *A. niger* (31±1.61). *V. leucoxylon* was more active against *A. flavus* (31.11±1.18) and *Fusarium* sp. (32±1.39).

A substantial antifungal activity was observed in the case of petroleum ether extract of *V. altissima* against *Fusarium* sp. (27.62±0.83) followed by *A. flavus* (26.92±2.13) and *A. niger* (25.71±1.16). The methanolic extract of *V. altissima* also showed activity against five of the tested fungi with high sensitivity towards *A. flavus* (26±2.27) and *A. niger* (23.14±0.19).

S. jamaicensis petroleum ether extract was effective against only two of the tested fungi such as *Curvularia* sp. (13.71±0.71) and *Penicillium* sp. (12.11±0.88). On the other hand, *Curvularia* sp. (18.70±1.08), *Penicillium* sp. (13.09±1.04) and *Fusarium* sp. (13.01±0.08) was sensitive towards methanolic extract. The widespread use of *S. jamaicensis* in the treatment of a number of diseases is documented from time immemorial. The antimicrobial activity and the presence of phytochemicals such as phenolic compound, tannin, saponin, terpenoids and flavonoid was reported earlier (Putera, 2010) with notable variation in activity of root and leaf extracts. The current observation also referred the same, though antifungal reports of the plant are less.

C. inermis petroleum ether and methanolic extracts were effective against six of the tested fungi with notable variations. *C. serratum* petroleum ether extract was effective against three while methanolic extract was effective against four of the tested fungi. *C. inerme* possess antifungal potential irrespective of extracting solvents. The antifungal activity of *C. inerme* was thoroughly

Table 3. Sensitivity pattern of essential oil of Vitex sp. (mm)

S.No.	Plants tested	*A. flavus*	*A. niger*	*A. fumigatus*	*Verticillium sp.*	*Curvularia sp.*	*Penicillium sp.*	*Mucor sp.*	*Fusarium sp.*
1.	*V. trifolia*	30.11± 2.19	33.04± 0.21	25.90± 1.91	15.08± 0.07	-	16.73± 0.86	28.07± 1.98	26.79± 2.07
2.	*V. negundo* var. *negundo*	22.18± 3.29	16.07±1.05	21.09±0.98	-	13.17± 0.86	18	17.50±0.67	-
3.	*V. altissima*	25.27±0.18	20± 0.15	19.16±0.64	-	12	-	-	12.0.16±0.08
4.	*V. leucoxylon*	28.16±1.79	24.17±2.06	21.06±0.13	12.90±0.30	-	15.05±0.17	-	-

explored (Anitha and Kannan, 2006). In Indian systems of medicine, *C. inerme* are used for treating fever, cough, skin rashes and boils, and also used to treat umbilical cord infection and for cleaning the uterus in local medicine (Nadkarni, 1976). Tharmaraj *et al.* (2012) highlighted the phytochemical and bioefficacy potential of the plant.

In an overall analysis, the genus *Vitex* showed prominent sensitivity pattern against all of the tested fungi. Thus, an attempt was also done to evaluate the antifungal activity of essential oil of the genus. The results were summarized in Table 3. This clearly demonstrated that *V. trifolia* was active against seven of the tested fungi followed by *Vitex negundo* var. *negundo* (6 species), *V. altissima* and *V. lecucoxylon* (five species each).

The genus *Vitex* (*V. trifolia, V. negundo* var. *negundo, V. negundo* var. *pubescens, V. altissima, V. leucoxylon, V. pinnata*) is well known for a cornucopia of treatment attributes (Padmalatha *et al.*, 2009). An array of phytochemicals are also detected and purified from the plant by different methods (Meena *et al*., 2011). The present results also inferred the widely accepted notion about the plant. Concerted efforts to characterize and evaluate antimicrobial effects were also noticed in concordance with the available results (Srinivas *et al*., 2010; Panda *et al*., 2011; Sathish *et al*., 2012; Natheer *et al*., 2012).

C. serratum also showed commendable antifungal activity in the present investigation. Ethno-medicinal grandness of the plant has been reported in various indigenous systems of medicines like Ayurveda, Siddha and Unani for the treatment of various grievous diseases such as syphilis, typhoid, cancer, jaundice and hypertension (Shrivastava and Patel, 2007). The plant was rich in D-mannitol, hispidulin, cleroflavone, apigenin, scutellarein, serratagenic acid, acteoside, verbascoside, oleanolic acid, clerodermic acid, γ-sitosterol, β-sitosterol, cholestanol, clerosterol, campesterol and 24-ethyl cholesterol which paves the way for the plants antimicrobial effects (Singh *et al*., 2012).

CONCLUSION

Establishing the antifungal activity of these plants will impart the systematic scientific probe of indigenous medicinal plants in hunting of novel drugs. It is concluded that *P. serratifolia, L. nodiflora, V. trifolia, V. negundo* var. *negundo, V. negundo* var. *pubescens, V. altissima, V. leucoxylon, V. pinnata, S. jamaicensis, C. inermis* and *C. serratum* do possess antifungal properties and that this activity is largely dependent on phytochemicals. However, further studies are required in order to gain more lucidity as to the specificity and biochemical mechanisms creditworthy for the antifungal properties of these plants. Fungal diseases are emerging and there is an urgent need in developing newer antifungals to combat fungal diseases. The phytochemical compounds should be subjected to animal and human trials to ascertain effectiveness, toxicity and effects on normal microbiota. More systematic exploration and interpretation would be facilitated to achieve targets.

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