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# Antifungal Activity of Some Essential Oils

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Thirteen essential oils recovered by steam distillation from Indian herbs were analyzed for their chemical compositions using GC and GCMS. The antifungal activity against plant and Food mold rot were examined in vitro using poison food technique. The essential oil from cymbopogan exhibited control over all the plant and food mold rot tested. The bioactive compound in the oil and its minimum inhibitory concentration were determined using TLC bioautography.

**KEYWORDS: Essential oils; antifungal activity; plant and food mold rot; TLC bioautography**

## INTRODUCTION

The search for simple bioactive compounds of plant origin against fungi has been the target of interest for ecologically safe products. As the essential oils are known to contain a natural cocktail of monoterpenes, diterpenes and hydrocarbons, with a variety of functional groups, leading to antifungal (*1*−*4*) and antimicrobial (*5*−*9*) activities. A study on the antimicrobial efficacy of *Limonia acidissima* L. (Wood Apple) Hindi Keith, a member of the family Rutaceae, which is of wide occurrence in Indian subcontinent, revealed its utility in inhibiting the growth of four fungi and four bacteria with pure oil. The zone of inhibition and dilution of oil exhibited negative association, because an increase in dilution of oil reduces the zone of inhibition (*10*). Essential oil from Tagetes patual Linn. was evaluated for fungicidal properties against the five strains *Aspergillus niger*, *Penicillium feniculosa*, *Fusarium solani*, *Rhizomucor* sp., and *Trichoderma viride*. The oil concentrations, 1600 and 3200 ppm, were fully inhibitive, whereas at concentrations 10, 200, 400, and 800 ppm, there was a dose-dependent decrease in fungal growth rates. Among the five strains, *Aspergillus niger* was the least inhibited, while the growth of *Fusarium solani* was most inhibited (*11*). The turmeric leaves (*Curcuma longa* Linn.) having antimycotic/antifungal activity of both rhizome oil and leaf oil against human pathogens were studied, where in leaf oil showed better activities in comparison to rhizome oil. Turmeric leaf oil (neat) demonstrated fungicidal activity, which compares well with standard antifungal drugs (*12*). These oils could be of choice for developing lead compounds to control plant diseases.

The purpose of the study is to screen a number of essential oils and identify the lead compounds having remarkable antifungal activity. The results are presented in this paper.

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## MATERIALS AND METHODS

**Essential Oils and Cultures Used.** Steam-distilled essential oils were obtained from an essential oil manufacturing company. (M/s Venuss Herbo Aromatic Pvt.Ltd., Rajapalayam, Tamilnadu, India)

The following fungi obtained from the TRG Centre for Natural Products, SPIC Science Foundation were used. *Pyricularia oryzae*, *Drechslera oryzae*, *Rhizoctonia solani*, *Colletotrichum lindemuthianum*, *Colletotrichum capsici*, *Macrophomina phaseolina*, *Alternaria alternata*, *Phyllosticta* sp., *Pestalotia theae*, *Curvularia lunata*, *Fusarium oxysporum*, *Granaria uvicola*, *Sclerotinia sclerotiorum*, *Mycosphaerella* sp., *Botrytis cinerea*, *Aspergillus niger*, *Aspergillus flavus*, *Botryodiplodia theobromae*, *Cercospora nicotiana*, and *Phoma* sp. All the fungi were stored at 4 °C. Fungi were activated on Czapek-Dox agar plates grown at room temperature.

**Poison Food Technique.** This technique (*13*) plays a significant role in bioassay methods to evaluate the bio-efficacy of the compound. This involves identifying a synthetic nutrient medium in which the pathogen grows, incorporating the target compound in to the medium, inoculating the test organism, and incubating for the required time. The mycelial growth of the fungus is estimated by measuring the diameter of the radial growth of the mycelial tips. A comparison with the control in which only the nutrient medium is taken indicates the bio-efficacy of the compound. If there is no growth, then the compound is said to be a poison. The details of the experiment are given below.

A weighed quantity of compound was added to the molten Czapek-Dox Agar (CDA) medium (~45 °C) to yield the desired concentration. For initial screening, 100 mg of the compound incorporated in 100 mL of CDA medium at the concentration of 1000 ppm was used for a screening test, and the other without compound served as control. The pathogen of interest from the growing tips (punched in fungal mat grown on CDA medium in sterile Petri plates) was placed at the center and allowed to grow, and all plates were incubated at room temperature. Radial growth in terms of diameter (mm) was examined at 72 h, and every 24 h therafter for 240 h. Five replicate plates were used, and the entire experiment was repeated three times.

**Bioautography Method.** This technique (*14*) was used to ascertain the active constituent. The sample was loaded on TLC precoated silica gel Merck 60 F 254 (0.02 mm), followed by separation of the constituents, and then spraying of conidia on to the developed TLC plate. The essential oil was spotted using micro-capillaries on two TLC plates and allowed to develop using hexane/ethyl acetate at a ratio of

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**Table 1.** Major Components of Essential Oils Tentatively Identified by GC and GCMS

| sample no. | name of the tested oils | botanical names | plant part used | lead compounds tentatively identified |
|---|---|---|---|---|
| 1 | carrot seed | *Daucus carota* | dried seeds | carotol-40% |
| 2 | juniperous | *Juniperus virginiana* | needles, stems, berries | cedrol- 31.6% |
| 3 | dill seed | *Anethum graveolens* | seeds | carvone[a]–45% |
| 4 | ginger | *Zingiber Officinale* | root | zingiberene-25% |
| 5 | nutmeg | *Myristica fragrans* | seeds | linalool[a]–20% |
| 6 | orange | *Citrus sinensis* | fresh peel of the fruit | d-limonene[a]–80% |
| 7 | tagetes | *Tagetes patula* | herb | tagetone-50% |
| 8 | costus root | *Saussurea lappa* | root | linalool[a]–15% |
| 9 | calamus | *Acorus calamus* | root | $\alpha$, $\beta$-thujone-50% |
| 10 | rukhus | *Vetiveria Zizanioides* | root | vetiverone-60% |
| 11 | ajowan | *Tachyspermum copticum* | seed | thymol[a]–40% |
| 12 | jatamanshi | *Nardostachys Jatamansi Oil* | flower | bornyl acetate[a]–40% |
| 13 | cymbopogan | *Cymbopogan martini* | grass | geraniol[a]–80% |

[a] Indictes components identified with reference standards.

**Table 2.** Percentage Inhibition Zones of *Colletotrichum lindemuthianum* and *Alternaria alternata* Tested against Different Essential Oils at 1000 ppm conc

| sample no. | essential oil used | percentage inhibition zones[l] | |
|---|---|---|---|
| | | *Colletotrichum lindemuthianum* | *Alternaria alternata* |
| 1 | carrot seed | 0[k] | 0[k] |
| 2 | juniperous | 0[k] | 0[k] |
| 3 | dill seed | 25[e] | 69[c] |
| 4 | ginger | 0[k] | 20[f] |
| 5 | nutmeg | 25[e] | 16.50[h] |
| 6 | orange | 0[k] | 0[k] |
| 7 | tagetes | 0[k] | 6.6[j] |
| 8 | costus root | 0[k] | 0[k] |
| 9 | calamus | 17.5[gh] | 18[g] |
| 10 | rukhus | 0[k] | 17[gh] |
| 11 | ajowan | 36.7[d] | 73[b] |
| 12 | jatamanshi | 16.4[h] | 3.3[j] |
| 13 | cymbopogan | 100[a] | 100[a] |

[a–k] Mean followed by common letters are not significant at 5% level by DMRT. [l] Mean of three replicates. CD (0.05) = 12.4447.

**Table 3.** Percentage Inhibition Zones of Different Food Mold Rots Affected against Different Essential Oils

| sample no. | essential oils used | percentage inhibition zones[s] | | | |
|---|---|---|---|---|---|
| | | *A. niger* | *A. flavus* | *Penicillium* sp. | *Rhizopus* sp. |
| 1 | carrot seed | 0[u] | 8.52[r] | 37.16[de] | 24.22[j] |
| 2 | juniper berry | 10.47[pq] | 0[u] | 12.84[o] | 3.82[s] |
| 3 | dill seed | 45[c] | 37.94[d] | 50.88[b] | 3.43[s] |
| 4 | ginger | 0[u] | 1.47[t] | 9.31[qr] | 0[u] |
| 5 | nutmeg | 11.67[op] | 26.17[i] | 27.75[h] | 20.69[l] |
| 6 | orange | 0[u] | 0[u] | 35.97[ef] | 9.31[qr] |
| 7 | tagetes | 0[u] | 0[u] | 22.67[k] | 19.90[l] |
| 8 | costus root | 0[u] | 26.17[l] | 36.37[ef] | 0[u] |
| 9 | jatamanshi | 22.25[k] | 16.37[m] | 35.58[f] | 14.41[n] |
| 10 | rukhus | 8.52[r] | 32.05[g] | 100[a] | 0[u] |
| 11 | cymbopogan | 100[a] | 100[a] | 100[a] | 100[a] |
| 12 | ajowan | 100[a] | 100[a] | 100[a] | 100[a] |

[a–r] Mean followed by common letters are not significant at 5% level by DMRT. [s] Mean of three replicates. CD (0.05) = 1.40610.

9:1, v/v. One plate was dipped into Pancal-d reagent followed by heating to 100 °C to visualize the spots separated of the compounds. The other plate was used for the bioautography assay system. Growing tips of *Botrytis cinerea* were harvested using 10% Czapek dox broth taken in an atomizer and sprayed over the developed TLC plate. Then the plate was kept in a humid chamber to monitor the activity of the compound. The inhibition zone was noticed after 72 h of incubation in humid chamber.

**General Experimental Procedure (GC and GCMS Analysis).** Each essential oil was first analyzed by GC-FID and the number of components and their relative percentages were obtained from the chromotogram. Then under identical conditions, GC-MS analysis was performed to determine the identity of the major components. The experimental details are as follows:

The GC analysis was carried out in a Shimadzu GC-17A. A Shimadzu J&W capillary column (30m × 0.25 mm id., 0.25-$\mu$m film thickness) was used with nitrogen as the carrier gas. The temperature program used was 70 °C for 10 min, 10−260 °C for 20 min. The injection and detector temperatures were 250 °C and 300 °C, respectively. The percentage of each component was calculated from FID chromatograms. The compounds were identified by mass spectra using Shimadzu QP 5000 system using the same column and temperature program. The split ratio was adjusted to 60:40 for MS. The electron impact mass (from *m*/*z* 40−700) were recorded at 70 eV.

**Table 4.** Percent Inhibition Zones of Different Phytopathogenic-Fungi-Affected Cymbopogan Oil

| | | | percentage inhibition[t] | | | |
|---|---|---|---|---|---|---|
| | | | treated (ppm) | | | |
| sample no. | fungus used | control[u] | 25 | 45 | 90 | 135 |
| 1 | *Pyricularia oryzae* | 42.6 ± 3.5 | 26[t] | 100[a] | 100[a] | 100[a] |
| 2 | *Drechslera oryzae* | 36.0 ± 2.6 | 30[r] | 100[a] | 100[a] | 100[a] |
| 3 | *Rhizoctonia solani* | 85.0 ± 0 | 32[q] | 100[a] | 100[a] | 100[a] |
| 4 | *Colletotrichum lindemuthianum* | 85.0 ± 0 | 36[o] | 87.8[c] | 100[a] | 100[a] |
| 5 | *Colletotrichum capsici* | 85.0 ± 0 | 30[r] | 100[a] | 100[a] | 100[a] |
| 6 | *Macrophomina phaseolina* | 85.0 ± 0 | 28[s] | 100[a] | 100[a] | 100[a] |
| 7 | *Alternaria alternata* | 85.0 ± 0 | 45j[k] | 91[b] | 100[a] | 100[a] |
| 8 | *Phyllosticta sp.* | 85.0 ± 0 | 30[r] | 100[a] | 100[a] | 100a |
| 9 | *Pestalotia theae* | 85.0 ± 0 | 28[s] | 100[a] | 100[a] | 100[a] |
| 10 | *Curvularia lunata* | 67.6 ± 2.5 | 32[q] | 100[a] | 100[a] | 100[a] |
| 11 | *Fusarium oxysporum* | 85.0 ± 0 | 44[k] | 85[d] | 100[a] | 100[a] |
| 12 | *Granaria uvicola* | 60.6 ± 4 | 52[h] | 100[a] | 100[a] | 100[a] |
| 13 | *Sclerotinia sclerotiarum* | 85.0 ± 0 | 46[j] | 100[a] | 100[a] | 100a |
| 14 | *Mycosphaerella sp.* | 57.0 ± 8.6 | 48[i] | 66[f] | 70[f] | 70[f] |
| 15 | *Botrytis cinerea* | 85.0 ± 0 | 28[s] | 100[a] | 100[a] | 100[a] |
| 16 | *Aspergillus niger* | 85.0 ± 0 | 34[p] | 100[a] | 100[a] | 100[a] |
| 17 | *Aspergillus flavus* | 85.0 ± 0 | 38[n] | 100[a] | 100[a] | 100[a] |
| 18 | *Botryodiplodia theobromae* | 72.3 ± 11.1 | 40[m] | 100[a] | 100[a] | 100[a] |
| 19 | *Cercospora nicotiana* | 30.3 ± 4.7 | 42[l] | 100[a] | 100[a] | 100[a] |
| 20 | *Phoma sp.* | 85.0 ± 0 | 44[k] | 80 [e] | 84[d] | 84[d] |

[a–s] Mean followed by common letters are not significant at 5% level by DMRT. [t] Mean of three replicates CD (0.05) = 1.61251. [u] The values for control are respected as mycelial growth (mm ± SD).

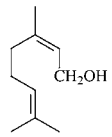
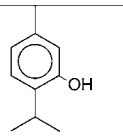
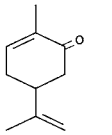
## RESULTS AND DISCUSSION

The GCMS analysis of essential oils indicated the presence of different chemicals, of which some of them are tentatively identified and others were identified using reference standards obtained from Aldrich (**Table 1**). The results recorded in **Table 2** for the antifungal properties reported as mean mycelial growth (±SD) and percentage inhibition against *Colletotrichum lindemuthianum* and *Alternaria alternata* against 13 different essential oils. Cymbopogan oil showed remarkable antifungal activity, followed by ajowan oil and dill seed oil. Other essential oils had no visible effect on the mycelial growth of test organisms. An interestingly similar trend was obtained when these oils were tested against food mold rot, and the results are shown in **Table 3**. Because cymbopogan oil showed total mycelial growth inhibition against *C.lindemuthianum* and *A. alternata*, we therefore tested this oil for broad spectrum against plant pathogens. The results obtained for 20 pathogens are presented in **Table 4**. Remarkably, cymbopogan oil exhibited inhibition of total mycelial growth of the tested fungi at higher doses (90 and 135 ppm). However, at a concentration of 45 ppm, the reduction in mycelial growth was 91% against *A. alternata*, 87.8% for *C. lindemuthianum*, 80% for *Phoma* sp., and only 66% for *Mycosphaerella* sp.

It is noticed that sensitivity to cymbopogan oil is different in *Mycosphaerella* sp. and *Cercospora* sp., the two different morphotypes. This is a new and highly relevant observation emerging from this study. It is very important to keep in mind the above observation while formulating natural biocides and evaluating their field of efficacy for control of different pathogens.

As three essential oils showed significant inhibition of mycelial growth, it was of interest to identify the active components and their MIC using the TLC bioautography test. The presence of an inhibition zone corresponding to three separated compounds (geraniol, thymol, and carvone) was noticed, and the results showed an excellent antifungal activity of cymbopogan oil compared to dill seed oil and ajowan oil and suggest that it can serve as a broad spectrum fungistatic compound for the control of phytopathogenic fungi. Also, the active ingredients geraniol, thymol, and carvone can be used as lead structures for the development of antifungal components. (**Tables 5** and **6**)

**Table 5.** Active Components of Selected Essential Oils Corresponding to the Inhibitory Zones in TLC Bioautography

| Sample No. | Oils used | Active Components | $R_f$ value* |
|---|---|---|---|
| 1 | Cymbopogan |  Geraniol | 0.45 |
| 2 | Ajowan |  Thymol | 0.50 |
| 3 | Dill Seed |  Carvone | 0.80 |

*Solvent system used Hexane/Ethyl Acetate 9:1ratio(v/v). $R_f$ value corresponding to the inhibitory zone.

Thus, an approach toward discovery of a lead compound has been made using essential oils as the source. Thirteen essential oils were screened; of these, three essential oils (viz., cymbopogan, ajowan and dill seed oil) were found to be more potent. The sensitivity of *Mycosphaerella* sp. and *Cercospora* sp., the two different morphotypes to cymbopogan, has indicated the need for application of natural biocides in the field. Using TLC bioautography, the bioactive ingredients have been identified

**Table 6.** MIC of Active Components by Poison Food Technique and TLC Bioautography

| sample no. | sample used | MIC/poison food technique | MIC/TLC bioautography ($\mu$g mL$^{-1}$) |
|---|---|---|---|
| 1 | cymbopogan | 45 ppm | 160 |
| 2 | geraniol | 160 ppm | 160 |
| 3 | ajowan | | 200 |
| 4 | thymol | 200 | 200 |
| 5 | dill seed | | 225 |
| 6 | carvone | 225 | 225 |

as simple monoterpenes (geraniol, thymol, and carvone). Synthetic modifications can be made in these molecules, especially geraniol, to obtain optimum antifungal product with lipophilicity.

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