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Antifungal Activity of *Artemisia Nilagirica* Essential Oil from the Western Ghats Nilgiris against Food Borne Fungi

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Abstract

Commercial antifungal drugs lead to develop fungal resistance and cause their own side effects to humans. Alternative use of plant essential oil as antifungal agent was attempted. The present study revealed that the extracted essential oil from *Artemisia nilagirica* was assessed for its chemical constituents and antifungal activity against four food borne fungi. Forty compounds were present in the essential oil out of which thujone was the major compound followed by γ- curcimene, α- caryophyllene, lavundulol and germacrene. Minimum inhibitory concentration for *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum* and *Penicillium notatum* was 57.33, 32.66, 87.66 and 13.66 µl/L air respectively. Results obtained showed that the Essential oil has more potency against *P. notatum* followed by other fungal species at a slightly higher concentration.

Keyword: *Artemisia nilagirica*; Food borne fungi; Essential oil; Antifungal activity

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Introduction

Fungal infections are spread all over the environment, especially on the fruits, vegetables and other food products stored for longer period. These food borne fungi not only spoil the stored food products, but they are also responsible for various deadly diseases. In this respect, essential oil from plant was effective against the food borne fungi and act as an alternative to synthetic antifungal products [1,2] and used as herbal medicine against the disease caused by fungi [3]. They have wide application against variety of fungal infections, treatment and prevention [4] and also helpful in effective management of fungi in buildings and indoor environment [5]. These fungi are not only responsible for the spoilage of food, they also produce certain toxins such as alfatoxins ochratoxin-A, which is the secondary metabolites produced by the *Aspergillus* species [6]. Synthetic fungicides have been used for the control of pathogenic fungi, however, their potential toxicological problems and considerable deterioration of the environmental quality and human health have generated considerable interest in preservation of grains by using naturally occurring compounds [7]. In connection in our study we focused on finding novel antifungal agent from natural source (plant essential oil) and its food preservative potential. Therefore *A. nilagirica* essential oil can be used as biocontrol agent against stored-product insects. Even though essential oil from *Artemisia* species have been reported for its insecticidal activity, yield of plant essential oil and its chemical composition may vary based on the location, season, plant part and method of extraction. Therefore our plant may be a novel source for its unique chemical composition, yield and insecticidal activity. Favorable eco-toxicological properties of essential oil make them potentially suitable for control of food borne fungi.

Materials and Methods

Plant collection

Artemisia nilagirica was collected from Kagguchi village (latitude- 11.33691°N, longitude- 76.81061°E), Western Ghats, The Nilgiris, based on the information provided by the local tribes. The collected plant was identified and authenticated by Botanical Survey of India, Coimbatore (authentication number – BSI/SRC/5/23/2012-13/Tech.1741).

Extraction of essential oil

Essential oil was extracted from dried leaf powder (20 g) by hydro-distillation at 96°C for 4 hours. Extracted essential oil was stored at 4°C until use.

Analysis of essential oil components

Qualitative analysis of *A. nilagirica* essential oil was performed

using Gas Chromatography and Mass Spectroscopy (Shimadzu, GC-MS QP 2010+). Various constituents of essential oil were identified by comparing their mass spectra with those stored in NIST MS search 2.0 (MS library).

Antifungal activity

Well diffusion method: *Aspergillus flavus* (KUMBF07), *Aspergillus niger* (KUMBF13), *Fusarium oxysporum* (KUMBF56) and *Penicillium notatum* (KUMBF57) were obtained from Karpagam Microbial Culture Collection Center (KMCC), Karpagam University, Coimbatore. Fungal spore suspensions (1.5 x 10^5 CFU ml^{-1}) were spread separately on sterile potato dextrose agar medium. Three wells were cut in each plate. First well is loaded with 50 µl of essential oil (sample), second well with 50 µl of ketaconazole (50 µg-standard drug), and third well with 50 µl of methanol (negative control). Zone of inhibition was observed after 60 h of incubation.

Broth macro dilution method: Sterile potato dextrose broth containing tween-20 at a concentration of 4% was aliquated into five tubes with 900 µl each. To the first tube 100 µl of Essential oil was added, mixed well and 100 µl was taken and added to second tube, thus all tubs were serially diluted, similar steps were followed for the four fungal species with replicates. Fungal spore suspension- 10 µl (1.5 x 10^5 CFU ml^{-1}) was added to all the test tubes and incubated at room temperature (27°C). Minimum inhibitory and fungicidal concentrations of essential oil were observed after 60 h.

Results and Discussion

GC-MS studies enabled the detection of forty compounds in *A. nilagirica* essential oil **(Table 1)** out of which, α-thujone (34.24%) is the major compound followed by γ- curcimene (11.40%), α- caryophyllene (9.97%), lavundulol (4.03%) and germacrene (3.81%). Essential oil of *A. nilagirica* from northern hilly areas of India which contained forty three compounds, out of which, α-thujone (36.35%) is the major compound followed by β-thujone (9.37%), germacrene (6.32%), 4-terpineol (6.31%), camphene (5.47%), β-caryophyllene (5.43%) and borneol (4.12%) to those obtained by Sati et al. [8]. In comparison, the analyzed *A. nilagirica* essential oil from two extreme origin of India has nearly same number of compounds and their major compound percentage have slightly varied. From this study we concluded that the bioavailability of *A. nilagirica* was widely present in the hilly regions of India with similar chemical constituents. *A. nilagirica* essential oil was required 5.7% v/v for inhibition of *A. niger*, but against *P. notatum* it required only 1.3% v/v **(Table 2)**. Maximum zone of inhibition was found to be against *P. notatum* 27.66 ± 0.43 **(Figure 1)**. The minimum inhibitory concentration of essential oil required is varied based on the fungal species and also the type of oil **(Figure 2)**. Similarly, the isolated essential oil from Indian medicinal plants showed their minimum inhibitory concentration between 0.5 and >4% v/v against *A. niger* [2].

Table 1 Chemical composition of *Artemisia nilagirica* essential oil.

Compound	R Time	Area %	Compound	R Time	Area %
Sabinene	6.596	0.94	Elemene	15.367	0.56
1-octen-3-ol	6.667	1.22	**α-Caryophyllene**	15.773	**9.97**
Cymene	7.679	0.37	**Germacrene**	16.115	**3.81**
Limonene	7.778	0.52	`β-Sesquiphellandrene	16.320	2.23
1,8- Cineol	7.848	0.69	β-Humulene	16.614	1.35
γ-Terpinene	8.428	0.60	Neoallocimene	16.726	0.45
α- Thujone	9.625	**34.24**	**γ-Curcumene**	17.231	**11.40**
Thujol	10.366	1.45	Lepidozene	17.471	2.22
Cis verbenol	10.465	0.85	Cedrene	17.533	0.52
Furandione	10.525	1.75	γ-Cadinene	17.793	0.68
Geraniol	10.876	2.96	4-Octylphenol	18.338	0.52
Terpinol	11.132	1.29	Palustrol	18.608	0.39
β- Terpinol	11.369	0.90	Ledol	19.240	0.42
Cis-Carveol	11.907	0.43	Cadinol	10.043	1.80
Perilaladehyde	13.106	1.77	Eudesmol	20.158	0.74
Lavundulol	13.342	**4.03**	Iodine	20.302	0.73
Perillol	13.616	1.88	Bisabolol	20.462	1.37
Peugenol	14.668	0.84	Triene	23.135	0.66
Copaene	15.098	1.03	Rimuen	24.765	0.45
Zingiberene	15.278	0.60	Phytol	26.477	0.74

R Time-Retention Time. Indicated in bold font- Major compounds

Table 2 Antifungal activity of *Artemisia nilagirica* essential oil against food borne fungi.

Test fungi	Zone of inhibition (mm)		MIC	MFC
	Ketaconazole (50 µg)	Essential oil (50 µl)		
A. flavus	11.83 ± 0.54	10.33 ± 0.43	57.33 ± 0.43	93.33 ± 0.39
A. nigerr	11.83 ± 0.43	12.66 ± 0.43	32.66 ± 0.62	56.33 ± 0.43
F. oxysporum	13.33 ± 0.43	10.66 ± 0.43	87.66 ± 0.43	142.33 ± 0.43
P. notatum	10.33 ± 0.43	27.66 ± 0.43	13.66 ± 0.62	24.66 ± 0.43

MIC: Minimum Inhibitory Concentration, MFC: Minimum Fungicidal Concentration, Values are based on six determinations and expressed as mean ± standard error

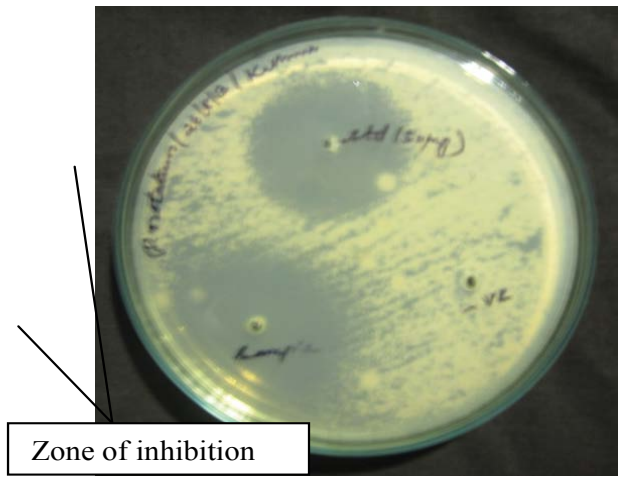

Figure 1 Zone of inhibition of *A. nilagirica* essential oil against the susceptible strain *Penicillium notatum* by well diffusion method.

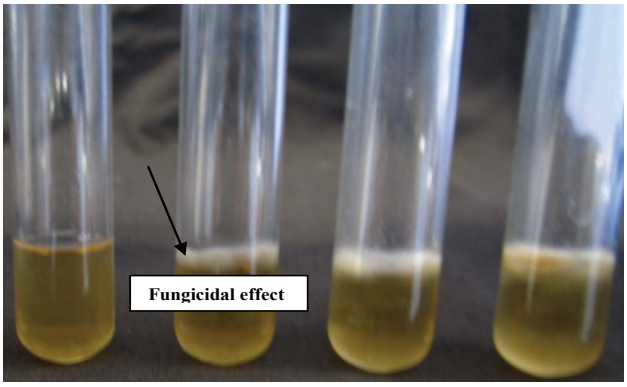

Figure 2 Fungicidal effect of *A. nilagirica* essential oil against *Penicillium notatum* in broth macrodilution method.

Conclusion

The present study revealed that *Artemisia nilagirica* essential oil showed effective antifungal activity against all the food borne fungi tested at tested concentrations. This plant is a weed and abundantly present all around the hilly regions of India, therefore it can be used as economically and environmentally safe alternative to the synthetic fungicides. Further bioactive compound present in the essential oil responsible for the antifungal activity will be predicted, which may lead to development of commercial bio-fungicide formulation against food borne fungi. The bioactive compounds present in this essential oil can be used as fumigants in the warehouses were rice, wheat, cereals and pulses are stored for longer duration.

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