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Note

Antifungal Substance in the Essential Oil of Anise (*Pimpinella anisum* L.)†

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Previously, we reported the antifungal properties of some essential oils of angiosperms.[1~3] In the course of our continuing search for antifungal activity in higher plants, we recorded strong antimycotic activity in the essential oil of anise (*Pimpinella anisum* L. of family Apiaceae), the fruits of which are used as a diuretic, a carminative and as an agent to prevent flatulence and colic.[4] In the present communication, we report the various antifungal properties of the oil of anise. The antifungal principle in the oil was also isolated, identified and studied for its fungitoxic efficacy.

Pure cultures of the test fungi, namely *Aspergillus flavus* and *Penicillium italicum*, were obtained from the Division of Mycology and Plant Pathology at the Indian Agricultural Research Institute of New Delhi. The fruits of *Pimpinella anisum* were obtained from local stockists and washed with 70% ethanol, then repeatedly with sterilized water. The sterilized fruits were then subjected to steam distillation in a Clevenger's apparatus in order to collect the water-immiscible essential oil.[5] This oil was subjected to antifungal testing against both test fungi by the poisoned food technique.[6] The fungitoxic properties of lethal dose for absolute mycelial inhibition, nature of toxicity, the effect of temperature, storage and increased inoculum on the antifungal activity, and the range of the antifungal spectrum of the oil and the active principle were undertaken by the techniques described in our recent report.[2] In order to standardize the quality of the oil used in the present studies, various physico-chemical properties of the oil were also determined by the techniques previously described.[7]

In order to isolate the active antifungal constituent, 10 ml of the oil was vigorously and repeatedly shaken with 10% sodium hydroxide solution. The white sodium hydroxide-insoluble and yellow sodium hydroxide-soluble portions were separated and subjected to antifungal testing by the modified paper disc technique.[8] Since the white sodium hydroxide-insoluble portion exhibited antifungal activity, only this was investigated further.

The sodium hydroxide-insoluble portion was acidified with 10% acetic acid to decompose the sodium salts of the phenolic compounds. The mixture was then extracted with ether. The etherial extract and ether-insoluble (residual, acidified sodium hydroxide) portions were subjected to antifungal testing by the modified paper disc technique.[8] The etherial extract exhibited antifungal activity, while no activity could be detected in the ether-insoluble portion.

The antifungal etherial extract was dried over anhydrous sodium sulphate, and the ether was removed under reduced pressure. The oily liquid thus collected (6 ml) was subjected to TLC using silica gel plates and benzene-petroleum ether (75:25) as the solvent. A single circular spot with *Rf* 0.83 was identified on the TLC plate when the chromatogram was developed in an iodine chamber. The liquid, when kept below 0°C for 48 hr, was converted into white crystals. These crystals when exposed to room temperature for a few minutes were converted to a oily liquid, whose boiling point was determined and spectrum analyzed.

Fruits (100 g) yielded 2.2 ml of the oil, whose recovery was 2.2%. The lethal dose of the oil needed for absolute mycelial inhibition of both the test fungi was 1000 ppm, the nature of toxicity being fungistatic. The antifungal activity of the oil was not affected by a heating treatment upto 100°C for 1 hr, not by storage upto 365 days. The oil sustained a heavy inoculum density and inhibited the growth of as many as 16 discs of 5 mm diameter of the test fungi. The oil at 1000 ppm concentration exhibited the inhibition of growth of 28 other fungi (Table II). The various physico-chemical properties of the oil are listed in Table I.

The chemical investigation and antifungal testing of each fraction resulted in the isolation of an oily liquid which crystalized at temperatures below 0°C. The crystals melted at 22°C, and hence at room temperature (27°C)

TABLE I. PHYSICO-CHEMICAL PROPERTIES OF THE ESSENTIAL OIL OF *Pimpinella anisum* L.

Parameter	Result
Specific gravity[a]	0.9693
Acid value	8.77
Saponification value	296.97
Ester value	261.20
Carbonyl percentage[b]	39.58
Refractive index	1.5467
Specific viscosity	0.127
pH	4.03
Specific rotation[a]	−60°
Phenolic test	Positive

[a] At 25°C.
[b] In terms of anisaldehyde (molecular weight 136.14).

† This work forms a part of the Ph.D. programme of the senior author.

TABLE II. RANGE OF ANTIFUNGAL ACTIVITY OF THE OIL OF *Pimpinella anisum* L. AND ANETHOLE

Name of fungus	Percentage mycelial inhibition			
	Oil		Anethole	
	500 ppm	1000 ppm	400 ppm	600 ppm
1	2	3	4	5
Alternaria alternata (Fr.) Keissler	80.0	100	76.2	100
A. tenuissima (Nees ex Fr.) Wiltshire	80.0	100	73.3	100
Aspergillus awamori Nakazawa	80.0	100	80.0	100
A. fumigatus Fres.	78.0	100	73.3	100
A. nidulans (Eidam) Wingate	74.0	100	77.5	100
A. niger Van Tiegh	90.0	100	75.0	100
A. ochraceus Withelm	75.0	100	80.0	100
A. parasiticus Speari	82.0	100	90.0	100
A. sydowi (Bain & Sartories) Thom & Church	90.0	100	73,3	100
A. tamarii Kita	75.0	100	87.5	100
A. terreus Thom	80.0	100	87.5	100
Botryodiplodia theobromae Pat.	82.0	100	82.5	100
Cladosporium herbarum (Pres.) Link	80.0	100	73.3	100
Colletotrichum capsici (Syd.) Butler & Bisby	90.0	100	81.2	100
Curvularia lunata (Wakker) Boedijn	90.0	100	90.0	100
C. pallescens Boedijn	82.0	100	82.5	100
Epicoccum nigrum Link	85.0	100	80.0	100
Fusarium accuminatum Ell. and Ev.	88.0	100	75.0	100
F. equisiti (Corda) Sacc.	82.0	100	77.5	100
F. moniliforme Shildon.	85.0	100	66.6	100
F. oxysporum Schlecht.	80.0	100	66.6	100
F. semitectum Beak and Rav.	85.0	100	73.3	100
F. udum Butler	91.0	100	87.5	100
Macrophomina phaseoli (Maubh) Ashby	85.0	100	70.0	100
Nigrospora oryzae (Berk & Br.) Petch	90.0	100	90.0	100
Penicillium chrysogenum Thom	90.0	100	66.6	100
P. citrinum Thom	85.0	100	75.0	100
Rhizopus nigricans Ehrenb.	90.0	100	77.0	100

they were in the liquid form. The boiling pont of the liquid was found to be 232°C, which is in accordance with the boiling point of anethole.[9] The UV, IR and NMR spectra further revealed the identity of the isolated liquid to be anethole. An authentic sample of anethole procured from Sigma Chemicals (U.S.A.) gave a superimposible IR spectrum and, when mixed with the isolated liquid, produced no depression in the boiling point. Anethole exhibited abolute toxicity against the test fungi at 600 ppm, the nature of toxicity being fungistatic. It sustained heavy inoculum density to inhibit the growth of as many as 16 discs of 5 mm diameter of the test fungi. It also inhibited the growth of 28 other fungi at 600 ppm (Table II).

Various volatile substances like methyl chavicol, *p*-methoxyphenyl acetone, terpenes and a few sulphur-containing compounds have been isolated from the essential oil of *Pimpinella anisum*.[9] For the first time, anethole present in the essential oil was found to be responsible for its antifungal activity.

Essential oils are produced by higher plants either to attract insects or to repel enemies, and are generally the "waste products" of the metabolic pathway.[10] During recent years, many essential oils have been found to be non-phytotoxic but potent fungitoxic agents.[11] The utilization of essential oils for the fungus-free storage of food has also been successfully established.[12,13] The present investigation on some aspects of the mycotoxicity of the oil of *Pimpinella anisum* that are reported here indicate its possibility for protecting food and feed from fungal deterioration during storage, especially in view of the fact that *Pimpinella anisum* appears to be nontoxic to humans from its established use as a condiment.[4] However, the

effect of its use in practice must await the results of work on the method of its application.

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