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Antifungal Activities and Essential Oil Constituents of Some Spices from Pakistan

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Summary

Hydrodistillation of *Amomum subulatum*, *Cinnamomum verum*, *Coriandrum sativum*, *Cuminum cyminum*, *Elettaria cardamomum*, *Myristica fragrans* (Mace), and *Myristica fragrans* (Nutmeg) purchased from local spice markets yielded essential oils which contained 1,8-cineole (72.7%), cinnamaldehyde (79.8%), linalool (78.1%), cuminaldehyde (37.4%), 1,8-cineole (30.7%), terpinen-4-ol (20.0% and 31.3%), respectively, as main constituents determined by GC/MS. Antifungal activity of the resulting essential oils against various pathogenic fungi (*Aspergillus flavus*, *A. niger*, *Candida albicans*, *Fusarium oxysporum* var. *lycopersici*, *Microsporum canis*, *Pseudallescheria boydii*, *Trichopyton mentagrophytes* and *T. simii*) was investigated.

Introduction

The common spices have a long history of use in Eastern Cultures as food flavours, perfumes and medicinal values. The traditional Pakistani dishes are enriched with a number of exotic spices and herbs.

The commonly known large cardamom (*Amomum subulatum* Roxb.) and the small cardamom (*Elettaria cardamomum* (L.) Maton) are related species from the same family (Zingiberaceae). *Amomum subulatum* is grown in Eastern Himalayas with large cultivation in Nepal, India, Bhutan and in West Bengal (1). The seeds of *Amomum subulatum* yield an essential oil obtained by distillation which has the characteristic odour of 1,8-cineole. It is used as an aromatic stimulant, acting as a stomachic, carminative, astringent and diuretic. It is also known to reduce inflammations (2). The small cardamom is mainly cultivated in Nepal, Bhutan, Sri Lanka, India and other countries and used as a flavouring agent. Medicinally it is used as carminative in dyspepsia, flatulence and in gastrointestinal complaints. Depressions, headache and epilepsy are also reported to be treated by its oil (1-6).

Cinnamomum verum Presl. (Syn. *C. zeylanicum* Breyn.) (Lauraceae), is known as "Ceylon Cinnamon" because it is indigenous to Sri Lanka. The oil is mainly distilled from the bark in Europe and USA. Bark oil is used as a food flavouring agent in the industry. The essential oil is also widely used as a basic note in perfumery with a sweet - spicy

smell. It is used in pharmaceutical industry to mask unpleasant taste of certain medicines (1).

Coriandrum sativum Linn. (coriander) (Umbelliferae) is native to the Mediterranean region and is cultivated in plains and hills of Pakistan, India, Morocco and in Europe. Its fruits are used in gastrointestinal complaints such as dyspepsia and flatulence. It is useful as poultice for ulcers and carbuncles. Essential oil is widely used in food industry and in the flavouring of alcoholic beverages, as well as in perfumery (1, 2).

The spice *Cuminum cyminum* Linn. (cumin) (Umbelliferae) is widely cultivated in Pakistan-India subcontinent; Iran, Egypt, Turkey, Morocco, China, Russia, Japan and Algeria. Medicinally dried fruits are commonly used in carminative and digestive preparations. The essential oil is known to be an effective antiseptic besides other uses (1, 2).

Myristica fragrans Houtt (Myristicaceae) is used to obtain “nutmeg oil” from dried kernels of the ripe seed, by hydro-distillation. It is used for flavouring food products and to cure various gastro-intestinal complaints, psychological disorders and urinary diseases (2-5).

In this study the hydro-distillation of the spices *Amomum subulatum*, *Cinnamomum verum*, *Coriandrum sativum*, *Cuminum cyminum*, *Elettaria cardamomum*, *Myristica fragrans* (Mace), and *Myristica fragrans* (Nutmeg) purchased from local markets were carried out to obtain the resulting oils. The essential oils were analyzed by GC/MS. The antifungal properties of each oil were evaluated against a number of pathogenic fungi.

Experimental

Plant Material: The spices were purchased from the local market in Karachi, Pakistan. The essential oils were obtained by water-distillation, using a Clevenger-type apparatus. The details of the plant materials with their respective oil yields are given in Table-1.

Table-1: Percentage Yields of Essential Oils from Food Spices

No	Botanical Name	Local Name	Common Name	Plant Part	Yield (%)
1	<i>Amomum subulatum</i>	Ilaichi Kalan, Qaqla Kabar	Large Cardamom	Fruit	1.71
2	<i>Cinnamomum verum</i>	Dar Sheini	Ceylon Cinnamon	Bark	0.05
3	<i>Coriandrum sativum</i>	Dhanya, Kishneez	Coriander	Fruit	0.20
4	<i>Cuminum cyminum</i>	Safid Zira, Jeero	Cumin	Fruit	2.53
5	<i>Elettaria cardamomum</i>	Ilaichi Khurd, Chota Nanda	Cardamom	Fruit	3.95
6	<i>Myristica fragrans</i>	Jawatri	Mace	Seed	12.00
7	<i>Myristica fragrans</i>	Jaiphal	Nutmeg	Seed	2.28

Analysis: The oils were analysed on a Hewlett-Packard GCD system. Innovax FSC column (60 m x 0.25 mm with film thickness) by using Helium as the carrier gas. Injector temperature was 250⁰C. Split flow was 1 ml/min. The GC oven temperature was kept at 60⁰C for 10 mins and programmed to 220⁰C at a rate of 4⁰C/min and then kept constant at 220⁰C for 10 mins to 240⁰C at a rate of 1⁰C/min. MS were taken at 70 eV and a mass range of 35-425. Library search was carried out using Wiley GC/MS Library and *TBAM Library of Essential Oil Constituents*. Relative percentage amount were calculated from TIC by the computer. The composition of each oil is given in the Table-2. Major components are listed in Table-3.

Antifungal Activity: Stock solutions of crude essential oils were freshly prepared in 1 ml dimethylsulfoxide (DMSO).

These solutions were diluted into sterile molten Sabouraud dextrose agar (SDA) medium to reach a final concentration of 200 mg/ml separately. Test tubes were kept at room temperature for solidification. Medium containing DMSO was used as negative control. Fungal cultures were cut to 4x4 mm from 1 week grown plates and then inoculated onto the slant. After an incubation period of 7-10 days at 29⁰C, tubes were examined for the growth inhibition.

Growth on the media containing compound was determined by measuring the linear growth (mm) of fungal culture. Growth inhibition (%) was calculated with reference to the negative control (Table-4).

Results and Discussion

The chemical compositions of *Amomum subulatum*, *Cinnamomum verum*, *Coriandrum sativum*, *Cuminum cyminum*, *Elettaria cardamomum*, *Myristica fragrans* (Mace) and *Myristica fragrans* (Nutmeg) essential oils have been studied. The main constituents of each oil and their relative percentages is summarized in Table-3.

Amomum subulatum oil previously was reported to have 1,8-cineole (74%) as main constituent (1). Gurudutt and co-workers (4) reported the occurrence of 1,8-cineole (61.3%) The main constituent from the oil of large cardamom of Indian origin obtained in 2.5% yield. In the present work, 1,8-cineole (72.7%) and a-terpineol (13.3%) were found to be the major constituents of the large cardamom oil in agreement with the previous reports.

Cinnamomum verum bark oil was reported to contain cinnamaldehyde (60-82%) by Lawrence (7). Main component in the present was also found to be cinnamaldehyde (79.8%). The remaining twenty-four components were also characterized representing 95.5% of the total components detected in the oil.

Linalool (59.6-71.6%) has been reported as the main constituent of the essential oil of coriander fruits (1,8). The highest yield of linalool (83%) was achieved by CO₂ extraction from the total oil (9). Linalool (78%) was also characterized as the main component among other twenty-four compounds identified, representing 99.8% of the total oil in the present work.

Previous work by Karim and co-workers on cumin oil from *Cuminum cyminum* of Pakistani origin have yielded 10 compounds with cuminaldehyde (22.4%) and cuminyl alcohol (72.2%) are the main constituents. In an early work by our group, compositions of cumin seeds obtained from different localities of Turkey and abroad were reported. Cuminaldehyde (19.6-27.0%), *p*-mentha-1,3-dien-7-al (4.3-12.3%), *p*-mentha-1,4-dien-7-al (24.5-44.9%), *g*-terpinene (7.1-14.1%), *p*-cymene (4.6-12.0%) and *b*-pinene (2.9-8.9%) were identified as major components by GC and GC/MS (11). Lawrence reviewed the previous work on cumin oil (12). The present work showed cuminaldehyde (37.4%) and *p*-cymene (16.5%) as main constituents. Twenty compounds characterized comprised 95.8% of the total oil.

The essential oil from *Elettaria cardamomum* fruits were reported to contain 1,8-cineole (74%) as the main constituent (1). In another previous study, 1,8-cineole (54.4%) and a-terpinyl acetate (24%) were reported as main constituents (6). Noleau *et al* investigated the composition of the essential oils of cardamom cultivars. 1,8-cineole (31.8%) and a-terpinyl acetate (39.3%) was reported as major characteristic constituents of the *alpha*-minor variety. The *beta*-major variety was reported to contain large amounts of a-pinene, 4-thujanol and terpinen-4-ol (13). Twenty-five compounds were detected in the present work, comprising 97% of the total oil. Main components were identified as 1,8-cineole (30.7%) and a-terpinyl acetate (30.6%), matching to the previously reported *alpha*-minor variety.

Nutmeg essential oil (*Myristica fragrans*) and its composition was reported by Masada. The major components by GC/MS were identified as a-pinene (26.7%), b-pinene (20.7%), sabinene (14.5%), limonene (9.4%) and terpinen-4-ol (4.4%) in nutmeg essential oil (14). Lawrence reviewed the previous work on nutmeg and mace essential oils (15). In the present work thirty-three and thirty-seven constituents have been identified representing 99.9% and 99.3% of the total mace and nutmeg essential oils, respectively. Major component was terpinen-4-ol (20.0% and 31.3%), respectively in mace and nutmeg essential oils.

Antimicrobial activity of commercial cardamom, cinnamon, cumin and nutmeg oils against various pathogenic fungi and bacteria were investigated in a number of previous papers (16-18). In the present paper, the antifungal property of

the essential oils was screened against pathogenic fungi at 200 ml concentration using agar tube dilution method (19, 20). Results were reported in percentage inhibition compared to standard antifungal drugs as given in Table- 4. *Cuminum cyminum* oil showed the most significant fungicidal activity against *P. boydii* (88%). The same fungus was also inhibited by nutmeg oil (77%). Animal pathogen *M. canis* was inhibited by mace oil (70%). Resistant fungi *A. flavus* was inhibited by *C. cyminum* and *E. cardamomum* essential oils, some oils however, moderately stimulated the growth of some fungi such as *A. subulatum* oil promoted the growth of *T. simii*.

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Table-2: Composition of the Essential Oils in various spices

Compound	1	2	3	4	5	6	7
a-pinene	1.1	-	2.5	0.4	0.5	4.9	5.3
a-thujene	-	-	-	0.1	0.1	1.3	0.9
camphene	-	-	-	-	-	0.1	-
b-pinene	2.7	-	0.3	9.8	0.1	4.6	4.9
sabinene	-	-	-	0.1	0.8	1.9	2.5
d-3-carene	-	-	-	-	-	0.6	0.9
myrcene	0.3	-	0.1	0.4	0.6	1.6	1.4
a-phellandrene	-	-	-	-	-	0.6	0.6
a-terpinene	-	-	-	-	-	3.5	3.2
limonene	2.9	-	-	0.2	3.7	3.2	2.7
1,8-cineole	72.7	-	-	0.1	30.7	0.1	0.1
b-phellandrene	-	-	-	-	-	2.7	2.8
g-terpinene	0.4	-	3.4	8.1	-	7.8	5.6
p-cymene	0.3	-	1.6	16.4	1.3	6.5	2.4
terpinolene	tr	-	-	-	-	2.4	2.0
trans-linalool oxide (<i>furanoid</i>)	-	-	0.4	-	0.2	-	-
a-p-dimethyl styrene	-	-	-	-	-	0.1	0.1
cis-linalool oxide (<i>furanoid</i>)	-	-	0.3	-	0.1	-	-
a-copaene	-	0.3	-	-	-	0.1	0.1
decanal	-	-	0.2	-	-	-	-
camphor	-	-	0.2	-	-	-	-
benzaldehyde	-	0.2	-	-	-	-	-
linalool	-	-	78.1	-	8.7	0.4	0.2
octanol	-	-	0.9	-	-	-	-
linalyl acetate	-	-	-	-	0.1	-	-
trans-p-menth-2-en-1-ol	-	-	-	-	-	0.2	0.3
cis-isopulegone	-	-	-	0.2	-	-	-

bornyl acetate	-	-	-	-	-	-	0.2
terpinen-4-ol	4.7	0.1	0.5	0.4	4.2	31.3	20.0
b-caryophyllene	-	-	-	-	-	-	0.4
cis-p-menth-2-en-1-ol	-	-	-	-	-	0.1	0.2
(E)-2-decenal	-	-	0.1	-	-	-	-
d-terpineol	1.0	-	-	-	-	-	-
trans-piperitol	-	-	-	-	-	0.1	-
a-terpineol	13.3	1.8	0.9	0.6	11.5	5.2	3.5
g-murolene	-	tr	-	-	-	-	-
a-terpinylacetate	-	2.5	-	-	30.6	0.1	0.2
borneol	-	-	-	-	-	0.1	-
cis-1,2-epoxy-terpin-4-ol	-	-	-	-	-	1.1	-
a-murolene	-	1.0	-	-	-	-	-
nerylacetate	-	-	-	-	0.2	-	-
geranial	-	-	-	-	0.3	-	-
phellandral	-	-	-	0.2	-	-	-
carvone	-	-	-	-	0.1	-	-
cis-piperitol	-	-	-	-	-	0.1	-
geranylacetate	-	-	3.8	-	0.8	0.2	0.1
d-cadinene	-	0.4	-	-	-	-	0.1
citronellol	-	-	0.3	-	-	-	-
ar-curcumene	-	0.2	-	-	-	-	-
p-methylacetophenone	-	-	-	-	0.1	-	-
3-phenylpropylaldehyde	-	0.7	-	-	-	-	-
cuminaldehyde	-	0.2	0.5	37.4	-	-	-
nerol	-	-	0.1	-	0.1	-	-
p-mentha-1,3-dien-7-al	-	-	0.2	15.0	-	-	-
p-mentha-1,4-dien-7-al	-	-	-	5.5	-	-	-
trans-carveol	-	-	-	-	0.1	-	-
calamenene	-	0.8	-	-	-	-	-
geraniol	-	-	1.4	-	1.4	-	-
p-cymen-8-ol	-	-	-	0.1	0.3	0.3	-
(E)-2-dodecanal	-	-	0.5	-	-	-	-
safrole	-	-	-	-	-	2.0	3.4
a-calacorene-I	-	0.3	-	-	-	-	-

methyleugenol	-	-	-	-	-	0.8	13.3
cinnamaldehyde	-	79.8	-	-	-	-	0.3
(E)-nerolidol	0.5		-	-	0.4	-	-
b-caryophyllene alcohol	-	0.9	-	-	-	-	-
epicubenol	-	0.5	-	-	-	-	-
cumin alcohol	-	-	-	0.3	-	-	-
cis-p-menth-4-en-1,2-diol	-	-	-	0.2	-	-	-
cinnamyl acetate	-	0.8	-	-	-	-	-
pentadecanol	-	0.1	-	-	-	-	-
cis-p-menth-3-en-1,2-diol	-	-	-	-	-	0.2	-
T-cadinol	-	0.2	-	-	-	-	-
eugenol	-	0.9	-	-	-	0.2	0.7
trans-methylisoeugenol	-	-	-	-	-	0.1	2.3
T-muurolol	-	1.5	-	-	-	-	-
d-cadinol	-	1.2	-	-	-	-	-
a-bisabolol	-	0.5	-	-	-	-	-
p-isopropylphenol	-	-	-	0.2	-	-	-
cadalene	-	0.6	-	-	-	-	-
elemicin	-	-	-	-	-	4.8	4.7
myristicin	-	-	-	-	-	7.1	14.4
decanoic acid	-	-	0.2	-	-	-	-
tetradecanoic acid	-	-	1.5	-	-	2.9	-
hexadecanoic acid	-	-	2.1	-	-	-	-
	99.9	95.5	99.8	95.7	97.0	99.3	99.9

1: *Amomum subulatum*, **2:** *Cinnamomum verum*, **3:** *Coriandrum sativum*, **4:** *Cuminum cyminum*, **5:** *Elettaria cardamomum*, **6:** *Myristica fragrans* (Nutmeg), **7:** *Myristica fragrans* (Mace)

Table-3: Main Components of the Essential Oils.

Spice Name	Identified Compounds	(%)	Main Component	(%)
<i>Amomum subulatum</i>	12	99.9	1,8-cineole	72.7
<i>Cinnamomum verum</i>	24	95.5	cinnamaldehyde	79.8
<i>Coriandrum sativum</i>	24	99.8	linalool	78.1
<i>Cuminum cyminum</i>	20	95.7	cuminaldehyde	37.4
<i>Elettaria cardamomum</i>	25	97.0	1,8-cineole	30.7

<i>Myristica fragrans</i> (Mace)	33	99.9	terpinen-4-ol	20.0
<i>Myristica fragrans</i> (Nutmeg)	37	99.3	terpinen-4-ol	31.3

Table- 4: Antifungal Activity of the Essential Oils.

Name of Fungus	Inhibition (%)								Standard	(%)
	1	2	3	4	5	6	7			
<i>Aspergillus flavus</i>	18.2	14.0	22.7	66.7	58.1	-19.4	-	Miconazole	100	
<i>A. niger</i>	8.9	8.6	8.9	1.9	7.6	15.2	24.5	Miconazole	100	
<i>Candida albicans</i>	19.6	11.0	10.8	11.0	14.3	5.5	16.5	Miconazole	100	
<i>Fusarium oxysporum</i> var. <i>lycopersici</i>	-2.1	-	-3.2	19.2	-18.2	-12.1	1.5	Benlate, Nabam	100	
<i>Microsporum canis</i>	6.1	14.9	36.4	51.6	-12.9	-32.3	70.4	Miconazole	100	
<i>Pseudallescheria boydii</i>	-31.3	17.6	31.3	88.2	23.5	77.6	7.0	Amphotericin	100	
<i>Trichopyton mentagrophytes</i>	-	-	-	-	-	-	44.4	Ketoconazole	100	
<i>T. simii</i>	-66.6	50.0	-66.7	25.0	41.7	1.7	31.4	Miconazole	100	

1: *Amomum subulatum*, **2:** *Cinnamomum verum*, **3:** *Coriandrum sativum*, **4:** *Cuminum cyminum*, **5:** *Elettaria cardamomum*, **6:** *Myristica fragrans* (Nutmeg), **7:** *Myristica fragrans* (Mace)

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