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Chemical composition, antibacterial and antifungal activities of *Azadirachta indica* and *Mentha spicata* L. leave essential oils

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Abstract

The current workout investigated the chemical symphony and bioactivities of essential oils of *Azadirachta indica* and *Mentha spicata*. The essential oils of both herbs were acquired by soxhlet extraction apparatus and a brief chemical examination was conducted by gas chromatography-mass spectrometry (GC-MS) and GC (gas chromatography). Total 13 constituents from the essential oils of *Azadirachta indica* had been identified among them the major components are 11-octadecanoic acid methyl ester (87.76%), docosanoic acid (4.93%) and 9-octadecanoic acid ethyl ester (3%). Sixteen constituents from *Mentha spicata* essential oils were also observed among them the major ones are E-Piperitol (42.68%), Caryophyllene oxide (7.79%), 4-terpinol (4.39%), octanoic acid (4.24%), Ascaidole (3.8%), Carvacrol (3.3%), Anethole (3.2%), bicyclo-germacene (2.75%), Caryophyllene (2.73%), Thymol (1.99%), 2-decadienal (1.64%), D-carvone (1.58%), Piperitol (1.38%) and Linollyl acetate (1.34%). The essential oils were screened for their antimicrobial activities. The essential oil of *Azadirachta indica* was active against 5 Gram positive and 2 Gram negative bacteria showed activity against 1 fungi. Similarly *mentha spicata* essential oils were also active against 3 Gram positive and 2 Gram negative bacteria.

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Introduction

Azadirachta indica (1) is commonly known as Neem belongs to the family mahogany. Neem typically cultivated in the sub-tropical and tropical regions of Asian and African countries (Prashanth GK, *et al.,* 2014). The common constituents which have been reported from the neem are flavonoids, carotenoids, alkaloids and triterpenoids. Generally, neem is utilized as Ayurvedic pharmaceutical framework for the treatment of hopeless diabetes (Prieto P, *et al.,* 1999). Neem is herb which is healthy and nutritious historically utilized for the treatment of polygenic disorder, infectious disease and Respiratory diseases. Its unrefined concentrates from bark and leaves have been utilized as a part of people solution to control maladies such as infection, respiratory framework and intestinal helminthiasis (Bandyopadhyay U, *et al.,* 2004). It also showed antibacterial, antigastric ulcer, antifungal, antipyretic, antiarthritic hypoglycemic, and antitumor activities (Sultana B, *et al.,* 2007, Ebong PE, *et al.,* 2008, Mahapatra S, *et al.,* 2012, Paul R, *et al.,* 2011).

Mentha spicata (2) belongs to the family Labiatae which is usually known as spearmint. This plant vastly grows in temperate areas of the world like Asia, Europe, Australia and Africa as of now cultivation was carried out throughout all regions of the world. The essential oil of dried and fresh plants can be utilized majorly in confectionary food, chewing gum, cosmetics, paste and pharmaceutical industries. Strong antimicrobial, insecticidal, antioxidant and mutagenic activities have been showed by these essential oils as well as larvicidal activities of the essential oil of *M. spicata*. It is a well-known and observed fact that polyphenolic constituents (Yasser S 2015).

Keeping in view the medicinal properties this study was conducted to find out the phytocomponents of *Azadirachta indica* and *Mentha spicata* essential oils. To find out the antibacterial activity against some pathogenic bacteria and these oils were also evaluated for their antifungal activities.

Material and methods

Plant Material

Fresh leaves of *Azadirachta indica* and *Mentha spicata* L. were collected from the botanical garden of the Karachi University during the month of December, 2017.

Isolation of the essential oil

Firstly, we have sampled the neem and spearmint leaves that we have selected for our workout and then left it for few days approximately 5-10 days for drying purpose and when they were dried completely we utilized them in the extraction of oils by Soxhlet extraction apparatus. The oils of *Azadirachta indica* and *Mentha spicata* were examined by gas chromatography (GC-MS and GC-FID). The compounds were recognized by comparing with reported mass spectral analysis.

Gas Chromatography (GC)

"Gas activity was disbursed pattern FID (flame ionization detector) on the less polar capillary column Zebron™ ZB-5 (60m x zero.53mm x 0.25µm film thickness of 5% – Phenyl – 95% Dimethylpolysiloxane) place in on a Shimadzu 17-AGC system. The analysis was performed with dual temperature program an initial temperature 75°C for three minutes then ramped at a rate of 9°C/minutes to a final temperature one 200°C with operating time of five minutes and yet again ramped at a rate of 7°C/ minutes to a final temperature two 240°C with holding time of 30 minutes Injector with a splitting ratio of 1:10 was set at 240°C and FID at 260°C. Carrier and compose gas nitrogen was maintained at a flow of 10.939mL/minutes with pressure of 89 kPa" (Table 1).

Gas Chromatography-Mass Spectrometry (GC-MS)

For GC-MS experiments Agilent Technologies 7000 GC/MS Triple Quad gas chromatograph, had been combined with a Jeol and equipped with ZB-5MS (30m x 0.32 ID and 0.25µm film thickness), JMS-600H mass spectrometer operating in EI mode with ion source at 250°C and 70eV electron energy. The amount of carrier gas was adjusted between 1.0-5.0mL/min based on the detector response (Table 2).

Antibacterial Activity

A widely utilized method of disk diffusion had been utilized for analyzing antibacterial activities of the sample (Ayub, *et al., 2017)*. 10mg/ml (essential oils)

of stock solution in DMSO of each sample had been developed. Sterilized filter discs consisting of 10 µl of stock solution were utilized for performing activities. The Iso sensitest agar (Oxoid) plates had been seeded by 24 hours old culture (containing around 1-2x108 CFU/ml) developed in Mueller Hinton broth (Oxoid). The discs developed had been fixed on top of the agar surfaces at various plates and points had been nurtured at 37°C for 24 hours. Outcomes had been established through analyzing and observing inhibition zones in mm (Table 3).

Table 1. Identification of compounds through GC/GC-MS analysis of petroleum ether soluble fraction of *Azadirachta indica* essential oil.

S. No	Names of compounds identified	Rt (min)	Molecular weight	Formula	Peak%
1	Hexadecanoic acid methyl ester	14.76	270	$C_{17}H_{34}O_2$	0.21
2	9-octadecanoic acid methyl ester	18.44	296	$C_{19}H_{36}O_2$	0.38
3	11-octadecanoic acid methyl ester	20.67	296	$C_{19}H_{36}O_2$	87.76
4	9-octadecanoic acid ethyl ester	21.86	310	$C_{20}H_{38}O_2$	3.00
5	Methyl stearate	22.47	298	$C_{19}H_{38}O_2$	0.12
6	Methy-18-methyl nonane decanoate	22.57	326	$C_{21}H_{42}O_2$	0.54
7	Behenic acid	23.83	340	$C_{22}H_{44}O_2$	0.12
8	Docosanoic acid, methyl ester	24.01	354	$C_{23}H_{46}O_2$	4.93
9	Arachidic acid	26.16	312	$C_{20}H_{40}O_2$	0.05
10	Stearic acid	26.68	284	$C_{18}H_{36}O_2$	0.83
11	Oleic acid	26.95	282	$C_{18}H_{34}O_2$	0.55
12	Palmitic acid	27.65	256	$C_{16}H_{32}O_2$	0.07
13	Linoleic acid	28.61	280	$C_{18}H_{32}O_2$	0.1

Table 2. GC/GC-MS analysis of petroleum ether soluble fraction of *Mentha spicata* essential oil.

S. No.	Name of constituents identified	Rt (min)	Molecular weight	Formula	Peak%
1	Linollyl acetate	25.30	196	$C_{12}H_{20}O_2$	1.34
2	4-Terpenol	26.09	154	$C_{10}H_{18}O$	4.39
3	Caryophyllene	27.15	220	$C_{15}H_{24}O$	2.73
4	B-Farnesene	27.88	204	$C_{15}H_{24}$	1.09
5	Piperitol	28.61	154	$C_{10}H_{18}O$	1.38
6	D-Carvone	28.82	150	$C_{10}H_{14}O$	1.58
7	E-Piperitol	29.42	154	$C_{10}H_{18}O$	42.68
8	Bicyclo germacene	30.37	204	$C_{15}H_{24}$	2.75
9	2-Decadienal	30.78	162	$C_{10}H_{26}O$	1.64
10	Anethole	31.39	148	$C_{10}H_{12}O$	3.2
11	P-Cymene-8-ol	33.91	150	$C_{10}H_{14}O$	0.9
12	Ascaidole	33.93	184	$C_{10}H_{16}O_3$	3.8
13	Caryophyllene oxide	34.85	220	$C_{15}H_{24}O$	7.79
14	Octanoic acid	35.36	144	$C_8H_{16}O_2$	4.24
15	Thymol	35.58	150	$C_{10}H_{14}O$	1.99
16	Carvacrol	41.87	150	$C_{10}H_{14}O$	3.39

Table 3. Antibacterial activities of *Azadirachta indica* and *Mentha spicata* essential oils.

Bacteria Tested	1	2
Gram Positive		
Bacillus cereus	14	11
Bacillus subtillus	13	12
Bacillus thuringiensis	12	11
Corynebacterium diptheriae	11	9
Corynebacterium hoffmanii	11	9
Corynebacterium xerosis	10	9
Micrococcus luteus	10	8
Staphylococcus aureus	9	9
Staphylococcus aureus AB, 188	9	8
Staphylococcus epidermidis	9	8
Staphylococcus faecalis	8	7
Staphylococcus saprophyticus	8	7
Gram Negative		
Escherichia coli	13	11
Escherichia coli ATCC 8739	11	12
Escherichia coli Multi drug resistant	10	9
Klebsiella pneumonia	8	9
Proteus mirabilis	7	8
Pseudomonas aeroginosa	7	7
Pseudomonas aeroginosa ATCC 9027	0	0
Shigella dysenteriae	0	0

Antifungal Activity

Antifungal activities were furthermore analyzed by means of disc diffusion method as described above (Ayub, *et al., 2017)*. Vastly, little quality of culture had been transferred to 2-3mL normal saline or distilled water in a screw capped tube with little glass beads (1mm in diameter) and vortexes for 5-10 minutes in order to prepare a homogeneous suspension of fungal culture. Sabouraud dextrose agar (SDA) plates had been seeded along with this deferment holding about 1-2 x 108CFU/ml. Sterilized filter discs (containing concentration of 1000µg/disc of extract/500µg/disc of fractions and 100µg/disc of pure compounds) were fixed on the surfaces at various positions. Plates had been incubated at room temperature for 1 week. Outcomes had been confirmed by analzing inhibition zones in mm (Table 4).

Results and discussion

In the present studies phytoconstituents of essential oils of *Azadirachta indica* and *Mentha spicata* were studied by GC and GC-MS techniques and these oils were also examined to identify the antibacterial and antifungal activities.

The volatile constituents present in the extract of *Azadirachta indica* had been analyzed by using GC and

GC-MS. Thirteen compounds had been identified in this oil the components which were identified along with their peak percentages are mentioned in table 1. Among them the major components are 11-octadecanoic acid methyl ester (87.76%), docosanoic acid (4.93%) and 9-octadecanoic acid ethyl ester (3%).

Similarly *Mentha spicata* essential oil was also analyzed by GC and GC-MS techniques to find out the phytoconstituents. Sixteen constituents were identified in *Mentha spicata* in which the major components are E-Piperitol (42.68%), Caryophyllene oxide (7.79%), 4-terpinol (4.39%), octanoic acid (4.24%), Ascaidole (3.8%), Carvacrol (3.3%), Anethole (3.2%), bicyclo-germacene (2.75%), Caryophyllene (2.73%), Thymol (1.99%), 2-decadienal (1.64%), D-carvone (1.58%), Piperitol (1.38%) and Linollyl acetate (1.34%).

Both of these oils were also evaluated for their antibacterial and antifungal activities. The *Azadirachta indica* essential oil were found effective against 3 out of 12 Gram positive bacteria and 1 out of 8 Gram negative bacteria and this is effective against the Gram positive bacteria *Bacillus cereus*, *Bacillus sibtilus* and *Bacillus Thuingiensis* and a Gram negative bacteria *Escherichia coli*. *Mentha spicata* was found to be active against 1 (*Bacillus sibtilus*) out of 12 Gram positive bacteria and 1 (*Escherichia coli* ATCC 8739) out of 8 Gram negative bacteria (Table 3).

Table 4. Antifungal activities of *Azadirachta indica* and *Mentha spicata* essential oils.

Fungi Tested	1	2
Filamentous		
Aspergillus flavus	10	8
Aspergillus terreus	9	7
Rhizopus sp.	9	8
Penicillium sp.	8	7
Dermatophytes		
Trichophyton rubrum	11	8
Trichophyton mentagrophytes	10	9
Trichophyton tonsurans	9	8
Microsporum canis	8	7
Microsporum gypseum	8	8
Fusarium sp.	7	7
Other Fungi		
Candida albicans	10	7
Candida albicans ATCC 0383	9	8
Saccharomyces cerevisiae	8	7
Helimanthosporum sp.	0	0

The antifungal activities of both the plant *Azadirachta indica* and *Mentha spicata* were also examined but both the oils showed marginal activity. In both of the essential oils, *Azadirachta indica* was found to be effective against three fungi Aspergillus flavus, Trichophyton rubrum, Trichophyton mentagrophytes, Candida albicans (Table 4).

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