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ORIGINAL ARTICLE

FT-IR, GC-MS/MS Analysis of Essential Oil from *Coriandrum sativum* Seeds, Antibacterial Assay

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ABSTRACT
Infectious diseases caused by bacteria are the major causative factor for disease. Hence, it is essential to kill the bacteria either by natural products or by chemically formulated products. Considering the side effects and therapeutic efficacy between these two, it will be better to opt for natural products even if the expected result is moderate to greater. In order to find a better natural product, the present work was designed and essential oil was extracted from Coriandrum sativum dry seed black variety through hydrodistillation process. The yield obtained was around 0.70ml±0.01/100gm. To know, whether, the oil extracted contains functional groups in order to proceed further, functional groups analysis was performed using Fourier transform infrared analysis. As, we got positive results with FT-IR, the phytochemicals present in the oil was assessed through GC-MS/MS analysis. Highest peak was observed with n-Hexadecanoic acid and moderate peak was with 9,12-Octadecadienoic acid (Z,Z)-. The percentage of compound present was found to be lower for the rest of the compounds. The gram positive B.subtilis showed significant antibacterial activity. While among the five different gram negative bacteria tested, the zone of inhibition was highest with E. coli followed by S.aureus, S.enterica, P.aeruginosa. No zone of inhibition was observed with K. pneumonia. From the results obtained, it is concluded that the phytochemicals present in the essential oil of Coriandrum sativum dry seeds black variety might be responsible for its antibacterial activity. Hence, Coriandrum sativum essential oil could be used in pharmacy towards a novel synthesis of drug.
Key words: *Analytical, Antibacterial, Essential oil, GC-MS, Microbes.*

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INTRODUCTION

Coriandrum sativum L. belonging to family Umbelliferae is a spice crop and also an aromatic plant grows up to 2 feet in height with branching stems, green, soft, hairless, bi and trilobed leaves. It was widely distributed in Southeastern Europe, Middle East, China, India, Turkey [1] Morocco, USSR, Hungary, Poland, Romania, Mexico, USA. In India major production centers are Rajasthan, Maharashtra, Gujarat and Karntaka [2]. Mature plants have small light pink color flowers which turn into seeds of about 4-6 mm in diameter with hollow cavity able to hold 1-2% of essential oils contributing for its antibacterial, antifungal, antioxidant activities [3]. The hydrophobic nature of essential oils permit them to detach bacterial cell wall lipids thereby disturbing the overall structure which makes it more permeable and death of bacteria [4]. Coriander otherwise called as cilantro (west) or Chinese parsley, has been used as a foodstuff since 10th century [5]. Aqueous seed extract was used to relieve anxiety, insomnia, sedativeness, hypotensiveness. It is a very good muscle relaxant, hypoglycemic [6,7] hypolipidemic agent [6,8,9]. The property of being liquid at room temperature and gaseous state at higher temperature helps to attract insects protects plants from heat or cold. Hence, the present study was carried out to extract essential oil and to characterize through FT-IR, GC-MS/MS, as well as to analyses its antibacterial activity through disc diffusion assay.

MATERIALS AND METHODS

Sample collection

The dry *coriandrum sativum* seeds Black variety was purchased from a shop at Salem, Tamil Nadu, India. The seeds were cleaned and made dust, stones free. The cleaned seeds were used for essential oil extraction process.

Essential oil extraction

The essential oil was extracted with 100gm of dried *Coriandrum Sativum* seeds black variety by hydro-distillation process for 4hrs at 100°C. The obtained essential oil was dried over anhydrous sodium sulphate and stored at 4°C until tested and analyzed.

Microbial strains

The essential oil of *C. sativum* was tested against six bacteria *Bacillus subtilis* MTTC 8114*, Escherichia coli* MTCC 1692*, Klebsiella pneumonia* MTCC 7403, *Pseudomonas aeruginosa* MTCC 2581*, Staphylococcus aureus* MTCC 7443*, Salmonella enterica* MTCC 8587*.*

ANALYTICAL TECHNIQUES

Fourier transform infrared analysis

The essential oil was analyzed by KBr pellet method [10]. Here, potassium bromide an alkali halide was used which becomes plastic on application of pressure and forms a transparent sheet in the infrared region. 0.1 % sample was mixed into 200 to 250 mg of KBr powder,then finely pulverized and kept in a pellet-forming die. The Fourier transform infrared spectrum was recorded using Bruker Tensor 27 spectrometer in the wavelength range 400-4000 cm^{-1} by potassium bromide pellet technique with a resolution and scanning speed of 4 cm^{-1} and 2 mm/sec respectively.

Gas Chromatography-Mass Spectrophotometry/Mass Spectrophotometry

Gas Chromatography-Mass Spectrophotometry was performed on a Scion 436-GC Bruker carrying Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl95% Dimethyl poly siloxane), 30m x 0.25mm ID x 0.25m df. The column oven temperature program was as follows: 80°C hold for 2 min, Up to 160°C at the rate of 20°C/min-No hold, Up to 280°C at the rate of 5°C / min-No hold, Up to 300°C at the rate of 20°C/min-10 min hold, Injector temperature 280°C, Total GC running time was 36min. The inlet temperature was set at 280°C, source temperature 250°C; ionization mode, ionization at 70-eV ionization energy; For single scan analysis, the scan range was set from m/z 40 to 600; Solvent Delay: 0-3.5 min; and the injection volume was 2µl. The GC-MS/MS was performed by the Institute of crop processing technology, Tanjavur.

ANTIMICROBIAL ACTIVITY

Disc Diffusion Assay

The disc diffusion method for antibiotic susceptibility testing was performed [11]. *In vitro* antimicrobial activity was screened using Mueller Hinton Agar obtained from Himedia. The agar plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5min and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. In the Kirby-Bauer test, small filter disks soaked with different concentration of essential oil (5µl,10µl,15µl) as well as standard antibiotic (Chloramphenicol 30µg/disc) was placed on the surface of the solidified agar, then allowed to diffuse for 5min and the plates were kept for incubation at 37°C for 24 h, for diffusion into the surrounding agar, displaying a zone of inhibition. The inhibition zones formed around the disc were measured using transparent scale in millimeter. Each concentration was repeated thrice.

RESULTS AND DISCUSSION

Yield

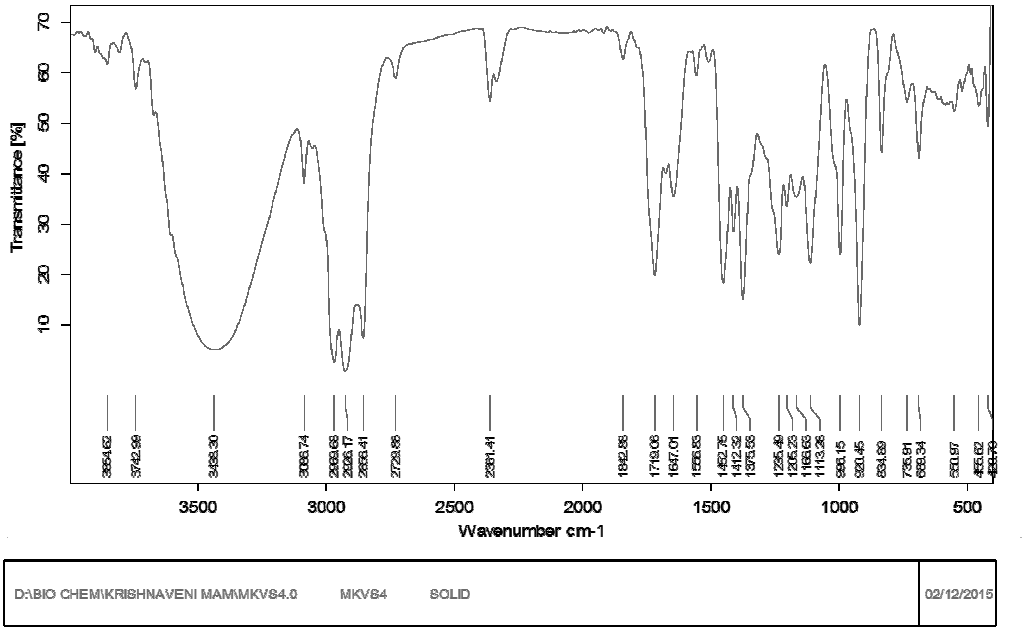
The yield of essential oil was found to be 0.70±0.01ml/100gm.

Fourier transform infrared analysis

The results of Fourier transform infrared analysis are shown in Fig.1. Fourier transform infrared spectrum was used to identify and assess the stability of chemical constituents, functional groups of the active components based on the peaks obtained through stretching and bending vibrations in the region of infra red radiation. Different peaks are obtained due to the shifts in the Fourier transform infrared spectra. IR absorption below 1000cm^{-1} corresponds to C-H bending vibrations, the absorptions from 997 to 1130cm^{-1} were attributed to stretching vibrations of C-O of monosaccharides, oligosaccharides, carbohydrates, while the absorbtion from 1150- 1270cm^{-1} corresponds to stretching vibrations of carbonyl C-O, or O-H bendings. The absorptions from 1300 – 1450cm^{-1} corresponded to stretching vibrations of C-O amide and of C-C stretching from the phenyl groups. While the absorptions from 1500-1600cm^{-1} corresponded to aromatic domain and the N-H bending vibrations. The absorptions in the complex range from 1600- 1760cm-1 corresponded to the bending vibrations of N-H amino acids, C=O

stretching –aldehyde, ketone, esters. The absorptions from 2800-2900 cm^{-1} were attributed to C-H stretching vibrations specific to CH_3 and CH_2 from lipids and methoxy derivatives and C-H aldehydes as well as from cis-double bonds. In addition, the absorptions from 3350- 3360cm^{-1} shows stretching vibrations of the OH groups from water, alcohols, phenols, carbohydrates etc as well as from amides. This technique is well known for its simplicity in sample preparation and speed of analysis.

Fig.1 Fourier transform infrared Spectrum of Essential oil from *Coriandrum sativum* seeds



Gas chromatography and Mass spectrophotometry/Mass Spectrophotometry

Table.1. Components in the ethanol extract of *Coriandrum sativum* seeds

S.No	RT	Name of the compound	Molecular Formulae	MW	Peak area %
1	3.64	γ-Terpinene	$C_{10}H_{16}$	136	0.66
2	3.98	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	154	0.32
3	5.56	Citronellal	$C_{10}H_{18}O$	154	2.12
4	5.64	Camphor	$C_{10}H_{16}O$	152	0.88
5	5.81	endo-Borneol	$C_{10}H_{18}O$	154	5.76
6	6.02	L-α-Terpineol	$C_{10}H_{18}O$	154	1.89
7	6.38	Citronellyl butyrate	$C_{14}H_{26}O_2$	226	0.12
8	6.66	Geraniol	$C_{10}H_{18}O$	154	1.73
9	6.90	Citral	$C_{10}H_{16}O$	152	2.69
10	7.17	2-n-Octylfuran	$C_{12}H_{20}O$	180	1.68
11	7.66	Myrtenyl acetate	$C_{12}H_{18}O_2$	194	5.98
12	7.97	6-Octen-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{22}O_2$	198	0.29
13	8.34	Geranyl acetate	$C_{12}H_{20}O_2$	196	3.12
14	8.95	Cyclododecanol	$C_{12}H_{24}O$	184	0.78
15	9.75	n-Decanoic acid	$C_{10}H_{20}O_2$	172	1.14
16	9.98	2-n-Heptylfuran	$C_{11}H_{18}O$	166	2.10
17	11.13	Dodecanoic acid	$C_{12}H_{24}O_2$	200	2.37
18	11.67	trans-2-Dodecenoic acid	$C_{12}H_{22}O_2$	198	0.13
19	11.83	9,12-Octadecadien-1-ol, (Z,Z)-	$C_{18}H_{34}O$	266	1.19
20	12.18	Tridecanoic acid	$C_{13}H_{26}O_2$	214	0.41
21	12.64	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	1.20
22	12.84	sedanolide	$C_{12}H_{18}O_2$	194	4.47
23	13.65	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.38
24	14.41	Palmitoleic acid	$C_{16}H_{30}O_2$	254	0.64
25	14.90	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	4.25
26	16.05	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	31.61
27	17.61	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	1.34

28	18.59	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	18.00
29	19.08	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.15
30	21.66	cis-11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	0.21
31	23.60	Succinic acid, di(3,7-dimethyloct-6-en-1-yl) ester	$C_{24}H_{42}O_4$	394	0.11
32	24.08	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	$C_{12}H_{20}O_2$	196	0.06
33	24.53	Hexadecanoic acid, cyclohexyl ester	$C_{22}H_{42}O_2$	338	0.05
34	26.33	Heptacosane	$C_{27}H_{56}$	380	0.16

MF- Molecular Formulae, MW- Molecular Weight, PA-Peak Area percent

The results of Gas Chromatography –Mass Spectrophotometry/Mass spectrophotometry obtained for the essential oil extracted from *Coriandrum sativum* is shown in Table. 1. The oil was dissolved in solvent ethanol for the analysis.The 34 peaks were obtained in a total retention time of 3.64 to 26.33. Highest peak was observed with n-Hexadecanoic acid (31.61 %) followed by 9,12-Octadecadienoic acid (Z,Z)-(18.00%). The compounds that was found to be moderate are as follows: endo-Borneol (5.76%), sedanolide (4.47%), Pentadecanoic acid (4.25%), Geranyl acetate (3.12%) , Dodecanoic acid (2.37 %), Citral (2.69 %), Octadecanoic acid (2.15 %), 2-n-Heptylfuran(2.10 %), L-α-Terpineol (1.89 %), Geraniol (1.73 %), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-(1.20%), 9,12-Octadecadien-1-ol,(Z,Z)-(1.19%), n-Decanoic acid (1.14%), Heptadecanoic acid (1.34 %). Certain compounds present in trace amount ranging from 0.05 to 0.88% are: Hexadecanoic acid , cyclohexyl ester (0.05%) , 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)- (0.06%), Succinic acid, di(3,7-dimethyloct-6-en-1-yl) ester (0.11%), trans-2-Dodecenoic acid (0.13%), Citronellyl butyrate (0.12 %), cis-11,14-Eicosadienoic acid, methyl ester (0.21%), 6-Octen-1-ol, 3,7-dimethyl-, acetate (0.29%), Tetradecanoic acid, ethyl ester (0.38%), Tridecanoic acid (0.41%), Cyclododecanol (0.78%), 1,6-Octadien-3-ol, 3,7-dimethyl-(0.32%), γ-Terpinene(0.66%), Camphor (0.88%). The *coriandrum sativum* essential oil components contain alcohols, hydrocarbons, ketones, esters. The monoterpene hydrocarbons are usually present in higher concentration. The strong odor that is developed was mainly due to the essential oil content of the dry seed. The composition of essential oil vary from place to place, climatic condition, variety, maturity of seeds.

Antibacterial assay

Table.2. Antibacterial activity of Essential oil from *Coriandrum sativum* seeds

	Standard	**Essential oil from *C. sativum* concentration (µl)**		
Bacteria studied	**Chloramphenicol (30µg/disc) (ZOI in mm)**	**5 µl (ZOI in mm)**	**10µl (ZOI in mm)**	**15µl (ZOI in mm)**
B. subtilis	0.80±0.00	0.60±0.00	0.70±0.20	0.70±0.20
E. coli	1.30±0.00	0.46±0.05	1.10±0.00	1.10±0.20
K. pneumonia	0.76±0.05	0.00±0.00	0.00±0.00	0.00±0.00
P. aeruginosa	0.90±0.00	0.26±0.05	0.40±0.00	0.60±0.00
S. aureus	1.46±0.05	0.60±0.00	0.80±0.20	1.06±0.05
S. enterica	1.56±0.05	0.40±0.00	0.56±0.05	0.90±0.20

Values are Mean ± SD for three experiments, ZOI- Zone of Inhibition

The results of antibacterial activity are depicted in Table.2. From the results it is observed that**,** the *coriandrum sativum* essential oil was found to be active against the selected gram positive and gram negative bacteria such as *B. subtilis* -MTTC 8114*, E.coli* MTCC 1692*, P.aeruginosa* MTCC 2581*, S.aureus* MTCC 7443*, S.enterica* MTCC 8587 except *K.pneumonia* might be due to its resistance nature. The zone of inhibition was higher at higher concentration among the three concentrations selected. Compared to gram positive bacteria *B.subtilis,* the obtained results were good with gram negative bacteria. Our reports were similar to previous work reported by Filomena Silva et.al 2011 [12]. The diameter of zone developed around each organism depends on the sensitivity of microbe, the rate of diffusion of essential oil/antibiotic through the agar as well as the depth of the agar. *In vitro* and *In vivo* studies prove the use of aromatic substances, alcohol as an anti-microbial agents [13, 14]. The increased hydroxylation from phenolic susbstances are directly proportional to their toxicity towards microbes [15]. The higher zone of inhibition was reported for *Coriandrum sativum* fruit essential oil [16]. Whereas, in our study, the zone

of inhibition was lesser. According to Dorman and Deans [17], geraniol present in the essential oil also play a role in antibacterial property. The mechanism attributed in the inhibition of microorganism by aromatic, phenolic compounds in essential oil was mainly due to their hydrophobicity, which gain entry into the cell membrane's lipid bilayer, making it more permeable, alteration in structure and function causing leakage of bacterial cell contents and death [18,19,20].

CONCLUSION

The reduced toxicity of essential oil is the main cause for its use in traditional medicine. Since, essential oils were endowed with phytochemicals required for antibacterial activity. Apart from its antimicrobial property, it is widely applied in creams, pharmaceutical preparations, perfumes, detergents, surfactants, emulsifiers etc. Thus, an in depth study is required towards the analysis of properties essential oil and its pharmacological benefits.

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CONFLICT OF INTEREST

No conflict of interest

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