Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(3):1846-1850

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antibacterial activity of essential oil extracted from *Coriandrum sativam* (L.) and GC-MS analysis

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ABSTRACT

Extraction of essential oils from locally available plant Coriandrum sativam (L.) was carried out using steam distillation followed by ether extraction. Dried and purified extracted oils were screened for their antibacterial activity of both gram positive (Staphylococcus aureus) and gram negative (Enterobacter aerogenes, Klebsiella pneumoniae, Vibrio cholerae and Salmonella typhi) bacterial strains by Agar well diffusion method. Coriandrum sativam shows antibacterial activity against the above given five bacterial strains. GC-MS was done on Coriandrum sativam oil. These results support that this plant oil can be used to cure bacterial infections and may also have role as pharmaceuticals and preservatives.

Keywords: *Coriandrum sativam*, GC-MS, Essential oil, Antibacterial activity and Extraction.

INTRODUCTION

Natural products have been a major source of new drugs [19].Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries [7].Current research on natural molecules and products primarily focuses on plants. Since they can be sourced more easily and be selected based on their ethno-medicinal uses [2]. A wide range of medicinal plants parts is used to extract as raw drugs and they possess varied medicinal properties while some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used many other raw drugs are collected in larger quantities and traded in the market as the raw materials for many herbal industries. Plant used for traditional medicine contains a wide range of substances that can be used to treat chronic as well as infectious diseases [18].

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years [8].These purposes vary from the use of rosewood and cedar wood in perfumery, to flavouring drinks with lime, fennel or juniper berry oil [9], and the application of lemongrass oil for the preservation of stored food crops [12]. In the antimicrobial activity of plant oils and xtracts has formed the basis of many applications including raw and processed food preservation pharmaceuticals, alternative medicine and natural therapies [17,11].

Essential oils of herbs and their components, which are products from the secondary metabolism of plants have many applications in ethno-medicine food, flavouring and preservation as well as in the fragrance and pharmaceuticals industries [5].The antimicrobial properties of essential oils have been described [15], and because

of the growing demand on antimicrobials for preventing microbial food spoilage and bacterial infections, there is an increasing interest in medicinal plants as an alternative to synthetic preservatives and antibiotics [4].Many essential oils are already used in the food industry as flavouring agents and some are known to exert antimicrobial activity ,but the mechanism of action is often not entirely understood. *Coriandrum sativam* (L.) is a well known herb widely used as a spice, in folk medicine and in the pharmacy and food industries [3].Coriander seed oil is one of the major essential oils in the world market [10], and it is known to exert antimicrobial activity however, its mechanism of action is still unclear.

EXPERIMENTAL SECTION

Selection of Microorganisms

Totally five human pathogenic bacteria were selected for the present investigation. Among them, five bacterial strains such as, *Staphylococcus aureus. Enterobacter aerogenes, Klebseilla pnemoniae, Salmonella typhi* and *Vibrio cholerae* . The human pathogenic bacteria were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and used for present investigation.

Preparation of Microbial Inoculums

The young microbial inoculum culture were prepared and used during the research period. The nutrient broth (NB) was prepared and poured into several tubes. Then these tubes were sterilized. The pure microbial cultures were collected from the institute (either solid or liquid medium) and included in the tubes by using inoculation loops. After these tubes were incubated at 37^{o}C for 24-48hrs. After incubation the cultures were used for the experiments.

Media Preparation

Composition of Nutrient Agar Medium

Peptone	-	5gm
Beef extract	-	3gm
NaCl	-	5 gm
Agar	-	15gm
Distilled water	-	1000ml
pH	-	6.8

Preparation of Nutrient Agar Medium

The ingredients (peptone – 5g; beef extract – 3g; Nacl -5g) were weighed and taken in a conical flask contains 1000ml distilled water. Then pH of the medium was adjusted to 6.8 using a pH meter by the addition of either acid (or) alkali. The flask were sterilized in an autoclave at 121ºC for 15 lbs pressure for 15 minutes and allowed to cool.

Procedure for of oil extraction process

About 750 g of dried fruits of coriander accurately weighed and transferred to 1 litre distillation flask (Clevenger Apparatus) together with 900ml of water .Added few pieces of porcelain to it in order to avoid bumping during distillation. Distillation tank kept on the heating mantle and set the distillation assembly. Graduated receiver filled with water avoiding any air bubbles. The out let near the upper end of the receiver was not tightened instead, loosely packed with cotton. Heating mantle was switched on and continued distillation for four hours at a rate which keeps the lower end of the condenser cool. The distillate allowed being collected in the graduated receiver in which the aqueous portion of the distillate was automatically separated and returned to the distillation flask. Measured the volume of volatile oil which separated out as the upper layer in the graduated tube and calculated the percentage v/w of isolated oil on a dry weight basis. The volume of isolated volatile oil from the given sample of Coriander fruits 2.2ml.

Screening for Antibacterial Activity assay

The antibacterial activities of the Coriander oil were analyzed by agar - well diffusion method. The Coriander oil was tested against the selected bacterial strains. The petriplates were washed and placed in an autoclave for sterilization. After sterilization, nutrient agar medium was poured into each sterile petriplates and allowed to solidify in a laminar air flow chamber. After solidification, using a sterile cotton swabs, fresh bacterial culture with known population count was spread over the plate by spread plate technique. Then one well of 5mm size made in the agar plates with the help of sterile cork borer, the wells were loaded with 200µl of oil. All the plates were incubated at 37°C for 24-48hours. After incubation, the plates were observed for formation of clear inhibition zone

around the well indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well.

Antibiotic sensitivity test on microbes (Positive control)
The antibiotic sensitivity test using standard antibiotics (ampicillin, Kanamycin and tetracycline for bacteria) were analyzed by following the method of Bauer *et al*., 1996.

GC-MS analysis
The GC-MS analysis was carried out using a clarus 500 perkin –Elmer (Auto system XL).Gas chromatography equipped and coupled to a mass detector turbo mass Gold –perkin Elmer turbo mass 5.1 spectrometer with an Elite 1(100% Dimethyl poly siloxane) 30m x0.25 mm IDX 1mm of capillary column. The instrument was set to an initial temperature of 110˚C and maintained at this temperature for 2 minutes.At the end of this period the over temperature was rose up to 280˚C at the rate of an increaseof 5˚C /min and maintained for 9 min.Infection part temperature was ensured as 250˚C and Helium flow rates as 1ml/min.The ionization voltage was Foev. The samples were infected in split made as 10:1 mass spectral scan range was set at 45-450(m/2) using computer searchers on a NIST Ver 2.1 Ms data library and comparing the spectrum obtained through GC-MS the compounds present in the samples were identified.

RESULTS

Antibacterial activity of *Coriandrum sativam* essential oil
Antibacterial activities of *Coriandrum sativam* oil was analyzed by agar well diffusion method against human pathogenic bacterial strains such as, *Staphylococcus aureus, Enterobacter aerogenes, Klebsiella pneumoniae, Salmonella typhi* and *Vibrio cholerae.* Essential oil extracted from dried fruits of *Coriandrum sativam* was analysed for antibacterial activity.

The essential oil of *Coriandrum sativam* has highest antibacterial activity against the *Staphylococcus aureus* (12mm), *Enterobacter aerogenes* (12mm), *Klebsiella pneumoniae* (10mm), *Vibrio cholerae* (13mm) and *Salmonella typhi* (15mm) given in table-1 and fig-1.

Antibiotic sensitivity test on bacteria
The antibiotic sensitivity test using standard antibiotics viz., ampicillin, Kanamycin and tetracycline were tested against pathogenic bacteria. The results of antibiotic sensitivity test were presented in table-2.

GC-MS analysis of *Coriandrum sativam* essential oil
The essential oil of *Coriandrum sativam was analysed by* GC-MS and 13 components were indentified (Table-3 and Fig-3). The major components in the essential oil of *Coriandrum sativam* were Bicycle(4.1.0),heptanes,3,7,7-trimethyl-(1a,6a,3a),(6.12%) propanoic acid,2-methyl-3,7-dimethyl octadiennyl ester,(E)-(6.62%),2- undecenal (7.57%),2-Napthalene methanol,decahydro-a,a,4a-trimethyl-8-methylene- [2R-(2a,4aa,8aa)]- (9.87%).

Table -1: Antibacterial activity of *Coriandrum sativam* essential oil

S. No.	Test Organisms (Bacterial pathogens)	Zone of inhibition (diameter in mm)
		Essential oil
1.	*Enterobacter aerogenes*	12
2.	*Klebsiella pneumoniae,*	10
3.	*Salmonella typhi.*	15
4.	*Staphylococcus aureus*	12
5.	*Vibrio cholerae*	13

Table -2: Antibiotic sensitivity test on bacterial strains

S. No.	Test Organisms (Bacterial pathogens)	Zone of inhibition (diameter in mm)		
		Ampicillin	Tetracycline	Kanamycin
1.	*Enterobacter aerogenes*	11	10	9
2.	*Klebsiella pneumoniae,*	8	9	10
3.	*Salmonella typhi.*	7	8	9
4.	*Staphylococcus aureus*	6	10	11
5.	*Vibrio cholerae*	9	11	10

Table -3: GC-MS analysis of *Coriandrum sativam* essential oil

No.	RT	Name of the compound	Molecular	MW	Peak Area %
1.	3.26	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	154	85.89
2.	5.50	2-n-Octylfuran	$C_{12}H_{20}O$	180	0.23
3.	5.70	Undecanal	$C_{11}H_{22}O$	170	1.08
4.	6.12	Bicyclo[4.1.0]heptane, 3,7,7-trimethyl-, (1à,3à,6à)-	$C_{10}H_{18}$	138	0.40
5.	6.62	Propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (E)-	$C_{14}H_{24}O_2$	224	9.93
6.	6.91	Dodecanal	$C_{12}H_{24}O$	184	0.44
7.	7.14	Caryophyllene	$C_{15}H_{24}$	204	0.09
8.	7.57	2-Undecenal	$C_{11}H_{20}O$	168	0.59
9.	7.87	2-n-Heptylfuran	$C_{11}H_{18}O$	166	0.35
10.	8.04	Tridecanal	$C_{13}H_{26}O$	198	0.17
11.	9.02	Aromadendrene oxide-(2)	$C_{15}H_{24}O$	220	0.18
12.	9.87	2-Naphthalenemethanol, decahydro à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]-	$C_{15}H_{26}O$	222	0.48
13.	11.70	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.17

Fig -1 Antibacterial activity of *Coriandrum sativam* essential oil and standard antibiotics

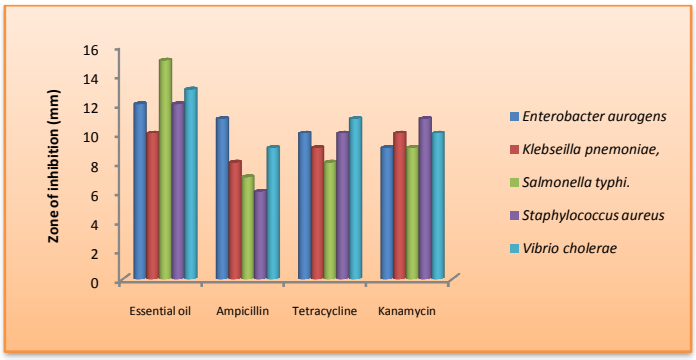
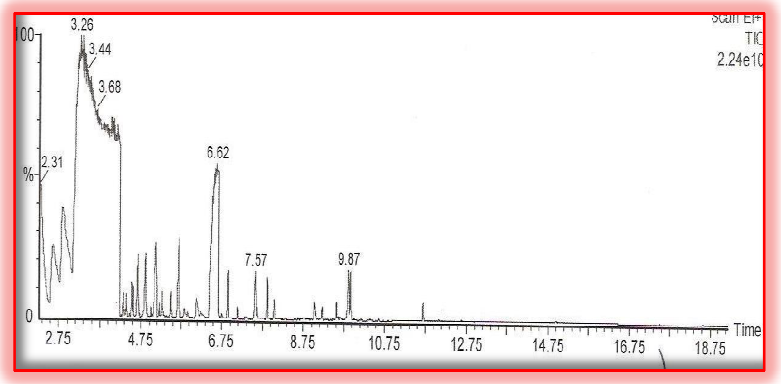


Fig -2 GC-MS analysis of *Coriandrum sativam* essential oil



DISCUSSION

Microorganisms are the concealed enemies to the mankind. They are small but cause a very profound damage in human body as well as other living organism. The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents or drugs. There are a lot of antimicrobial drugs of which some are discovered or established and some are hidden in the nature. Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management[1,16,13,20] and more natural antimicrobials have driven scientist to investigate the effectiveness of inhibitory compounds such as extracts from plants[14].There are several reports for antibiotics resistance of human pathogens to available antibiotics [6,21]. Bimolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens.

This study deals with five pathogenic bacterial strains in which four bacteria *Enterobacter aerogenes, Klebsiella pneumoniae, Vibrio cholerae and Salmonella typhi* are gram negative bacteria while *Staphylococcus aureus* is gram positive bacteria. In the present work antibacterial activity of *Coriandrum sativam* essential oil was found against five human pathogenic bacteria. All the five human pathogenic bacterial strains were sensitive to essential oil of *Coriandrum sativam. Coriandrum sativam* essential oil show highest inhibitory activity against *Salmonella typhi*. The antibiotic sensitivity test using standard antibiotics ampicillin, Kanamycin and tetracycline were tested against bacteria.

Acknowledgement
The authors are grateful to Sri Gowri Biotech Research Academy, Thanjavur (Dt),Tamilnadu. For their permission to utilized the laboratory facility.

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