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Analysis of Bioactive Components of Ethanolic Extract of *Coriandrum Sativum* L.

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ABSTRACT

Coriandrum sativum(L.) is a annual herb in the family Apiaceae, is a medicinal herb used in the treatment of various disease and disorders. The present investigation was carried out to determine the bioactive components from the ethanolic extract of ariel parts of *Coriandrum sativum* by GC-MS Technique. This analysis revealed that ethanolic extract of *Coriandrum sativum* contains 9-Octadecenoic Acid (Z)- ethyl ester(56.68%), Linoleic Acid ethyl ester(13.64%), Ethyl Hexadecanoate(7.69%), Alpha.-Monoolein(6.66%) in a high percentage. The most prevailing compound 9-Octadecenoic Acid (Z)- ethyl ester(ethyl oleate) used as a solvent for Pharmaceutical drug preparation, it act as a drug for intramuscular drug delivery, in some cases to prepare the daily doses of progesterone in support of pregnancy. Alpha Monolein act as food emulsifier for all kinds of food processing and medicine.The present study validates the traditional use of coriander has been credited with medicinal properties.

KEY WORDS: *Coriandrum sativum*, GC-MS analysis, TLC, Stigmasterol.

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INTRODUCTION

Plants are directly used as medicines by a majority of cultures around the world. Bioactive compounds in plants are of natural origin and serve as secondary metabolites. *Coriandrum sativum*(L.) is a annual herb in the family Apiaceae. A study found both the leaves and seed contains antioxidant, but the leaves were found to have a stronger effect[1]. Chemicals derived from coriander leaves were found to have antibacterial activity against *Salmonella choleraesuis*, and this activity was found to be chemicals acting as nonionic surfactants[2].The Essential oil and its fractions could be used as potential antimicrobial agents to treat or prevent *Candida* yeast infections[3]. Antimicrobial potential of aqueous infusions and aqueous decoctions of *Emblica officinalis* and *Coriandrum sativum* against 186 bacterial isolates belonging to 10 different genera of G +ve bacterial population and 2 isolates of *Candida albicans* isolated from urine specimens[4].Essential oils from *Allium tuberosum, Coriandrum sativum, Cymbopogon martini, Cymbopogon winterianus*, and *Santolina chamaecyparissus* was evaluated against *Candida spp*. isolates from the oral cavity of patients with periodontal disease[5]. *C.sativum* has been used as a folk medicine for the relief of anxiety and insomnia in Iran. Experiments in mice support its use as an anxiolytic[6]. *C.sativum* has been documented as a traditional treatment for diabetes. A study on mice found coriander extract had both insulin-releasing and insulin-like activity[7]. *C.sativum* seeds were found in a study on rats to have a significant hypolipidemic effect, resulting in lowering of levels of total cholesterol and triglycerides and increasing levels of high density lipoprotein . This effect appeared to be caused by increasing synthesis of bile by the liver and increasing the breakdown of cholesterol into other compounds[8]. *C.sativum* can produce an allergic reaction in some people[9,10]. *Coriandrum sativum* seeds, used to treat hyperglycemia and hyperlipidemia, on endocrine functions and structures[11]. Coriander has been shown to attenuate the development of streptozotocin induced diabetes in mice[12].Enhanced hepatic bile acid synthesis and the increased degradation of cholesterol to fecal bile acids and neutral sterols appeared to account for coriander's hypocholesterolemic effects[13]. It is reported that coriander seed oil contains linalool (60-70%) and 20% hydrocarbons and the composition of the herb oil completely differs from the seed oil[14]. Rastogi and Mehrotra reported detection of α-pinene, limonene, β-phellandrene, eucalyptol, linalool, borneol, β- caryophyllene, citronellol, geraniol, thymol, linalyl acetate, geranyl acetate, caryophyllene oxide, elemol and methyl heptenol in seed oil by TLC[15].

MATERIAL AND METHODS

Plant Material and Extraction Procedure

Coriandrum sativum were collected from local market in Chennai, Tamilnadu, India and it was Taxonomically identified and authenticated by Botanist, Department of Plant Biology and Biotechnology, Aringnar Anna Govt College, Walajapet, Tamilnadu. The plant was washed in fresh water and dried under shade at room temperature. The leaves and stem was cut into small pieces, powdered in a mixer grinder and stored in sterile containers for further use. Then this powdered sample (100gm/100ml) was soaked in ethanol extract for overnight at room temperature, Soxhlet apparatus are used for this extraction[16,17]. The extract were evaporated at 50^0c until solvent layer evaporated completely.

Thin Layer Chromatography

TLC was used to monitor the identity of each extracts and fractions, additionally to screen the qualitative purity of the isolated compound. It was also developed to optimize the solvent system that would be applied for column chromatography. Analytical TLC was performed qualitatively on precoated TLC plates with Silica gel plate(Himedia, India) using diverse solvent systems for mostly semi-polar compounds. However, solvent system containing Chloroform:Methanol(19:1) was used. The compound was then detected by their UV transilluminator at wavelength 254 and 366nm.

Gas Chromatography- Mass Spectrum Analysis(GC-MS)

GC-MS technique was used in this study to identify the bioactive components present in the extract. GC-MS technique was carried out at Sargam laboratory, Chennai, Tamil Nadu. GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25mm, Film thickness : 0.25 µm Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 1ml was employed (split ratio: 10). Injector temperature 240°C; Ion source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 5 minutes with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component

was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The Name, Molecular weight, Molecular formula and Structure of the component of the test material was ascertained.

RESULT AND DISCUSSION

Thin layer chromatography

In order to identify the compounds, the extract was subjected to fractionation and purification of its components. Hence TLC was performed on aluminum sheets precoated with silica gel using capillary tube (2-5 l) and allowed the plates to dry. The plate was developed in Chloroform: Methanol(19:1) and visualized under UV illuminator. The color change indicates the presence of bioactive components in ethanolic extract of *Coriandrum sativum*.

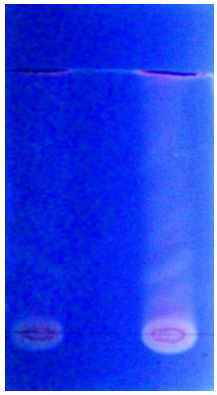

Fig.1 : TLC of Ethanolic extract of *Coriandrum sativum* visualized under uv transilluminator

Eighteen compounds were identified in ethanolic extract of *Coriandrum sativum* by GC-MS analysis. The active principle Molecular Weight (MW), Concentration (%), Molecular Formula (MF), Retention Time (RT) and their bioactivity are presented in Figure 2, Table1&2. The prevailing compounds were 9-Octadecenoic Acid (Z)-, Ethyl Ester(56.68%), Linoleic Acid Ethyl Ester(13.64), Ethyl Hexadecanoate(7.69%), Alpha.-Monoolein(6.66%).

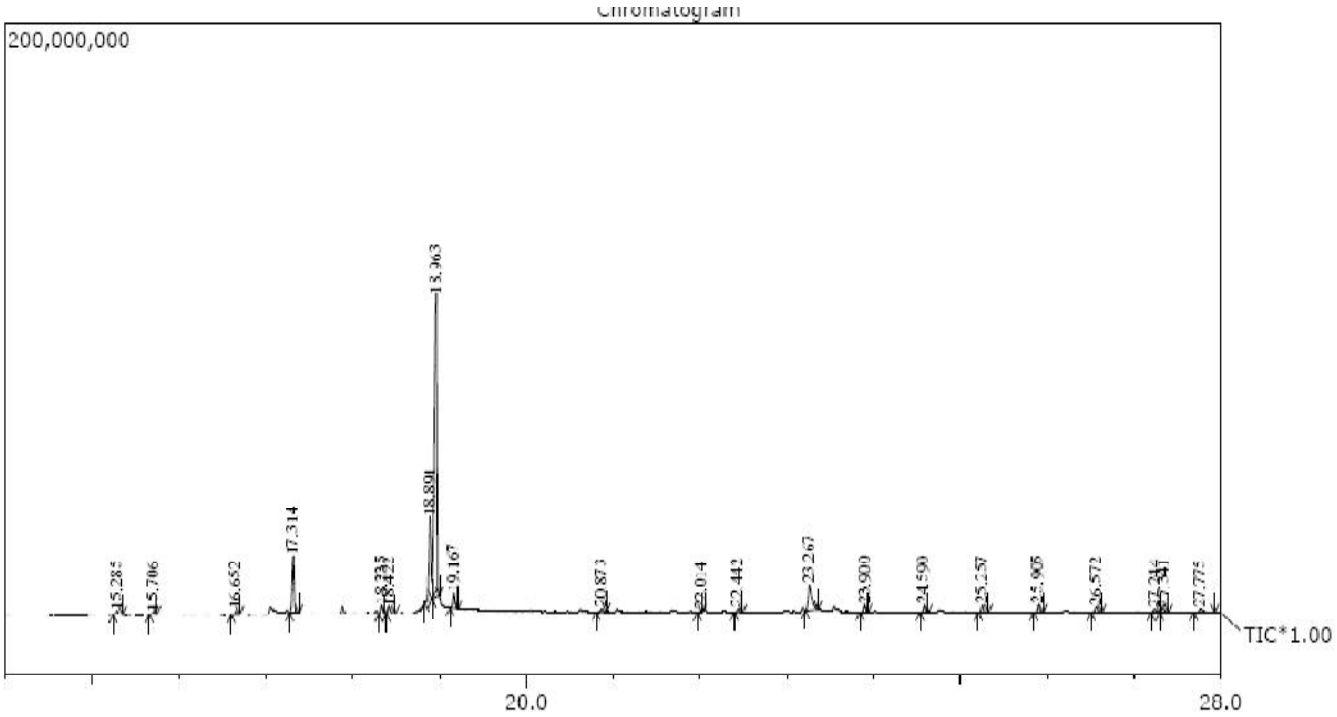

Figure 2: Chromatogram obtained from the GC- MS with the ethanolic extract of *Coriandrum sativum*

DISCUSSION

Myristic acid is a saturated fatty acid commonly found in animal and vegetable fats that is frequently used in cosmetics, soaps, perfumes and flavorings. It increases low density lipoprotein cholesterol making and it is one of the most hypercholesterolemic of the saturated fatty acids[18]. Myristic acid ethyl ester is a more hydrophobic form of the free acid. It is a marker of excessive ethanol consumption that can be isolated from the hair of an individual[19]. Neophytadiene is an enzyme inhibitor. It contains up to 15% hexanes and up to 5% of the analogous dimer by NMR[20,21,22]. Saturated fatty acids are synthesized by both plants and animals from acetyl coenzyme A as a form of long-term energy storage. Palmitic acid is a common 16-carbon saturated fat that represents 10-20% of the normal human dietary fat intake, and approximately 25% of the total plasma fatty acids in plasma lipoproteins[23] Saturated free fatty acids induce the expression of cyclooxygenase-2[24].Palmitic acid methyl ester(MP) is a fatty acid ester whose concentration in cells is modulated by methanol. In studies with isolated Kupffer cells, MP inhibits phagocytosis and decreases cell viability. In cells treated with lipopolysaccharide, it also decreases secretion of interleukin-10, TNF-α, nitric oxide, and prostaglandin E_2. This effect is thought to occur by the inhibition of NF-κB[25].Elaidic acid is the major trans fat found in hydrogenated vegetable oils and occurs in small amounts in caprine and bovine milk (very roughly 0.1 % of the fatty acids)[26] and some meats. It is the trans isomer of oleic acid. The name of the elaidinization reaction

comes from elaidic acid. Elaidic acid increases CETP activity, which in turn raises VLDL and lowers HDL cholesterol[27].

Table 1: Total ionic chromatogram (GC-MS) of *Coriandrum sativum* obtained with 70eV using an Elite -1 fused silica capillary column with He gas as the carrier.

No	RT	Name of the compound	Molecular Formula	Molecular weight	Peak area %
1.	15.285	Tetradecanoic Acid, Ethyl Ester	$C_{16}H_{32}O_2$	256	0.65
2.	15.706	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{20}H_{38}$	278	0.40
3.	16.652	Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	270	0.46
4.	17.314	Ethyl Hexadecanoate	$C_{18}H_{36}O_2$	284	7.69
5.	18.335	9-Octadecenoic Acid, Methyl Ester, (E)-	$C_{19}H_{36}O_2$	296	1.17
6.	18.422	Phytol	$C_{20}H_{40}O$	296	1.16
7.	18.891	Linoleic Acid Ethyl Ester	$C_{20}H_{36}O_2$	308	13.64
8.	18.963	9-Octadecenoic Acid (Z)-, Ethyl Ester	$C_{20}H_{38}O_2$	310	56.68
9.	19.167	Stearic Acid, Ethyl Ester	$C_{20}H_{40}O_2$	312	2.11
10	20.873	Docosanoic Acid, Ethyl Ester	$C_{24}H_{48}O_2$	368	0.54
11.	22.014	1,2-Benzenedicarboxylic Acid, Diisooctyl Ester	$C_{24}H_{38}O_4$	390	0.17
12.	23.267	Alpha.-Monoolein	$C_{21}H_{40}O_4$	356	6.66
13.	23.900	Docosane	$C_{22}H_{46}$	310	0.99
14	24.590	Nonacosane	$C_{29}H_{60}$	408	0.97
15.	25.905	Tetracontane	$C_{40}H_{82}$	562	1.21
16.	27.244	Stigmasterol	$C_{29}H_{48}O$	412	0.76
17.	27.341	N-Tetratetracontane	$C_{44}H_{90}$	618	0.91
18.	27.775	Gamma.-Sitosterol	$C_{29}H_{50}O$	414	1.02

Table 2: Biological activity of Phytocomponents identified in *Coriandrum sativum*

No	Name of the Compound	Molecular formula	Common name	Compound nature	Biological activity
1.	Tetradecanoic Acid, Ethyl Ester	$C_{16}H_{32}O_2$	Myristic acid	Fatty acid	hypercholesterolemic
2.	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{20}H_{38}$	Neophytadiene	-	Enzyme inhibitor
3.	Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester	Fatty acid	expression of cyclooxygenase-2 inhibition of NF-κB.
4.	Ethyl Hexadecanoate	$C_{18}H_{36}O_2$	Ethyl palmitate	Fatty acid	expression of cyclooxygenase-2 increase cytosolic Ca2+
5.	9-Octadecenoic Acid, Methyl Ester, (E)-	$C_{19}H_{36}O_2$	Elaidic acid methyl ester	Fatty acid	Raises Vldl And Lowers Hdl Cholesterol
6.	Phytol	$C_{20}H_{40}O$	-	alcohol	Manufacture of vitamin e and k activate the transcription factors PPAR-alpha and retinoid X receptor
7.	Linoleic Acid Ethyl Ester	$C_{20}H_{36}O_2$	Ethyl linoate	Fatty acid	pharmaceuticals, soaps, cosmetics, and food packaging.
8.	9-Octadecenoic Acid (Z)-, Ethyl Ester	$C_{20}H_{38}O_2$	Oleic acid, ethyl ester;	Fatty acid	steroids and primer pheromone.
9.	Stearic Acid, Ethyl Ester	$C_{20}H_{40}O_2$	Ethyl stearate	Fatty acid	perturbs the cell cycle and induces apoptosis in Hep-G_2 cells
10	Docosanoic Acid, Ethyl Ester	$C_{24}H_{48}O_2$	Behenicacid ethylester		-
11.	1,2-Benzenedicarboxylic Acid, di isooctyl Ester	$C_{24}H_{38}O_4$	Diisooctyl phthalate	-	-
12.	Alpha.-Monoolein	$C_{21}H_{40}O_4$	-	-	-
13.	Docosane	$C_{22}H_{46}$		alkane	-
14.	Nonacosane	$C_{29}H_{60}$	Celindiol deoxy	Alkane	pheromone of female *Anopheles stephensi* mosquito
15.	Tetracontane	$C_{40}H_{82}$	-	Alkane	-
16.	Stigmasterol	$C_{29}H_{48}O$	Phytosterol	Alcohol	manufacture of semisynthetic progesterone synthesis of cortisone
17.	N-Tetratetracontane	$C_{44}H_{90}$	-	Alkane	-
18.	Gamma.-Sitosterol	$C_{29}H_{50}O$			

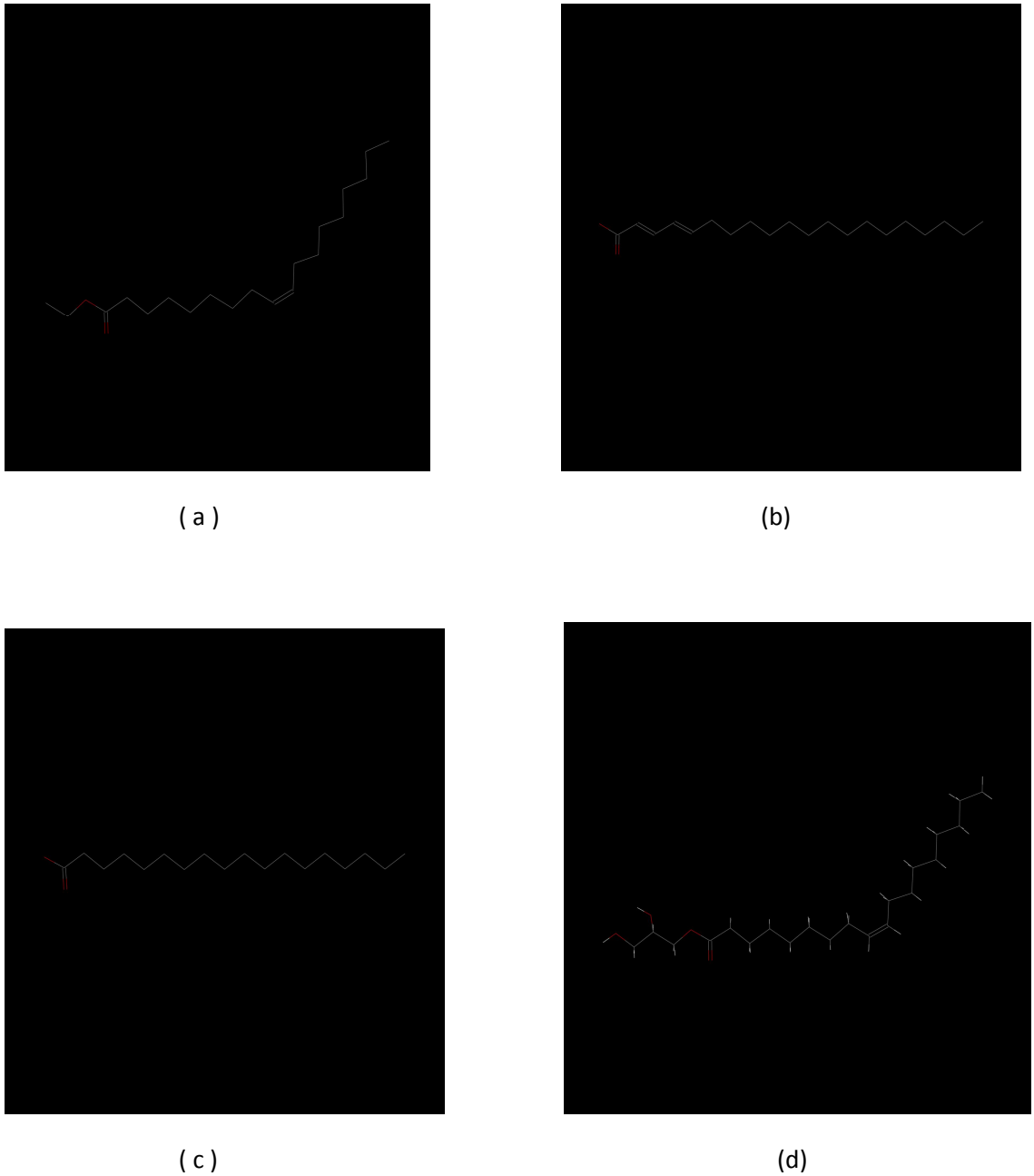

Figure 3. Bioactive compounds of peak area a. 9-Octadecenoic Acid (Z)- ethyl ester b. Linoleic Acid ethyl ester c. Ethyl Hexadecanoate d. Alpha. Monoolein.

Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E[28] and vitamin K1[29]. In ruminants, the gut fermentation of ingested plant materials liberates phytol, a constituent of chlorophyll, which is then converted to phytanic acid and stored in

fats[30]. Phytol and its metabolites have been reported to bind or activate the transcription factors PPAR-alpha[31] and retinoid X receptor(RXR)[32]. Phytol is used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents[33]. Ethyl oleate is a fatty acid ester formed by the condensation of oleic acid and ethanol. It is a colorless to light yellow liquid. Ethyl oleate is produced by the body during ethanol intoxication[34]. Ethyl oleate is used as a solvent for pharmaceutical drug preparations involving lipophilic substances such as steroids[35]. It also finds use as a lubricant and a plasticizer. Ethyl oleate has been identified as a primer pheromone in honeybees[36]. Ethyl oleate is one of the fatty acid ethyl esters (FAEE) that is formed in the body after ingestion of ethanol. There is a growing body of research literature that implicates FAEEs such as ethyl oleate as the toxic mediators of ethanol in the body (pancreas, liver, heart, and brain)[37,38,39]. Among the speculations is that ethyl oleate may be the toxic mediator of alcohol in fetal alcohol syndrome[40]. The oral ingestion of ethyl oleate has been carefully studied and due to rapid degradation in the digestive tract it appears safe for oral ingestion[41]. Ethyl oleate is not currently approved by the U.S. Food and Drug Administration for any injectable use. However, it is used by compounding pharmacies as a vehicle for intramuscular drug delivery, in some cases to prepare the daily doses of progesterone in support of pregnancy. Studies which document the safe use of ethyl oleate in pregnancy for both the mother and the foetus have never been performed. Stearic acid is a saturated fatty acid commonly found in animal and vegetable fats that is frequently used in cosmetics, candles, soaps, plastics, oil pastels, and for softening rubber. Stearic acid ethyl ester (ethyl stearate) is the neutral, more lipid soluble form of the free acid. It perturbs the cell cycle and induces apoptosis in Hep-G_2[42] cells and is a marker of excessive alcohol consumption that can be isolated from an individual's hair. Nonacosane is a straight-chain hydrocarbon it plays a role in the chemical communication of several insects, including the female *Anopheles stephensi* mosquito[43] Nonacosane occurs naturally and has been identified within several essential oils. It can also be prepared synthetically[44].

Stigmasterol also known as Wulzen anti-stiffness factor is one of a group of plant sterols, or phytosterols, that include β-sitosterol, campesterol, ergosterol (provitamin D_2), brassicasterol, delta-7-stigmasterol and delta-7-avenasterol, that are chemically similar to animal cholesterol. Phytosterols are Stigmasterol is used as a precursor in the manufacture of semisynthetic progesterone[45] a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D_3[46]. The Upjohn

company used stigmasterol as the starting raw material for the synthesis of cortisone[47] insoluble in water but soluble in most organic solvents and contain one alcohol functional group. Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast, and colon cancers. Studies have also indicated that a diet high in phytoesterols may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption. Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23% and 30%, respectively, over a 6-week period. It was demonstrated that it inhibits several pro-inflammatory and matrix degradation mediators typically involved in osteoarthritis-induced cartilage degradation[48]. It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties[49].

CONCLUSION

The result of this work suggest that the ethanolic extract of *Coriandrum sativum* has number of bioactive components. These bioactive components acts as a drug for various diseases. The ethanolic extract of Coriandrum sativum reveals the presence of eighteen bioactive components from gc-ms technique, each components has specific functions and act as a drug for various disease. Further in future these components can be isolated and invivo studies of animal model can be performed.

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