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GC-MS ANALYSIS OF *CURCUMA ZEDOARIA* TUBER EXTRACTS AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT: This study investigates the presence of bioactive compounds present in the tubers of *Curcuma zedoaria* using GC-MS analysis. Different compounds, including alkaloids, tannins, flavonoids, and proteins, were identified using the preliminary phytochemical screening for aqueous, ethanol and methanol extracts. The present study reports the antimicrobial activity analysis using aqueous, ethanol and methanol extracts. The results show that the ethanol and methanol extracts showed relatively enhanced results. The extracts were subjected to GC-MS analysis, and antibacterial properties for the extracts were analyzed. Bioactive compounds such as 1,2 cyclopentanedione, 2(5H)-furanone, hexadecanoic acid, succinic acid, oleic acid, pyrrolidine *etc*., were identified through GC-MS. These compounds exhibited various therapeutic functions such as anticancer, anti-inflammatory, and anti-microbial activity. The antibacterial assay showed a considerable zone of inhibition for the ethanolic extracts in the case of *K. pneumonia* and *B. subtilis*. Hence, these crude extracts can be used as a better and alternative source, which can be used in herbal medicines. The study concluded that *C. zedoaria* tuber contains various bioactive compounds. Therefore, this tuber can be considered of phytopharmaceutical importance.

INTRODUCTION: Bioactive compounds / secondary metabolites are major sources in conventional medicine. The most expensive resource for some potential medications is thought to be plants [1-4]. Even though the utilization of plants as medication has been known for centuries, nowadays its remedial worth is picking up centrality. *Curcuma zedoaria* (*C. zedoaria*) belongs to the class curcuma and family Zingiberaceae [5-8]. It is predominantly known as kichilikizhangu locally and white turmeric [9-10]. Pharmacological evaluation reports suggest this plant has antifungal, antiamoebic, antiulcer, anticancer, analgesic, and anti-asthmatic activity [11-16].

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In ancient days, the rhizome of *C. zedoaria* was used to treat bad breath, skin problems, menstrual irregularities, and digestive disorders [17-24]. The gas chromatography-mass spectrometry (GC-MS) study is worth identifying and quantifying small molecular metabolites, including sugars, acids, unsaturated fats, drugs, sterol and toxins [18-24]. This technique can easily analyze the secondary metabolites of both plant and non-plant species. As a result, the current study's main objective is to assess the various bioactive substances in the aqueous, ethanol, and methanol extracts of *C. zedoaria* using GC-MS along with the investigation of antibacterial activity for the same [25-30].

MATERIALS AND METHODS:

Collection of Plants: The local market in Chennai, Avadi, where the plant material was purchased. To get rid of any adhering material, it was washed in running water, dried at RT for a week in the shade. An airtight container was used to store the dried

plant materials after they had been ground into a fine powder

Preparation of Plant Extract: The coarse powder of *C. zedoaria* was extracted using water, ethanol and methanol solvents in a ratio of 1:5 and kept in an orbital shaker for 48 h. The extracts were filtered using Whatman no.1 filter paper and were dried in distillation unit [31].

The prepared extracts were used to check antibacterial efficacy, screen the preliminary phytochemical compounds, and perform GC-MS analysis to determine the pharmacological compounds.

Phytochemical Screening: The *C. zedoaria* extracts were assessed for the existence of phytochemical compounds such as alkaloids, phenol, flavonoids, saponins, tannins, terpenoids, protein, carbohydrate and cardiac glycosides by using the standard protocol [31].

Antibacterial Activity of *C. zedoaria*: The methanol and ethanol extracts were tested for antibacterial activity in a agar well diffusion method. Three bacterial species namely, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia* were grown on nutrient agar plates using spread plate technique.

Four wells were bored in 0.5 cm in diameter and 0.5 µL of the plant extract was added in the bored wells. The plates were then incubated at 37°C for 24 hours. The observation was done based on different antibiotics, and the zone of inhibition was measured using the scale around the discs [32].

GC-MS Analysis: Bioactive compounds of methanol and ethanol extracts were determined by GCMS analysis (model-GCMS-QP 2010 plus). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.9%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 µl was employed (split ratio of 1:10); injector temperature 280°C; ion-source temperature 250°C.

The oven temperature was programmed from 50°C (isothermal for 1 min), with an increase of 10°C/min, to 200°C, ending with a 5 min isothermal at 300°C. Mass spectra were taken at 70 eV in a scan-interval of 0.30 s. The identification of each compound was compared by their relative retention time and mass spectra.

GCMS interpretation was carried out by the NIST (National Institute Standard and Technology) database with 62000 patterns. The unknown compounds were compared with the NIST library is known compounds and its name, molecular mass, molecular weight, and chemical structure have been ascertained.

Based on the GCMS analysis, the peak in ethanol and methanol extracts of the *C. zedoaria* plant showed the presence of bioactive secondary metabolites [33].

RESULTS AND DISCUSSION:

Phytochemical Screening: Preliminary phytochemical analysis of *C. zedoaria* revealed the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, terpenoids, proteins, and reducing sugars, phenolic compounds, and saponins were absent. The phytochemical screening of methanol extract, ethanol extract, and aqueous extract has been listed in **Table 1**, with their corresponding test method.

TABLE 1: PHYTOCHEMICAL SCREENING OF *C. ZEDOARIA* USING DIFFERENT EXTRACTS

Sl. no.	Compound	In methanol extract	In ethanol extract	In aqueous extract	Test	Ref
1	Alkaloids	+	+	-	Mayer's Test	34
2	Phenolic compounds	+	+	+	Ferric chloride Test	35
3	Glycosides	+	+	-	Borntrager's Test	34
4	Flavonoids	++	++	+	NaOH Test	36
5	Tannins	-	-	-	Ferric chloride Test	36
6	Reducing sugars	++	++	-	Fehling's Test	37
7	Terpenoids	+	+	-	Salkowski Test	38
8	Saponins	-	-	-	Foam Test	39
9	Proteins	-	+	-	Biuret Test	40

++ denotes highly present, + denotes mildly present and - denotes absence.

Gas Chromatography and Mass Spectroscopy: Gas chromatography and mass spectroscopy of ethanol and methanol extracts of *C. zedoaria* were carried out to detect the constituents of esters, volatile matter, long-chain, and alcohol acids in **Fig. 1 & 2.**

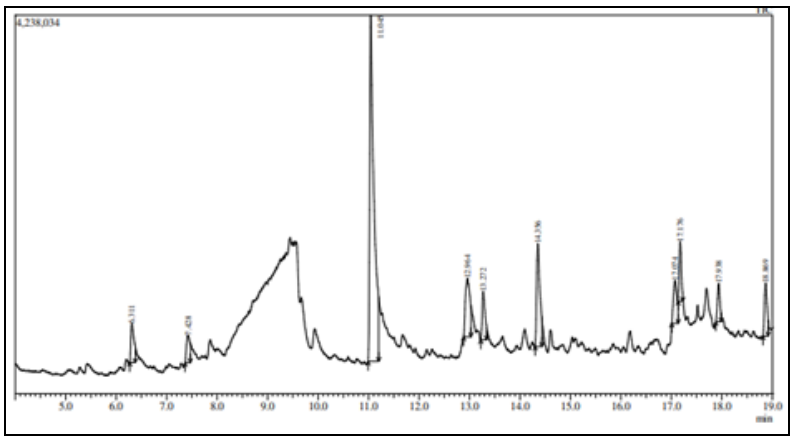

FIG. 1: GC-MS CHROMATOGRAM ANALYSIS OF *C. ZEDOARIA* ETHANOLIC EXTRACT

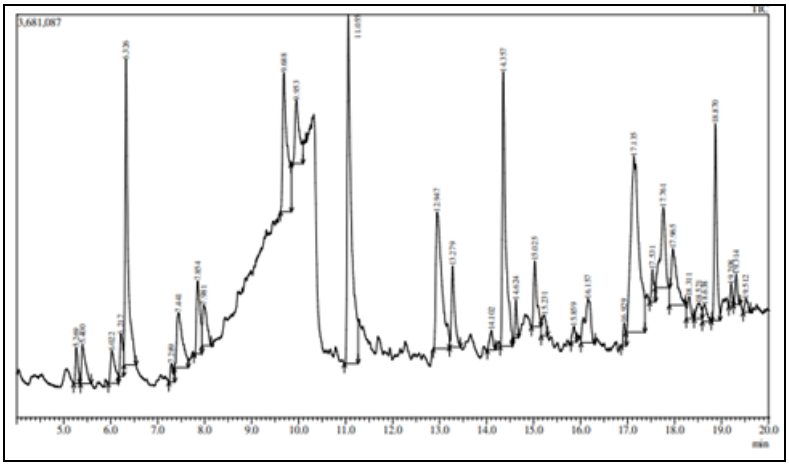

FIG. 2: GC-MS CHROMATOGRAM ANALYSIS OF *C. ZEDOARIA* METHANOLIC EXTRACT

GC-MS chromatogram analysis of *C. zedoaria* ethanolic extract is shown in **Fig. 1** and methanolic extract in **Fig. 2. Fig. 1** and **2** illustrate the GC-MS spectrum and confirm the presence of various bioactive components with different retention times. The mass spectrometer analyzed the nature and structure of these compounds were the compounds eluted at different times [41].

The GC-MS study of *C. zedoaria* had shown the presence of many phytochemicals which added their contribution to the medicinal importance of that plant. The results showed ten major peaks in ethanolic extract and eight in methanolic extract.

The properties of the phytochemical compounds are listed in **Table 2** and **Table 3** for ethanolic and methanolic extract, respectively. The various phytochemical identified here contributes to medicinal activities. The compound name, molecular formula, retention time, and biological activity have been. Many of the compounds proved to have antimicrobial, antioxidant, anti-inflammatory, and antitumor activities [42-44].

Among the compounds identified, the most predominant compounds acknowledged in ethanolic compounds are 2(3H)-furanone, allyl (ethoxy) dimethylsilane, hexadecanoic acid ethyl ester, ethyl 9-hexadecenoate, docosanoic acid, cyclopentane carboxylic acid, ethyl p-methoxycinnamate, propanoic acid, succinic acid and 3-bromocholest-5-ene.

Moreover, the compounds including cyclopent-4-ene, cylopentanone, pyrrolidine, 2(3H)-furanone, 5 methyl 2-hydroxycyclopent-2-en-1-one, 1-butene, 4-isothiocyanato, phenol, formic acid 2-propenyl ester were identified in methanol extract of *C. zedoaria.*

TABLE 2: PHYTOCOMPONENTS IDENTIFIED IN THE ETHANOLIC EXTRACT OF *C. ZEDOARIA* BY GC-MS

Sl. no.	Compound	Molecular Formula	Retention Time	Peak Area%	Activity
1	2(3H)-furanone	$C_5H_6O_2$	6.31	0.48	Anti-inflammatory
2	Allyl(ethoxy) dimethylsilane	$C_7H_{16}OSi$	7.43	0.37	Antioxidant
3	Hexadecanoic acidEthyl Ester	$C_{18}H_{36}O_2$	27.765	2.12	Anti-inflammatory
4	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	29.885	1.14	Antibacterial
5	Docosanoic acid	$C_{24}H_{48}O_2$	34.405	0.61	Antimicrobial &Antioxidant
6	Cyclopentane carboxylic acid	$C_{17}H_{26}O_2$	35.615	2.57	Antitumor
7	Ethyl p-methoxycinnamate	$C_{12}H_{14}O_3$	35.72	0.41	Anti-inflammatory
8	Propanoic acid	$C_3H_6O_2$	35.88	0.24	Antipyretic & Anti-inflammatory
9	Succinic acid	C_4H_6O	36.965	0.68	Antiketogenic
10	3-bromocholest-5-ene	$C_{27}H_{45}Br$	38.475	0.43	Antifungal

TABLE 3: PHYTOCOMPONENTS IDENTIFIED IN THE METHANOLIC EXTRACT OF *C. ZEDOARIA* BY GC-MS

Sl. no.	Compound	Chemical Formula	Retention Time	Peak Area%	Activity
1	Cyclopent-4-ene	C_5H_4O	5.4	0.46	Antiasthmatics
2	Cylopentanone	C_5H_8O	6.02	0.43	Anti-Melanogenesis
3	Pyrrolidine	C_4H_9N	6.215	0.37	Anticancer
4	2(3H)-Furanone,5 methyl	$C_5H_6O_2$	6.28	0.19	Anti-microbial
5	2-hydroxycyclopent-2-en-1-one	$C_5H_6O_2$	7.86	0.76	Antineoplastic
6	1-butene,4-isothiocyanato	C_5H_7NS	7.289	0.19	Myrosinase
7	Phenol	C_6H_5OH/ C_6H_6O	7.854	0.76	Antioxidant& Antimicrobial activity
8	Formic acid,2-propenyl ester	$C_4H_6O_2$	7.981	0.54	Antioxidant

Since, the aqueous extract did not respond to much of the compounds, the antibacterial study was carried out for ethanol and methanol extracts. The zone of inhibition was observed around the well in the plates containing microorganisms *Escherichia coli, Bacillus subtilis,* and *Klebsiella pneumonia.* The results were compared with standard antibiotics such as ampicillin and streptomycin. Compared to ethanol and methanol extract, ethanol extract exhibited the highest zone of inhibition against *Bacillus subtilis* (30 mm) and *Klebsiella pneumonia* (35 mm). From the tested bacteria, *E. coli* shows resistance towards the plant extract even at higher concentrations. In the case of the plant extracts, depending on the bacterial zone of inhibition, they can either act alone or in combination to inhibit bacterial growth as it is due to the presence of active metabolites [45]. Plant-derived medicines are vital to human health and are effective against microbial infections.

TABLE 4: ZONE OF INHIBITION BY THE WELL DIFFUSION METHOD

		Zone of inhibition (*mm*)			
Sl. no.	Microorganism	Ethanolic extract of *C. zedoaria*	Methanolic extract of *C. zedoaria*	Streptomycin	Ampicillin
1	*Escherichia Coli*	~20	~13	~25	~27
2	*Klebsiella Pneumonia*	~28	~19	~28	~29
3	*Bacillus subtilis*	~22	~18	~23	~28

CONCLUSION: Traditional & home remedial medicine use a wide variety of natural materials. Among these, medicinal plants are widely used to treat various diseases caused by microorganisms. Based on this, the current study has tried to reveal the importance of *C. zedoaria* tubers extracts. GC-MS studies have shown various bioactive compounds in *C. zedoaria* tubers extracts. Moreover, these bioactive compounds importance have been conformed via antibacterial assay. The antibacterial activities against the growth of the selected organisms have been confirmed will the formation of clear zones. The study confirms the effectiveness of *C. zedoaria* ethanolic extract on *K. Pneumonia* and *B. subtilis* compared to *E. coli.* Moreover, the *C. zedoaria* tubers extract's antibacterial activity might be linked with alkaloids, terpenoids, flavonoids, and other bioactive compounds present in the *C. zedoaria* tuber. From the results, it is concluded that *C. zedoaria* tuber contains various bioactive

compounds. Therefore, this tuber can be considered as phytopharmaceutical importance.

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