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RHIZOME ESSENTIAL OIL COMPOSITION OF *ZINGIBER CERNUUM* AND ITS ANTI MICROBIAL ACTIVITY

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ABSTRACT: *Zingiber cernuum* belongs to family Zingiberaceae is a perennial herb, also called as Curved stem ginger. The rhizomes of *Zingiber cernuum* is steam distilled and the composition of essential oil was studied. The essential oil obtained was yellow in colour and the yield was about 0.007%. The chemical profile of the oil was obtained from their GC-MS analysis. The major components present in the oil are *trans*-Caryophyllene, α-Humulene and δ-3-Carene. The antimicrobial activity of the essential oil was studied using disc diffusion method. The essential oil was tested against four pathogenic stains of gram positive and gram negative bacteria. The essential oil of *Zingiber cernuum* show considerable antibacterial activity. The oil was found to be active against both gram positive and gram negative bacteria. The anti bacterial activity is mainly due to the major components present in the oil or due to the synergistic effect of the major and minor components.

INTRODUCTION:

Zingiber cernuum Dalzell Common name**:** Curved-Stem Ginger, Nodding-stem ginger. Family: *Zingiberaceae* (Ginger family) Curved-Stem Ginger is a large perennial herb, 1-2 m tall, with curved stem. Leaves are 15-30 cm, narrow-elliptic, long-pointed. Flowers are borne in spikes 5-10 cm long, directly from the rootstock, rising just above the ground. Bracts are 2-3 cm long, greenish-yellow. Sepal cup is shortly 3-lobed. Stamen is one with short filament. Style is threadlike. Capsules are 1 cm long, smooth with red, channeled seeds. Flowers are creamish, variegated with red, with the lib broad and 3-lobed. Flowers open at night. Curved-Stem Ginger is found in the evergreen forests of Western Ghats, India. Kulkarni and coworkers identified flavanoid and tannins from the phytochemical investigation of *Z. cernuum* [1]. This species shows an appropriate amount of iron, manganese and low amount of molybdenum, sulphur, nitrate in rhizome and leaf [2]. Phytochemical screening of *Z. cernuum* shows flavnoids which shows antioxidant activity [3].

In the present investigation the composition of essential oil from the rhizome of *Z.cernuum* was studied and the chemical profiles of the oil were obtained from their GC-MS analysis.

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MATERIALS AND METHODS:

Extraction: The plant material was collected from Malappuram district, Kerala, India. The rhizome (*Z.cernuum*-1.1kg) was ground into a paste and subjected to steam distillation separately for 5 hours. The oils were extracted with diethyl ether. The ether extract was dried using anhydrous

sodium sulphate and ether evaporated. The pure oil weighed 80 mg.

Analysis of the oil:

GC-FID analysis was carried out using a Perkin Elmer Clarus 500 GC equipped with a 30 m × 0.32 mm Elite-5MS capillary column (0.32 µm film thickness). 1 µl of each sample was diluted with 300 µl of Et_2O and injected (0.5 µl) in the "split" mode (1:30) with a column temperature programme of 40°C for 5 min, then increased to 280°C at 4°C/min and finally held at this last temperature for 10 min. Injector and detector were set at 250 and 300°C, respectively, and the carrier gas was He with a head pressure of 12.0 psi.

GC/MS analyses were carried out using a Perkin Elmer Clarus 500 GC equipped with a Clarus 500 mass spectrometer using the same capillary column and chromatographic conditions as for the GC/FID analysis. Mass spectra were acquired over 40-500 amu range at 1 scan/sec. The identification of essential oil components was performed by means of their retention indices (RI), by a peak matching library search [4] and by comparison with authentic reference compounds as well as with published mass spectra [5, 6]. Retention indices (RI) were calculated using a n-alkane series (C_6-C_{35}) under the same GC conditions as for the samples. The relative amount (%) of individual components of the oil is expressed as percent peak area relative to total peak area from the GC/FID analyses of the whole components.

Anti microbial activity:

The antibacterial screening of the extract was carried out by determining the zone of inhibition using standard method [7]. The essential oil was tested against four pathogenic bacterial strains of gram positive and gram negative organism by disc diffusion method [8]. The test microorganisms of gram positive bacteria: *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, Staphylococcus albus*. gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Protieus vulgaris, Klebsiella aerogenes*. Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5°C for 1h to permit good diffusion and then transferred to incubator at 37°C for 24h. After completion of 24h, the plates were inverted and placed in an incubator set to 37°C for 24h.

RESULTS: The essential oil obtained from *Z.cernuum* was yellow in colour. The yield is 0.007% of fresh weight. The details of compounds identified by GC-MS analysis are given in **Table 1**

Table 1: CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *Z.CERNUUM*

RI*	RI**	Compound	*Z.cernuum*(%)
924	923	α-Thujene	0.31
932	929	α-Pinene	1.39
945	944	Camphene	0.21
970	970	Sabinene	4.78
974	972	β-Pinene	1.15
988	989	Myrcene	1.82
1002	1003	α-Phellandrene	1.29
1008	1006	δ-3-Carene	14.91
1014	1014	α-Terpinene	0.93
1020	1022	*p*-Cymene	1.85
1025	1027	β-Phellandrene	2.83
1086	1056	γ-Terpinene	2.92
1065	1068	*cis*- Sabinene hydrate	0.12
1086	1082	Terpinolene	0.82
1098	1098	*trans*-Sabinene hydrate	0.12
1095	1100	Linalool	0.11
1118	1122	*cis* -p- Menth -2-en- 1-ol	0.15
1141	1141	Camphor	0.11
1166	1162	*p*-Mentha1,5 dien 8-ol	0.05
1165	1168	Borneol	0.07
1174	1179	Terpinen-4-ol	6.77

1186	1193	α-Terpineol	0.14
1287	1281	Bornyl acetate	0.04
1374	1370	α-Copaene	0.17
1389	1385	β-Elemene	0.07
1408	1398	*cis*-Caryophyllene	0.06
1409	1401	α-Gurjunene	0.52
1418	1418	*trans*- Caryophyllene	32.01
1432	1430	α-*trans*- Bergamotene	0.12
1452	1451	α-Humulene	11.86
1458	1454	*allo*-Aromadendrene	0.32
1500	1498	BicycloGermacrene	0.67
1511	1514	δ-Amorphene	0.78
1561	1560	*trans*-Nerolidol	0.55
1577	1572	Spathulenol	0.51
1582	1576	Caryophyllene oxide	4.45
1608	1602	Humulene epoxide II	0.84
1638	1636	*epi*- α-Cadinol	0.26
1650	1650	α- Cadinol	0.44
1740	1737	Mint sulphide	0.70
		Total	97.22

RI*: R.P. Adams, Identification of essential oil components by Gas Chromatography/mass spectrometry 4th edition (2007) Allured Publishing Corporation, Carol Stream, IL

RI**: Calculated by GC/MS using n-alkane series under the same conditions as for the Sample

The major (above 10%) compounds constituting the oil are *trans*-Caryophyllene, α-Humulene and δ-3-Carene from *Z.cernuum*. These and the other compounds present in the oil from different plant sources exhibit diverse biological properties. *trans*-Caryophyllene and α-Humulene show anti-inflammatory properties [9]. δ-3-Carene present in *Z. cernuum* is found to exhibit antimicrobial properties [10]. Terpinen-4-ol was able to induce caspase-dependent apoptosis of melanoma cells and this effect was more evident in the resistant variant cell population. It also shows anti-inflammatory properties [11, 12, 13]. The activity of essential oil against the microorganism under study can be concluded from their respective zone of inhibition diameter which is given in **Table 2**.

TABLE 2: ANTIMICROBIAL SCREENING OF ESSENTIAL OILS

Sl.No.	**Test organisms**	**Diameter of zone of inhibition (mm) at different concentrations**			
		Zingiber cernuum			**STD**
	Gram +ve bacteria	**1 mg/l**	**2.5 mg/l**	**5 mg/l**	**2µg/disc**
1	*Staphylococcus aureus*	10	12	15	20
2	*Bacillus subtilis*	10	11	15	19
3	*Streptococcus faecalis*	11	12	14	19
4	*Staphylococcus albus*	10	12	15	18
	Gram-ve bacteria	**1 mg/l**	**2.5 mg/l**	**5 mg/l**	**2µg/disc**
1	*Escherichia coli*	09	10	10	18
2	*Pseudomonas aeruginosa*	10	11	11	19
3	*Klebsiella aerogenes*	10	11	12	19
4	*Protieus vulgaris*	11	13	14	19

Standard (STD) – Ciprofloxacin 2µg/disc

Solvent – DMSO (Shows nil effect against the micro organisms under test)

It was observed that the essential oil exhibit biological activity, hampering the growth of one or the other organism. It shows susceptibility to both gram +ve and gram -ve bacteria. **Table 3** shows the minimal inhibitory concentration.

TABLE 3: MINIMAL INHIBITORY CONCENTRATION

MIC - DETERMINATION							
Sl.No.	**Tested organism**	**Diameter of zone of inhibition (mm) at different concentrations(_µg/ml)**					
		Zingiber cernuum					**STD**
	Gram +ve bacteria	800	600	400	200	100	
1	*Staphylococcus aureus*	07	07	06	06	06	20
2	*Bacillus subtilis*	07	07	05	05	04	19
3	*Streptococcus faecalis*	08	07	07	06	06	19
4	*Staphylococcus albus*	07	07	06	06	06	18
	Gram –ve bacteria	800	600	400	200	100	
1	*Escherichia coli*	07	06	NI	NI	NI	18
2	*Pseudomonas aeruginosa*	07	05	03	NI	NI	19
3	*Klebsiellaaerogenes*	07	06	04	NI	NI	19
4	*Protieus vulgaris*	08	05	03	NI	NI	19

Standard (STD) – Ciprofloxacin 2µg/disc
NI – No Inhibitory effect

DISCUSSION: Essential oil from *Z.cernuum* shows good activity. This activity can be attributed to the compounds present in the oils. The higher antimicrobial activity in essential oil may be due to the presence of major components or due to the synergistic effect of the major and minor components. Usually the major components are responsible for the antimicrobial activity of plant essential oil, but the minor components also play major role making the whole oil more active than the combination of major components in synergism.

CONCLUSION: It can be concluded that the essential oil from the rhizomes of *Z.cernuum* growing in Kerala have major components such as *trans*-Caryophyllene, α-Humulene and δ-3-Carene which were already reported for their potential biological properties. The oil also possesses considerable anti-bacterial activity against gram positive and gram negative bacteria *in vitro*. The component present in the oil is responsible for its antibacterial activity. .

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