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Rhizome Essential Oil Composition of *Costus Speciosus* and its Antimicrobial Properties

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Abstract
The essential oil from the rhizome of *Costus speciosus* (Koen) Smith collected from Calicut University, Kerala, were obtained by steam distillation and analyzed by GC-FID and GC-MS. A total of twenty six compounds were identified from the oil accounting for 97.82% of its contents. The essential oil was found to consist only of different sesquiterpenoids among which α-Humulene and Zerumbone dominating. Medicinal use of the plant could be related with the biological properties of the individual compounds present in the oils.

Key words: *Costus speciosus, α-Humulene, Zerumbone*

Introduction
Costus speciosus (Koen.) Smith (Syn.*Cheliocostus speciosus*) commonly known as Channakkoova in Kerala and Keu in Hindi belongs to the family *Costaceae*. *Costus speciosus* or crape ginger is possibly the best known cultivated species of the genus *Costus*. This plant is native to Southeast Asia, especially on the Greater Sunda Islands in Indonesia. *Costus* differs from the common ginger by having only one row of spirally arranged leaves. The leaves of this species are less fleshy and have an acrid taste. The rhizomes are cooked and eaten. About seven species of the *Costus* Linn are known in India in which *Costus speciosus* (spiral or wild ginger) is the main species, found wild and cultivated in gardens. It is cultivated in India for its medicinal uses and elsewhere as an ornamental. In wild habitat, however it is now a rare species. According to Ayurveda the rhizomes are bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant and tonic and useful in burning sensation, constipation, leprosy, worm infection, skin diseases, fever, asthma, bronchitis, inflammations and anemia. Phytochemical and antimicrobial studies of rhizomes of the medicinal plant *Costus speciosus* have been carried out by Saraf and coworkers [1]. The essential oil composition and bacteriostatic property of *Costus speciosus* have been investigated [2]. The composition of essential oil from the rhizome of *Costus speciosus* was studied. The chemical profiles of the oil were obtained from their GC-MS analysis. The crystalline solid Zerumbone that separated out from the oil of *Costus speciosus* was isolated and identified by spectroscopic analysis. This is the first time that such a high percentage of Zerumbone is found to be present in a *Costus* species. Antimicrobial study against four gram positive and four gram negative bacteria was conducted.

Materials and Methods
Extraction
The plant material was collected from Calicut university campus, Malappuram district, Kerala, India. It was identified by Dr.Sabu, Department of Botany, University of Calicut and a specimen voucher is deposited in the specially maintained Herbarium of Chemistry department of University of Calicut. The rhizome (*Costus speciosus*-1kg) were ground into a paste and subjected to steam distillation for 5 hours. The oil was extracted with diethyl ether. The ether extract was dried using anhydrous sodium sulphate and ether evaporated. The pure oil weighed about 2 g of fresh weight.

Analysis of the oil
GC-FID analysis of the essential oil 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 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 used was helium with a head pressure of 12.0 psi.
GC/MS analysis was 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 [3] and by comparison with authentic reference compounds as well as with published mass spectra [4, 5] . Retention indices (RI) were calculated using n-alkane series (C_6-C_{35}) under the same GC conditions as for the sample. 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.

Isolation of Zerumbone from the essential oil

The solid separated from *Costus.speciosus* essential oil was recrystallized from ethanol and confirmed as zerumbone by spectroscopic analysis. Zerumbone melted at 67-69^{o}C. UV absorption shows 248 and 325nm and a shoulder peak at 233nm. IR shows characteristic peaks at 3005 cm^{-1} (Olefinic C-H), 1638 cm^{-1} (α, β unsaturated carbonyl). Its ^{1}H NMR and ^{13}C NMR data were found to be identical with the reported values for zerumbone [6].

^{1}H NMR(DMSO-d_6) : δ 1.07(s,3H,CH_3 at C 11),1.21 (s,3H,CH_3 at C 11), 1.54 (s,3H,CH_3 at C3), 1.80 (s, 3H, CH_3 at C7), 1,85-1.93(m,1H, H at C1), 2.22-2.35 (m,4H,H at C1,4 and 5), 2.42-2.48(m,1H,H at C5), 5.23(bd,1H,H at C2), 5.85(d,1H, H at C 10), 5.97(d,1H,H at C9), 6.01 (bd,1H,H at C6)

^{13}C NMR (DMSO-d_6): δ 11.8(CH_3 at C 7), 15.2 (CH_3 atC3), 24.2 (CH_3 at C 11), 24.4 (C5), 29.4 (CH_3 at C11), 37.86(C11), 39.4(C4), 42.43(C1), 125.0(C2), 127.16(C9), 136.26(C3), 137.9(C7), 148.82 (C6), 160.78 (C10), 204.38 (C8).

Biological study

The antibacterial screening of the extract was carried out by determining the zone of inhibition using standard method [7]. The oil was tested against four pathogenic bacterial strains of gram positive and gram negative organism by disc diffusion method [8]. 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 and Discussion

The essential oil obtained from *Costus.speciosus* was yellow in colour. The yield is about 0.18% of fresh weight. The details of compounds identified by GC-MS analysis are given in Table 1. The major (above 10%) compounds constituting the oils are α-Humulene and Zerumbone. These and the other compounds present in the oil from different plant sources exhibit diverse biological properties. α-Humulene show anti-inflammatory properties [9].

Zerumbone, a potential anticancer natural compound is a cyclic sesquiterpenoid. It shows activity on rat bone marrow cells, T-acute lymphoblastic leukemia cells, Hela cells, HL-60 cells, P-388D1 cells and apoptosis in HepG2 cells – human liver cancer cells. Cancer is one of the leading causes of death around the world. Several chemotherapeutic, cytotoxic and immunomodulating agents are available in Western medicine to treat cancer. Besides being enormously expensive, these drugs are associated with serious side effects. Still the search continues for an ideal treatment that has minimal side effects and is cost effective. A few members of *Zingiberaceae* species have been studied for their potential anticancer activity. Curcumin, a yellow pigment isolated from C*urcuma longa* (turmeric) has been shown to have anticancer activity *invitro* and/or in chemically induced animal cancer models including colon, skin, lung, liver and breast. As *Costus.speciosus* is a good source of zerumbone it can be grown for this anticancer agent. It is also a promising chemopreventive agent [10,11,12] as well as anti HIV [13,14].Camphene and Sabinene present in this oil also show biological properties [15,16].This is the first time that such a high percentage of Zerumbone is identified in a *Costus* species. The antimicrobial properties of the oil has been evaluated (*in vitro)* by using disc diffusion method. The results are tabulated in Table 2 and Table 3. The oil was found to be active against the four gram positive bacteria namely *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, and Staphylococcus albus* and gram negatives namely *Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes, Protieus vulgaris.* The activity may be due to the presence of individual components like α-Humulene and Zerumbone 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 oils, but the minor components also play

major role making the whole oil more active than the combination of major components in synergism [17].

Conclusion

It can be concluded that the essential oil from the rhizomes of *Costus speciosus* growing in Kerala, India shows considerable variation in composition to those belonging to other global regions.

The major components of the oil are α-Humulene and Zerumbone which were already reported for their potential anticancer properties. The oil also possesses considerable anti-bacterial activity against gram positive bacteria and gram negative bacteria *in vitro*.

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Table 1: Chemical composition of essential oil of *Costus.speciosus*

RI*	RI**	Compound	*Costus.speciosus*(%)
921	917	Tricyclene	0.05
932	929	α-Pinene	1.02
945	944	Camphene	4.96
974	972	β-Pinene	0.04
988	989	Myrcene	0.21
1002	1003	α-Phellandrene	0.12
1008	1006	δ-3-Carene	0.58
1020	1022	*p*-Cymene	0.13
1024	1026	Limonene	0.51
1026	1027	1,8-Cineole	1.73
1086	1082	Terpinolene	0.12
1095	1100	Linalool	0.40
1141	1141	Camphor	2.11
1165	1168	Borneol	0.23
1174	1179	Terpinen-4-ol	0.09
1186	1193	α-Terpineol	0.11
1287	1281	Bornyl acetate	0.06
1389	1385	β-Elemene	0.04
1418	1418	*trans*- Caryophyllene	1.40
1452	1451	α-Humulene	20.55
1511	1514	δ-Amorphene	0.07
1582	1576	Caryophyllene oxide	1.38
	1592	Sesquiterpene $C_{15}H_{24}O$	4.19
1608	1602	Humulene epoxide II	2.34
1649	1647	β -Eudesmol	0.27
1735	1735	Zerumbone	55.11
		Total	97.82

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

Table 2: Antimicrobial screening of essential oil

Sl.No.	Test organisms	Diameter of zone of inhibition (mm) at different concentrations			
		Costus speciosus			STD
	Gram+ve bacteria	**1 mg/l**	**2.5 mg/l**	**5 mg/l**	2µg /disc
1	*Staphylococcus aureus*	16	17	18	20
2	*Bacillus subtilis*	16	17	18	19
3	*Streptococcus faecalis*	16	17	18	19
4	*Staphylococcus albus*	15	16	18	18
	Gram -ve bacteria	**1 mg/l**	**2.5 mg/l**	**5 mg/l**	2µg /disc
1	*Escherichia coli*	10	11	12	18
2	*Pseudomonas aeruginosa*	11	13	14	19
3	*Klebsiella aerogenes*	12	13	14	19
4	*Protieus vulgaris*	13	14	15	19

NI – No Inhibitory effect
Standard (STD) – Ciprofloxacin 2µg/disc
Solvent – DMSO (Shows nil effect against the micro organisms under test)

Table 3: Minimal inhibitory concentration

MIC - DETERMINATION : µg/ml

Sl.No.	Tested organism	Diameter of zone of inhibition (mm) at different concentrations					
		Costus speciosus					STD
	Gram +ve bacteria	800	600	400	200	100	
1	*Staphylococcus aureus*	15	15	15	15	15	20
2	*Bacillus subtilis*	15	15	15	15	15	19
3	*Streptococcus faecalis*	15	15	15	15	15	19
4	*Staphylococcus albus*	14	14	14	14	14	18
	Gram –ve bacteria	800	600	400	200	100	
1	*Escherichia coli*	05	05	04	03	02	18
2	*Pseudomonas aeruginosa*	05	04	04	03	03	19
3	*Klebsiella aerogenes*	06	06	05	05	04	19
4	*Protieus vulgaris*	05	05	04	03	03	19

Standard (STD) – Ciprofloxacin

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