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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF MEDICINALLY USEFUL ESSENTIAL OIL FROM THE RHIZOMES OF *ALPINIA ALLUGHAS* ROSC.

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ABSTRACT

To obtain much better information about the medicinally useful essential oil of the rhizome of *Alpinia allughas* Rosc., the oil from the rhizome collected from Guwahati, Assam, has been isolated by hydrodistillation and analysed by GC and GC/MS. Twenty-eight compounds representing 95.14% of the oil have been identified. Norisoprenoid related antioxidant β-ionol is the major component (15.53%). In a rare observation, the sesquiterpenes & derivatives (39.47%) and monoterpenes & derivatives (39.42%) are almost in equal amounts. Among the monoterpenes & derivatives, the terpinen-4-ol (11.51%), 1,8-cineole (8.91%) and fenchyl acetate (7.55%) are main. Among sesquiterpenes & derivatives, the major components are found to be β-selinene (13.77%) and γ-elemene (6.30%). The oil shows good antibacterial activity against *Staphylococcus aureus*.

KEYWORDS :*Alpinia allughas, Alpinia nigra, Zingiber nigrum,*Zingiberaceae essential oil composition β-ionol

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INTRODUCTION

Alpinia allughas Rosc. (Zingiberaceae), syn. *Alpinia nigra*, *Zingiber nigrum* (Fig: 1) is a plant that grows naturally in some parts of North-Eastern India and cultivated mostly in South Konkan[1]. It also occurs in some parts of Sri Lanka, Bhutan and Thailand. Rhizomes are used in rheumatism, bronchial catarrh and in dyspepsia and gout and colic disease[2] ,food wrappers etc. They are also useful in respiration troubles, especially of children[1]. Aqueous solution of the rhizome has a good antibacterial activity[3] against Streptococcus and Staphylococcus spp. Alcoholic extract of the shoot has been reported to have flukicidal activity[4]. Certain pharmacognostical studies on the rhizome of Chinese sample have been carried out by Chunfeng et al.[5]. The dichloromethane/methanol extract of the rhizome is reported to show good antioxidant activity[6] and good nutritive value[7]. An elementary report[8] on essential oil composition from *A. allughas* fruit of a Japanese sample is available but not of the rhizome. From Indian sample, in the beginning only an elementary work, through column chromatography, towards composition of essential oil from rhizome was carried out by Purohit and Devi[9]. Latter on, a good work was carried out by Prakash et al.[10] and they identified as much as 22 constituents from the rhizome oil from Uttarakhand. In a Guwahati rhizome, we got success in identifying further large number of components through GC/MS, results being quite different and interesting. Antimicrobial study has also been carried out.

MATERIALS AND METHODS

Authentic rhizomes (Fig-2 :reddish brown) were collected from Guwahati, Assam and authenticity further verified by Forest Research Institute, Dehradun. The rhizomes were washed with luke-warm water and air dried for several days, avoiding any direct sunlight.

Isolation of essential oil

The oil was obtained by hydrodistillation using a Clevenger type apparatus. The lighter than water transparent oil was re-extracted with diethyl ether and dried over anhydrous Na_2SO_4. A light brown semi transparent oil having a characteristic sharp odour was obtained with yield 0.10% (w/w); d^{25} 0.9576 g mL^{-1}; n^{25} 1.362.

Gas Chromatography

The essential oil sample was subjected to GC analysis. Perkin Elmer Gas Chromatograph Clarus 500 equipped with DB-5 MS (60 m × 0.32 mm; film thickness 5 µm) was used. Oven temperature was kept at 65°C for 4 min and programmed to 250°C at a rate of 4°C/min and kept constant at 250°C for 20 min. Carrier gas: nitrogen; flow rate: 1.5 mL/min; split ratio: 1:10; injection temperature: 240°C; detector temperature: 250°C. The percentage compositions were computed electronically from the GC peak areas.

Gas Chromatography/Mass Spectrometry

GC/MS analysis of the oil was carried out on Shimadzu GC-2010, GCMS-QP 2010 equipped with similar column and operated under similar conditions as GC. Carrier gas: helium; flow rate: 24 cm/s (linear velocity); split ratio: 1:5, detector voltage: 5 KV; ion source temperature: 200°C. The oil components were identified by comparing their retention indices and mass fragmentation pattern with those of standard and MS library (NIST 12 and NIST 62).

Antibacterial Studies

A collection of three microorganisms was used including the Gram(+) bacteria *Staphylococcus aureus,* and *Bacillus subtilis* and the Gram(-) *Escherichia coli.* The test pathogenic bacteria were maintained on nutrient agar medium (NAM) slants at 4°C. The paper disc diffusion method[11] was used to determine inhibitory zone of pure essential oil and the chloramphenicol standard (30% aqueous solution) for antibacterial studies.

Figure 1
***ALPINIA ALLUGHAS* ROSC.**

Figure 2
Rhizomes of Alpinia Allughas Rosc.

RESULTS

The yield of essential oil obtained from rhizomes of *Alpinia allughas* is 0.10% w/w. 28 constituents are identified representing 95.14% of the oil. They are summarised in Table 1. Results of antibacterial studies are given in Table 2.

Table 1
Chemical composition (as percentages) of Alpinia allughas rhizome oil

Compound	RI	Percent
2,6-dimethyl-1-heptene	821	0.51
isobutyric acid	872	0.07
α-pinene	936	0.61
β-pinene	970	1.12
1,8-cineole	1030	8.91
β-linalool	1088	0.14
trans-2-menthenol	1140	1.49
β-terpineol	1158	2.10
methyl 4,6-dimethyloctanoate	1168	0.59
terpinen-4-ol	1175	11.51
α-fenchyl acetate	1227	7.55
p-cymen-7-ol	1286	3.02
unidentified	1322	2.18
4-acetonylcycloheptanone	1398	2.38
β-gurjunene	1418	2.86
γ-muurolene	1441	3.71
γ-elemene	1454	6.30
elixene	1466	2.55
β-ionol	1480	15.53
β-selinene	1502	13.77
trans-nerolidol	1546	1.58
caryophyllene oxide	1592	1.10
longifolenaldehyde	1625	1.69
cubenol	1642	1.15
1-[2-(2,2,6-trimethylbicyclo [4.1.0]hept-1-yl)ethyl]vinyl acetate	1655	1.19
bulnesol	1674	0.61
6-isopropenyl-4.8a-dimethyl-1,2,3, 5, 6,7,8,8a- octahydronaphthalen-2-ol	1704	0.67
trans,trans-farnesyl acetate	1859	2.29
unidentified	2055	0.40
unidentified	2162	1.50
unidentified	2178	0.78
thunbergol	2198	0.14

RI- relative to n-alkanes on the DB-5 column.

Table 2
Antibacterial activity of essential oil from rhizome of Alpinia allughas

Microorganism	Inhibition zone (mm)	
	Essential oil	Standard
Staphylococcus aureus (+)	14	31
Bacillus subtilis (+)	Nil	52
Escherichia coli (-)	Nil	36

Standard was Chloramphenicol, 30 µg/disc
Results are mean of three determinations

DISCUSSION

The remarkable observation is the presence of the norisoprenoid related β-ionol as the major component (15.53%) of the oil. β-Ionol is a good antioxidant and has been found to be useful in preventing the toxic effect of thiophenol on rats[12]. Among the identified 28 constituents, 27 are terpenes and terpenopids. Of them, 11 are monoterpene hydrocarbons and derivatives (including 9 oxygenated, but excluding β-ionol) representing 39.42% of the total essential oil. The major monoterpenes and derivatives are terpinen-4-ol (11.51%), 1,8-cineole (8.91%) and α-fenchyl acetate (7.55%). Monoterpenes are analgesic, expectorant and stimulant[13]. Some have been found to possess antiviral activities and some help breakdown gall stones[14]. They also help in mucocilary clearance in patients suffering with chronic obstructive pulmonary[15]. Terpinen-4-ol is antihypertensive[16] and suppresses the inflammatory mediator production by activated human monocytes[17]. 1,8-Cineole is a stimulating expectorant in cases of chronic bronchitis. Its cardiovascular effects in normotensive rats have also been reported[18]. Its use in room sprays, lotions and in all kinds of cosmetic preparations is well known. α-Fenchyl acetate is mainly applied in herbal-pine fragrances. In alcohol form, it is used as solvent, as an odorant and in flavoring[19]. Among the identified monoterpenoids, the presence of methyl 4,6-dimethyloctanoate (0.59%) and 4-acetonylcycloheptanone (2.38%) are novel. Methyl 4,6-dimethyloctanoate is among the pheromones[20] while presence of 4-acetonylcycloheptanone has been reported earlier in fresh flowers of *Wistaria sinensis*[21]. Presence of 2,6-dimethyl-1-heptene (0.51%) is also rare. Earlier its presence in essential oil of certain flowers has been noticed[22]. Sesquiterpene hydrocarbons and derivatives (39.47%) are found to be almost in same percentage as the monoterpene hydrocarbons and derivatives in the present work. Sesquiterpenes are anti-inflammatory and good stimulants for liver and gland[13]. They go beyond the brain blood barriers, increasing oxygen around the pineal and pituitary glands[13]. They are useful also for the characterization of mountain cheeses[23]. 15 sesquiterpenes and derivatives (including 9 oxygenated) are identified in the present investigation. The prominent among them are β-selinene (13.77%) and γ-elemene (6.30%). β-Selinene is antimalarial and antiplasmodial[24]. γ-Elemene is reported to have antitumor activity[25]. Among the sesquiterpene hydrocarbons the observed presence of elixene (2.55%) is rare. Earlier its natural occurrence has been reported in *Bixa orellana*[26] and *Fusarium sambucinum*[27]. Presence of the terpenoid 1-[2-(2,2,6-trimethylbicyclo[4.1.0]hept-1-yl)ethyl]vinyl acetate (1.19%) is also a rare observation. Its presence has earlier been reported among volatile constituents of *Vitex rotundifolia*[28]. The sesquiterpenoid 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-ol is commonly occurring one, the recent finding being in *Scapania verrucosa*[29] and in certain leaf essential oils[30]. Another important observation is the presence of the diterpenoid thunbergol (0.14%). Its presence is a little bit rare. Fokielakis et al. reported its presence in Euphorbia spp.[31]. The presently investigated essential oil of *Alpinia allughas* rhizome has been tested for its antibacterial properties against certain important bacteria (Table 2). It shows inhibition towards the growth of *Staphylococcus aureus* but no activity against *Bacillus subtilis* and *Escherichia coli*, indicating selective inhibition which is important at times.

CONCLUTION

To Study the rhizomes much better information about the medicinally useful essential oil of the rhizome of *Alpinia allughas* Rosc. Twenty-eight compounds representing 95.14% of the oil have been identified. Norisoprenoid related antioxidant β-ionol is the major component. In a rare observation, the sesquiterpenes & derivatives and monoterpenes & derivatives are almost in equal amounts. Among sesquiterpenes & derivatives, the major components are found to be β-selinen and γ-elemene. The presently investigated essential oil of *Alpinia allughas* rhizome has been tested for its antibacterial properties against certain important bacteria. The oil shows good antibacterial activity against *Staphylococcus aureus* .It shows inhibition towards the growth of *Staphylococcus aureus* but no activity against *Bacillus subtilis* and *Escherichia coli*, indicating selective inhibition which is important at times.

CONFLICT OF INTEREST

Conflict of interest declared none.

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