Research Article

PHYTOCHEMICAL AND ANTIOXIDANT STUDIES ON THE ESSENTIAL OIL OF THE RHIZOME OF *CURCUMA AERUGINOSA* ROXB.

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ABSTRACT

The genus *Curcuma*, of Zingiberaceae, comprises of 80 species, some of which have been used in traditional systems of medicine (Ayurveda, Siddha, Unani) for a long time. The present investigation was conducted to examine the chemical composition and *in vitro* antioxidant activity of essential oil of *Curcuma aeruginosa* Roxb. The GC-MS analysis of the oil has shown a profile of 18 compounds. Ethoxybenzene, Santolinatriene, Eucalyptol and Camphene are the two major components. The antioxidant activity was done by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, Ferric reducing antioxidant power and nitric oxide scavenging assay. The IC₅₀ value of essential oil revealed that the oil had potent antioxidant activity, so this study has proved that the essential oil could provide a significant bio-resource of antioxidants for using in food and pharmaceutical industry.

Keywords: *Curcuma aeruginosa* Roxb., Essential oil, GC-MS Analysis, antioxidant

INTRODUCTION

Medicinal plants are a rich source of pharmacologically active molecules. In India, family Zingiberaceae is well-known for its medicinal values and it is distributed widely. Zingiberaceae are usually aromatic in all or most parts or at least one of the plant parts and many species are known to be rich in terpenoids. The medicinal properties of the rhizome have been widely discussed and accepted worldwide. *Curcuma* is one of the most valuable genus which has been studied for decades for their chemical and biological properties. *Curcuma aeruginosa*, a rhizomatous herbaceous species is commonly known as 'kali haldi'. Fresh rhizomes are aromatic and deep blue or bluish black coloured cortex with pungent odor¹. In India, it occurs in West Bengal, Madhya Pradesh, Orissa, Bihar and Uttar Pradesh and is used by the tribals to cure various ailments². Isolation of new compounds with excellent medicinal properties is still going on with these plants. Isolation of two guaiane derivatives isolated from the rhizomes of *C. aeruginosa*³, diaryl derivatives from the root tuber of *C. longa*⁴ and labdanoid terpenes from *C. comosa* with fetal hemoglobin induction potency⁵ are a few recent developments to be mentioned. Essential oils are potential sources of antimicrobial compounds, comprising of mixtures of monoterpenes, sesquiterpenes, and various aliphatic hydrocarbons⁶.

The rhizomes and leaves of most of the *Curcuma* species are aromatic, indicating the presence of volatiles/essential oils. Essential oils are commercially important plant volatiles employed extensively in pharmaceutical, flavouring and perfumery industries and possess a wide range of pharmacological properties⁷. The essential oil of *C. longa* has been well studied and reported to contain ar-turmerone, turmerone, turmerol and zingiberene as the major constituents. Essential oils from *C. longa* and *C. zedoaria* possess antioxidant, antimicrobial, anti-inflammatory and cytotoxic properties⁸⁻¹³. Most of the other tuber rising *Curcuma* species produce aromatic rhizomes which are rich in essential oils varying in chemical constituents but which remain unexplored for their pharmacological properties.

Studies on their biological activity would be beneficial in medicinal applications. Mango ginger (*Curcuma amada* Roxb.) is a perennial herb, which morphologically resembles the ginger (*Zingiber officinale*) but, it imparts mango (*Mangifera indica*) flavour. The mango ginger starch constitutes 43% of amylose and resembles the characteristic of both *Curcuma longa* and *Zingiber officinale* starch¹⁴. Essential oil from *Curcuma amada* Roxb. could serve as an important bio -resource of antioxidants for using in food and pharmaceutical industry¹⁵.

Antioxidants have great importance because they can reduce oxidative stress which could cause damage to biological molecules. Antioxidant compounds play a crucial role in the treatment of various diseases related to degenerative disorders, namely, cardiovascular and brain diseases, arthritis, diabetes, cancer and immune system decline, by acting as free radical scavengers, and thus decreasing the extent of oxidative damage. Furthermore, studies about antioxidant substances in foods and medicinal natural sources have attracted increased interest in the recent decades. In addition, the use of plant materials in lipids and lipid-containing foods is important because the plant potentials of decreasing rancidity, delaying the formation of toxic oxidation products, maintaining nutritional quality and increasing the shelf life of food products. Hence, evaluation of radical scavenging properties and antioxidant activity are of commercial interest to the pharmaceutical and food industries as a source of natural antioxidants¹⁶⁻²¹. The objectives of the present study were to identify chemical composition as well as assess the antioxidant properties of the essential oil of the rhizome of *Curcuma aeruginosa* using gas chromatography combined with mass spectrometry (GC-MS) and flame ionization detector.

MATERIALS AND METHODS

Collection of Plant Sample

Curcuma aeruginosa was collected from Kottayam and Poonjar (Kerala, India). They were identified and authenticated by Dr. S. John

Britto, the Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (*Autonomous*), Tiruchirappalli, Tamilnadu, India. The voucher specimen (RHT 65182) was deposited at Rapinat Herbarium.

Extraction of Essential oil

The fresh rhizomes of plants were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate (Na_2SO_4) and preserved in a sealed vial at 4°C until further analysis.

GC-MS analysis

The analysis of the essential oil was performed using a Hewlett Packard 5890 II GC equipped with a FID detector and HP-5 ms capillary column (30m' 0.25mm, film thickness 0.25 μm). For GC-MS detection, an electron ionization system was used with ionization energy of 70 eV. Helium was the carrier gas, at a flow rate of 1ml/min. Injector and MS transfer line temperature were set at 220 and 290°C respectively. Column temperature was initially at 50°C , and then gradually increased to 150°C at a $3^\circ\text{C}/\text{min}$ rate, held for 10 min and finally increased to 250Vc at 10Vc/min. Diluted samples (1/100 in petroleum ether) of 1.0 μl were injected manually and split less. The components were identified based on the comparison of their relative retention time and mass spectra with those of Wiley 7N Library data and standards of the main components.

Antioxidant activity

DPPH Radical Scavenging activity

Radical scavenging activity was measured by using DPPH scavenging method²². A solution of DPPH in methanol (24 $\mu\text{g}/\text{ml}$) was prepared and 2ml of this solution was added to oil at different concentrations (10- 50 $\mu\text{g}/\text{ml}$). Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity were calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. Ascorbic acid was used as reference compound.

Total antioxidant capacity assay

The total antioxidant capacity assay was determined as described by Prieto et al.²³. Different concentrations of the essential oil (10-50 $\mu\text{g}/\text{ml}$) were taken and added 1.0 ml of the reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

Reducing power assay

The reducing power of extract was determined by the method of Yen and Duh²⁴. Different concentrations of essential oil (10-50 $\mu\text{g}/\text{ml}$) were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1 % Potassium ferri-cyanide. The mixtures were incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% Trichloroacetic acid were added to the mixtures, followed by centrifugation for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1 % Ferric chloride and the absorbance of the resultant solution were measured at 700 nm.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was measured spectrophotometrically²⁵. The essential oil was added to different test-tubes in varying concentrations (10-50 $\mu\text{g}/\text{ml}$). Sodium

nitroprusside (5mM) in phosphate buffer was added to each test tube to make volume up to 1.5ml. Solutions were incubated at 25°C for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% Sulphanilamide, 0.1% Naphthylethylenediamine dichloride and 3% Phosphoric acid) was added to each test tube. The absorbance was measured immediately at 546 nm and the percentage of scavenging activity was measured with reference to ascorbic acid.

RESULT AND DISCUSSION

Generally, the reliability of medicinal plant for its usage is evaluated by correlating the phytochemical compounds with their biological activities²⁶. The GC-MS study of *C. aeruginosa* has shown many phytochemicals which contributes to the medicinal activity. The *C. aeruginosa* rhizome's essential oil contains about 18 phytochemical compounds such as *Camphene*, Eucalyptol, (+)-2-Bornanone, Santolinatriene, (E)- β -Farnesene, Elemene, Phenol, 3-phenoxy, Caryophyllene, and other compounds. These 18 compounds are responsible for antimicrobial, antifungal, sedative, antitumor, antioxidant and insecticidal in this plant. Camphene is used as stimulant; Eucalyptol used as antibacterial, anti-inflammatory and analgesic properties; 2-Bornanone and Santolinatriene were used as Anticancer Agent; Caryophyllene is used as antifungal; β -Farnesene is used as inflammation and Elemene used as Anti-Lung-Cancer Activity (Table.1) (Figure 1).

Free radical scavenging property and antioxidant capacity are useful for medicinal applications and as pharmaceutical industries. So, in the present study, the antioxidant capacity of *C. aeruginosa* was evaluated using DPPH radical scavenging method by comparing with the activity of the ascorbic acid as a known antioxidant. In this experiment, the concentrations range from 10-50 $\mu\text{g}/\text{ml}$ and highest percentage of inhibition was 77% at 50 $\mu\text{g}/\text{ml}$. IC_{50} and EC_{50} values were receptively 28 $\mu\text{g}/\text{ml}$ and 30 $\mu\text{g}/\text{ml}$ (Figure 2).

The total antioxidant assay of the essential oil was determined by phosphormolybdenum with using Ascorbic acid as standard. In phosphormolybdenum assay, the concentrations range from 10-50 $\mu\text{g}/\text{ml}$, essential oil showed higher percentage of activity was 64.3% at 50 $\mu\text{g}/\text{ml}$, IC_{50} and EC_{50} values were receptively 45 $\mu\text{g}/\text{ml}$ and 30 $\mu\text{g}/\text{ml}$ (Figure 3).

The result obtained was confirmed by the high potency of essential oil towards the transition metal ions. The reducing power assay was found to be 2.54 at 50 $\mu\text{g}/\text{ml}$ in essential oil. This result showed that ascorbic acid exhibited excellent reducing power activity than *C. aeruginosa* essential oil (Figure 4).

Nitric Oxide (NO) scavenging assay is based on the scavenging ability of essential oil, as well as ascorbic acid, which is used as standard. The scavenging of NO was found to increase in dose dependent manner. Maximum inhibition of NO was observed in the extracts of highest concentration (50 $\mu\text{g}/\text{ml}$) for both the samples. At this maximum concentration, inhibition was found to be 76.8% for ascorbic acid, which serves as the standard. For *C. aeruginosa* essential oil inhibition was found to be higher 72.3% (Figure 5).

The plants cells own extremely effective antioxidative defense system, which get rid of the harmful effect of oxidative stress.²⁷ Mau et al²⁸ also reported that the essential oil of *C. zedoaria* was good in reducing power and excellent in DPPH scavenging activity. Lowest activity was seen in *C. raktakanta* and *C. malabarica* followed by other species including *C. sylvatica* oil and *C. amada*. Many curcuma species had oleoresins, that also exhibited high DPPH radical scavenging activity and ferric reducing power, which had good correlation with phenolic content²⁹ Essential oils of *C. aeruginosa* have been known for its antifungal activity³⁰. Rhizome of *C. aeruginosa* contains great coloring agents i.e. curcumin which acts as antioxidant as well as cytotoxic and tumour reducing properties³¹

Free radicals are the cause for several major disorders. So, evaluation of antioxidant activity in plants could result in the discovery of natural antioxidants with pharmacological and food value. The importance of phenol compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known^{32,33}. Therefore, these observations could help in developing new

drugs for the therapeutic use in human-beings. Therefore, the antioxidant properties of essential oil could play a valuable role in the food conservation and also in the prevention of oxidative damage related to the pathophysiology of many diseases, including significant and prevalent neurodege.

Table: 1 Chemical composition of Essential Oil from the Rhizome of *C. aeruginosa*

No	Name	Molecular formula	Molecular weight	Structure	RT	%Area	Uses
1	Camphene	C ₁₀ H ₁₆	136.23		3.682	1.26	Insecticides
2	Eucalyptol	C ₁₀ H ₁₈ O	154.249		5.410	6.44	antibacterial, anti-inflammatory and analgesic properties
3	(+)-2-Bornanone	C ₁₀ H ₁₆ O	152.2334		8.517	3.10	Anticancer Agent
4	Santolatriene	C ₁₀ H ₁₆	136.2340		15.612	7.28	Anticancer effect
5	Caryophyllene	C ₁₅ H ₂₄	204.36		16.316	1.65	anaesthetic, antifungal, antiseptic and antibacterial
6	(E)-β-Farnesene	C ₁₅ H ₂₄	204.3511		17.243	3.99	Antifungal
7	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)	C ₁₅ H ₂₄	204.3511		17.930	3.36	immune enhancement
8	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-	C ₁₅ H ₂₄	204.3511		18.055	1.42	anti-inflammatory
9	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans	C ₁₅ H ₂₀ O	216.3187		18.342	7.15	immune enhancement
10	10s,11s-Himachala-3(12),4-diene	C ₁₅ H ₂₄	204.3511		19.789	1.29	perfume industry and traditional medicine

11	Ethoxybenzene	$C_6H_5OC_2H_5$	122.16		21.157	33.44	"Perfume, insect "Pheromone"
12	Isolongifolene, 4,5-dehydro	$C_{15}H_{22}$	202.33518		21.552	3.34	various chronic diseases
13	Elemene	$C_{15}H_{24}$	204.35		23.062	4.02	Anti-Lung-Cancer Activity
14	4,6-Dimethyldibenzothiophene	$C_{14}H_{12}$	212.310		24.235	5.27	Catalysts
15	Phenol, 3-phenoxy	$C_{12}H_{10}O_2$	186.2066		28.716	1.58	antimicrobial
16	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{16}O$	152.2334		24.310	2.30	Perfumes
17	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)	$C_{10}H_{16}O$	152.2334		24.996	4.46	antimicrobial
18	4-amino-N-(2-phenylethyl)-1,2,5-oxadiazole-3-carboxamide	$C_{11}H_{12}N_4O_2$	4.46		25.208	8.62	Inhibitors of protein kinases

Figure 1: GC- Chromatogram of essential oil of *Curcuma aeruginosa* rhizome

Figure 2: DPPH Scavenging assay of essential oil of *Curcuma aeruginosa* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean $\pm$ standard deviation (n=3).

Figure 3: Total antioxidant assay of essential oil of *Curcuma aeruginosa* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean $\pm$ standard deviation (n=3).

Figure 4: Reducing power assay of essential oil of *Curcuma aeruginosa* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean $\pm$ standard deviation (n=3).

Figure 5: Nitric oxide scavenging of essential oil of *Curcuma aeruginosa* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean $\pm$ standard deviation (n=3).

CONCLUSION

On the basis of results obtained in the present study, the following salient findings are summarized. Quantitative analyses of the chemical composition of the investigated essential oils of *Curcuma aeruginosa* were tested. Gas chromatography/mass spectrometry (GC-MS) analyses revealed the presence of 18 major chemicals were present in the oils. Chemical identification of the oil constituents was conducted based on their retention time (tR), retention indices (KI) and mass spectral data, as well as by computer search of mass spectral databases. The chemical structures and medicinal properties also identified. The sample was subjected to screening for their possible antioxidant activity by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, Ferric reducing antioxidant power and nitric oxide scavenging assay. Results showed that the essential oil possessed a strong degree of antioxidant activity.

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