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In vitro antioxidant potential of the essential oil and leaf extracts of *Curcuma zedoaria* Rosc.

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ABSTRACT

In this study, we examined the chemical composition of the essential oil and tested the antioxidant potential of the oil and leaf extracts of *Curcuma zedoaria* Rosc. The chemical compositions of the oil were analysed by GC-MS. Twenty-four compounds representing 92.4% of the total oil was identified. The antioxidative potential was evaluated using two separate methods, inhibition of free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radicals scavenging activities assay. In the first case, the IC_{50} value of the oil was 14.8 ± 2.2. Among the extracts, the strongest activity was exhibited by the ethyl acetate extract (IC_{50} = 17.56 ± 1.6 µg/ml). In the superoxide radicals scavenging activities assay, ethyl acetate extract was superior to all other extracts (IC_{50} = 23.47 ± 1.2 µg/ml). Furthermore, the amount of total phenolic compounds was also determined as gallic acid equivalent. Thus, the natural products produced from *C. zedoaria* may be used in food and pharmaceutical industries.

INTRODUCTION

Free radicals are responsible for aging and causing various human diseases. A study shows that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. By donating hydrogen radical, the primary radicals are reduced to non radical chemical compounds and are converted to oxidize antioxidant radicals (Jadhav *et al.,* 1995; Yamaguchi *et al.,* 1998). This action helps in protecting the body from degenerative disease. Widely used artificial antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are very effective in their role (Chan, 1987). However, their use in food products has been falling off due to their instability, as well as due to a suspected action as promoters of carcinogenesis (Namiki, 1990; Pokorny, 1991). For this reason, there is a growing interest in the studies of natural additives as potential antioxidants.

Many medicinal plants contain large amounts of antioxidants such as poly phenols, which have been widely used as additives to avoid the degradation of foods. Also, antioxidants have an important role in preventing a variety of stress-related diseases because these are closely related to the active oxygen and lipid per oxidation (Noguchi and Niki, 1999). *Curcuma zedoaria* Rosc. is a medicinal properties-bearing Zingiberaceae from which rhizomes are commercially exploited. Natural products from this species are widely used in perfumary, in food industry as condiment and dye, and medicine as well. In addition to the well known effect of zedoary as a stomachical, it has been recently studied by its anti tumor (Kim *et al.,* 2000), hepatoprotective (Matsuda *et al.,* 2001), anti inflammatory (Jang *et al.,* 2001) and analgesic (Navarro *et al.,* 2002) effects. In the Ayrvedic system of medicine, *C. zedoaria* is known as zedoary while its conventional name is aadaa or ginger. *C. zedoaria* grows up to 1.2 m in height. The leaves of zedoary are oblong and can be up to 81 cm long and 18 cm wide. There are many species belonging with this genus but only four species namely *C. aeruginosa*, *C. phaeocaulis*, *C. pallida* and *C. zedoaria* were recorded as medicinal species.

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The plant is native to India and Indonesia. It was introduced to Europe by Arabs around the sixth century. In Bangladesh, it is found mostly in Chittagong, Dhaka, Srimangal and Dinajpur. It is also been cultivated in home yards and gardens. (Staples and Herbst, 2005). Therefore, the aim of this study was to determine the chemical composition of the essential oil from leaves of *C. zedoaria* by GC–MS and to evaluate the antioxidative properties of the essential oil and various organic extracts.

MATERIALS AND METHODS

Chemicals and Reagents

Gallic acid, kojic acid, L-ascorbic acid, L-tyrosine, Folin–Ciocalteau's phenol reagent, 1, 1- diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA),tyrosinase, and xanthine oxidase (XOD) were obtained from Sigma–Aldrich (St. Louis, MO). Nitrotetrazolium blue chloride (NBT) was purchased from Fluka (Buchs, Switzerland). All other chemicals and solvents were of highest commercial grade.

Plant materials

The leaves of *Curcuma zedoaria* Rosc. were collected from Gopalgonj, Jhenaidah and Kushtia of Bangladesh in January 2010 and identified by Dr. Oliur Rahman, Bangladesh National Herbarium, Dhaka, where a voucher specimen (DACB 35212) has been deposited.

Isolation of the Essential Oil

The air-dried leaves (200 g) of *C. zedoaria* were subjected to hydro distillation for 3h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4°C for further analysis.

Preparation of Organic Extracts

The air-dried leaves *C. zedoaria* were first pulverized into powdered form. The dried powder (50 g) was then extracted with hexane, chloroform, ethyl acetate and methanol separately at room temperature for 7 days and the solvents were evaporated by vacuum rotary evaporator temperature at 50°C. The extraction process yielded hexane (7.3 g), chloroform (6.2 g), ethyl acetate (7.4 g) and methanol (6.5 g) extracts respectively. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, St. Louis, MO, USA).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS was carried out using total ion monitoring mode on a Varian 3800 gas chromatograph interfaced to a Varian Saturn ion trap 2200 GC-MS spectrometer. The temperatures of transfer line and ion source were 280°C and 275°C respectively. Ions were obtained by electron ionization mode. The VF-5 capillary column (30 m length, 0.25 mm I.D. and 0.25 µm film thickness) was used. A 20% split injection mode was selected with a solvent delay time of 3 min with injection volume 0.2 µl. The initial column temperature was started at 50°C for 1 min, programmed at 8°C/min to 200°C and heated until 280°C at 10°C/min. Injection port was set at 250°C. Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. Molecular ions (mass range: 40-500 m/z) were monitored for identification. The relative percentage of the oil constituents was expressed as percentage by peak area normalization. Identification of components of the essential oil was based on their retention indices, relative to a homologous series of *n*-alkane (C_8 - C_{20}) on the VF-5 capillary column under the same operating conditions and computer matching with the GC-MS spectra from the Wiley 6.0 MS data and literature data (Adams, 2007).

Determination of antioxidant activity

DPPH-Radical Scavenging Activity

DPPH radical scavenging activity of the extracts was determined by the method described by (Archana *et al.*, 2005). Various concentrations of test extracts were added to 2.9 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 min of incubation period at room temperature, the absorbance was measured against a blank at 517 nm. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation. Synthetic antioxidant reagents, butylated hydroxyanisole (BHA) and L-ascorbic acid were used as reference positive controls. Inhibition free radical DPPH in percent (I %) was calculated in following way:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation.

Superoxide anion ($O_2^{\bullet-}$) scavenging activity

Superoxide radicals were generated in vitro by the xanthine oxidase (XOD). The scavenging activity of the sample was determined using the nitro-blue tetrazolium (NBT) reduction method. In this method, O_2 reduces the yellow dye (NBT^{2+}) to produce the blue formazan, which was measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the blue NBT formation (Cos *et al.*, 1998). The results were calculated as the percentage of inhibition according to the following formula:

I (%) = 100[1-(S-SB) / (C-CB)] where S, SB, C, and CB are the absorbance of the sample, the blank sample, the control, and the blank control, respectively.

Determination of total phenolics

Total phenolic constituents of the aforementioned extracts were determined by Folin-Ciocalteu reagent in alkaline medium (Lister and Wilson, 2001) and was expressed as gallic acid equivalents (GAE). The absorbance of samples was measured at 760 nm and the results were expressed in mg/g (GAE) of dry weight of samples.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The hydrodistillation of dried leaves of *C. zedoaria* Rosc. gave yellowish essential oil (yields ~ 0.8%, w/w). The identified compounds, qualitative and quantitative analytical results by GC-MS, according to their elution order on a VF-5 capillary column are shown in Table 1.

Table. 1: Chemical composition of the essential oil of *Curcuma zedoaria* Rosc.

Sl. No.	Compound	RI[a]	% RA[b]	Identification[c]
1.	Cyclohexane	719	1.2	RI, MS
2.	*cis*-4-Heptenal	884	0.6	RI, MS
3.	Acetic acid	895	1.5	RI, MS
4.	Cyclopentylmethanol	903	tr	RI, MS
5.	l-Octen-3-ol	961	12.4	RI, MS
6.	Benzyl alcohol	1036	2.6	RI, MS
7.	Eucalyptol	1059	22.4	RI, MS
8.	Borneol	1088	1.6	RI, MS
9.	Naphthalene	1196	tr	RI, MS
10.	1,2-Dihydro-1,1,6-trimethyl-naphthalene	1224	0.4	RI, MS
11.	1-Methylnaphthalene	1289	1.5	RI, MS
12.	Bornyl acetate	1292	0.8	RI, MS
13.	Cycloheptanol	1306	1.6	RI, MS
14.	Azulene	1386	1.3	RI, MS
15.	β- Elemene	1388	9.6	RI, MS
16.	Benzofuranone	1426	1.2	RI, MS
17.	γ-Elemene	1430	0.8	RI, MS
18.	α-Caryophyllene	1447	17.2	RI, MS
19.	Ledol	1530	1.6	RI, MS
20.	(-)-Spathulenol (Spathulenol)	1550	2.2	RI, MS
21.	Caryophellene oxide	1561	8.3	RI, MS
22.	Aromadendrene oxide-(II)	1664	0.8	RI, MS
23.	Hexahydrofarnesylacetone	1816	1.0	RI, MS
24.	Pentadecanoic acid	1832	1.8	RI, MS
	Total identified		92.4%	

[a]Retention index relative to *n*-alkanes on VF-5 capillary column, tr: trace amount (< 0.2%).
[b]Relative area (peak area relative to the total peak area);
[c]Identification: MS, comparison of mass spectra with MS libraries.
RI, comparison of retention index with bibliography.

Twenty-four constituents accounting for 92.4% of total oil compositions were identified. The oil contains a complex mixture consisting of mainly oxygenated mono- and sesquiterpenes, and mono- and sesquiterpene hydrocarbons. The major compounds detected in the leaves oil were eucalyptol (22.4%), α- caryophyllene (17.2%), 1-octen-3-ol (12.4%), β-elemene (9.6%) and caryophyllene oxide (8.3%). Besides, benzyl alcohol (2.6%), ledol (1.6%), cycloheptanol (1.6%) and pentadecanoic acid (1.8%) were also found to be the minor components of *Curcuma zedoaria* oil. On the other hand, the previous research shows that the oil of *C. zedoaria* was made up mainly of mono- and sesquiterpenoids, monoterpene hydrocarbons (2.3%), oxygenated monoterpenes (26%), sesquiterpene hydrocarbons (38%), and oxygenated sesquiterpenes (13.5%). α-terpinyl acetate (8.4%), isoborneol (7%), dehydrocurdione (9%) and selina-4(15),7(11)-dien-8-one (9.4%) were the major constituents of the leaf oil (Garg *et al.,* 2005). However, it is noteworthy that the composition of the essential oils from a particular species of plant can differ between harvesting seasons, extraction methods, and geographical sources, and that those from the different parts of the same plant can also differ widely (Burt, 2004). Several papers reported that all these compounds possess significant antioxidant activity in several model systems (Ruberto and Baratta, 2000; Cabrera and Prieto, 2010). It is also possible that the minor components might be involved in some type of synergism with the other active compounds. Also, the essential oils are, from the chemical point of view, quite complex mixtures constituted by several tens of components, and this complexity makes it often difficult to explain the aforesaid activities. If we exclude the case of some phenolic components, whose antimicrobial and antioxidant activity is well known and widely documented (Helander *et al.,* 1998; Yanishlieva *et al.,* 1999) and some other examples of pure compounds nothing is known about the effectiveness of most components (Aeschbach *et al.,* 1994).

Determination of antioxidant activity

Scavenging activity of DPPH radical

The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants (Sánchez-Moreno, 2002). The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.,* 1997). The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The DPPH radical scavenging activity of the essential oil and the organic extracts are shown in Table 2.

Table. 2: Free radical scavenging activity (DPPH) of the oil and extracts of *Curcuma zedoaria* R.

Sample	IC_{50} (µg/ml)
Essential oil	14.8 ± 2.2
Methanol extract	25.11 ± 1.5
Hexane extract	117.79 ± 2.2
Chloroform extract	50.13 ± 1.1
Ethyl acetate extract	17.56 ± 1.6
Ascorbic acid (Control)	6.55 ± 0.9
BHA (Control)	18.27 ± 1.4

Values are given as mean ± S.D. of triplicate experiments.
BHA: butylated hydroxyanisole.

Lower IC_{50} value indicates higher antioxidant activity. Polar extracts exhibited stronger activity than non-polar extracts. Of all samples studied, the essential oil and ethyl acetate extract had the strongest free radical-scavenging activity with an IC_{50} value of 14.8 ± 2.2 and 17.56 ± 1.6 µg/ml, respectively. The methanol (IC_{50} = 25.11 ± 1.5 µg/ml) and the chloroform extract (IC_{50} = 50.13 ± 1.1 µg/ml) showed moderate DPPH radical scavenging activity, while hexane extract showed little activity (IC50 = 117.79 ± 2.2 µg/ml) as compared to positive controls L-ascorbic acid (IC_{50} = 6.55 ± 0.9 µg/ml) and BHA (IC_{50} = 18.27 ± 1.4 µg/ml). These results indicated that organic extracts of *C. zedoaria* leaves have a noticeable effect on scavenging free radical.

Superoxide anion ($O_2^{-\bullet}$) scavenging activity

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The scavenging activities of the extracts on superoxide radicals are shown in Table 3.

Table. 3: Superoxide anion ($O_2^{-\bullet}$) scavenging activity of various leaf extracts of *Curcuma zedoaria* R.

Sample	IC_{50} (µg/ml)
Essential oil	ne
Methanol extract	34.41 ± 1.5
Chloroform extract	51.76 ± 1.7
Ethyl acetate extract	23.47± 1.2
Kojic acid	8.27 ± 1.4

Values are given as mean ± S.D. of triplicate experiments.
ne: not examined.

All the extracts exhibited noticeable superoxide radical scavenging activities. The highest superoxide radical scavenging activities was found in ethyl acetate extract (IC_{50} = 23.47 ± 1.2 µg/ml). Methanol and chloroform extract exhibited IC_{50} values 34.41 ± 1.5 and 51.76 ± 1.7 µg/ml, respectively. However, those values were significantly higher than the value of the positive control kojic acid (IC_{50} = 8.27 ± 1.4 µg/ml). These results imply that organic extracts of *C. zedoaria* leaves are superoxide scavengers and their capacity to scavenge superoxide may contribute to their antioxidant activity.

Total phenolics content

The phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano *et al.,* 1989). Based on the absorbance values of various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, as described above, total phenolics are shown in Table 4.

Table. 4: The amount of total phenolic contents mg/g (GAE) d/w of various leaf extracts of *Curcuma zedoaria* R.

Extracts	Total phenolic (mg/g (GAE) d/w)
Essential oil	ne
Methanol extract	122.12 ± 1.6
Hexane extract	40.2 ± 1.2
Chloroform extract	61.15 ± 1.1
Ethyl acetate extract	141.31 ± 1.5

Values are given as mean ± S.D. of triplicate experiments.
ne: not examined.

Among the organic extracts, the amount of total phenolics was higher in the ethyl acetate extract (141.31 ± 1.5 GAE mg/g sample) as compared to methanol (122.12 ± 1.6 GAE mg/g sample) and chloroform extracts (61.15 ± 1.1 GAE mg/g sample). The lowest value was exhibited by the hexane extract (40.2 ± 1.2 GAE mg/g sample). The key role of phenolic compounds as scavengers of free radicals is emphasised in several reports (Madsen *et al.,* 1996; Moller *et al.,* 1999). Phenolic antioxidants are products of secondary metabolism in plants, and the antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals, inhibiting lipoxygenase and scavenging free radicals (Decker, 1997). Some mechanisms are available for the mode of action of phenolic compounds in antioxidant activity test systems. One of them has been put forward by Ramirez-Anguiano *et al.,* 2007. According to this group, the oxidation of diphenols to quinines is a very fast reaction, which might occur in seconds. Even when only a few quinines are formed before the preparation of the extract, they become to the low molecular weight compounds and might react spontaneously with other phenols, generating molecules like dopachrome, indolic compounds, catechol dimers and other higher polymers yielding radical scavenging degradation products. Therefore, it could be concluded that the phenolic compounds were highly involved in the antioxidant activity found in organic extracts of *C. zedoaria* leaves and also able to enhance or complement their activity.

CONCLUSION

This study concludes that the strong antioxidant and radical scavenging activity of the essential oil and extracts of *C. zedoaria* leaves points towards its strong protective role against oxidative diseases. The strong antioxidant activity indicates a possible use of residue oil as a natural antioxidant, food supplement and potential pharmaceutical application. However, further research is needed in order to establish the real application of *C. zedoaria* leaves essential oil or extracts in food or pharmaceuticals.

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