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Chemical Composition, Antioxidant and Antibiosis Activities of Leaves Essential Oil of *Curcuma phaeocaulis*

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ABSTRACT —***The essential oil from Curcuma phaeocaulis leaves, The chemical constituents of essential oil were extracted from the leaves of Curcuma phaeocaulis by applying steam distillation. The chemical constituents were analysed and identified by using gas chromatography-mass spectrometry (GC-MS). The essential oil in the leaves of Curcuma phaeocaulis was extracted and from 36 compounds were identified (77.41% of the total essential oil). The major compounds that were identified by GC–MS were Eucalyptol (26.63%), L-linaloool (6.32%), Camphor (5.22%) and beta- Pinene (4.60%). The antioxidant properties have been assessed by inoxidizability and antibacterial action, find the essential oil from Curcuma phaeocaulis leaves essential oil from Curcuma phaeocaulis leaves and antibacterial are high.***

Keywords —*Curcuna phaeocaulis* essential oil, GC-MS, Antioxidant activity, Antibacterial activity

1. INTRODUCTION

Curcuma L. is wide spread in Asia, Africa and Australia, which composed of approximately 70 species (Purseglove, 1974). About 10 *Curcuma* species are distributed in China (Xiao et al., 1997; Li et al., 2001; Ye et al., 2008). *Curcuma phaeocaulis* Valeton was used as Chinese folk herbal medicine more than a thousand years ago. The rhizomes of *C. phaeocaulis* was named as Rhizoma Curcumae in Traditional Chinese Medicine (TCM), which is officially recorded in Chinese Pharmacopoeia (2010) as one of original plants of Rhizoma Curcumae. The extract of *C. phaeocaulis* rhizomes exhibits anti-inflammatory, anticancer and HIV-1 protease inhibitory activity (Moussavi et al., 2006).

Lipids are rich source of energy and constitute an important part of our balanced diet. However, oxidation with atmospheric oxygen and lipolysis are responsible for the rancidity of lipids and lipid containing food products (Allen and Hamilton, 1983). Lipid peroxidation causes a decrease in nutritional value, safety and appearance of foods. It also initiates other undesirable changes in food, affecting its nutritional quality, color, flavor and texture. Auto-oxidation of polyunsaturated lipids involves a free radical chain reaction, generally initiated by exposure to light, heat, metal ions, etc. Therefore, the inhibition of free radical oxidation by incorporating antioxidants is of great practical importance in preserving lipids from deterioration.

Antioxidants have been widely used as additive to provide protection against oxidative degradation of foods (Gulcin et al., 2004). Although many synthetic chemicals, such as phenolic compounds are found to be strong radical scavengers, they usually have serious side effects (Imaida et al., 1983). In view of this, antioxidant substances obtained from natural sources will be of great interest. There are many herbs and spices which have been used for long ago in folk medicines. *Curcuma longa* L. commonly known as turmeric, is one such perennial herb. The oils of its rhizomes are very useful. It is extensively used as spice in domestic cooking. In combination with other natural dyes, it is also used as a coloring agent for textiles, pharmaceuticals, confectionary and cosmetics (Singh et al., 2003). In Indian system of medicine, turmeric rhizomes are used in stomachache, as a blood purifier, carminative, appetizer and tonic. Turmeric is also used in drugs against cancer, dermatitis, AIDS and high cholesterol level (Kuttan et al., 1985; Ammon and Wahl, 1991; Azuine and Bhide, 1992). Beyond that, the essential oil extracted from turmeric possesses anti-inflammatory, antifungal, antihepatotoxic and antiarthritic activities (Arora et al., 1971; Kiso et al., 1983; Palasa et al., 1992; Behura et al., 2000).

The objective of this study is to analyze the chemical components of essential oils in leaves of *C. phaeocaulis*, and to compare their antioxidant and antibacterial activity.

2. MATERIALS AND METHODS

2.1 Materials

C. phaeocaulis were collected from Chongzhou, Sichuan Province, China. It was identified by Prof. Ruiwu Yang of Sichuan Agricultural University. Gas chromatography mass spectrometry instrument comes from island ferry company GCMS-QP2010. Essential oil extractor, n-hexane, sodium sulphate anhydrous were purchased from the Chengdu Kelong Chemical Factory (Chengdu, China). All chemicals were of analytical grade.

2.2 Extraction of essential oil

The leaves of *C. phaeocaulis* were washed, air dried and thinly grated, sun dried and pulverized into a fine powder. The chemical components were analyzed by GC-MS. The materials were placed in a Soxhlet extraction apparatus for 5 hours and extracted three times with hexane. The light yellow colored oil obtained was dried over minimum amount of anhydrous sodium sulfate and stored at 4℃.

2.3 Chemical investigations

Chemical composition of essential oil of *C. phaeocaulis* was analyzed by GC-MS technique using gas chromatography mass spectrometry instrument island ferry company GCMS-QP2010 and a Perkin Elmer Elite-5MS capillary column (5% phenylmethyl siloxane; length 30 m×inner diameter 0.25 mm×film thickness 0.25 µm). The injector, interphase, ionsource and selective mass detector temperatures were maintained at 280℃, 280℃, 230℃ and 150℃, respectively. Helium (He) was used as a carrier gas at a flow rate of 1.0 ml/min. The oven temperature was programmed as follow:

For essential oil of leaves: at 60℃ for 1 min; then increased from 60℃ to 185℃ at the rate of 1.5℃/min and held at 185℃ for 1 min; then again increased from 185℃ to 275℃ at the rate of 9℃/min and held at 275℃ for 2 min.

The percentage composition of the essential oil was computed by the normalization method from the GC peak areas, assuming identical mass response factor for all compounds. Results were calculated as mean values of two injections from essential oil, without using correction factors. All determinations were performed in triplicate and averaged.

2.4 Antioxidant properties

2.4.1 DPPH free radical scavenging activity

The DPPH assay was carried out according the previously described method (Yang et al., 2009) with some modifications. Briefly, the reaction solution consisted of 2.0 mL DPPH solution (0.1 mmol/L in 95% ethanol), 2.0 ml of related tested samples with different concentrations (0, 2, 4, 6, 8, 10 mg/mL in 95% ethanol) in the tubes. After 60 min at room temperature, the absorbance was measured at 517 nm with a spectrometer (UV-1750; Shimadzu, Kyoto, Japan). This experiment was repeated 3 times, the data was averaged. V_C as a control. The capability to scavenge the DPPH radical was calculated by using the following equation:

Scavenging ability (%) = $(1-A_{sample}/A_{DPPH}) \times 100$

Where A_{sample} was the absorbance of a mixture of sample and DPPH solutions, and A_{DPPH} was the absorbance of the control reaction in which the sample was replaced by ethanol.

2.4.2 Hydroxyl radicals scavenging assay

Scavenging activity against hydroxyl radical was determined according to Fenton's reaction (Wu et al. 2012). Briefly, the reaction solution consisted of 1.0 mL sample solution (0, 2, 4, 6, 8, 10 mg/mL in 95% Ethanol), 1.0 mL phenanthroline (5 mmol/L), 2 mL of PBS (pH 7.4), 1 mL of $FeSO_4$ (0.75 mmol/L), 1 mL H_2O_2 (0.1 %,). The final mixture was incubated for 60 min at 37℃. The absorbance of the mixture was measured at 510 nm with a spectrometer (UV-1750; Shimadzu, Kyoto, Japan). This experiment repeated 3 times, the data averaged. V_C as a control. The capability to scavenge the hydroxyl radical was calculated by using the following equation:

scavenging ability (%) = $(A_{sample} - A_{blank})/(A_{control} - A_{blank}) \times 100$

where $A_{control}$ was the absorbance of the control (blank, without of H_2O_2), A_{blank} was the absorbance in the absence of sample, and A_{sample} was the absorbance in the presence of sample.

2.4.3 Superoxide anion scavenging activity assay

The superoxide radical scavenging activity was conducted according to the previously described method (Liu et al. 2009) with some modifications. Briefly, the reaction solution consisted of 1 mL Tris-HCl (16 mmol/L, pH 8.0) containing 468 µmol/L NADH, 1 mL Tris-HCl (16 mmol/L PH 8.0) containing 468 µmol/L NBT, and 1 mL Tris-HCl (16 mmol/L,

pH 8.0) containing 60 μmol/L PMS, and 1.0 mL sample solution (0, 2, 4, 6, 8, 10 mg/mL in 95% ethanol). The reaction mixture was incubated at 25℃ for 5 min, and the absorbance was measured at 560 nm with a spectrometer (UV-1750; Shimadzu, Kyoto, Japan). This experiment repeated 3 times, the data averaged. V_C as a control. The capability to scavenge the superoxide radical was calculated by using the following equation:

Scavenging ability (%) = $(1- A_{sample}/A_{blank}) \times 100$ %

where A_{sample} was the average absorbance value of the sample, and A_{blank} was the average absorbance in the absence of sample

2.4.4 Ferrous ion chelating activity

The reducing power was determined referring to the ferric-reducing antioxidant power (FRAP) assay (Yuan et al. 2005) with some modifications. Briefly, the reaction solution consisted of 2.5 mL sample solution (0, 1, 5, 10, 15, 20 mg/mL in 95% ethanol), 2.5 mL PBS (0.2 mol/L, pH 6.6), 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v). The mixture was incubated at 50℃ for 20 min. Then 2.5 mL trichloroacetic acid [TCA] (10%, w/v) was added after the reaction mixture cooling, which was centrifuged at 3 000 r/pm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of absolute ethyl alcohol and 0.5 mL ferric chloride [$FeCl_3$] (1%, w/v) in a test tube. After 10 min reaction, the absorbance of the resulting solution was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. This experiment repeated 3 times, the data averaged. V_C as a control.

2.5 Antimicrobial activity

The zedoary turmeric oil were tested against two Gram-positive bacterial strains (*Staphylococcus aureus* and *Bacillus cereus*), two Gram-negative bacterial strains (*Salmonella* and *Escherichia coli*) through agar well diffusion method. All these bacterial strains were obtained from the Department of Chemistry (Biochemistry), Sichuan agriculture University, Yaan, Chian.

One liter nutrient agar aqueous solution was made by dissolving 18 g nutrient agar in distilled water and the constant volume was 1 000 mL with the pH adjusted to 7.0. The media and glass wears (Petridishes, test tubes, cotton swabs, distilled water alcohol) needed for further use were sterilized by autoclave at 15 psi at 121℃ for 20 minutes. Essential oil were accurately weighted and dissolved in sterile 95% ethanol to give appropriate dilutions of about 2, 4, 6, 8 and 10 mg/mL to yield the required concentrations. The stock solutions were stored at -20℃. The Zedoary turmeric oil obtained from rhizomes of *C. phaeocaulis* showed antimicrobial activity by using the agar well diffusion method. The inoculation was carried out in a laminar air-flow. Briefly 25ml quantities of nutrient agar were plated into the Petri dishes and allowed them to cool and solidified for 40 minutes. After solidification of the media, the bacterial strains were inoculated by swabbing method. Wells of 6 mm in diameter and 4 cm apart were made in the culture media by using sterilized cork borer to make four uniform wells in each plate. A drop of molten nutrient agar was used to seal the bases of each well. These wells were filled with 50μl of essential oil by using micropipette and were allowed to diffuse for 40 minutes. The antimicrobial activities were determined after 24 hours of incubation at 37℃ in incubator. The antimicrobial activities were measured from the diameter of the inhibition zone form by the essential oil around the well. The zone diameter of inhibition is produced by the essential oil inhibition zone. Each sample was used in triplicate for the determination of antimicrobial activity (Burt, S. 2004).

3. RESULTS AND DICUSSION

3.1 Chemical composition of the essential oils from C. phaeocaulis leaves

The essential oil yield of *C. phaeocaulis* obtained from hydrodistillation of leaves was 0.93%. (Gauvin et al. 2004) have quantified the yield of leaves essential oils, which were between 0.1% and 0.3%. The yield of our work was far superior to that of (Gauvin et al. 2004).

As shown in the Table 1, 36 compounds, representing about 77.41% of the essential oils were characterized. The oil contained a complex mixture mainly of monoterpene hydrocarbons and oxygen containing mono- and sesquiterpenes. The major compounds that were identified by GC–MS were Eucalyptol (26.63%), L-linaloool (6.32%), Camphor (5.22%) and beta- Pinene (4.60%), along with some other minor components presented in trace amounts. Volatile oils are very complex mixtures of compounds. The constituents of the oils were mainly monoterpenes and sesquiterpines, which are hydrocarbons with the general formula (C5H8)n. The antioxidant activity of essential oils is of considerable interest as these may preserve foods from the toxic effects of oxidants (Maestri et al. 2006). Moreover, essential oils have the ability to scavenge free radicals and play an important role in the prevention of some diseases such as brain dysfunction, cancer, heart disease and immune system decline (Kamatou et al. 2010). The high concentration of a-pinene in *C. phaeocaulis* oil makes it potentially useful in medicines because this exhibits antibacterial, antifungal, anti-inflammatory, insecticidal and antioxidant properties, and it is used traditionally as flavoring agent and antimicrobial material in food (Hajji et al. 1993;

Tantaoui-Elaraki et al. 1993). The present results agree with those of Ayyanar and Subash-Babu (Ayyanar et al. 2012) who also reported that the essential oils isolated from the freshly collected *C. phaeocaulis* leaves contain a-pinene, camphene, b-pinene, myrcene and limonene as a major compounds.

Table 1.Chemical composition of the leaves from *Curcuma phaeocaulis*

No.	Compounds	Mol.Form	%Area	Identification
1	alpha-Pinene	$C_{10}H_{16}$	2.68	MS, RI, co-GC
2	beta-Myrcene	$C_{10}H_{16}$	2.42	MS, RI, co-GC
3	beta- Pinene	$C_{10}H_{16}$	4.60	MS, RI, co-GC
4	Camphene	$C_{10}H_{16}$	1.43	MS, RI, co-GC
5	1,4-Cyclohexadiene	$C_{10}H_{16}$	0.82	MS, RI, co-GC
6	2-Nonanone	$C_9H_{18}O$	0.33	MS, RI, co-GC
7	2-Decanone	$C_{10}H_{20}O$	0.33	MS, RI
8	2-Dodecanone	$C_{12}H_{24}O$	0.33	MS, RI
9	Cyclohexene	$C_{10}H_{16}$	1.44	MS, RI
10	Thujone	$C_{10}H_{16}O$	1.08	MS, RI
11	Bicyclo	$C_{10}H_{16}O$	1.72	MS, RI
12	Borneol	$C_{10}H_{18}0$	0.72	MS, RI
13	3-Cyclohexen-1-ol	$C_{10}H_{18}0$	0.96	MS, RI
14	2-Adamantanone	$C_{10}H_{14}O$	0.20	MS, RI
15	2-(4-Methylphenyl)propan-2-ol	$C_{10}H_{14}O$	0.24	MS, RI
16	alpha-Terpineol	$C_{10}H_{18}O$	2.12	MS, RI
17	D-limonene	$C_{10}H_{16}$	2.86	MS, RI
18	Eucalyptol	$C_{10}H_{18}O$	26.63	MS, RI
19	L-linaloool	$C_{10}H_{18}O$	6.32	MS, RI
20	Camphor	$C_{10}H_{16}O$	5.22	MS, RI
21	alpha-Farnesene	$C_{15}H_{24}$	2.46	MS, RI
22	Cyclohexane	$C_{15}H_{24}$	3.51	MS, RI
23	Caryophyllene	$C_{15}H_{24}$	1.76	MS, RI
24	gamma-Elemene	$C_{15}H_{24}$	1.21	MS, RI
25	1,3-Cyclohexadiene	$C_{15}H_{24}$	0.36	MS, RI
26	Eudesma-4(14),11-diene	$C_{15}H_{24}$	0.23	MS, RI
27	Caryophyllene oxide	$C_{15}H_{24}$	0.07	MS, RI
28	Geranyl bromide	$C_{10}H_{17}Br$	1.46	MS, RI
29	Linalool oxide trans	$C_{10}H_{18}O_2$	1.40	MS, RI
30	2,4,6-Octatriene	$C_{10}H_{16}$	0.24	MS, RI
31	1,3-Cyclohexadiene,1,3,5,5-tetramethyl	$C_{10}H_{16}$	0.25	MS, RI
32	2(10)-Pinen-3-one	$C_{10}H_{14}O$	0.32	MS, RI
33	Isoborneol	$C_{10}H_{18}O$	1.08	MS, RI
34	Cyclooctadiene	$C_{12}H_{18}$	0.26	MS, RI
35	Isopinocarveol	$C_{10}H_{16}O$	0.23	MS, RI
36	beta-Elemene	$C_{15}H_{24}$	0.12	MS, RI
	Total		77.41	

The retention index (RI) was calculated using a homologous series of n-alkanes C9–C15.
Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector.
[a]Co-GC: co-injection with an authentic sample.

3.2 Antioxidant activities

3.2.1 Hydroxyl radical scavenging activity of essential oil

The DPPH is a stable free radical with a characteristic absorption maximum at 517 nm and thus, it is widely accepted as a tool for estimating the free radical-scavenging activity of antioxidants, the reaction mixture contained 0.1 mL of test samples and 0.1mL of a 1mmol/L solution of DPPH in ethyl alcohol. 0.1mL of 10 mmol/L solutions of ascorbic acid was used as positive control, (Hu et al., 2004; Li et al., 2011). Briefly, 2 mL of polysaccharide solution in the different concentration range was added to 2 mL of DPPH solution (0.2 mmol/L, in 95% ethanol solution). The mixture was shaken and reacted for 30 min at 25◦C in the dark. (Wang,Yang, & Wei, 2012; Yu et al., 2007) . The mechanism of scavenging DPPH radical is caused by the fact that natural compounds can transfer either an electron or a hydrogen atom

to DPPH (Naik et al., 2003). At this stage of reaction, absorbance is decreased and the solution changes from purple to light yellow.

The DPPH radical scavenging activity of essential oil were measured, and the results are shown in Fig. 1 (a), which depicts DPPH scavenging abilities of different concentrations of essential oil and is compared with Vc as a control standard. The scavenging ability of the essential oil were evident at all tested concentrations, and well correlated with increased concentration up to 10 mg/mL. The sample had weaker activities than Vc. At 10 mg/mL, the scavenging activities were at 84.31%.

3.2.2 Hydroxyl radical scavenging activity of essential oil

Hydroxyl radical, an extremely potent oxidant, has an ability to traverse the cell membranes, readily react with most biomolecules, and may cause damage to cell and tissue by producing hydroxyl radical via the Fenton reaction. Thus, removal of hydroxyl radicals protects tissues from oxidative damage (Yuan et al., 2008; Huang et al., 2012). Fig. 1(b) indicates scavenging activity is concentration dependent, with sample concentration ranging from 0 mg/ml to 10 mg/mL. After concentration of 10 mg/mL, scavenging ability on hydroxyl radical are keeping balance at 77.23%. The results indicated that essential oil had strong capability of scavenging hydroxyl radical, which was close to Vc.

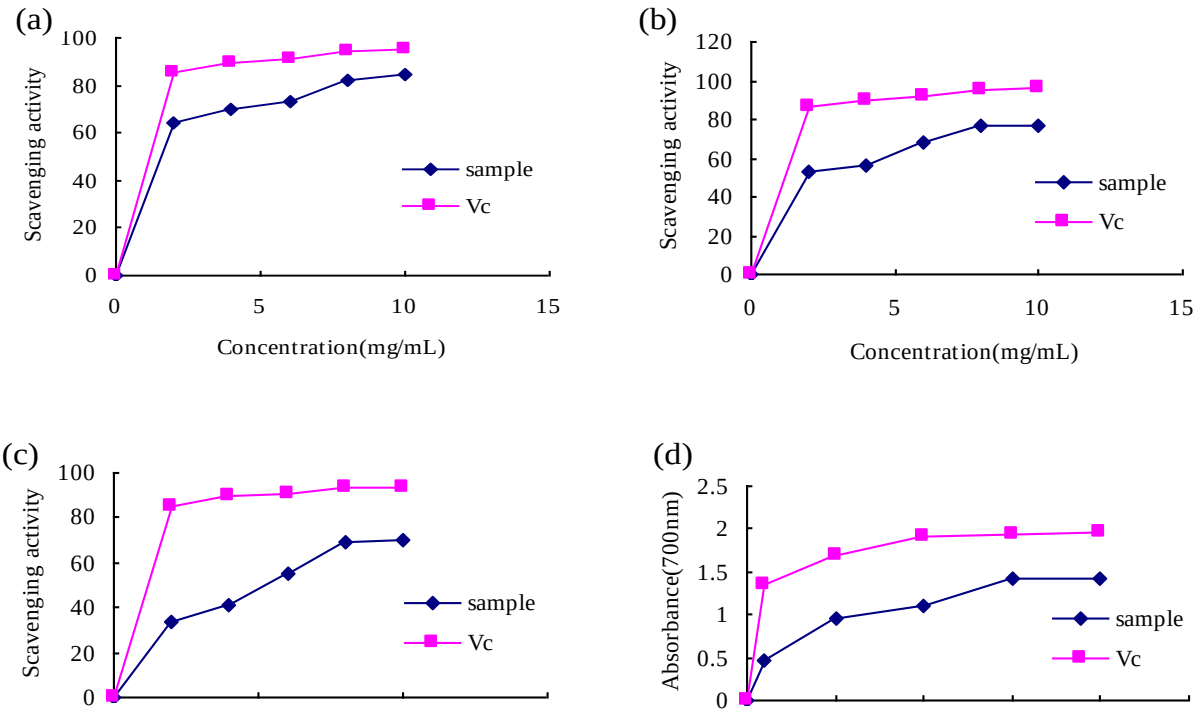

Fig.1(a) Radical scavenging effect of *Curcuma phaeocaulis* essential oil on DPPH, **(b)** Radical scavenging effect of *Curcuma phaeocaulis* essential oil on hydroxyl radical, **(c)** Radical scavenging effect of *Curcuma phaeocaulis* essential oil on superoxide anion, **(d)** Fe^{2+} chelating effect of *Curcuma phaeocaulis* essential oil.

3.2.3 Superoxide anion-scavenging activity of essential oil

Superoxide anion is generated first among different reactive oxygen species, it is one of the precursors of singlet-oxygenand hydroxyl radicals, which possess greater oxidative and oleophilic ability. By contributing to the production of highly reactive radical species, superoxide anions cause extensive cellular damage, induce lipid peroxidation (Athukorala, Kim, & Jeon, 2006). and induce pathological incidents, such as arthritis and Alzheimer's disease (Liu et al.,2010). Thus, scavenge superoxide radical is necessary.

The superoxide anion-scavenging activities are illustrated in Fig. 1(c). The superoxide anion-scavenging ability was measured by a PMS/NADM system for assay in the reduction of NBT. Scavenging activities of essential oil followed a dose-dependent manner at all tested concentrations. The superoxide radical scavenging effects of essential oil and Vc were 70.02% and 93.46%, respectively, at concentration of 10 mg/mL. Moreover, the results indicated that essential oil had a noticeable superoxide radical scavenging activity.

3.2.4 Reducing power of essential oil

The reducing power is an indicator of the molecule potential antioxidant potential (Kallithraka, Bakker, & Clifford, 2001). Higher absorbance value means stronger reducing power of samples, and the color of the mixture will be change from yellow to various of blue or green depending upon the reducing power of compound. As shown in Fig. 1(d), reducing power of essential oil and Vc was depicted. The reducing power of essential oil gradually increased with concentration, but was lower than that of Vc. The essential and Vc possessed strong reducing power values were 1.4332 and 1.9565, respectively, at the concentration of 10 mg/mL. Obviously, reducing power of essential oil was significant.

3.3 Antimicrobial activities of the essential oil samples against bacteria

Essential oil from the rhizome of *C. phaeocaulis* was applied to test the antibacterial activities against two different strains of Gram positive bacterial strains (*Staphylococcus aureus* and *Bacillus cereus*) and Gram negative bacterial strains (*Salmonella* and *Escherichia coil*). Essential oils have high resistance to bacteria from Table 2. The essential oil of fresh rhizomes showed stronger activity than that of dry rhizomes. Resistance of essential oils on the *Bacillus cereus* and *Escherichia coil* is relatively weak.

In the present investigation the methanol extract was found to be more effective on both Gram positive and Gram negative bacteria, and especially against Gram positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis*. Additionally, *Shyamala* and *Vasantha*, (Shyamala et al. 2010) stated that the *C. phaeocaulis* leaf extract showed activity against *Escherichia coli* and *Staphylococcus aureus* and the authors rendered the activity to the presence of essential oil constituents in the leaf extract.

Table 2.Antimicrobial activity of the essential oil of *Curcuma phaeocaulis* leaves

Microorganism used	**Different concentrations of oil the size of inhibition zone (mm)**				
	2(mg/mL)	**4(mg/mL)**	**6(mg/mL)**	**8(mg/mL)**	**10 (mg/mL)**
Staphylococcus aureus	8.67±0.02[a]	9.68±0.04[b]	11.08±0.03[c]	12.29±0.08[d]	15.34±0.02[e]
Bacillus cereus	9.32±0.04[a]	10.46±0.08[b]	12.43±0.02[c]	13.14±0.04[d]	14.86±0.05[e]
Salmonella	9.87±0.02[a]	10.32±0.05[b]	13.35±0.04[c]	14.83±0.06[d]	15.33±0.06[e]
Escherichia coli	6.67±0.03[a]	10.12±0.04[b]	10.15±0.03[b]	11.73±0.04[c]	13.91±0.03[d]

4. CONCLUSION

The findings of this study support the view that certain medicinal plants are promising sources of potential antioxidants. The extracts obtained using a high polarity solvent (ethanol), indicating that antioxidant or active compounds of different polarity could be present in leaves of *C. phaeocaulis*. There is a need for more research on the use of *C. phaeocaulis* essential oils and extracts as preservative agents in different foods. The results presented here should encourage the use of *C. phaeocaulis* leaves for medicinal health, functional food and nutraceuticals applications, due to their antioxidant and antibacterial properties. Those results can be considered as a preliminary study in order to show the importance and the originality of this endemic species.

5. ACKNOWLEDGEMENT

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