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ORIGINAL ARTICLE

Chemical composition and antioxidant properties of the essential oil of *Cinnamomum altissimum* Kosterm. (Lauraceae)

Siddig Ibrahim Abdelwahab [a,*], Abdalbasit Adam Mariod [b,*], Manal Mohamed Elhassan Taha [a], Faridah Qamaruz Zaman [c], Adil Hassan Ahmed Abdelmageed [d], Shamsul Khamis [c], Yasodha Sivasothy [e], Khalijah Awang [e]

[a] *Medical Research Centre, Jazan University, Jazan, Saudi Arabia*
[b] *College of Sciences & Art-Alkamil, King Abdulaziz University, Jeddah, Saudi Arabia*
[c] *Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia*
[d] *Faculty of Agriculture, University of Khartoum, Khartoum, Sudan*
[e] *Department of Chemistry, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia*

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Abstract The essential oil of the bark of *Cinnamomum altissimum* Kosterm. obtained by hydrodistillation was analyzed by capillary GC and GC–MS. The oil was characterized by linalool (36.0%), methyl eugenol (12.8%), limonene (8.3%), α-terpineol (7.8%) and terpinen-4-ol (6.4%). The content of total phenolics in the extracts was determined spectrometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalents (GAE). A total phenolic compound was 50.41 ± 0.98 μg GAE/mg oil. The extract displayed antioxidant activities, with an IC_{50} value of 38.5 ± 4.72 μg/ml, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and 345.2 ± 14.8 μM Fe (II)/g dry mass using ferric reducing/antioxidant power (FRAP) assay.

1. Introduction

Cinnamomum (Family: Lauraceae) is a genus of evergreen trees and shrubs. This genus contains about 250–350 species worldwide, distributed in tropical and subtropical regions of North America, Central America, South America, Southeast Asia, and Australia (Jayaprakasha et al., 2003; Rana et al., 2009; Wang et al., 2009). Approximately 21 species of this genus have been recognized in Peninsular Malaysia

* Corresponding authors. Address: Department of Biology, College of Sciences & Arts-Alkamil, King Abdulaziz University, P.O. Box 110, Alkamil 21931, Saudi Arabia. Tel.: +966 543524074.
E-mail addresses: siddigroa@yahoo.com (S.I. Abdelwahab), basitmariod@yahoo.com, aalnadif@kau.edu.sa (A.A. Mariod).
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(Kochummen, 1989). A number of these species are used in traditional medicine and as spices, especially in food, fragrances, fumigants, and traditional medicines (Burkill, 1966). The genus *Cinnamomum* commercially is known as cinnamon, and is considered as one of the oldest spices in the world (Jayatilaka et al., 1995). Cinnamon oil is widely used in the food processing, cosmetic, flavorings, confectionaries and pharmaceutical industries (Jayatilaka et al., 1995), to treat inflammatory diseases (Yu et al., 2007), and antifungal diseases (Jantan et al., 2008). The main compound in the cinnamon oil is cinnamaldehyde, which was found to have hypothermic and antipyretic actions. Broadhurst et al. (2000) stated that the cinnamon extracts impersonate the function of insulin, while, Jarvill-Taylor et al. (2001) noted that cinnamon extracts can potentiate insulin action in isolated adipocytes and also enhanced the insulin receptor function.

Cinnamomum altissimum Kosterm. (Lauraceae) is a medium size to fairly tall tree reaching 39 m in height and 1.5 m in girth. It is indigenous to Peninsular Malaysia and Sumatra and widely distributed in lowland and hill forests and rarely in mountain forests, up to 1800 m altitude (Kochummen, 1989). The leaves are opposite or sub-opposite, tri-nerved with a stalk 1–1.5 cm in length. The blade is thick, leathery, shiny above, slightly glaucous, elliptic or oblong, apex pointed or blunt, base cuneate or rounded with midrib and secondary nerves raised on the upper surfaces and restrictions faintly to distinctly visible below (Kochummen, 1989).

The composition of the essential oil of different *Cinnamomum* species has been widely investigated (Jantan and Goh, 1990; Jantan and Goh, 1992; Jantan et al., 2003; Rana et al., 2009; Abdelwahab et al., 2010; Geng et al., 2011). The oils were found to contain cinnamaldehyde, linalool, camphor, terpinen-4-ol and 1,8-cineole, eugenol, safrole, γ-muurolene, α-cadinol, germacrene D, α- terpineol, α-cadiene, 1,6-octadien-3-ol,3,7-dimethyl and 1-phenyl-propanr-2,2-diol diethanoate as major compounds (Jantan and Goh, 1990; Jantan and Goh, 1992; Jantan et al., 2005; Abdelwahab et al., 2010). Spasthulenol was found as the major compound in leaf oil of *C. altissimum* (Jantan et al., 2003).

The antioxidant activity of some *cinnamomum* species has been extensively investigated (Lin et al., 2003; Jayaprakasha et al., 2006; Ho et al., 2008; Prasad et al., 2009; Norazah et al., 2010; Abdelwahab et al., 2010). Several studies on the antimicrobial properties of the essential oils of some *cinnamomum* species has been reported (Jantan et al., 1994; Ali et al., 2002; Jantan et al., 2008; Abdelwahab et al., 2010). In addition, Jantan et al. (2005) reported that *C. altissimum* showed significant inhibitory effects on platelet aggregation. Although, the chemical constituents of leaf and bark essential oils of *C. altissimum* have been studied (Jantan et al., 2003), the antioxidant properties have yet not been studied. Hence, in the present work, an attempt has been made to explore the possible antioxidant properties by different methods which can give more comprehensive information. The present study aimed to examine the chemical composition of essential oil from *C. altissimum* bark and to investigate their antioxidant activities.

2. Materials and methods

2.1. Plant material

The bark of *C. altissimum*, identified by Assistant Professor Shamsul Khamis at the Unit of Biodiversity, Institute of Bioscience, Universiti Putra Malaysia, Malaysia, was collected from the Pahang state, Malaysia in 2011 and a voucher specimen (CA2011) has been deposited in the herbarium of Universiti Putra Malaysia.

2.2. Solvents and chemicals

Pentane (GC–MS grade) and the homologous series of *n*-alkanes (C_6–C_{30}) were purchased from Merck (Germany) and Dr. Ehrenstorfer Gmbh (Germany), respectively. Chemicals used in the antioxidant assay were obtained from Sigma Aldrich (Singapore).

2.3. Isolation of essential oils

Fresh homogenized bark (200 g) was hydrodistilled for 4 h in an all-glass apparatus similar to that described in the British Pharmacopoeia, using pentane as the collecting solvent. The solvent was carefully removed using a gentle stream of N_2, yielding colorless aromatic oil. The oil yield (w/w) was 1.9%, all on a fresh weight-basis.

2.4. Plant material

Fresh bark of *C. altissimum* was collected from the Pahang state, Malaysia in 2011. The plant was identified by Assistant Professor Shamsul Khamis at the Unit of Biodiversity, Institute of Bioscience, Universiti Putra Malaysia, Malaysia. The voucher specimen under the plant's name was deposited in the unit herbarium.

2.5. Solvents and chemicals

All solvents used were of analytical grade. Methanol, ethyl acetate, hexane, chloroform, butylated hydroxytoluene (BHT) and Folin–Ciocalteau reagent were obtained from Merck (Merck, Darmstadt, Germany).

2.6. Isolation of essential oils

Fresh bark (200 g) was separately hydrodistilled for 4 h in an all-glass apparatus similar to that described in the British Pharmacopoeia, using pentane as the collecting solvent. The solvent was carefully removed using a gentle stream of nitrogen gas, yielding yellow aromatic oils in each case. The oil yields (w/w) were 1.99%.

2.7. Gas chromatography (GC)

GC analysis was carried out using an Agilent 7890A GC System equipped with a FID and an Agilent 7683B Series auto-injector.

A HP-5MS UI (30 m × 0.25 mm id, film thickness 0.25 μm) thickness 0.25 μm) fused-silica capillary column (J.W. Scientific) was employed. Operating conditions were as follows: initial oven temperature, 50 °C for 5 min, then to 150 °C at 4 °C min^{-1} and held for 5 min, then to 250 °C at 4 °C min^{-1} and held for 10 min; injector and detector temperatures, 275 °C; carrier gas, 1.0 ml min^{-1} N_2; injection volume, 0.2 μL; split ratio, 50:1. Quantitative data were obtained electronically from FID area percent without the use of correction factors.

2.8. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was performed using a Shimadzu GCMS-QP 2010 equipped with a Combi Pal auto-injector coupled to a Shimadzu GCMS-QP 2010 Plus Mass Detector and the same capillary GC conditions as described above. Carrier gas used was helium. Significant MS operating parameters were: ionization voltage, 70 eV; ion source temperature 230 °C; mass range 50–600 u.

2.9. Identification of constituents

Constituents were identified by comparison of their mass spectra with those of authentic compounds or with reference spectra in the computer library (NIST 08), and confirmed by comparison of their retention indices with those of authentic compounds or with data in the literature (Adams, 2001; Sivasothy, et al. 2011).

2.10. Total phenolic content

TPC of *C. altissimum* was determined using the Folin–Ciocalteu method (Taga et al. 1984). Oil was prepared at a concentration of 10 mg/mL in methanol. Five microliters of this solution was transferred to a 96-well mircoplate (TPP, USA). To this, 80 μL of Folin–Ciocalteu reagent (1:10) was added and mixed thoroughly. After 5 min, 160 μL of sodium bicarbonate solution ($NaHCO_3$ 7.5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. Absorbance was measured at 765 nm using microplate reader (Molecular Devices, Sunnyvale, USA). The TPC was expressed as gallic acid equivalent (GAE) in μg/mg oil, obtained from the standard curve of gallic acid. The gallic acid standard curve was established by plotting concentration (mg/mL) versus absorbance (nm) ($y = 0.001x + 0.055$, $R^2 = 0.9975$), where y is absorbance and x is concentration in GAE ($n = 3$).

2.11. Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was modified from the method of Benzie and Strain (1996). The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl; and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ, and 2.5 ml of $FeCl_3{\cdot}6H_2O$. The temperature of the solution was raised to 37 °C before use. Extracts (10 μL) were allowed to react with 190 μl of the FRAP solution for 30 min in the dark. Colorimetric readings of the product ferrous tripyridyltriazine complex were taken at 593 nm. The standard curve was linear between 200 and 1000 μM $FeSO_4$. Results are expressed as μM Fe (II)/g dry mass and compared with those of positive controls.

2.12. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The scavenging activity of *C. altissimum* on DPPH was determined using the method of (Choi et al., 2002). Oil was tested at final concentrations ranging 0–50 μg/mL in methanol. One milliliter of 0.3 mM DPPH ethanol solution was added to 2.5 mL of sample solution of different concentrations to make the test solutions; while 1 mL of methanol was added to 2.5 mL of samples to make the blank solutions. The negative control (blank) consisted of 1 mL of DPPH solution plus 2.5 mL of methanol. These solutions were allowed to react at room temperature for 30 min in the dark. The absorbance values were measured at 518 nm and converted into percentage antioxidant activity using the following equation:

$$\%\text{Inhibition} = [(A_B - A_A)/A_B] \times 100$$

where: A_B: absorption of blank sample; A_A: absorption of tested samples. The IC_{50} of DPPH scavenging activity was determined.

2.13. Statistical analysis

Experimental values were expressed as the means ± standard deviation (SD) of the number of experiments indicated in the legends. Statistical significance was assessed using a one way analysis of variance (ANOVA) followed by a multiple comparison test (Tukey's post hoc test), where $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Composition of the essential oil

Table 1 lists the constituents identified in the bark oil of *C. altissimum*, the relative GC peak areas of these constituents and their experimental retention indices on the HP-5 MS UI column. Forty-nine compounds, constituting 92.9% of the sample were identified. The 27 monoterpenoids clearly dominated the volatile profile, contributing 76.3%, accounting for more than half of the sample, although this figure was largely due to linalool (36.0%), limonene (8.3%), α-terpineol (7.8%), terpinen-4-ol (6.4%), γ-terpinene (3.5%), α- terpinene (2.3%) and 1,8-cineole (2.3%). Except for methyl eugenol (12.8%), the remaining constituents were present at less significant amounts. A previous investigation of the bark oil by Jantan et al. (2003) revealed a higher content of sesquiterpenoids (42.9%). They identified fifty-one constituents among which twenty-three were common to the present study, and found much lower levels of linalool (25.3%), terpinen-4-ol (0.8%), α-terpineol (0.8%) and methyl eugenol (0.4%). They did not detect limonene, but reported the presence of spathulenol (8.9%), β-eudesmol (9.7%) and γ-eudesmol (3.8%). These marked differences in the composition of the bark oil determined by Jantan et al. (2003) from that of the present study could be attributed to the source, cultivation, vegetative stage and the growing season of the plant under investigation (Sari et al., 2006).

Table 1 Constituents present in the essential oils of the bark of *Cinnamomum altissimum* Kosterm.

No.	Constituent	RI (HP 5- MS)	Area (%)[a]
1	α-Thujene	926	0.3
2	α-Pinene[b]	932	1.6
3	Camphene[b]	947	0.1
4	Sabinene	972	1.7
5	β-Pinene[b]	975	1.2
6	Myrcene[b]	990	0.9
7	α-Phellandrene	1003	0.3
8	α-Terpinene[b]	1015	2.3
9	p-Cymene[b]	1023	0.4
10	Limonene	1028	8.3
11	1,8-Cineole	1030	2.3
12	Cis-β-ocimene	1037	0.1
13	Trans-β-ocimene	1047	0.1
14	γ-Terpinene	1058	3.5
15	Cis-sabinene hydrate[b]	1066	0.1
16	Trans-linalool oxide (furanoid)	1072	t
17	Terpinolene[b]	1087	1.0
18	Linalool[b]	1102	36.0
19	Fenchol[b]	1114	t
20	Trans-sabinene hydrate	1121	0.3
21	Trans-p-mentha-2-en-1-ol	1139	0.2
22	Borneol[b]	1167	0.1
23	Terpinen-4-ol[b]	1178	6.4
24	α-Terpineol[b]	1192	7.8
25	Trans-piperitol	1196	0.1
26	Nerol	1227	0.3
27	Geraniol[b]	1254	0.9
28	δ-Elemene[b]	1338	t
29	Eugenol	1357	1.4
30	α-Copaene	1376	0.1
31	10-Undecanal	1397	0.1
32	Methyl eugenol[b]	1403	12.8
33	β-Caryophyllene[b]	1420	0.4
34	Trans-α-bergamotene	1433	0.1
35	α-Humulene[b]	1453	0.8
36	Alloaromadendrene[b]	1461	0.2
37	γ-Muurolene	1476	0.1
38	Germacrene D	1482	0.3
39	β-Selinene[b]	1488	0.3
40	α-Selinene[b]	1498	1.1
41	Trans,trans- α-farnesene[b]	1507	0.1
42	γ-Cadinene	1514	0.1
43	δ-Cadinene[b]	1522	0.3
44	Trans-nerolidol[b]	1558	0.3
45	Caryophyllenyl alcohol	1569	0.1
46	Viridiflorol	1592	0.2
47	α-Muurolol	1641	0.6
48	α-Cadinol	1646	0.1
49	α-Bisabolol	1686	0.2
			92.9

[a] Percentage of total FID area obtained on HP-5 MS UI column, t = (< 0.05%).
[b] Previously reported by Jantan et al. (2003).

3.2. Antioxidant capacity

Hydro-distillation of fresh bark of CP, afforded colorless pleasant-smelling essential oil. The total phenolic content (TPC) of this essential oil was determined using Folin–Ciocalteu method and expressed in μg GAE/mg. Results presented in Table 1 showed that *C. altissimum* had TPC of 50.41 ± 0.98 μg GAE/mg oil. Mariod et al. (2012), reported a total phenolic of 170.4, and 167.9 g/kg plant extract as GAE for of *Annona squamosa*, and *Catunaregam nilotica* bark methanolic extracts, respectively. And 593.0 mg/g^{-1} for the methanolic extract from *Sclerocarya birrea* bark (Mariod et al. 2008).

Table 2 Antioxidant activities of *C. altissimum*.

Samples	FRAP value (μM Fe (II)/g dry mass)	TPC (μg GAE/mg oil)	DPPH scavenging activity (IC_{50} μg/ml)
Sample	345.2 ± 14.8	50.41 ± 0.98	38.5 ± 4.72
Gallic acid	2885.6 ± 164.2	–	8.25 ± 3.81
Ascorbic acid	461.1 ± 28.5	–	16.4 ± 2.71
Rutin	825.0 ± 38.4	–	12.5 ± 0.92
Querciten	2561.1 ± 15.7	–	7.13 ± 0.51

Sample and positive control were done in triplicate (n = 3).

3.2.1. FRAP

A simple, automated test measuring the ferric reducing ability of the essential oils, the FRAP assay, is presented as an accurate method for assessing "antioxidant power." Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. From Table 2 FRAP values of the sample is 345.2 ± 14.8 (μM Fe (II)/g dry mass). The FRAP value of the methanol extract of the bark of *C. altissimum* was significantly lower than that of gallic acid, ascorbic acid, quercetin and rutin. FRAP assay is widely used in the evaluation of the antioxidant component in dietary polyphenols (Luximon-Ramma et al., 2005). The FRAP assay is inexpensive, reagents are simple to prepare, results are highly reproducible, and the procedure is straightforward and speedy.

4. Conclusion

The composition of the essential oil of *C. altissimum* bark was linalool (36.0%), limonene (8.3%), α-terpineol (7.8%), terpinen-4-ol (6.4%), γ-terpinene (3.5%), α- terpinene (2.3%) and 1,8-cineole (2.3%) and methyl eugenol (12.8%). The extract showed high total phenolic with good antioxidant activity.

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