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Composition of essential oil and antioxidant activity of Khat (*Catha edulis* Forsk), Ethiopia

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In this study, we determined the chemical composition and antioxidant activities of the essential oils from two different varieties of khat (*Catha edulis* Forsk) cultivated in Ethiopia. The essential oils were extracted by hydrodistillation using the Clevenger type apparatus, identifications of compounds were made by gas chromatography and gas chromatography-mass spectrometry (GC-MS). Seventy seven different compounds were identified from essential oils of the two different khat cultivars. The essential oils in the samples from Bahir Dar and Wendo were composed of 50 and 34 compounds, respectively. The major compound identified in khat essentials oils include: limonene, 1-phenyl-1,2-propanedione, 1-hydroxy,1-phenyl-2-propanone, camphor, (sulfurous acid)-2-propylundecyl ester, hexadecane, O-mentha-1(7), 8-dien-3-ol, heptadecane, 10-methylnonadecane, (phthalic acid)-isobutyl octadecyl ester, and tritetracontane. The antioxidant and free radical scavenging activity of the oils were assessed by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. The scavenging activities of the oils were 23.5-23.6 µg AAE/kg of fresh khat sample.

Capsule Summary: The chemical composition of essential oils and free radical scavenging activities of two different cultivars of khat (*Catha edulis* Forsk) from Ethiopia were investigated.

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INTRODUCTION

Khat is the name generally used for (*Catha edulis* Forsk), a young leaves and shoots of a flowering evergreen tree or shrub that grows at high altitudes in the region extending from eastern to southern Africa, as well as in the Arabian Peninsula (Lamina, 2010; Sikiru, 2012). The leaves have an aromatic odor and the taste is astringent and slightly sweet (Cox and Rampes, 2003; Feyissa and Kelly, 2008). The most favored part of the plant is leaves, particularly the young shoots near the top of the plant. However, leaves and stems at the middle and lower sections are also used. Khat is chewed by millions of people on a daily basis for its stimulating property due to the presence of the phenylpropylamino alkaloids: cathinone, cathine, and norephedrine (Patel, 2000; Al-Thobhani et al., 2008; Al-Motarreb et al., 2010; Atlabachew et al., 2010).

In Ethiopia, depending upon geographical location, various chemotypes of khat are cultivated and these

chemotypes differ in color, size, height and size of the young shoots of the leaves and the plant as a whole and marketed under different names such as Gelemso, Gurage, Beleche, Wendo, Sebeta, Bahir Dar, Kuto, Berdaye, Anferara, Awedi, Abo Mismar, Colombia, Debo, Gurbicho, Magna (Atlabachew et al., 2011). Among all, some of them are regularly available in Addis Ababa and exported to the neighboring countries, while the others are chewed by the local people (Atlabachew et al., 2010). In Yemen, a study has been carried out on the geographical variability of more than 40 genotypes of khat using randomly amplified polymorphic DNA markers and found that some populations from different geographical regions and growth habitats showed a clear genetic differentiation and they were categorized in to three major clusters (Al-Thobhani et al., 2008). Thus, it is evident about the existence of different chemotypes of khat and hence quality of *Catha edulis*, depending on the geographical origin.

In addition to the stimulant alkaloids, khat contains several chemical constituents which includes terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and mineral (Feyissa and Kelly, 2008; Al-Thobhani et al., 2008; Al-Zubari et al., 2008; Atlabachew et al., 2014). Furthermore, there is one report published on the analysis of the essential oil of *Catha edulis* leaves from Yemen on only one chemotypes/genotype (Algabr et al., 2014). However, there is no report on essential oils composition of khat leaves from Ethiopia and elsewhere and their antioxidant activities. Herein, we aim to determine the chemical composition of the essential oils and antioxidant activity of khat (*Catha edulis* Forsk) from two different parts of Ethiopia.

MATERIAL AND METHODS

Plant material

Two varieties (Bahir Dar and Wendo) of fresh khat leaves were purchased at a local market of Addis Ababa. These two samples were cultivated at different geographical locations of the country. Cultivars were chosen due to their commercial relevance and for their potential increase in production and consumption by the local people in the country. The season of collection was from the beginning of January to March, 2013. Samples were processed in the laboratory of Chemistry Department, Addis Ababa University and stored in the refrigerator.

Isolation procedure

The essential oils were extracted according to the reported method (Meshkatalsadat et al., 2009). The leaves and the tender stem parts of the two khat samples (500 g) were subjected to hydrodistillation for 3 hours, using a Clevenger type apparatus. The isolated essential oils were dried over anhydrous sodium sulfate. The yields of essential oils isolated by hydrodistillation from the leaves of the two varieties of khat studied were very low (≤ 0.01%, v/w dry weight). Samples were stored in a refrigerator until gas chromatography-mass spectrometry (GC-MS) analysis (Atti-Santos et al., 2005; Wang et al., 2006; Meshkatalsadat et al., 2009).

Identification of components

GC-MS were used for identification and determination of the relative composition of each component. Essential oil constituents were identified based on their relative retention indeces and the relative peak area for individual constituents was determined in each of the two khat samples cultivated in Ethiopia (Atti-Santos et al., 2005).

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry analyses were performed on a GC-MS instrument (7890A, Agilent-Technologies, USA) with a HP-5MS Phenyl Methyl Silox (27 m in length, 250 µm in diameter, 0.25 µm in film thickness) with a stationary phase 5 MS SS and using He as carrier gas (1 mL/min). The mass spectrometer was operated in the 70 eV EI mode with scanning from 50 to 450 amu at 0.5 s, and mass source was set 250 ^{o}C, MS Quad, 200 ^{o}C. The identification of volatile constituent were based on GC retention indices (relative to n-alkanes from C_8 to C_{20}) and computer matching of their mass spectra fragmentation patterns with those stored in the Wiley mass spectra data base.

Antioxidant activity measurement

Antioxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method (Samarth et al., 2008). Solutions of 1.45 mM DPPH in methanol were prepared by adding 28.6 mg of DPPH to a 50 mL volumetric flask and diluting to the volume with methanol. In addition to this 20 mg of ascorbic acid standard was prepared by dissolving it into 150 mL distilled water and obtained 0.13 mg/mL of ascorbic acid. The calibration curve was constructed using five standard solutions of ascorbic acid (25, 50, 75, 100, and 200 µL) prepared in methanol. The ascorbic acid solutions mixed with DPPH reagent and methanol were transferred in a glass test tube. One hour later, the absorbance of the standard sample was measured at 517 nm using single beam UV spectrophotometer. The absorbance decreases because of a color change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule. Finally, the radical scavenging activities of the standards were measured.

Solutions of 30 µL of the sample essential oils in 3.47 mL of methanol were mixed with 0.5 mL of a 0.004% methanol solution of DPPH in a test tube to give a volume of 4.0 mL. After 1 hour incubation period at room temp.

Table 1: Chemical composition of the essential oils of two khat cultivar from Ethiopia

No.	Compounds	Relative index	Peak area (%)	
			Bahir Dar	Wendo
1	α-Phellandrene	1004	0.6	-
2	1R-α-Pinene	1025	0.6	-
3	Limonene	1026	30	-
4	cis-β-Ocimene	1040	0.9	-
5	Hexyl pentyl ether	1042	-	2.2
6	2,2,8-Trimetyl-decane	1121	-	2.4
7	δ-2-Carene	1134	0.5	-
8	3-Carene	1147	2.2	-
9	Oxalic acid, isohexyl pentyl ester	1162	-	1.0
10	2-Piperidinone, N-[4-bromo-N-butyl]	1163	-	1.4
11	1-Phenyl-1,2-propanedione	1171	11.6	-
12	n-Dodecane	1200	0.6	0.9
13	3,9,-Dimethyl-undecane	1233	-	2.5
14	γ-Terpinene	1245	1.3	-
15	1-Hydroxy,1-phenyl-2-propanone	1265	1.9	8.1
16	1-Chloroundecane	1279	-	1.2
17	1-(Ethenyloxyl)-octadecane	1316	-	0.9
18	Carvyl acetate, trans-	1337	0.6	-
19	Undecane, 3-Cyclohexyl-	1347	-	1.7
20	Ditetrdecyl ether	1354	-	0.6
21	α-Copaene	1376	0.6	-
22	7-Octenyl-oxirane	1398	-	0.6
23	Tetradecane	1400	0.7	-
24	1-Ethynyl-cyclohexanol	1403	-	1.6
25	Aromadendrene	1439	0.9	-
26	1-Chloro-dodecane	1446	-	0.9
27	Methoxyacetic acid,2-tetradecyl ester	1446	0.8	-
28	(+)-Epi-bicyclosesquiphellandrene	1458	1.6	-

Table 1: Continue…

No.	Compound	Relative index	Peak area (%)	
			Bahir Dar	Wendo
29	α-Cubebene	1460	1.5	-
30	2-Dodecanol	1469	-	0.6
31	Hexyl octyl ether	1497	-	0.9
32	Camphor	1515	6.3	-
33	β-Bourbonene	1523	2.4	-
34	2-Butyl-1-octanol	1528	-	1.5
35	β-Cubebene	1542	0.5	-
36	Sulfurous acid, 2-propylundecyl ester	1562	-	5.5
37	3-Hexen-1-ol benzoate	1569	0.3	-
38	Caryophyllene, Z-	1588	3.9	-
39	Oxirane,dodecyl	1590	0.2	-
40	2-Ethyl-1-dodecanol	1590	-	2.6
41	β-Elemene	1591	1.0	-
42	Caryophyllene, (E)-	1598	1.4	-
43	α-Caryophyllene	1599	0.6	-
44	Hexadecane	1600	1.9	5.7
45	2-*p*-Menthen-1-ol	1652	1.7	-
46	α-Humulene	1667	0.6	-
47	β-Humulene	1673	1.6	-
48	O-Mentha-1(7),8-dien-3-ol	1674	8.5	-
49	C-Muurolene	1690	1.7	-
50	Heptadecane	1700	1.0	5.1
51	Germacrene D	1708	0.3	-
52	α-Muurolene	1723	0.4	-
53	Cyclododecanol	1724	0.4	-
54	Valencene	1728	0.5	-
55	2,6,10,14-Tetramethyl-pentadecane	1755	0.5	-
56	δ -Cadinene	1756	0.9	-

Table 1: Continue…

No.	Compound	Relative index	Peak area (%)	
			Bahir Dar	Wendo
57	4-Cyclohexyl-undecane	1770	-	1.9
58	Octadecane	1779	0.4	-
59	2,10-Dimethyl-9-undecenol	1834	-	4.0
60	1-Nonadecene	1883	0.1	-
61	Nonadecane	1900	1.7	-
62	Nonadecane	1900	0.4	4.4
63	10-Methylnonadecane	1943	-	5.3
64	2-Chloro-acetophenone	1949	0.4	-
65	1-Chloro-octadecane	2099	-	1.4
66	Phytol	2121	0.1	-
67	Phthalic acid, isobutyl octadecyl ester	2157	0.3	6.0
68	1,1'-Oxybis-octane	2167	-	0.7
69	Hexadecanoic acid, methylester	2170	0.3	-
70	1-Eicosanol	2252	-	1.1
71	Phthalic acid, buthyl tetradecyl ester	2273	0.2	2.8
72	Acetic acid, trifluoro-tetradecyl ester	2277	-	1.8
73	(E)-5-Eicosene	2293	-	2.6
74	10-Methyl nonadecene	2404	0.1	-
75	n-Octacosane	2800	-	1.6
76	Tritetracontane	4300	-	12
77	Tetratetracontane	4301	0.2	-
	Total		97.4	93.5

The absorbance of the test sample was read against a blank at 517 nm. Samples were analyzed in triplicates. The radical scavenging activities of the tast samples were determined. Data were expressed as mean±SD.

RESULTS AND DISCUSSION

Chemical composition of khat essential oils are presented in Table 1. Seventy seven different compounds were identified in the essential oils from the two khat cultivars, accounting of 93.5–97.4% of the oils. The essential oils in the samples from Bahir Dar and Wendo type khat cultivars were composed of 50 and 34 identified compounds, respectively. It seems that there were little similarities but wide variations among the compositions of the studied essential oils obtained from the two varieties of the khat. The variation in the compositions of the essential oils might be attributed due to climatic and geographical variability of the sampling sites.

As shown in Table 1, 50 compounds were identified in the Bahir Dar type khat essential oil, of which, the major

Table 2: Scavenging effect (%) and concentrations (X±SD µg/kg) of khat essential oils (n = 3, AAE)

Name of khat samples	Scavenging effect (%)	µg AAE/kg khat
Bahir Dar	29.1 ± 1.2	23.5 ± 0.7
Wendo	29.5 ± 0.6	23.6 ± 0.4

AAE = Ascorbic Acid Equivalent.

constituents were limonene (30%), 1-phenyl-1,2-propanedione (11.6%), camphor (6.3%), O-mentha-1(7),8-dien-3-ol (8.5%), Z-caryophyllene (3.9%), β-bourbonene (2.4%), 3-carene (2.2%), β-humulene (1.6%) and α-cubebene (1.5%). While 34 compounds were identified in the Wendo type khat essential oils, of which the major components were hexyl pentyl ether (2.2%), 2,2,8-trimetyl-decane (2.4%), 3,9,-dimethyl-undecane (2.5%), 1-hydroxy,1-phenyl-2-propanone (8.1%), (sulfurous acid)-2-propylundecyl ester (5.5%), 2-ethyl-1-dodecanol (2.6%), hexadecane (5.7%), heptadecane (5.1%), 2,10-dimethyl-9-undecenol (4%), nonadecane (4.4%), 10-methylnonadecane (5.3%), phthalic acid, isobutyl octadecyl ester (6%), (phthalic acid)-buthyl tetradecyl ester, (2.8%), (E)-5-eicosene (2.6%) and tritetracontane (12%). The compositions of essential oils from khat from the two different parts of Ethiopia are different from each other and that reported from the Yemen (Algabr et al., 2014).

The antioxidant activities of essential oils of the two varieties of the khat were determined using DPPH radical scavenging assay method. Results were expressed as ascorbic acid equivalent (AAE) and shown in Table 2. From the results in Table 2, both types of khat had relatively good antioxidant activity (23.5 and 23.6 µg AAE/kg of dry matter). These results demonstrated that essential oil of khat has good antioxidant activity.

Reddy et al. (2012) reported that antioxidant activities of essential oils from medicinal plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them. Thus, even though the two types of the khat have different chemical composition, they have almost the same antioxidant activity.

CONCLUSIONS

The composition and antioxidant activity of essential oils from the leaves of khat cultivated in Ethiopia were evaluated. Seventy seven different compounds were identified in the essential oils from two different varieties of khat by GC-MS. The chemical compositions of essential oils from Ethiopian khat are quite different from that reported from Yeman. This study further revealed that both Bahir Dar and Wendo khat essential oil have comparable antioxidant activity.

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