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Antioxidant Activity and Characterization of Volatile Constituents of Taheebo (*Tabebuia impetiginosa* Martius ex DC)

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Volatiles were isolated from the dried inner bark of *Tabebuia impetiginosa* using steam distillation under reduced pressure followed by continuous liquid−liquid extraction. The extract was analyzed by gas chromatography and gas chromatography−mass spectrometry. The major volatile constituents of *T. impetiginosa* were 4-methoxybenzaldehyde (52.84 μg/g), 4-methoxyphenol (38.91 μg/g), 5-allyl-1,2,3-trimethoxybenzene (elemicin; 34.15 μg/g), 1-methoxy-4-(1*E*)-1-propenylbenzene (*trans*-anethole; 33.75 μg/g), and 4-methoxybenzyl alcohol (30.29 μg/g). The antioxidant activity of the volatiles was evaluated using two different assays. The extract exhibited a potent inhibitory effect on the formation of conjugated diene hydroperoxides (from methyl linoleate) at a concentration of 1000 μg/mL. The extract also inhibited the oxidation of hexanal for 40 days at a level of 5 μg/mL. The antioxidative activity of *T. impetiginosa* volatiles was comparable with that of the well-known antioxidants, α-tocopherol, and butylated hydroxytoluene.

KEYWORDS: Natural antioxidants; taheebo; volatile antioxidants; pau d'arco

INTRODUCTION

Tabebuia spp. (Bignoniaceae) are native to tropical rain forests throughout Central and South America. The herbal products obtained from the bark of tabebuia trees are called "taheebo", "lapacho", "pau d'arco", and "ipe roxo" (*1*). Taheebo is reported to be an astringent, anti-inflammatory, antibacterial, antifungal, diuretic, and laxative (*2*−*6*). Traditionally, taheebo has been used for treating ulcers, syphilis, gastrointestinal problems, candidiasis, cancer, diabetes, prostatitis, constipation, and allergies. Major constituents in bark extracts of *Tabebuia* spp. have been reported several times. They include furano-naphthoquinones (*7*−*9*), quinones (*10*), naphthoquinones (*11*), benzoic acid, benzaldehyde derivatives (*12*), cyclopentene dialdehyde (*13*), and flavonoids (*14*). Many studies on the biological and pharmacological effects of *Tabebuia* spp. extracts and their isolated compounds have been detailed. The antineoplastic and antitumor-promoting effects of *T. avellanedae* Lor. ex Griseb. (*15*, *16*), the cytotoxicity of *T. cassinoides* (*17*), and the antitumor activity of *T. barbata* (*18*) have been described. Also, the chloroform extract of *T. ochracea* ssp. *neochrysanta* showed cytoxicity and antimalarial activity (*9*). In addition, antinociceptive and antiedematogenic activities were reported for the aqueous extract of *T. avellanedae* Lor. ex Griseb (*19*).

Lapachol [2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione] and dehydro-α-lapachone (2,2-dimethylnaphtho[2,3-*b*]-pyran-5,10-dione), the major naphthoquinones of the heartwood of *T. avellanedae* Lor. ex Griseb, showed activity against different types of tumors (*20*−*22*). β-Lapachone (2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione) has also displayed activity against tumor cells (*23*). Its mechanism of action has been linked to reactive oxygen species generated by redox cycling (*24*, *25*), induction of apoptosis in tumor cells, and topoisomerase II-mediated DNA cleavage (*26*−*28*). A number of lapacho compounds have potent antiproliferative activity and cytotoxicity, whereas other lapacho-derived compounds appear to be promising as effective antipsoriatic agents (*29*).

To our knowledge, the only volatile constituents of taheebo that have been reported to date are 3,4-dimethoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde (vanillin), and 4-methoxybenzaldehyde (*12*). The antioxidant property of taheebo has also not been elucidated. In this study, the volatile constituents of an extract isolated from the dried inner bark of *T. impetiginosa* were identified by gas chromatography (GC) and gas chromatography−mass spectrometry (GC-MS). The antioxidant activity of the isolated volatiles was determined using two different methods.

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MATERIALS AND METHODS

Materials. The dried inner bark of *T. impetiginosa* Mart. ex DC was purchased from Frontier (Norway, IA).

Chemicals. Methyl linoleate (MeLo) was purchased from Nu-Chek-Prep, Inc. (Elysian, MN). *trans*-Anethole (99%), carvacrol (98%), *trans*-cinnamaldehyde (99+%), eugenol (99%), hexanal (98%), hexanoic acid (99.5+%), linalool (97%), menthol (99%), 2-methoxyphenol (98%), 4-methoxyphenol (99%), 2-methoxy-4-methytphenol (99%), thymol (98%), α-tocopherol (vitamin E; 97%), and undecane (99+%) were purchased from Aldrich Chemical Co. (Milwaukee, WI), and 2,6-di-*tert*-butyl-4-methylphenol (BHT; 99%) was obtained from Sigma Chemical Co. (St. Louis, MO). All of the previously mentioned chemicals were used without further purification. All organic solvents were received from Fisher Scientific Co., Ltd. (Fair Lawn, NJ).

Isolation of Volatile Constituents by Steam Distillation under Reduced Pressure (DRP) Followed by Continuous Liquid—Liquid Extraction. The dried inner bark of *T. impetiginosa* (50 g) was placed in a 3 L round-bottom flask with 1 L of deionized water. The solution was steam distilled at 55 °C for 3 h under reduced pressure (95 mmHg). The distillate (900 mL) was extracted with 100 mL of dichloromethane using a liquid−liquid continuous extractor for 6 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed in a rotary flash evaporator (Yamato Scientific, Tokyo, Japan). The distillation was stopped when the volume of extract was reduced to ~1 mL. The extract was transferred into a vial, the distillation flask was washed with a minimum amount of dichloromethane, and the washings were added to the vial. The solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.6 mL. This concentrated extract was used to determine antioxidant activities in both the conjugated diene and the aldehyde/carboxylic acid assays.

Determination of Total Volatile Constituents in Extracts. Dichloromethane was removed using a purified nitrogen stream until the total volume was reduced to ~100 μL. The mass of extract was measured using an analytical balance. The extract was then analyzed by GC, using a flame ionization detector (FID), to determine the percentage of total peak area of volatile components and solvent. The total mass of volatile components was calculated by multiplying the percentage representing the total peak area of components by the total mass of extract. Each experiment was repeated three times. The detector response to solvent was found to be linear over a range of 0.2−1.0 μL injected, with an R^2 value of 0.99. A Hewlett-Packard (HP) model 6890 GC equipped with a 30 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-Wax bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and an FID was used for analysis of total volatile components in each extract. The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 1:20. The injector and detector temperatures were 250 °C. The oven temperature was programmed from 50 to 180 °C at 3 °C/min and held for 20 min.

Identification of Volatile Constituents Isolated from Taheebo. Volatile compounds obtained by DRP followed by continuous liquid−liquid extraction were identified by comparison of the compound's Kovats index, *I* (*30*), and mass spectrum with that of a reference standard.

Capillary Gas Chromatography. An HP model 5890 GC, equipped with a 60 m × 0.32 mm i.d. (d_f = 0.25 μm) DB-1 bonded-phase fused-silica capillary column (J&W Scientific) and an FID, was used for determination of Kovats indices. The injector and detector temperatures were 170 and 250 °C, respectively. The oven temperature was programmed from 35 °C (4 min isothermal) to 230 °C (held for 25 min at final temperature) at 2 °C/min. The linear velocity of the helium carrier gas was 36 cm/s (30 °C) at a split ratio of 1:20. An HP model 5890 GC, equipped with a 60 m × 0.32 mm i.d. (d_f = 0.25 μm) DB-Wax bonded-phase fused-silica capillary column and an FID, was also used for determination of Kovats indices. The injector and detector temperatures were 170 and 250 °C, respectively. The oven temperature was programmed from 30 °C (4 min isothermal) to 170 °C (held for 25 min at final temperature) at 2 °C/min and then to 210 °C (held for 10 min at the final temperature) at 10 °C/min. The linear velocity of the helium carrier gas was 35 cm/s (30 °C) at a split ratio of 1:20.

Capillary Gas Chromatography—Mass Spectrometry (GC-MS). Two systems were employed. The first system consisted of an HP 6890 gas chromatograph coupled to an HP 5973 quadrupole mass spectrometer (capillary direct interface). A 60 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-1 bonded-phase fused-silica capillary column was used. Helium carrier gas was used at a column head pressure of 22 psi. The oven temperature was programmed from 35 °C (4 min isothermal) to 220 °C (held for 15 min at final temperature) at 2 °C/min. The second system consisted of an Agilent 6890 gas chromatograph coupled to an Agilent 5973N quadrupole mass spectrometer (capillary direct interface). A 60 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-Wax bonded-phase fused-silica capillary column was used. Helium carrier gas was used at a column head pressure of 22 psi. The oven temperature was programmed from 30 °C (4 min isothermal) to 170 °C (held for 25 min at final temperature) at 2 °C/min and then to 195 °C (held for 10 min at the final temperature) at 5 °C/min.

Oxidation of Methyl Linoleate. The test was performed as previously described (*31*) with some modification. Briefly, extracts were tested at various concentrations (125, 250, 500, and 1000 μg/mL) by addition to methyl linoleate (MeLo; 1 g) in 16 mL screw-cap amber glass vials (National Scientific, Lawrenceville, GA). Solvent was removed by a purified nitrogen stream (~20−25 s). Oxidation of MeLo was carried out in the dark at 40 °C in a shaking water bath (Lab-line Instruments, Inc., model 3545, Melrose Park, IL). Sample aliquots (10 mg) were taken at regular intervals and dissolved in 5 mL of 2,2,4-trimethylpentane (isooctane) for spectrophotometric measurements (Hewlett-Packard 8453 UV−vis spectrophotometer) of conjugated diene absorption at 234 nm. Isooctane was used as the blank. All analyses were carried out in triplicate. The amount of hydroperoxides was calculated using absorptivity of 26000 (*32*). The antioxidant activity was expressed as percentage inhibition of formation of conjugated diene hydroperoxides calculated at the times when the amount of hydroperoxides had reached 400 and 800 mmol/kg MeLo in the control sample. Moreover, α-tocopherol and BHT, known lipid antioxidants, were used in each experiment as control antioxidants.

Aldehyde/Carboxylic Acid Assay. The inhibitory effect of each extract on the oxidation of aldehyde to carboxylic acid was determined according to previously published methods (*33*). Various amounts of extract and components were added to 2 mL of a dichloromethane solution of hexanal (3 mg/mL) containing 0.2 mg/mL of undecane as a GC internal standard. The oxidation of the sample solution was initiated by heating at 60 °C for 10 min in a sealed vial. The vials were then stored at room temperature. The headspace of each vial was purged with pure air (1.5 L/min, 3 s) every 24 h for the first 10 days. The decrease in hexanal was monitored at 5-day intervals. Standards of BHT and α-tocopherol were also examined for their antioxidative activity using the same methodology. The quantitative analysis of hexanal was conducted according to an internal standard method (*34*). All analyses were carried out in triplicate. An HP model 5890 GC equipped with a 30 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-1 bonded-phase fused-silica capillary column and an FID was used for analysis of hexanal. The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 1:20. The injector and detector temperatures were 300 and 280 °C, respectively. The oven temperature was programmed from 40 to 180 °C at 4 °C/min and held for 10 min at the final temperature.

RESULTS AND DISCUSSION

Measurement and Identification of Volatile Constituents Isolated from Taheebo. The total yield of volatile constituents, isolated by DRP followed by continuous liquid−liquid extraction from taheebo, was 0.039 ± 0.002% (w/w; n = 3). Volatile constituents of *T. impetiginosa* were identified by comparison of the compound's Kovats index, *I*, and mass spectrum with that of a reference standard. **Table 1** shows the components identified and their concentrations. The concentration of each constituent was calculated using the following equation:

$$\text{concn } (\mu\text{g/g}) = \frac{\text{wt of volatiles} \times \text{GC peak area \%}/100 \text{ (mg)}}{\text{wt of dried inner bark of } \textit{T. impetiginosa} \text{ (50 g)}}$$

Table 1. Volatile Constituents Identified in the Dried Inner Bark of *T. impetiginosa*

constituent	I^{DB-Wax} exptl	I^{DB-Wax} ref	I^{DB-l} exptl	I^{DB-l} ref	% area[a] I^{DB-Wax}	concn[b] (μg/g)
1-octen-3-ol	1449	1448	969	962	0.352	1.37
2-furancarboxaldehyde (furfural)	1452	1456	798	800	0.632	2.45
1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (camphor)	1500	1513	1126	1118	0.179	0.69
benzaldehyde	1503	1516	922	926	0.245	0.95
3,7-dimethyl-1,6-octadien-3-ol[c] (linalool)	1545	1546	1078	1083	4.200	16.29
3,7-dimethyl-1,6-octadien-3-yl acetate (linalyl acetate)	1550	1552		1240	0.245	0.95
4-isopropyl-1-methyl-1-cyclohexen-4-ol (terpinen-4-ol)	1593	1600	1155	1159	0.706	2.74
5-methyl-2-(1-methylethyl)cyclohexanol[c] (menthol)	1632	1641	1152	1155	0.669	2.59
2-isopropylidene-5-methylcyclohexanone (pulegone)	1635	1645		1211	0.447	1.73
1-methoxy-4-(2-propenyl)benzene (estragole)	1659	1664	1170	1173	1.987	7.71
α,α,4-trimethyl-3-cyclohexene-1-methanol (α-terpineol)	1687	1687	1167	1170	0.371	1.44
1,2-dimethoxybenzene	1717	1721	1110	1111	0.946	3.67
2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one[c] (carvone)	1719	1733	1209	1213	5.370	20.83
1,4-dimethoxybenzene	1727	1735	1129	1131	1.710	6.63
4-isopropylbenzaldehyde	1765	1777		1210	0.446	1.73
1-methoxy-4-(1*E*)-1-propenylbenzene[c] (*trans*-anethole)	1815	1823	1255	1259	8.700	33.75
(*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol)	1845	1848		1234	0.506	1.96
2-methoxyphenol[c] (guaiacol)	1849	1855	1055	1058	0.893	3.46
benzenemethanol (benzyl alcohol)	1868	1874	1002	1004	0.519	2.01
benzeneethanol (2-phenylethanol)	1902	1910	1068	1081	1.318	5.11
2-methoxy-4-methylphenol[c]	1945	1952	1160	1164	1.307	5.07
caryophyllene oxide	1968	1968	1555	1566	0.389	1.51
phenol	1996	2000	963	957	0.939	3.64
4-allyl-1,2-dimethoxybenzene[c] (*O*-methyleugenol)	2004	2011	1368	1370	0.243	0.94
4-methoxybenzaldehyde[d]	2008	2024	1205	1211	13.620	52.84
3-phenyl-2-propenal[c] (cinnamaldehyde)	2021	2039	1222	1227	1.680	6.52
3-phenylpropanol	2035	2046	1195	1197	0.316	1.23
4-methylphenol[c]	2085	2085	1048	1051	0.244	0.95
4-allyl-2-methoxyphenol[c] (eugenol)	2152	2165	1323	1327	0.964	3.74
2-isopropyl-5-methylphenol[c] (thymol)	2178	2182	1268	1268	0.321	1.24
5-isopropyl-2-methylphenol[c] (carvacrol)	2206	2215	1276	1277	0.657	2.55
5-allyl-1,2,3-trimethoxybenzene (elemicin)	2217	2218	1516	1516	8.804	34.15
4-methoxybenzyl alcohol	2262	2282	1240	1244	7.807	30.29
3,4-dimethoxybenzaldehyde[d]	2384	2385	1423	1422	1.370	5.31
4-methoxyphenol[c]	2391	2400	1184	1185	10.030	38.91
3,4-dihydro-8-hydroxy-3-methylisocoumarin[e]	2440		1483		1.374	5.33

[a] Peak area percentage of total FID area excluding the solvent peaks. [b] Values are based on the dried weight of taheebo. [c] Tested for antioxidant activity with methyl linoleate. [d] Previously identified in *Tabebuia avellanedae* by Wagner et al. (*12*). [e] Tentatively identified.

Over 50 peaks were observed in the GC chromatograms of taheebo extract. Thirty-five volatile constituents were identified. The identified volatiles consisted of 11 monoterpenoids, 7 phenylpropanoids, 5 phenolics, 3 aromatic alcohols, 4 aromatic aldehydes, and 5 miscellaneous compounds. The most abundant monoterpenoid was carvone [(2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one) (20.83 μg/g)]. Other monoterpenoids were linalool (16.24 μg/g), terpinen-4-ol (2.79 μg/g), menthol (2.59 μg/g), carvacrol (2.55 μg/g), geraniol (1.96 μg/g), pulegone (1.73 μg/g), α-terpineol (1.44 μg/g), thymol (1.24 μg/g), linalyl acetate (0.95 μg/g), and camphor (0.69 μg/g). Seven phenylpropanoids, elemicin (34.15 μg/g), *trans*-anethole (33.75 μg/g), estragole (7.71 μg/g), cinnamaldehyde (6.52 μg/g), eugenol (3.74 μg/g), 3-phenylpropanol (1.23 μg/g), and *O*-methyleugenol (0.94 μg/g), were identified. Other phenolics identified included 4-methoxyphenol (38.91 μg/g), 2-methoxy-4-methylphenol (5.07 μg/g), phenol (3.64 μg/g), 2-methoxyphenol (3.46 μg/g), and 4-methylphenol (0.95 μg/g). Aromatic alcohols characterized were 4-methoxybenzyl alcohol (30.29 μg/g), 2-phenylethanol (5.11 μg/g), and benzyl alcohol (2.01 μg/g). Aromatic aldehydes were 4-methoxybenzaldehyde (52.84 μg/g), 3,4-dimethoxybenzaldehyde (5.31 μg/g), 4-isopropylbenzaldehyde (1.73 μg/g), and benzaldehyde (0.95 μg/g). Also, five miscellaneous compounds, 1,4-dimethoxybenzene (6.63 μg/g), 1,2-dimethoxybenzene (3.67 μg/g), 2-furancarboxaldehyde (2.45 μg/g), caryophyllene oxide (1.51 μg/g), and 1-octen-3-ol (1.37 μg/g), were identified.

Table 2. Inhibitory Effects of Different Concentrations of Taheebo Extract on the Oxidation of Methyl Linoleate at 40 °C

concn (μg/mL)	inhibitory effect[a] (%) day 5	inhibitory effect[a] (%) day 10
1000	86.0 ± 0.75	68.2 ± 0.87
500	65.2 ± 0.68	43.4 ± 1.63
250	34.9 ± 0.52	13.5 ± 0.95
125	16.6 ± 0.43	0.5 ± 1.09
α-tocopherol (10 μg/mL)	54.5 ± 1.07	31.7 ± 1.12
BHT (10 μg/mL)	97.9 ± 0.23	51.4 ± 1.17

[a] Values are mean ± standard deviation ($n = 3$).

Antioxidant Activity of Volatile Extract and Constituents of Taheebo in the Conjugated Diene Assay. Table 2 shows the inhibitory effects (percent) of various concentrations of taheebo extracts (125, 250, 500, and 1000 μg/mL) on the formation of conjugated diene hydroperoxides from MeLo. The values are mean ± standard deviation ($n = 3$). The extract exhibited dose-dependent inhibitory activity. At a concentration of 1000 μg/mL, the volatile extract of taheebo inhibited the formation of conjugated diene hydroperoxides by 86.0 and 68.2% after 5 and 10 days, respectively. A concentration of 500 μg/mL was relatively less active, inhibiting oxidation by 65.2 and 43.4% after 5 and 10 days, respectively. Taheebo extract showed much lower antioxidant activity than the control

Table 3. Inhibitory Effects of Various Taheebo Phenolics on the Oxidation of Methyl Linoleate at 40 °C

volatile constituent	concn (μg/mL)	inhibition[a] (%) day 5	inhibition[a] (%) day 10
4-methoxyphenol	500	95.2 ± 0.86	89.4 ± 0.96
	100	76.9 ± 0.42	31.0 ± 1.28
	50	43.6 ± 0.66	17.6 ± 1.16
	10	17.2 ± 1.52	6.8 ± 0.92
	5	8.1 ± 1.43	0.9 ± 0.37
2-methoxy-4-methylphenol	500	95.8 ± 0.64	91.1 ± 0.93
	100	93.0 ± 1.43	85.5 ± 1.85
	50	88.2 ± 0.25	52.4 ± 1.30
	10	31.3 ± 1.04	14.3 ± 2.31
	5	11.2 ± 0.77	6.4 ± 1.68
2-methoxyphenol	500	96.1 ± 0.33	90.3 ± 1.35
	100	74.8 ± 1.59	32.3 ± 1.20
	50	32.8 ± 1.95	15.5 ± 0.61
	10	9.5 ± 0.94	5.2 ± 0.95
	5	2.8 ± 0.63	
α-tocopherol	10	54.5 ± 1.07	31.7 ± 2.02
BHT	10	97.5 ± 0.19	51.4 ± 1.17

[a] Values are mean ± standard deviation ($n = 3$).

Table 4. Inhibitory Effects of Various Taheebo Phenylpropanoids on the Oxidation of Methyl Linoleate at 40 °C

volatile constituent	concn (μg/mL)	inhibition[a] (%) day 5	inhibition[a] (%) day 10
trans-anethole	500		
cinnamaldehyde	500		
4-allyl-2-methoxyphenol (eugenol)	500	96.2 ± 0.43	93.0 ± 0.51
	100	87.1 ± 0.48	63.4 ± 0.92
	50	65.3 ± 0.29	40.7 ± 1.15
	10	21.7 ± 0.31	16.4 ± 0.91
	5	16.6 ± 0.52	6.9 ± 0.55
4-allyl-1,2-dimethoxybenzene (*O*-methyleugenol)	500	59.3 ± 0.51	37.1 ± 1.04
	100	20.5 ± 0.67	15.6 ± 0.84
	50	12.2 ± 0.76	6.2 ± 1.62
	10	9.9 ± 1.11	1.8 ± 0.82
	5	6.5 ± 0.56	
α-tocopherol	10	54.5 ± 1.07	31.7 ± 2.02
BHT	10	97.5 ± 0.19	51.4 ± 1.17

[a] Values are mean ± standard deviation ($n = 3$).

antioxidants, α-tocopherol and BHT, in this assay. BHT had higher antioxidant activity than taheebo extract at a concentration 50 times lower (500 vs 10 μg/mL), inhibiting oxidation by 51.4% after 10 days, whereas α-tocopherol had slightly lower activity, inhibiting oxidation by 31.7% after 10 days.

The antioxidant activity of individual volatiles was tested to assess their contribution to overall activity. Of the three phenolic compounds tested, 2-methoxy-4-methylphenol had the strongest antioxidant activity, inhibiting the oxidation of MeLo by 91.1, 85.5, and 52.4% at 500, 100, and 50 μg/mL, respectively. 2-Methoxyphenol and 4-methoxyphenol possessed lower antioxidant activities. The antioxidant activity of various phenylpropanoids is shown in **Table 4**. Eugenol had an activity similar to that of 2-methoxy-4-methylphenol, inhibiting oxidation by 93.0, 63.4, and 40.7% at 500, 100, and 50 μg/mL, respectively. It was previously demonstrated that the antioxidant activity of clove extract is mainly due to its high content of eugenol (*35*). Methylation of the OH group had a dramatic effect on antioxidant activity as *O*-methyleugenol had ~10 times lower activity than eugenol. *trans*-Anethole and cinnamaldehyde were ineffective antioxidants in this assay. **Table 5** shows the antioxidant activity of various monoterpenoids. Carvacrol and thymol both displayed rather weak inhibitory effects despite the presence of a phenolic group. Carvacrol had a slightly higher antioxidant activity than thymol in this assay, in contrast to the results of Yanishlieva et al. (*36*), who found that thymol was a more effective and more active antioxidant than carvacrol in controlling the autoxidation of triglycerides at room temperature. Schwarz et al. (*37*) reported that thymol and carvacrol both possessed weak and similar antioxidant activities in both the Rancimat method (determines the induction period by measuring an increase in volatile acidic byproducts released from oxidizing fat) at 100 °C and the Schaal test (measures the formation of hydroperoxides) at 60 °C. Carvone, linalool, and menthol were ineffective in inhibiting the oxidation of MeLo.

Table 5. Inhibitory Effects of Various Taheebo Monoterpenoids on the Oxidation of Methyl Linoleate at 40 °C

volatile constituent	concn (μg/mL)	inhibition[a] (%) day 5	inhibition[a] (%) day 10
carvone	500		
linalool	500		
menthol	500		
2-isopropyl-5-methylphenol (thymol)	500	10.9 ± 1.27	2.5 ± 1.04
	100	1.7 ± 1.57	
	50	0.3 ± 0.56	
5-isopropyl-2-methylphenol (carvacrol)	500	18.9 ± 1.65	12.5 ± 1.29
	100	14.8 ± 0.28	9.4 ± 1.16
	50	7.8 ± 0.59	0.7 ± 1.01
	10	1.2 ± 0.84	
	5	0.4 ± 0.29	
α-tocopherol	10	54.5 ± 1.07	31.7 ± 2.02
BHT	10	97.5 ± 0.19	51.4 ± 1.17

[a] Values are mean ± standard deviation ($n = 3$).

Antioxidant Activity of Volatile Extract Isolated from Taheebo in the Aldehyde/Carboxylic Acid Assay. The aldehyde/carboxylic acid assay is a simple and robust assay for measuring the antioxidant activity of volatile chemicals or extracts. This method is based on the autoxidation of aldehydes to carboxylic acids with active oxygen species such as a hydroxy radical (*38*, *39*). In this assay, volatile extract dissolved in organic solvent, such as dichloromethane, can be easily measured for antioxidant potential. This assay is also suitable to evaluate long-term antioxidant potential of natural antioxidants because either aldehyde oxidation or carboxylic acid formation is monitored over 1 month. This method has been used to measure the antioxidant activity of natural volatile extracts isolated from beans, herbs, and spices (*35*, *40*). The method has also been validated with various volatile chemicals and typical antioxidants such as BHT and α-tocopherol (*41*, *42*).

The percentage of hexanal remaining in solutions treated with different amounts of volatile extract, α-tocopherol, and BHT is shown in **Table 6**. All samples exhibited dose-dependent inhibitory activity between concentrations (1−200 μg/mL) and antioxidant activity. After 30 days, control samples exhibited >94% oxidation of hexanal to hexanoic acid. Hexanal was completely oxidized to hexanoic acid on and after 40 days. The volatile extract of taheebo inhibited hexanal oxidation by >99% at concentrations from 5 to 200 μg/mL. However, the percentage of hexanal remaining at a concentration of 1 μg/mL decreased

Table 6. Inhibitory Effects of Different Amounts of Volatile Extract, α-Tocopherol, and BHT on the Oxidation of Hexanal throughout a Storage Period of 40 Days at Room Temperature

concn (μg/mL)	inhibitory effect[a] (%)		
	taheebo	α-tocopherol	BHT
0	4 ± 1.2	2 ± 0.2	5 ± 2.0
1	9 ± 2.1	20 ± 9.4	99 ± 3.0
5	99 ± 3.4	89 ± 0.6	99 ± 0.8
10	99 ± 5.4	92 ± 2.2	99 ± 0.7
20	100 ± 2.3	97 ± 2.3	100 ± 0.6
50	100 ± 1.3	98 ± 0.2	100 ± 0.1
100	100 ± 2.9	99 ± 1.3	100 ± 0.1
200	100 ± 5.8	100 ± 1.5	100 ± 0.1

[a] Values are mean ± standard deviation ($n = 3$).

to 9%. The inhibitory effect of taheebo at 5 μg/mL was comparable to that of BHT, which showed >99% inhibition at concentrations varying from 5 to 200 μg/mL. At concentrations of 5 and 10 μg/mL, the antioxidant activity of taheebo was greater than that of α-tocopherol, which inhibited hexanal oxidation by 89 and 92%, respectively.

Comparison of Taheebo Extract Activity in the Two Antioxidant Assays. Taheebo extracts displayed dose-dependent activity in both antioxidant assays. A higher concentration of extract was required to inhibit oxidation in the conjugated diene assay compared to the hexanal/hexanoic acid assay. Much of this effect can be explained by the large difference in substrate concentrations, methyl linoleate (3.4 mmol/mL) versus hexanal (30 μmol/mL), used in the two assays. This resulted in a 113-fold difference in molar substrate concentration between the two assays. However, the extract appeared to be more active in the hexanal/hexanoic acid assay than in the conjugated diene assay when compared to α-tocopherol and BHT. At extract concentrations of 5 mg/mL and above, oxidation was effectively inhibited in the hexanal/hexanoic acid assay, whereas at concentrations of 1 mg/mL the extract displayed about half of the inhibitory activity of α-tocopherol and 11 times less inhibitory activity than BHT. In contrast, despite a 12-fold higher concentration (125 vs 10 mg/mL), the extract displayed 63 times less activity than α-tocopherol and 100 times less activity than BHT in the conjugated diene assay.

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