

ESSENTIAL OIL AND SMOKE COMPONENTS OF *CARISSA SPINARUM*

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ABSTRACT. *Carissa spinarum* Linn. is an incense plant traditionally used in Ethiopia and other countries for the treatment of numerous diseases. It also exhibits different biological activities, and different classes of natural compounds were previously reported from the plant. In this study, the essential oil from the roots of *C. spinarum* together with medicinal smoke obtained from burnt roots of *C. spinarum* were analyzed by GC-MS. The essential oil of *C. spinarum* roots was predominantly composed of 2-hydroxyacetophenone (82.97%). The dominant components in the *n*-hexane soluble fraction of the smoke derived from the roots of *C. spinarum* were 2,6-dimethoxyphenol (14.16%), 2-methoxyphenol (10.34%) and 2-hydroxyacetophenone (9.51%). On the other hand, the major components in the MeOH-soluble fraction were 2,6-dimethoxyphenol (17.51%), 2-methoxyphenol (13.02%) and 2-hydroxyacetophenone (10.98%). The smoke derived from the roots of *C. spinarum* showed $92.60 \pm 0.34\%$ DPPH inhibition at concentration of 100 $\mu\text{g/mL}$. At the same concentration, standard ascorbic acid scavenged the DPPH radical by $96.09 \pm 0.16\%$. This result supports the traditional medicinal use of the plant material as a skin-care and wound healing agent most likely due to the presence of simple phenols and other biologically active compounds.

KEY WORDS: *Carissa spinarum* Linn., Medicinal smoke, Essential oil, Antioxidant activity, Phenolic compounds

INTRODUCTION

Carissa spinarum Linn. (Family: Apocynaceae) is a thorny, much-branched shrub or climber growing to 5 m high. *C. spinarum* is widely distributed in mountain bushland, at forest margins, along roadsides, riverbanks and rocky slopes at altitudes from 550–2500 m [1, 2]. *C. spinarum* is used as a traditional remedy for treatment of headache, chest complains, rheumatism, oedema, gonorrhoea, syphilis, rabies and it is also used as a remedy for fever, sickle cell anaemia, cough, ulcer, toothache, and worm infestation [3]. In pharmacological studies *C. spinarum* exhibited antiviral [4], anticonvulsant [5, 6], anti-cancer [7], antiplasmodial [8, 9], antimicrobial [10], analgesic [11], antipyretic [12], diuretic [13], hypoglycaemic [14], as well as wound healing activities [15]. *C. spinarum* is also used as a source of dye [16].

The genus *Carissa* is a rich source of different natural classes of compounds. A literature search for the phytoconstituents of *C. spinarum* indicated the isolation of lignans [17, 18], phenolic compounds [17, 19], sesquiterpenes [18, 20], chlorogenic acid derivatives [3, 21], flavonoids and their glycosides [3, 21], coumarins [17], triterpenes [3, 22] and cardiac glycosides [23]. *C. spinarum* is widely used traditionally as incense plant and for the treatment of various diseases in Ethiopia. Although the plant is widely used, there is no prior scientific report on the chemical composition and bioactivity of the smoke from the roots of this plant. Therefore, this study was undertaken to evaluate the antioxidant activity and phytochemical constituents of the essential oil and medicinal smoke derived from the roots of this plant.

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EXPERIMENTAL

General experimental procedures

UV-Vis and FTIR spectra were determined using a UV-T60 spectrophotometer and a Perkin-Elmer Spectrum 65 instrument in the range 4000–200 cm^{-1} , respectively. ^1H - and ^{13}C -NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at 400.13 and 100.60 MHz, respectively.

Plant material

The roots of *C. spinarum* were collected from Harbu city, Wollo, Ethiopia. The plant specimen was identified by the Department of Plant Sciences, Addis Ababa University, Addis Ababa, Ethiopia. A voucher specimen (No. Mel-004) was deposited at the National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia.

Extraction of essential oil from the roots of *C. spinarum* by hydrodistillation

The essential oil of the roots of *C. spinarum* was extracted by hydrodistillation following the procedure described by Costa *et al.* [24]. Thus, powdered roots of *C. spinarum* (300 g) and distilled water (500 mL) were placed in a distillation flask which was attached to a Clevenger apparatus and a condenser and the mixture was heated to boiling. After 8 h, the essential oil (1.5 mL, 1.28 g) was collected, dried over anhydrous Na_2SO_4 , filtered and then analyzed by GC-MS.

Collection of the smoke from burnt roots of *C. spinarum*

Dried and powdered roots of *C. spinarum* (300 g) were burned using an electrical stove (Figure 1) and the smoke was collected using an inverted funnel fitted with a rubber tube. The smoke was allowed to pass through a suction flask (250 mL) containing MeOH (100 mL) which in turn was connected to another 250 mL suction flask that contained *n*-hexane (100 mL). The side arm of the *n*-hexane-containing flask was attached to a water aspirator to help draw the smoke from the smoker into the solvents. The MeOH and *n*-hexane solutions were dried over anhydrous Na_2SO_4 , filtered and the solvents were removed under reduced pressure to yield methanol (2.5 g, 0.83%) and *n*-hexane (441 mg, 0.15%) residues which were then analyzed by GC-MS.

Retention index (*I*) was calculated for the different components in the smoke derived from the roots of *C. spinarum*. For retention index calculation, a mixture of *n*-alkanes (C_9 – C_{23}) was injected and analyzed using the same experimental condition as that of the essential oil analysis. The retention indices of the different components were then calculated according to the van den Dool and Kratz relationship (Equation 1) [26].

$$I = 100n + 100 \left(\frac{R_{t(\text{unknown})} - R_{t(n)}}{R_{t(n+1)} - R_{t(n)}} \right) \quad (1)$$

where *I* is retention index of the analyte, *n* is the number of carbon atoms of the *n*-alkane eluting immediately before the analyte, $R_{t(\text{unknown})}$ is the retention time of the analyte, $R_{t(n)}$ and $R_{t(n+1)}$ are the retention times of the reference *n*-alkanes eluting immediately before and after the analyte, respectively.

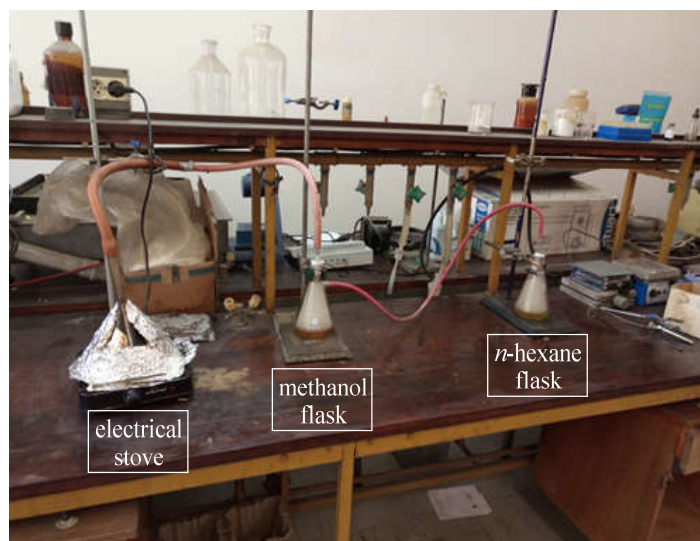


Figure 1. Smoke trapping setup [25].

GC-MS characterization of the essential oil and smoke derived from the roots of C. spinarum

The essential oil and smoke obtained by burning the roots of *C. spinarum* were analyzed by using an Agilent Technology 7820A GC system coupled with an Agilent Technology 5977E MSD equipped with an autosampler. The chromatographic separation was done on a DB-1701, (14%-cyanopropyl-phenyl)-methylpolysiloxane, column ($30 \times 0.25 \mu\text{m}$) at a pressure of 8 psi and a flow rate 0.97989 mL/min. Ultra-high pure helium (99.999%) was used as carrier gas at constant flow mode. An Agilent G4567A autosampler was used to inject $1 \mu\text{L}$ of the sample with a splitless injection mode into the inlet heated to 275°C with a total run time of 29.33 min. Oven temperature was programmed with the initial column temperature of 60°C and hold-time of 2 min. The column temperature was increased at a rate of $10^\circ\text{C}/\text{min}$ until the temperature reached 200°C and then heated again at the rate of $3^\circ\text{C}/\text{min}$ until the temperature reached 240°C . No mass spectra were collected during the first 4 min of the solvent delay. The transfer line and the ion source temperatures were 280°C and 230°C , respectively. The detector voltage was 1600 V and the electron energy was 70 eV. Mass spectral data were collected from 40–650 m/z . The names, structures, and qualities of peaks were determined through NIST 2014 library search and retention index (*I*) calculation.

DPPH radical scavenging assay

The DPPH radical scavenging assay is a simple method for quantifying antioxidants by measuring absorbance at 517 nm due to the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [27]. The radical scavenging activity of the MeOH-soluble fraction of the smoke derived from the roots of *C. spinarum* was assessed using DPPH according to the procedure described by Hoque *et al.* [28]. The extract was first dissolved in MeOH to afford 1.0 mg/mL solution. It was then serially diluted in MeOH to give concentrations of 500, 250, 125, 62.50, 31.25, 15.63 and 7.81 $\mu\text{g}/\text{mL}$. To 1.0 mL of each solution, 4.0 mL of 0.004% DPPH solution in MeOH was added to make 100, 50.0, 25.0, 12.5, 6.25, 3.13 and 1.56 $\mu\text{g}/\text{mL}$ solutions. The mixtures were shaken vigorously and left to

stand in the dark for 30 min. The absorbance of the samples was then recorded at 517 nm using a UV-Vis spectrophotometer. The radical scavenging activity of the extract was measured in relation to ascorbic acid (a known antioxidant) standards. All measurements were performed in triplicates and the percentage DPPH inhibition of the extract and ascorbic acid standards were calculated using the equation:

$$\% \text{ DPPH inhibition} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100 \quad (2)$$

where A_{control} is the absorbance of DPPH solution in MeOH and A_{extract} is the absorbance of the test sample plus DPPH solution.

RESULTS AND DISCUSSION

Extraction and characterization of compounds from the essential oil and the smoke derived from the roots of C. spinarum

The dried and powdered roots of *C. spinarum* were extracted by hydrodistillation and then analyzed by GCMS [24]. The total amount of extract obtained was calculated to be 0.43%. The essential oil was found to be predominantly composed of 2-hydroxyacetophenone (82.97%) by GC-MS analysis (Figure 2) as well as its UV-Vis, FTIR, ^1H -, ^{13}C - and DEPT135-NMR spectra (See Supplementary Information). 2-Hydroxyacetophenone was reported previously as the principal component of the DCM extract of the steam distillate of fresh root bark of *C. spinarum* [19].

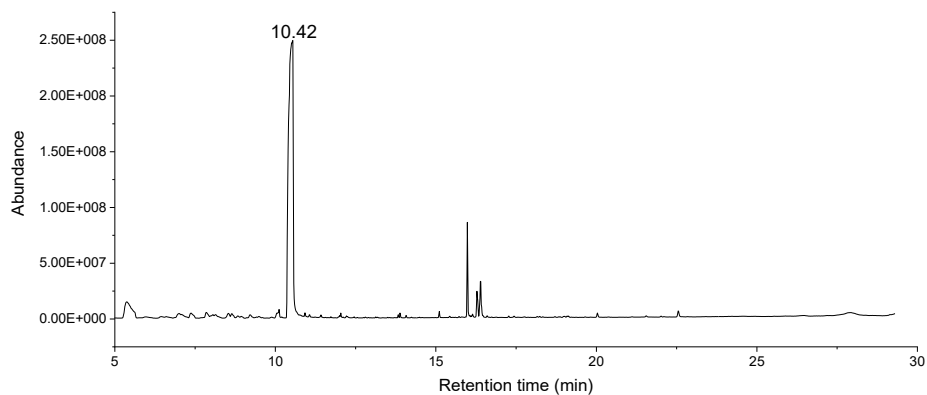


Figure 2. The GC-MS chromatogram of the essential oil from the roots of *C. spinarum*.

The smoke from burnt roots of *C. spinarum* was also collected and analyzed by GC-MS. A total of 31 compounds, 16 from the *n*-hexane fraction and 15 from the MeOH-soluble portion of the smoke obtained from burnt roots of *C. spinarum*, with qualities greater than 72% and area percentage greater than 2, were identified (Figure 3). The different components were characterized by matching their mass spectra with those of reference compounds recorded in NIST 2014 mass spectral library and confirmed by the retention indices obtained from a series of *n*-alkanes.

Table 1 shows that phenolic compounds, namely, 2,6-dimethoxyphenol (14.16%), 2-methoxyphenol (10.34%) and 2-hydroxyacetophenone (9.51%), were the dominant components

of the *n*-hexane fraction of the smoke derived from the roots of *C. spinarum*. Those three compounds were also the major compounds detected in the methanol fraction (2,6-dimethoxyphenol (17.51%), 2-methoxyphenol (13.02%) and 2-hydroxyacetophenone (10.98%). The percentage composition of the three major compounds was higher in the methanol fraction. Besides the compounds indicated in Table 1, the MeOH fraction contained cyclopropyl carbinol (Rt = 10.96, 6.80%), 9(*E*)-octadecene (Rt = 11.68, 2.65%), and vanillin (Rt = 14.79, 3.35%) which are absent from the *n*-hexane fraction. Similarly, four compounds (tetradecanal, guaiacylacetone, methoxyeugenol and α -cyperone) were only detected in the *n*-hexane fraction (Table 1).

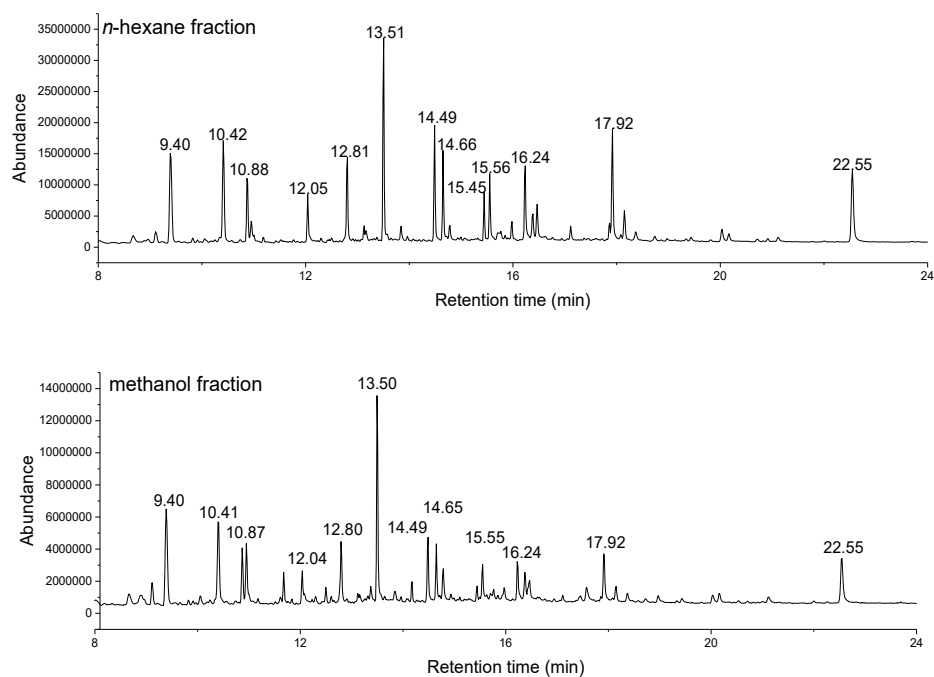
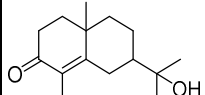


Figure 3. The GC-MS chromatogram of *n*-hexane- and methanol-soluble fractions of the smoke derived from the roots of *C. spinarum*.

Table 1. Major compounds identified from the *n*-hexane and methanol-soluble fractions of the smoke derived from the roots of *C. spinarum* by GC-MS analysis.

<i>n</i> -Hexane fraction							Methanol fraction
Pk	Rt	RI	Compound	Structure	Q	Area %	
1	9.40	1232.52	2-Methoxyphenol		95	10.34	D
2	10.42	1303.73	2-Hydroxyacetophenone		95	9.51	D

3	10.88	1337.5	Creosol		97	4.54	D
4	12.05	1424.05	4-Ethyl-2-methoxyphenol		91	3.71	D
5	12.81	1483.24	2-Methoxy-4-vinylphenol		91	6.98	D
6	13.51	1540.37	2,6-Dimethoxyphenol		97	14.16	D
7	14.49	1623.50	<i>trans</i> -Isoeugenol		98	7.36	D
8	14.66	1637.68	3,5-Dimethoxy-4-hydroxytoluene		97	5.18	D
9	15.45	1706.98	Tetradecanal		99	2.71	ND
10	15.56	1717.08	5- <i>tert</i> -Butylpyrogallol		80	4.37	D
11	16.24	1779.61	3',5'-Dimethoxyacetophenone		78	6.05	D
12	16.39	1793.11	Guaiacylacetone		80	2.21	ND
13	16.47	1800.90	Methoxyeugenol		93	2.69	ND
14	17.92	1917.92	4-Propenylsyringol		98	8.79	D
15	18.16	1935.24	α -Cyperone		99	2.64	ND

16	22.55	2220.94	Carissone		88	8.77	D
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Pk = peak number, Rt = retention time (min), Area % = area percentage, Q = quality, RI = retention index, D = Detected, ND = Not detected.

DPPH radical scavenging assay of the methanol-soluble fraction of the smoke derived from the roots of C. spinarum

The radical scavenging activity of the MeOH-soluble fraction of the smoke derived from the roots of *C. spinarum* was assessed using DPPH [27]. The MeOH-soluble fraction showed a 92.60% DPPH inhibition at concentration of 100.00 µg/mL which is comparable to that of ascorbic acid standard that exhibited a 96.09% DPPH inhibition at the same concentration (Table 2). The IC₅₀ value was also calculated to be 21.28 for the extract. The phenolic compounds in the smoke may be responsible for the observed antioxidant activity.

Table 2. Radical scavenging activities of the MeOH-soluble fraction of the smoke derived from *C. spinarum* and ascorbic acid standards.

Concentration (µg/mL)	% DPPH inhibition	
	MeOH fraction of the smoke from <i>C. spinarum</i>	Ascorbic acid standard
100	92.60 ± 0.34	96.09 ± 0.16
50.0	88.48 ± 0.31	96.29 ± 0.06
25.0	66.06 ± 0.71	96.26 ± 0.06
12.5	47.62 ± 0.11	96.06 ± 0.12
6.25	37.32 ± 0.81	91.30 ± 0.31
3.13	30.97 ± 0.95	54.06 ± 1.27
1.56	26.09 ± 0.15	35.00 ± 0.52

The results are reported as mean ± SD of three replicates.

CONCLUSION

In this work, the essential oil from the roots of *C. spinarum* together with the medicinal smoke obtained by burning the roots of *C. spinarum* were analyzed by GC-MS. The dominant components of the *n*-hexane- and MeOH-soluble fractions of the smoke derived from the roots of *C. spinarum* were found to be 2,6-dimethoxyphenol, 2-methoxyphenol and 2-hydroxyacetophenone. The presence of phenolic compounds in the smoke and essential oil of the plant may lend some credence to the traditional medicinal use of the plant. The antioxidant activity of the MeOH-soluble fraction of the smoke obtained from the roots of *C. spinarum* was also evaluated. The MeOH-soluble fraction of the smoke obtained from the roots of *C. spinarum* showed a 92.60% DPPH inhibition at concentration of 100 µg/mL. The observed antioxidant activity supports the traditional use of the plant for skin care.

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