

Investigations of chemical compositions and antioxidative potential of essential oils isolated from the leaves of two *Garcinia* species

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ABSTRACT

Garcinia quaesita and *Garcinia zeylanica* are Sri Lankan endemic plants with significant therapeutic potential and numerous health-care applications. Despite this, there are no adequate literatures reported on the chemical compositions (CCs) and antioxidative potential (AP) of leaves' essential oils (EOs). The purpose of this study was to extract EOs from the leaves and investigate the CCs and AP of the extracted EOs. The hydro-distillation technique was used to extract the EOs, and the CCs of the EOs were identified through gas chromatography–mass spectrometry analysis. Only those compounds that had a matching value of more than 90% were taken into consideration, and the AP of the extracted EOs was determined using the ferric-reducing antioxidant power (FRAP) assay. Hydro-distillation process yielded EOs in the same quantity, 0.12% (v/w) on a fresh weight basis for two varieties. About 33 CCs that were found in the extracted EOs were mainly sesquiterpenes. The most prevalent substances in the EOs were copaene (19.39%), caryophyllene (12.94%), alloaromadendrene (12.12%), α -humulene (11.24%), and α -cubebene (9.38%). It is interesting to note that copaene and alloaromadendrene were only found in *G. quaesita*, whereas α -cubebene was only found in *G. zeylanica*. Caryophyllene and α -humulene were identified in both EOs at different concentrations. The EO from *G. quaesita* showed high AP, presenting FRAP values $274.74 \pm 1.32 \mu\text{L Trolox Eq/L}$. This study is recognized as being the first to examine the CCs and AP of EOs, and the results may inspire the creation of new uses and high-value leaf products.

Key words: Antioxidants, essential oil, hydro-distillation, *Garcinia quaesita*, *Garcinia zeylanica*

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INTRODUCTION

Sri Lanka is among the renowned hot spots for biodiversity in tropical rainforests, with an estimated 70% of unique terrestrial evergreen crops.^[1] However, less studies have reported on some endemic medicinal plants grown in Sri Lanka. With this in mind, the current study considered two Sri Lankan endemic plants for scientific

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investigation for the purpose of novel discovery and the preparation of scientific data based on the utility of scientific community. *G. quaesita* (family: Clusiaceae) is a plant endemic to Sri Lanka and locally referred to as “Rath Goraka (Red-Goraka, red fruited)” in Sri Lanka, commonly called as “Goraka”^[2] and brindle berry in English.^[3] The other variety, *G. zeylanica*, also belongs to the same family as *G. quaesita* and is locally known as Ēla Goraka/Kaha Goraka (Yellow-Goraka, yellow fruited).^[3-5] The fruits of both *G. quaesita* and *G. zeylanica* are utilized as a spice or condiment and importantly in indigenous/ folk medicine. Normally, fruits are prescribed for ailments in Indian folk tradition, and rinds are used as Sri Lankan curry ingredients and condiments.^[3]

As very few studies are available on *G. zeylanica* and *G. quaesita* for their chemical compositions (CCs) and pharmacological activity.^[4,6] Kokilananthan *et al.* previously published two articles based on chemical analysis of leaves, which revealed that the methanolic and aqueous extracts contain several important phytochemicals with antioxidative potential (AP), such as flavonoids, saponins, alkaloids, phenolics, and terpenoids.^[7,8] As no previous studies on the essential oils (EOs) of these two plants’ leaves, and currently EOs are attracted considerable interest from scientific community, this work aimed to extract the EOs from the leaves of *G. zeylanica* and *G. quaesita* and analyze the CCs and the AP of them.

MATERIALS AND METHODS

Plant materials and chemicals

G. quaesita (GQ) and *G. zeylanica* (GZ) were plucked (each 1 kg) from a home garden in Matara, Sri Lanka (latitude 5.9478 °N, longitude 80.5483 °E). Plant materials have been authenticated in Bandaranaike Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka, and which were deposited there with Voucher Nos. 3006 and 3007, respectively.

Essential oil extraction

The hydro-distillation method was used to extract EOs from the leaves of both GQ and GZ, as described in the literature by Weli *et al.*^[9] The leaves were harvested manually, and the fresh mass of leaves per plant was determined before being stored in a refrigerator (approximately 4°C) until the extraction of the EO (around 5 h of refrigerated storage). The EO was extracted at the Department of Chemistry, University of Ruhuna, by hydro-distillation in a Clevenger apparatus for 3 h after the start of the boiling, using 200 g of slightly ground leaves (plant material-to-distilled water ratio = 1:5 w/v). The hydro-distillation process was tripled to ensure reproducibility in EO yields. By dividing the volume of water-free EO by the amount of fresh mass, the EO yield percentage was calculated. The extracted EOs were dehydrated with anhydrous Na₂SO₄, and the water-free EO

was stored in the refrigerator in a sealed, amber-colored vial for chromatographic analysis.

Gas chromatography–mass spectrometry analysis

GC/MS analyses were performed on an Agilent 7890A series gas chromatograph, directly coupled to Agilent 5975C series MSD version mass selective detector (Model No: 5975C TAD inert XL EI/CI MSD, Agilent Technologies, Palo Alto, CA, USA), equipped with an Agilent 19091S-433HP-5MS 5% Phenyl Methyl Silox capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature program: 70°C for 4 min, then 8°C/min to 270°C for 10 min; Run time: 39 min; Ion-source temperature: 230°C; Carrier gas: He (1 mL/min); Injection volume: 1 μL. The electron ionization (EI) mass spectra were acquired over the mass range of 33–550 m/z. Only those compounds that had a matching value of more than 90% were considered for this study.

Anti-oxidant activity

Ferric-reducing antioxidant power (FRAP) assay was used to characterize the antioxidant capacity of the extracted EOs as reported by Abraão *et al.*^[10] To measure the FRAP, EOs (100 μL) were placed in a test tube, followed by 3 mL of FRAP working solution (composed of 1 mL of TPTZ (2,4,6-tripyridyl-S-triazine, 10 mM dissolved in 40 mM HCl), 1 mL of FeCl₃ (20 mM in water), and 10 mL of acetate buffer (300 mM, pH – 3.6). The mixture was incubated at 37°C, protected from light, for 30 min. Afterward, the absorbance was measured at 593 nm. Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as a standard, and the results were expressed in μL Trolox Eq/L.^[11,12]

Statistical analysis

The data were analyzed and compared by an analysis of variance and a *t*-test (least significant difference). SAS OnDemand for Academics: Studio (SAS 9.4) software (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA.) was used for the statistical analysis. In terms of means and standard deviations, the data were given.

RESULTS

The EOs extracted from GQ and GZ leaves were light yellow in color and had a pleasant fragrance. The hydro-distillation of GQ and GZ leaves yielded the same amount of EOs, 0.12% (v/w) on a fresh weight basis for both varieties. The statistical analysis also revealed that the EOs of GQ and GZ were not statistically different at the 5% significance level.

The gas chromatography–mass spectrometry (GC-MS) analysis was used to identify the CCs of the extracted EOs. Despite the fact that a large number of CCs in each EO have been detected in GC-MS spectra, this study only includes matching values of more than 90% to the NIST MS library. About 33 CCs, mostly sesquiterpenes, have been

identified. All of the chemical structures of the identified CCs in both EOs have a higher than 90% matching value, as shown in Figures 1 and 2, and the detailed information of the identified CCs is tabulated in Table 1.^[13]

Both the EOs of GQ and GZ leaves exhibited a high concentration of sesquiterpenes (60.4% and 39.1%, respectively). The EO of GQ leaves showed the highest number of chemical constituents including copaene, (-)- α -gurjunene, caryophyllene, α -humulene, alloaromadendrene, α -elemene, β -maaliene, ionol, δ -cadinene, nerolidol, (-)-globulol, and heneicosane with the high concentration. The EO of GZ leaves rich in caryophyllene, α -humulene, ionol, δ -cadinene, tetracosane, 9-methylnonadecane, α -cubebene, γ -selinene, diethyl phthalate, humulol, methyl palmitate, 1-nonadecene, methyl elaidate, pentacosane, and heptadecane. Remarkably stated, both EOs of GQ and GZ leaves contained caryophyllene, α -humulene, ionol, δ -cadinene, tetracosane, and 9-methylnonadecane.

The antioxidant potential of both extracted EOs was determined using the FRAP assay. The EO of GQ evidently has more antioxidative power (274.74 ± 1.32 L Trolox Eq/L) than the EO of GZ (250.18 ± 3.31 L Trolox Eq/L).

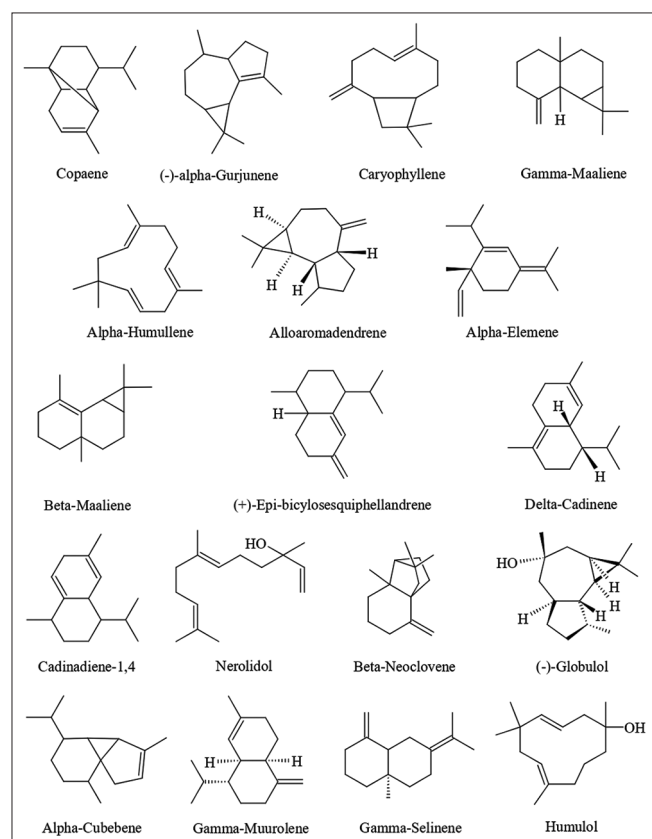


Figure 1: Sesquiterpenes and sesquiterpene alcohols which were discovered in both GQ and QZ EOs. GQ: *Garcinia quaesita*, GZ: *Garcinia zeylanica*, EO: Essential oil

Furthermore, statistical analysis confirmed the experimental findings, indicating that there are significant differences in antioxidative capacity at the 5% significance level.

DISCUSSION

As this is the first effort based on EOs of both GQ and GZ, there are no previous findings to compare yields, but which could be compared to earlier observations of other *Garcinia* species reported by Rameshkumar *et al.*^[14] The EO yield (0.12%, v/w) of both GQ and GZ belongs to the interval of values reported in the literature ranging between 0.75% and 0.01%.^[14]

According to the current study, the yield percentages of the two EOs are the same, and the number of accessible CCs and their quantity vary depending on the EO of the selected *Garcinia* varieties. A profound literature scan based on both GQ and GZ leaves has found that no reports are available on the same so far. This is, thus, the first report based on EOs of GQ and GZ leaves grown in Sri Lanka. The majority of the identified CCs have been known to exhibit a variety of pharmacological properties, which are listed in Table 2.

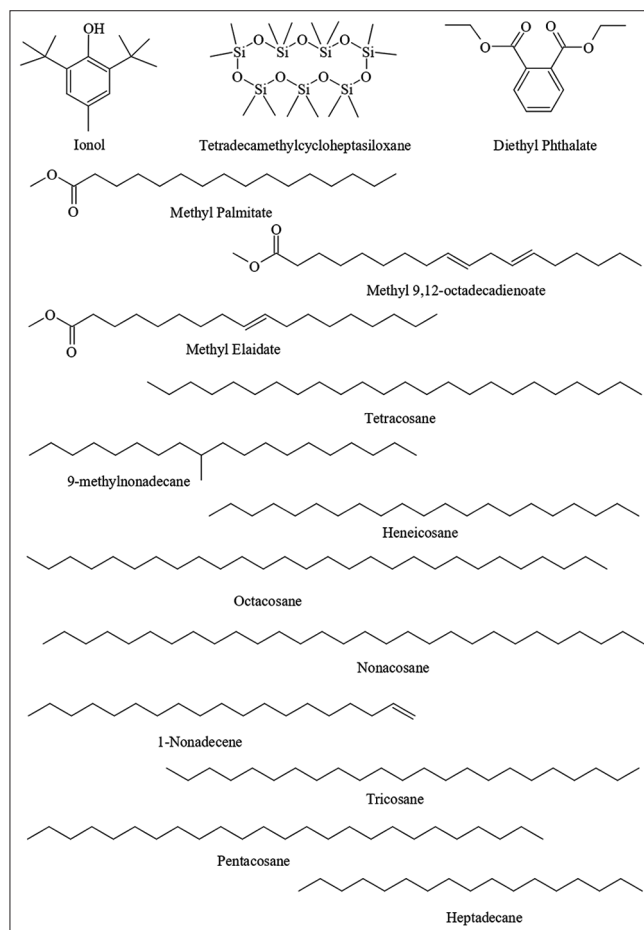


Figure 2: Hydrocarbons and other chemical compositions which were discovered in both GQ and QZ EOs. GQ: *Garcinia quaesita*, GZ: *Garcinia zeylanica*, EO: Essential oil

Table 1: Chemical compositions of the extracted essential oils from the leaves of *Garcinia quaesita* and *Garcinia zeylanica*

RT	Chemical name	MF	MW	RP (%)	
				GQ	GZ
12.86	α -cubebene ^a	C ₁₅ H ₂₄	204.35		9.4
12.86	Copaene ^b	C ₁₅ H ₂₄	204.35	19.4	
13.34	(-)- α -gurjunene ^b	C ₁₅ H ₂₄	204.35	1.3	
13.49	Caryophyllene ^c	C ₁₅ H ₂₄	204.35	6.7	12.9
13.59	γ -maaliene ^b	C ₁₅ H ₂₄	204.35	0.7	
13.94	α -humulene ^c	C ₁₅ H ₂₄	204.35	10.3	11.2
14.04	Alloaromadendrene ^b	C ₁₅ H ₂₄	204.35	12.1	
14.19	α -elemene ^b	C ₁₅ H ₂₄	204.35	1.4	
14.19	γ -muurolene ^a	C ₁₅ H ₂₄	204.35		0.7
14.31	Tetradecamethylcycloheptasiloxane ^a	C ₁₄ H ₄₂ O ₇ Si ₇	519.07		0.6
14.37	β -maaliene ^b	C ₁₅ H ₂₄	204.35	2.2	
14.47	γ -selinene ^a	C ₁₅ H ₂₄	204.35		1.2
14.60	Ionol ^c	C ₁₅ H ₂₄ O	220.35	2.0	4.6
14.69	(+)-Epi-bicyclosesquiphellandrene ^b	C ₁₅ H ₂₄	204.35	0.6	
14.77	δ -cadinene ^c	C ₁₅ H ₂₄	204.35	4.7	3.6
14.90	Cadinadiene-1,4 ^b	C ₁₅ H ₂₄	204.35	0.2	
15.18	Nerolidol ^b	C ₁₅ H ₂₆ O	222.37	3.4	
15.29	beta-neoclovene ^b	C ₁₅ H ₂₄	204.35	0.8	
15.58	Diethyl phthalate ^a	C ₁₂ H ₁₄ O ₄	222.24		7.6
15.59	(-)-Globulol ^b	C ₁₅ H ₂₆ O	222.37	1.7	
15.78	Humulol ^a	C ₁₅ H ₂₆ O	222.37		2.9
19.08	Methyl palmitate ^a	C ₁₇ H ₃₄ O ₂	270.50		1.4
20.61	1-nonadecene ^a	C ₁₉ H ₃₈	266.50		1.1
20.72	Methyl 9, 12-octadecadienoate ^a	C ₁₉ H ₃₄ O ₂	294.50		0.6
20.77	Methyl elaidate ^a	C ₁₉ H ₃₆ O ₂	296.50		1.7
22.49	Tricosane ^a	C ₂₃ H ₄₈	324.60		0.7
23.32	Tetracosane ^c	C ₂₄ H ₅₀	338.70	0.6	1.1
24.12	Pentacosane ^a	C ₂₅ H ₅₂	352.70		1.5
24.12	9-methylnonadecane ^c	C ₂₀ H ₄₂	282.50	0.9	5.9
25.95	Heneicosane ^b	C ₂₁ H ₄₄	296.60	1.0	
27.13	Heptadecane ^a	C ₁₇ H ₃₆	240.50		1.2
27.13	Octacosane ^b	C ₂₈ H ₅₈	394.80	1.0	
28.56	Nonacosane ^b	C ₂₉ H ₆₀	408.80	0.9	
Total				71.9	69.9
Esters (19, 22, 24, 25)					11.2
Hydrocarbons (10, 23, 26-33)				4.3	11.5
Lipophilic organic compounds (13)				2.0	4.6
Organosiloxane (10)					0.6
Sesquiterpene alcohols (17, 20, 21)				5.1	2.9
Sesquiterpenes (1, 2, 3-6, 7-9, 11, 12, 14-16, 18)				60.4	39.1

^aChemical constituents only present in EO of GZ leaves, ^bChemical constituents only present in EO of GQ leaves, ^cChemical constituents present in EOs of leaves of both GQ and GZ. RT: Retention time, MF: Molecular formula, MW: Molecular weight, RP: Relative percentage, GQ: *Garcinia quaesita*, GZ: *Garcinia zeylanica*, EO: Essential oil

The study discovered that *Garcinia* leaves, which are considered agricultural waste, contain active substances with good pharmacological activity, especially AP. As natural antioxidants are being introduced into the development of functional foods and nutraceuticals as a global interest, this study would be extremely beneficial in proposing these two EOs for incorporation into them as a natural antioxidant.

CONCLUSION

This study would be credited as the first to isolate EO, analyze CCs, and assess the AP of EOs from the leaves of GQ and GZ. About 33 CCs were identified from the EOs of two *Garcinia* varieties and their availability is not unique among them. The AP of GQ EO is shown to be higher than GZ. The majority of the chemical components

Table 2: Some of the chemical compositions of the extracted essential oils and its medicinal properties

Chemical name	Medicinal activity	References
Copaene	Cytotoxic, genotoxic/antigenotoxic, anticarcinogenic, and antioxidant activities	[15,16]
Aromadendrene	Antimicrobial property	[17]
Caryophyllene	Anticancer, analgesic, and anti-inflammatory activities	[18,19]
α -caryophyllene	Anti-inflammatory, analgesic, anti-catabolic, and pro-anabolic activities	[20,21]
BHT	Antioxidant activity	[22]
Nerolidol	Antioxidant, antifungal, and antiparasitic activities	[23-25]
(-)-Globulol	Antimicrobial activity	[26]
Tetracosane	Cytotoxic activity	[27]
Heneicosane	Antimicrobial activity	[28]
Octacosane	Mosquitocidal activity	[29]
α -cubebene	Anti-inflammatory, neuroprotective, and antioxidant activities	[30-32]
Diethyl phthalate	Toxic effect	[33]
Methyl palmitate	Anti-inflammatory and antifibrotic effects	[34]

BHT: Butylated hydroxytoluene

identified in *Garcinia* leaves EOs have been shown to have clear pharmacological effects. As a result, *Garcinia* leaves that are discarded as agricultural waste could be used to extract pharmacologically active compounds and develop functional foods and nutraceuticals.

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Conflicts of interest

There are no conflicts of interest.

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