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GC/MS and HPLC/PDA characterization of essential oils and phenolic compounds from the aerial parts of common rue (*Ruta graveolens*)

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Abstract Two different extraction methods were used to evaluate the medical value of common rue, *Ruta graveolens* L. (RGL). The results of our 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid assays indicated that the antioxidant activity of RGL essential oil extract obtained through steam distillation was very low, whereas ethanol (EtOH) extracts of RGL showed higher antioxidant activity. RGL essential oil was extracted by steam distillation and characterized by GC/MS analysis. Furthermore, EtOH extracts of RGL were obtained under reflux and analyzed by HPLC/PDA. The GC/MS results indicated that the ketone compounds 2-undecanol acetate, nonyl cyclopropanecarboxylate, and 2-nonanone accounted for more than 70% of the composition of RGL essential oil. The HPLC/PDA analyses indicated that the RGL extracts were rich in phenolic compounds such as protocatechuic acid, rutin, psoralen, xanthotoxin, and bergapten, among which rutin was the most abundant. Collectively, our results demonstrated that RGL contains high levels of phenolic compounds and could thus be commercialized as a valuable plant-derived antioxidant.

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Keywords Antioxidant activity · GC/MS · HPLC/PDA · *Ruta graveolens*

Introduction

Common rue, *Ruta graveolens* L. (RGL), is a medicinal plant belonging to the family Rutaceae, which is endemic to the Mediterranean coast, Crimea, and the Balkans [1]. RGL has a long history as an ancient medicinal herb and was historically used in alchemy and folk remedies [2]. RGL is known to have various pharmacological effects, including antioxidant and anti-inflammatory properties. Moreover, previous studies have reported that RGL possesses a wide variety of therapeutic properties, such as anti-microbial, anti-depressant, anti-hypertensive, anti-inflammatory, anti-tumor, and anti-cancer effects [3-5]. Additionally, many studies have demonstrated that RGL contains alkaloids, coumarins, and flavonoids, as well as essential oil [6].

A previous study extracted essential oil from RGL by steam distillation and analyzed their chemical composition via GC/FID and GC/MS, and the authors reported that 2-nonanone and 2-undecanone were the main compounds in the essential oil [7]. Essential oil from RGL has been used by humans since ancient times due to their sterilization, insecticide, and anti-parasitic activity. Therefore, RGL essential oil continues to be widely used in pharmacology even in modern times. This essential oil is also commonly used in cosmetics due to their low toxicity, biocompatibility, lack of allergic reaction, and pleasant aroma and taste [8]. RGL essential oil possesses strong anti-bacterial activity due to their coumarin, flavonoid, and furanocoumarin contents. RGL extracts have also been incorporated into skin drug formulations with promising results [9,10]. Interestingly, recent studies have reported that the methods used for the extraction and isolation of plant extracts can affect the biological activity of phytochemicals [11,12].

In phenolic compounds, protocatechuic acid, rutin, and

furanocoumarins are phenolic compounds present in RGL [13]. Both of these compounds are known to possess pharmacological and other bioactive properties, including anti-aging, anti-cancer, anti-oxidant, and anti-diabetic activities [14,15]. Psoralen, xanthotoxin, bergapten, and a group of furanocoumarins are widely used as skin re-pigmentation stimulants, as well as for the treatment of leukocytoma and psoriasis. Many other coumarins are also effective for the treatment of burns, rheumatoid diseases, psoriasis, and vitiligo [16-18]. Coumarins have attracted considerable attention due to their many potential health benefits [19]. Psoralen possesses anti-cancer, immunomodulatory, and estrogenic properties [20]. And bergapten has anti-proliferative effects and induces apoptotic responses in breast cancer cells [21-23].

Therefore, this study sought to characterize the composition and antioxidant activities of RGL essential oil and phenolic compounds obtained through different extraction methods.

Materials and Methods

Plant materials

This study was conducted using aerial parts collected from RGL plants cultivated in National Institute of Horticultural and Herbal Science, Eumseong, Korea. An authenticated voucher specimen (MPS004884) was deposited at the Herbarium of the Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, Eumseong 27709, Korea.

Instruments and reagents

GC/MS analysis was conducted using a 7890BGC/7010QQQ MS instrument (Agilent, Palo Alto, CA, USA) and a DB5-MS capillary column (30 m×0.25 mm, film thickness 0.25 μm). HPLC analysis was performed with an HPLC instrument (Waters Alliance 2695 Separations Module, Milford, MA, USA) equipped with a photodiode array detector (Waters 996 PDA Detector), a pump, and an auto-sampler with a YMC Pack Pro C18 column (4.6×250 mm, 5 μm). HPLC/grade solvents, including water, acetonitrile, and methanol (MeOH), were purchased from J. T. Baker (Philipsburg, Pennsylvania). Ethanol (EtOH) and acetic acid were purchased from Samchun Chemicals, (Pyeongtaek, Korea). An Epoch microplate spectrophotometer (BioTek, Winooski, VT, USA) was used in all assays requiring a microplate reader. The radical scavenging activity assay was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzo-thiazoline-6-sulphonic acid (ABTS), and potassium persulfate purchased from Sigma-Aldrich (Burlington, MA, USA).

Essential oil extraction by steam distillation

Essential oil was extracted from the aerial parts (i.e., leaves and stems) of RGL (4.07 kg) through conventional vapor distillation (Fig. 1A). Boiling distilled water was passed through the plant to generate steam loaded with essential oil, which was then transferred to the receiving flask via a condenser (Fig. 1B, 1C). In the separation funnel, the essential oil floated on top of the hydrosol (distilled water component) for separation. The faucet was then opened to allow the bottom water layer to slowly flow out of the funnel, while maintaining the essential oil (Fig. 1D).

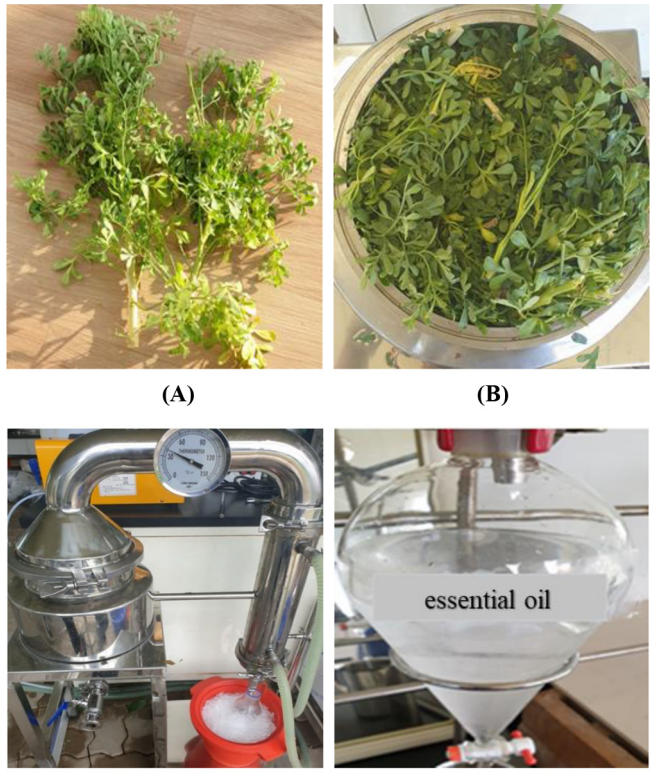

Fig. 1 RGL extraction procedure: collected RGL plants (A), RGL inside the extraction mantle (B), steam distillation (C), and essential oil and hydrosol (D)

Sample extraction and preparation

The dry RGL (10 g) was extracted using EtOH in reverse flow for 3 h and the procedure was repeated three times. The samples for HPLC/PDA analysis were dried using a decompression concentrator to obtain a 1.2 g extract. For quantitative evaluation, the RGL extract was dissolved in MeOH and sonicated for 30 min, then passed through a 0.45-μm polyvinylidene fluoride (PVDF) membrane filter. Stock solutions were also prepared and the composite content of all samples was measured using the test curve.

DPPH radical-scavenging assay

The DPPH assay was used to analyze the antioxidant capacity of the RGL extracts [24,25]. To prepare a DPPH working solution (0.2 mM), a 2 mM DPPH stock was diluted with EtOH. Afterward, 10 μL of extract and 200 μL of the 0.2 mM DPPH working solution were added to each of the wells of a 96-well plate and allowed to react in the dark for 30 min. The free radical

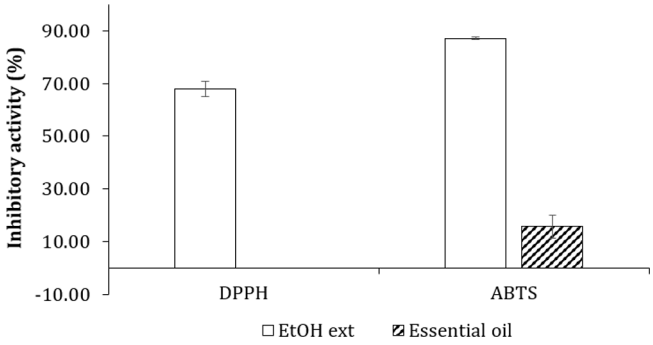

Fig. 2 Comparison of antioxidant activity of RGL essential oil and EtOH extract

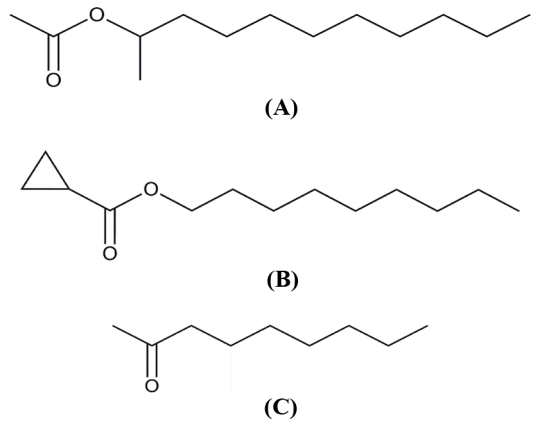

Fig. 3 Chemical structures of 2-undecanol acetate (A), cyclopropanecarboxylic acid, non-nonylester (B), and 2-nonanone

concentration was measured at 514 nm by a microplate reader (BioTek, Winooski, VT, USA).

ABTS antioxidant activity

The $ABTS^{+}$ assay was conducted to analyze the antioxidant activity of the RGL extracts as described in a previous study [26,27]. Briefly, ABTS and a potassium persulfate solution were mixed and diluted in distilled water to achieve an absorbance value of 1.0±0.04 mg/mL. After storage for 30 min in a dark room, each sample was added to the radical stock solution. After an additional 30 min, the residual radical concentration was measured at 734 nm by a microplate reader (BioTek, Winooski, VT, USA).

GC/MS conditions

GC/MS analysis was conducted with an DB5-MS capillary column (30 m×0.25 mm, film thickness 0.25 μm) using helium as a carrier gas at a flow rate of 1 mL/min. The injector port, ion source, and interface temperatures were 300, 230, and 300 ºC, respectively. The GC oven temperature was set up as follows: 40 ºC for 3 min, 40-230 ºC at 2 ºC/min, 230-300 ºC at 5 ºC/min, and 300 ºC for 15 min. The split ratio was 1:10 and masses were scanned from *m/z* 50 to 800.

HPLC conditions

Quantitative analysis of the chemical composition of RGL was performed using a reverse HPLC system. Chromatographic separation was conducted using a YMC Pack Pro C18 column (4.6×250 mm, 5 μm). The analysis was conducted via the gradient

Table 1 Chemical composition of RGL essential oil

Compound	t_R	Area (%)	RI	
			Observed [a]	Literature [b]
isovaleric acid	58.33	2.71	1641	1752
1-fluorododecane	53.22	0.43	1552	1187
trans-α-bergamotene	49.43	0.55	1488	1436
nonyl pivalate	46.85	1.78	1447	1590
2-undecanol acetate	45.88	22.90	1430	1416
2-decyloxirane	41.51	0.69	1361	1307
N-dodecyl fluoride	38.77	1.92	1319	1187
1-undecanol	38.13	1.78	1309	1372
2-undecanone	36.86	5.52	1290	1291
nonyl cyclopropanecarboxylate	33.04	14.47	1234	1483
2-decanone	29.96	0.88	1189	1192
geijerene	26.08	3.72	1134	1143
1-tridecanol	24.52	0.68	1112	1593
1-dodecene	23.98	0.55	1105	1192
nonanal	23.82	1.15	1102	1098
2-nonanone	22.9	33.90	1089	1091

[a]Retention index on column
[b]Retention index relative to literature value

method with 0.5% acetic acid in water (A) and acetonitrile (B). The elution system was as follows: 0-10 min 83% A; 40 min 30% A; 45 min 0% A; 50 min 83% A; 60 min 83% A. The temperature of the column was maintained at 30 ºC. The injection volume was 10 µL and the flow rate was set to 1.0 mL/min.

Calibration curve

A standard solution was prepared by dissolving five compounds in MeOH (1 mg/mL). The working solution used to construct the calibration curve was prepared by continuously diluting the standard solution to the desired concentration. RGL samples were dissolved in MeOH (10 mg/mL). The standard and sample solutions were then passed through a 0.45 µm PVDF filter, after which the compound content of RGL was determined from the calibration curve. The calibration function of the five compounds was calculated as peak area (Y), concentration (X, µg/10 µL), and average value (n=5) ± standard deviation.

Results and Discussion

RGL is a natural medicinal plant whose therapeutic properties

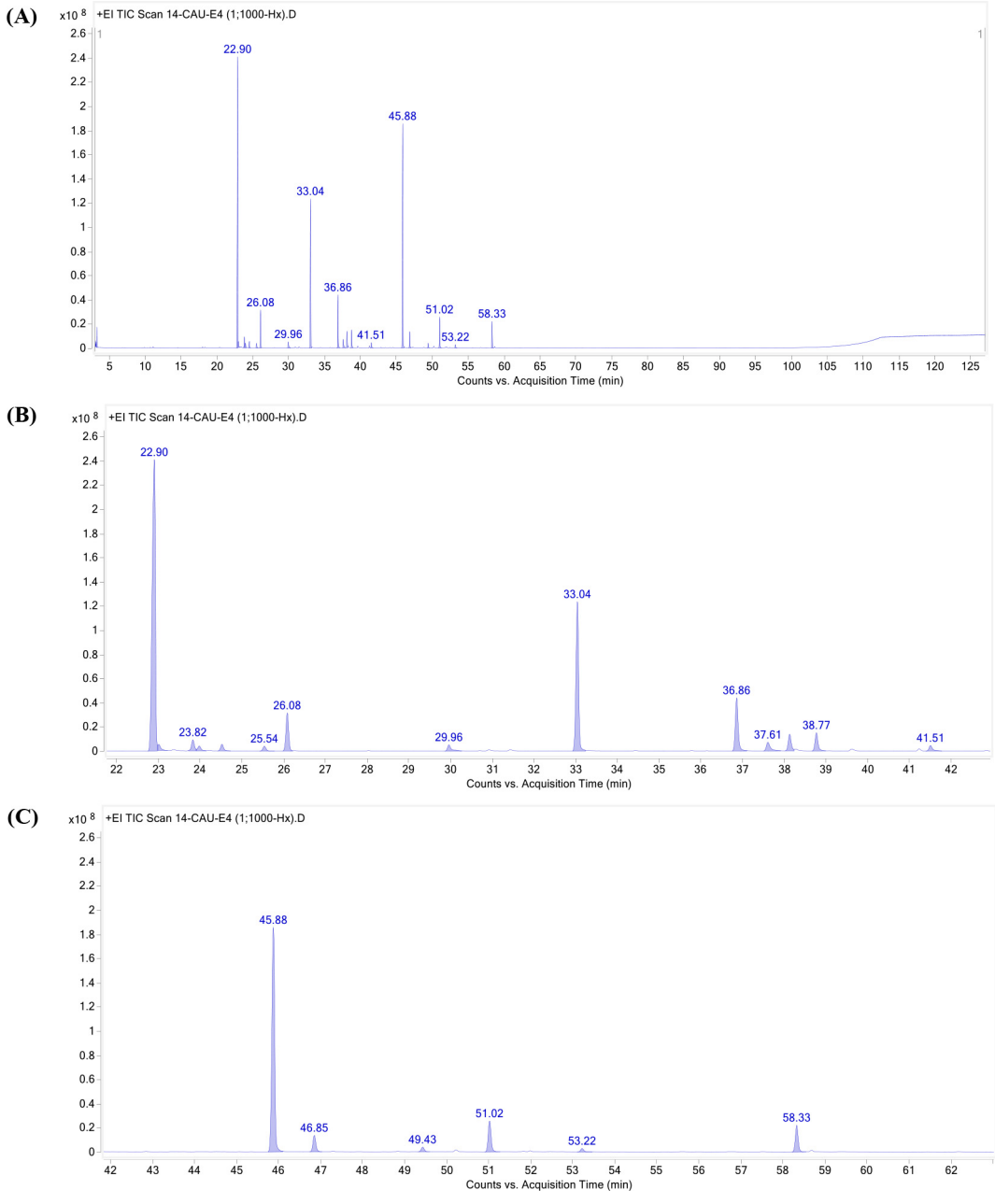

Fig. 4 GC/MS chromatograms of RGL essential oil (A) and expended chromatograms (B and C)

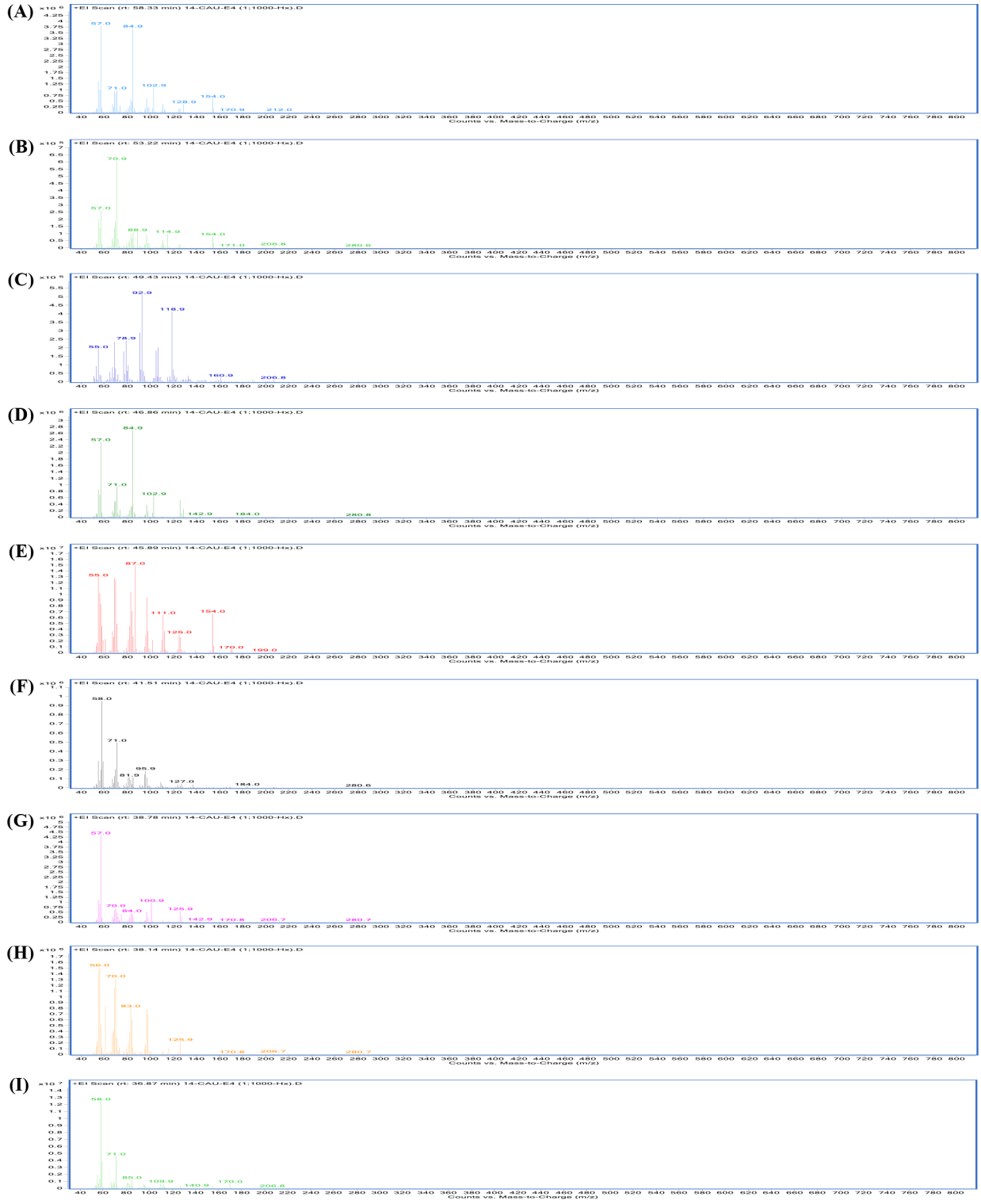

Fig. 5 GC/MS data of the 16 detected components of RGL essential oil: isovaleric acid (A), 1-fluorododecane (B), *trans*-α-bergamotene (C), nonyl pivalate (D), 2-undecanol acetate (E), 2-decyloxirane (F), N-dodecyl fluoride (G), 1-undecanol (H), 2-undecanone (I), nonyl cyclopropanecarboxylate (J), 2-decanone (K), geijerene (L), 1-tridecanol (M), 1-dodecene (N), nonanal (O), and 2-nonanone (P)

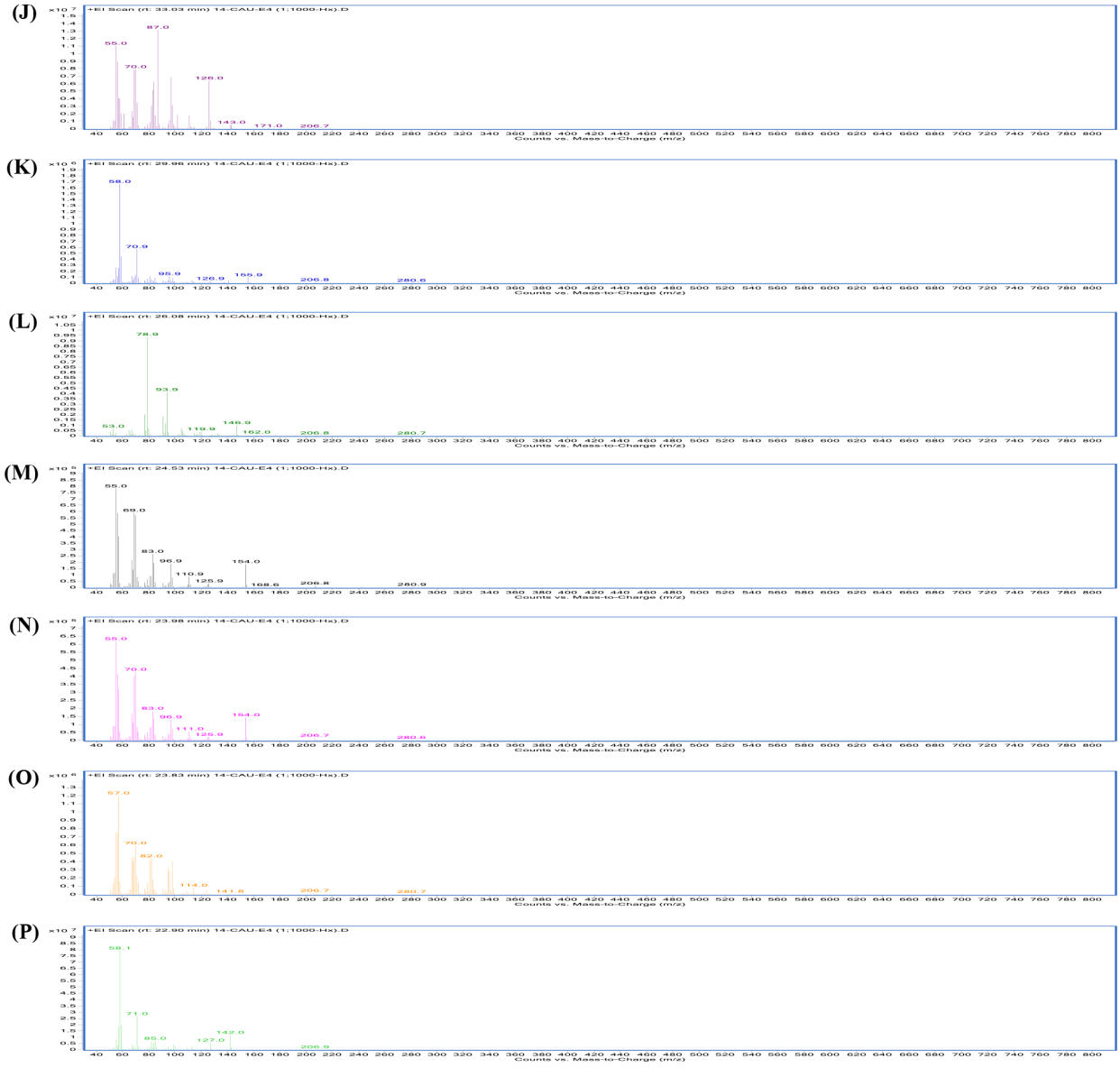

Fig. 5 Continued

have been linked to its high contents of bioactive phytocompounds such as coumarins, essential oil, alkaloids, and phenolic compounds. The extracts and essential oil of RGL are known to possess various pharmacological properties [28,29]. To evaluate the applicability of RGL extracts as antioxidants, this study compared the antioxidant activity of essential oil obtained by steam distillation and EtOH extracts. The EtOH extracts exhibited higher antioxidant activity (DPPH inhibitory activity =67.9%, ABTS+ inhibitory activity =87.1%) compared to the essential oil. A previous study has reported that the essential oil of RGL was mainly composed of 2-metacanone (32.8%), 2-nonanon (29.5%), and 2-nonanol acetate (18.2%), and exhibited weak DPPH radical inhibitory activity [30-32]. Similarly, our findings demonstrated that the antioxidative activity of RGL essential oil (DPPH inhibitory activity of 0% and ABTS inhibitory activity of 15.8%) was far lower than that of EtOH extracts (Fig. 2). Both the DPPH and ABTS radical-scavenging activity assays demonstrated that the radical scavenging activity of the EtOH extract at a concentration of 5,000 ppm was 50% higher than that of essential oil. Therefore, the higher antioxidant activity of the EtOH extract was due to a high level of phenolic compounds with various hydroxyl groups and sugars. Here, two extraction methods were investigated to measure the number and amounts of phenolic compounds in RGL.

Steam distillation and GC/MS analysis were performed to characterize the composition of RGL essential oil. An essential oil yield of 0.02% (v/w) was obtained from the aerial parts of RGL plants. Furthermore, the results of our GC/MS analyses (Table 1)

Table 2 Calibration curves of protocatechuic acid (**1**), rutin (**2**), psoralen (**3**), xanthotoxin (**4**), and bergapten (**5**)

Compound	t_R	Calibration equation[a]	Correlation factor, r^2 [b]
1	5.5	Y = 12372X – 3214.9	0.9997
2	18.7	Y = 12158X – 92793	1
3	30.7	Y = 16607X – 16975	1
4	31.9	Y = 26972X – 17440	0.9997
5	34.5	Y = 55406X – 79639	0.9999

[a]Y = peak area, X = concentration of standards (mg/mL)
[b]r^2 = correlation coefficient based on three data points in the calibration curves

Table 3 Content of protocatechuic acid (**1**), rutin (**2**), psoralen (**3**), xanthotoxin (**4**), and bergapten (**5**) in the MeOH extract of RGL

Sample	Content (mg/g DW)				
	1	**2**	**3**	**4**	**5**
RGL	0.050±0.001	5.222±0.003	0.308±0.001	1.181±0.001	0.466±0.001

indicated that the RGL essential oil was primarily composed of ketone compounds such as 2-nonanone (33.9%), 2-undecanol acetate (22.9%), and nonyl cyclopropanecarboxylate (14.5%) (Fig. 3). And our analyses confirmed the presence of 16 compounds in total (Figs. 4, 5). The profile of essential oil compounds observed in this study was consistent with previous results, which demonstrated that the primary components of RGL essential oil were 2-aliphatic ketones (2-nonanone and 2-undecanol acetate). However, there are also reports that 2-acetoxy tetradecanone (14.49%) was detected in RGL essential oil through GC/MS analysis [33,34]. Our GC/MS analyses detected 16 compounds in total. These findings are in line with previous studies that showed a similar profile of essential oil constituents, with 2-aliphatic ketones being the predominant components. However, there are also reports of 2-acetoxy tetradecanone being detected in the essential oil. Therefore, the composition of the essential oil in RGL may vary due to factors such as climate, geography, and harvest period.

Additionally, the EtOH extracts of RGL contain several polyphenols [13]. Particularly, our HPLC/PDA analyses indicated that the EtOH extracts of RGL contained large amounts of rutin, a type of flavonoid, and the presence of coumarin, psoralen, xanthotoxin, and bergapten was confirmed. The calibration curve of compounds **1**-**5** (Fig. 6) of RGL showed good linearity in Table 2. As illustrated in Fig. 7, each compound exhibited a unique retention time: 5.5 min for protocatechuic acid (**1**), 18.7 min for rutin (**2**), 30.7 min for psoralen (**3**), 31.9 min for xanthotoxin (**4**), and 34.5 min for of bergapten (**5**). A wavelength of 270 nm provided the most efficient response to the quantification of RGL compounds. This response was detected as a single peak for all compounds in the HPLC/PDA chromatogram of RGL, thus demonstrating that the proposed method was reliable. The contents of protocatechuic acid (0.05 mg/g DW), psoralen (0.31 mg/g DW), bergapten (0.47 mg/g DW), xanthotoxin (1.18 mg/g DW), and rutin (5.22 mg/g DW) were confirmed, and our results indicated that the EtOH extract of RGL contained high levels of rutin, with a content of 5.22 mg/g DW. The rutin contents observed in this study were slightly higher than those reported in previous studies (4.92 mg/g DW) [35]. The contents of other compounds such as protocatechuic acid (0.88 mg/g DW), psoralen (1.30 mg/g DW), bergapten (1.60 mg/g DW), and xanthotoxin (1.00 mg/g DW) were also analyzed in this study but were not substantially different from those reported in previous studies [36,37]. These findings provide valuable information on the

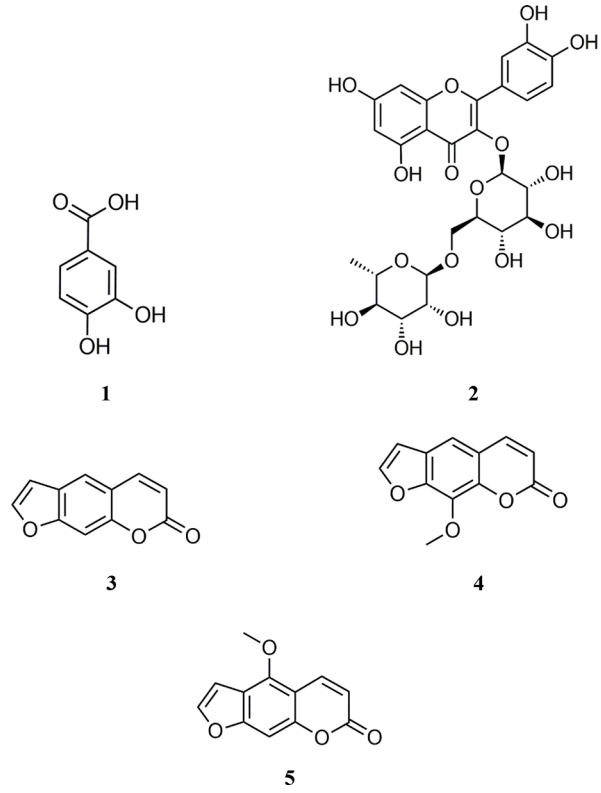

Fig. 6 Chemical structures of protocatechuic acid (**1**), rutin (**2**), psoralen (**3**), xanthotoxin (**4**), and bergapten (**5**)

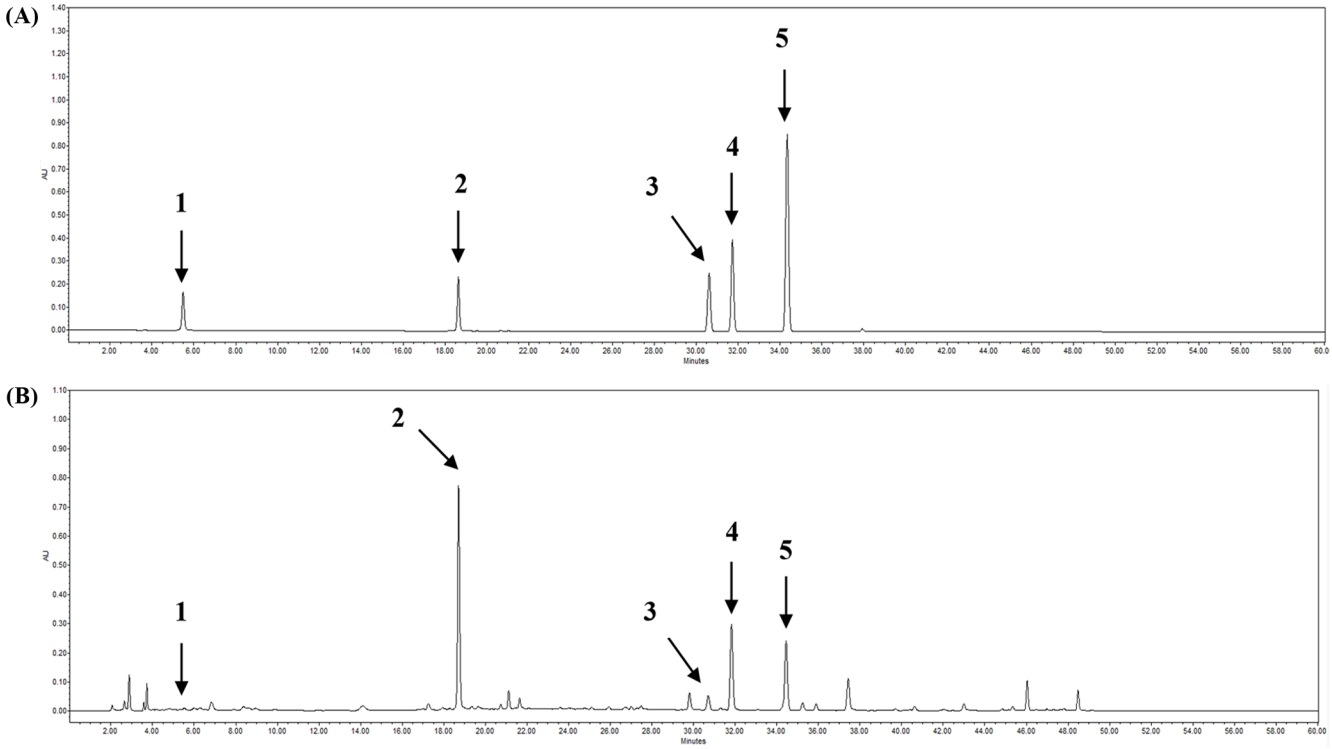

Fig. 7 HPLC/PDA chromatograms of compounds **1-5** (A) and the RGL EtOH extract sample (B): protocatechuic acid (**1**), rutin (**2**), psoralen (**3**), xanthotoxin (**4**), and bergapten (**5**)

composition of compounds **1-5** and highlight the potential of rutin as a valuable compound in RGL. However, further research is still needed to fully understand the implications of these results and to determine the potential applications of compounds **1-5**. Future studies could also explore the effect of different extraction methods on the content of these compounds and how this may impact their potential uses.

This study sought to evaluate the antioxidant activity and composition of RGL essential oil and EtOH extracts. Our results indicated that the EtOH extract of RGL had higher antioxidant activity compared to the essential oil. This was attributed to the presence of phenolic compounds containing various hydroxyl groups and sugars in the EtOH extract. Our GC/MS analyses confirmed the presence of 16 compounds in RGL essential oil, with 2-aliphatic ketones being its predominant components. HPLC/PDA analysis indicated that the EtOH extract of RGL contained several polyphenols, with rutin being the most abundant, followed by psoralen, xanthotoxin, and bergapten. Taken together, our findings provide evidence supporting the potential of RGL as a source of natural antioxidants. Therefore, RGL and its derivatives could be used as a complementary or alternative medicine in the future.

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