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Unlocking the potential antioxidant and anti-inflammatory activities of *Rhododendron molle* G. Don

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Abstract: *Rhododendron molle* G. Don is an important traditional Chinese medicinal plant, which has been applied to treat some inflammatory diseases. In the present study, ethanol extracts of *R. molle* flower (RFE) and leaf (RLE) were used for phytochemical, antioxidant and anti-inflammatory analysis. The antioxidant activity was investigated using the free radicals of 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl radical (OH^-)-scavenging activity, super oxide anion radical ($O_{2\cdot}^-$)-scavenging activity and iron reducing power (FRAP). Production of nitric oxide (NO) was an indicator to evaluate the anti-inflammatory activity. The results showed that compared with RFE, RLE was more active against DPPH (56.66%), FRAP (51.29%) and hydroxyl radical (OH-) (69.66%) at 100μg/mL. In the same time, RLE and RFE had significant anti-inflammatory activity which could reduce nitrite production from 8.76μM to 5.08μM and 6.01μM, respectively. In addition, GC-MS analysis showed that 43 compounds were identified in *R. molle.* Among them, 11 compounds had antioxidant and 5 compounds had anti-inflammatory effect. Results showed that ethanol extracts of *R. molle* have significant antioxidant and anti-inflammatory activity. These results would be helpful for further investigation on the anti-inflammatory mechanism of *R. molle.*

Keywords: *Rhododendron molle* G. Don, antioxidant activity, anti-inflammatory, phytochemical analysis.

INTRODUCTION

Inflammatory response is a defense mechanism which responses to noxious and non-noxious stimulation and whose regulation is strictly proceeded by pro-inflammatory and anti-inflammatory mediators (Abbadie *et al*., 1997). In this process, macrophages play a key role, which can secrete and cause an imbalance of chemokines, mediators and cytokines of inflammation when macrophages were activated (Lalenti *et al*., 1993). What's more, this imbalance can lead to significant cell or tissue damage and chronic inflammatory diseases such as rheumatoid arthritis, autoimmune diseases, neurodegenerative disorders and even cancers (Nathan, 2002; Jou *et al*., 2013). Lipopolysaccharide (LPS), a cell-wall component of bacteria, is a potent innate or natural immune factor, and it could be recognised by macrophages through toll-like receptor 4 (TLR4), and initiates inflammatory response (Hwang *et al*., 2017). LPS-stimulated macrophages cause morphological changes in cells, and activated macrophages could produce pro-inflammatory mediators and cytokines, such as nitric oxide (NO), lipid mediators, reactive oxygen species (ROS) and cytokines (Mongan *et al*., 2000; Huerre and Gounon, 1996). The abnormal expression of these inflammatory mediators was played an essential role in chronic inflammation-related disease (Jou *et al*., 2013). Therefore, reduction of these inflammatory mediators is a target to treat chronic inflammation-related disease.

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Oxidative stress is an imbalance condition between oxidation and antioxidation in the body, due to the excess production of reactive oxygen species (ROS) in cells (Videla *et al*., 2004). Oxidative stress could damage cellular macromolecules and triggers inflammatory responses and it involved in a variety of metabolic diseases (Schuster, 2010; Cheng *et al*., 2014). The nosogenesis of chronic inflammatory diseases may be related to oxidative stress. Nitric oxide (NO) and reactive oxygen species (ROS) almost play a critical role in the regulation immune responses at every stage (Guzik *et al*., 2004). Thus, natural antioxidants can be helpful in treating oxidative stress-related disorders, including inflammation.

Rhododendron molle G. Don (*R. molle*) is a *Rhododendron* plant native to China and Japan. It is one of high value medicinal (Cheng *et al*., 2016; Luo *et al*., 2010). As Chinese traditional medicinal plants, *R. molle* has been applied to treat some inflammatory diseases, including rheumatoid arthritis (RA) (Xia *et al*., 2002). In addition, the root, flower, and fruit of *R. molle* has been reported to contain flavonoids, coumarins, diterpenes, srerols and lignanoids (Zhang *et al*., 2012; li *et al*., 2000; Xia *et al*., 2002). However, previous studies about *R. molle* were focused on propagation and tissue culture, toxicity and genetic diversity (Luo *et al*., 2010; Xia *et al*., 2002). Research on the antioxidant and anti-inflammatory effect of *R. molle* is still very limited, which restricted the investigation and potential application of *R. molle*. Thus, the effects of antioxidant and anti-inflammatory of the extracts of *R. molle* were investigated in this study. The

phytochemical characterization of the extracts was also analyzed. These results would provide insights in further investigation of the anti-inflammatory mechanism, medicinal chemistry and phytochemistry of *R. molle*.

MATERIALS AND METHODS

Reagents and chemicals

Dulbecco's modified Eagle medium-high glucose (DMEM), lipopolysaccharides from *Escherichia coli* 055:B5 (LPS). Trypsin-EDTA 0.25%, L-glutamine, fetal bovine serum (FBS), penicillin/streptomycin, Phosphate buffered solution (PBS), 1,1-diphenyl-2-picrylhydrazyl (DPPH). All of the reagents and chemicals used in this study were analytical grade.

Sample collection and Preparation of extracts

The leaf and flower of *R. molle* were collected in Hunan province, south of China, which was authenticated by Prof. Wang MY, a taxonomist from Jiangxi Normal University. The samples were stored at -20°C for further analytical experiments.

The ethanol extract of *R. molle* was extracted according to the method described by Kang *et al*. (2012). Briefly, dried leaf and flower were pulverized and extracted with 75% ethanol (1:10, w/v) for approximately 1 h and repeated 3 times. The leaf extract (RLE) and flower extract (RLF) were filtered and dryness. Then, the RLE and RLF were dissolved in DMSO and stored at -20°C.

Hydroxyl radical (OH^-)-scavenging activity

The OH^- radical-scavenging activity of RLE and RFE was measured according to the method described by Cheng *et al*. (2014). Briefly, the different concentrations extract were mixed with 1mL of 0.75 mM phenanthroline alcohol solution, 2mL of phosphate buffered solution (0.2 mM PBS), 1mL of 0.75 mM $FeSO_4$, 1 mL of deionized water, and 1mL of H_2O_2 solution (0.01%). Incubated the mixture at 37°C for 60 min and read absorbance of the reaction mixture at 536 nm. OH^- radical-scavenging rate (%)= $(A_S - A_P)/(A_B - A_P) \times 100\%$. Among them, A_P represents the absorbance of control, A_S represents the absorbance of the tested sample, A_B represents blank sample absorbance.

Ferric reducing antioxidant power (FRAP) assay

The ability of reducing Fe^{3+} was estimated by the method according to Cheng *et al*. (2014). 1 mL of the sample solution was mixed with 1mL distilled water and 1mL potassium ferricyanide (1%, w/v). Then the mixture was incubated at 50°C for 20 min. Subsequently, 1mL trichloroacetic acid (10%, w/v) and 1mL ferric chloride solution (0.1%, w/v) was supplemented. Then the absorbance was recorded at 700 nm. FRAP (%)= $(A_S - A_B)/(A_P - A_B) \times 100\%$. Among them, A_S represents the absorbance of the tested sample, A_P represents the absorbance of the $FeCl_2$ which equates to the concentration of $FeCl_3$, A_B represents blank sample absorbance.

Superoxide anion radical (O_2^{-})-scavenging activity

The O_2^{-}-scavenging activity of RLE and RFE were assayed by the method of Cheng *et al*. (2014). 2.5 mL Tris-Hcl (pH 8.2) was mixed with 60% ethanol (0.5 mL) and 0.5 mL deionized water. After incubation for 20 min at 25°C, 0.5 mL different concentrations extract and 0.25 mL of pyrogallol were added. Then absorbance was read at 321 nm. O_2^{-}-scavenging rate (%)= $(A_0 - A_1)/A_0 \times 100\%$. Among them, A_0 represents the absorbance of control. A_1 represents absorbance in the presence of the tested sample.

DPPH free radical scavenging activity

DPPH radical scavenging activity was evaluated using a standard method described by Cheng *et al*. (2014). Briefly, extracts with different concentrations were mixed with 1.5 mL DPPH and incubated for 30 min in the dark. Then recorded at 517 nm. 1.5 mL ethanol replaced DPPH in blank samples. And the reagent blank samples which contained 1.5 mL DMSO and 1.5 mL DPPH. DPPH free radical scavenging rate (%)= $[R_a - (R_b - R_0)] / R_a \times 100\%$. Among them, R_a represents reagent blank absorbance, R_b represents sample absorbance, R_0 represents blank sample absorbance.

Cell viability assay

The cell viability was evaluated by MTT assay. RAW264.7 cells were seeded in 96-well plates. After incubated for 12h, the cells were treated with different concentrations extract for 24h. After incubation, MTT (10 μL) and DMEM (90 μL) were added to each well. After 4h incubation, the media solution was removed and DMSO (150 μL) was added to each well. Then absorbance of each well was read at 570 nm.

Nitric oxide assay

The concentration of nitric oxide (NO) in the cell culture medium was measured by Griess reaction method, as previously described (Cheng *et al*., 2017). The RAW264.7 cells were plated in 24-well plated. After 12h, the cells were treated with different concentrations extract in with or without LPS (1 μg/mL) for 24 h, respectively. After incubation, the culture medium (100 μL) was transferred into each well of 96-well plate and mixed with an equal volume of Griess reagent. After 10 min, absorbance was measured at 540 nm.

Gas chromatography-mass spectrometry (GC-MS) phytochemical analysis

The chemical composition of the *R. molle* leaf and flower ethanol extract was measured using Thermo's GC-MS system according to Alam *et al*. (2017). This GC-MS system fitted with a DB-5MS and coupled with a quadrupole mass detector. The oven temperature was programmed for 60°C for 1 min, with an increase of 8

°C/min to 285°C for 18 min. Mass scan range for 50 to 650 m/z. Individual compounds were identified by comparing fragmentation patterns in the mass spectra with those from the software database.

STATISTICAL ANALYSIS

In each experiment, the data recorded was from 3 replications (n= 3) and all the results are represented as mean±SD. One-way analysis variance (ANOVA) was performed to analyse the differences among groups. $P< 0.05$ was considered to be statistically significant.

RESULTS

Phytochemical analysis

The result of GC-MS analysis indicated that 35 compounds were found in the ethanol extract of *R. molle* flower (RFE) (table 1). β-Sitosterol (20.58%) and Palmitic acid (7.41%) were the major identified components. Cis-9, cis-12-Octadecadienoic acid, 2-Octyldodecan-1-ol, α-Linolenic acid and 5-Hydroxymethylfurfural were detected at high abundance (>3%). Similarly, in the ethanol extract of *R. molle* leaf (RLE) 31 compounds were identified (Table 2), in which β-Sitosterol was the highest abundance component (16.13%). Vitamin E, Phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Squalene, Palmitic acid and α-Linolenic acid were also detected at abundance, higher than 3%.

In total, 43 compounds were identified in the two kinds of extracts (RLE and RFE) by GC-MS analysis. These compounds could be classified into seven classes: phenolic compounds (PC), alcohols (ALC), alkanes and alkenes (AA), fatty acids (FA), phytosterols (PS), esters (ES) and minor groups (MG). Phytosterols (32.09%) and fatty acids (12.66%) were major compounds identified in the RFE (fig. 1A). And in the RLE, phytosterols (20.56%), fatty acid (17.31%) and alcohols (16.92%) were major identified compounds (fig. 1B).

Antioxidant activity

The OH^--scavenging activity of RFE and RLE were shown in fig. 2A. The results showed that the hydroxyl radical scavenging activity of RLE was significantly greater than that of RFE at the same tested concentration. The highest OH^--scavenging capacity of RLE was 69.66±2.12% at 100μg/mL (the concentration of the extracts). However, the OH^- radical scavenging rate of RFE was 49.85±3.31% at the same concentration.

The ability to reduce Fe^{3+} of the extracts was estimated by FRAP assay. The FRAP assay suggested that the FRAP value of RLE was significantly higher than that of RFE at the same tested concentration (fig. 2B). The highest FRAP value of RLE and RFE at 100 μg/mL with 51.29±1.02% and 24.71±1.00%, respectively.

The $O_2^{\cdot-}$-scavenging activities of RFE and RLE were presented in fig. 2C. The $O_2^{\cdot-}$-scavenging activity of RLE was significantly higher than that of RFE at the same concentration. The maximum scavenging capacity of super oxide anion radical was 10.19±0.64% for RLE and 8.52±0.68% for RFE at 100μg/mL, respectively.

Further analysis indicated that all the samples were dose-dependently scavenged DPPH radicals. The highest DPPH scavenging activity of RLE was to 56.66±0.45% at the concentration of 100μg/mL. However, the fig for RFE was 47.21±0.56% at the same concentration (fig. 2D).

Anti-inflammatory activity

The potential cytotoxicity of RLE and RFE was evaluated in RAW264.7 cells by MTT assay (fig. 3A). The results showed that low levels of RLE and RFE (≤50μg/mL) had no inhibitory effect on cell viability. Based on the above results, RLE of 1, 5, 10, 30 and 50μg/mL were used to investigate the anti-inflammatory effect in subsequent *in vitro* experiments.

The anti-inflammatory effect of RLE and RFE was determined by Griess reaction method (fig. 3B). The results indicated that the concentration of NO was 2.51 μM in control group and in LPS-induced group the NO production level was 8.76μM which was approximately 3.5 times higher than the control group. All tested concentrations of RLE and RFE could be dose-dependent reduced LPS-induced elevation of NO, and the NO concentration reduced to 5.08μM at 50μg/mL of RLE.

DISCUSSION

In order to confirm the use of *R. molle* in traditional drug treatment of inflammation, we assessed its in vitro anti-inflammatory, antioxidant potential and phytochemical composition. In this study, GC-MS analysis detected 43 compounds in *R. molle* flower and leaf extracts. Phytosterols and fatty acids were the main categories of compounds. In addition, β-Sitosterol was the major compound in RFE and RLE with 20.58% and 16.13%, respectively. β-Sitosterol and α-linolenic acid had been identified previously in *R. molle* (Wang *et al*., 2014). In this study, 9 of 43 compounds have been detected in Ericaceae, namely, Phenol,2,4-bis(1,1-dimethylethyl), α-Linolenic acid, cis-9,cis-12-Octadecadienoic acid, Phytol, Linolenic acid methyl ester, Hexadecanoic acid, Palmitic acid and n-Hexadecanoic acid methyl ester (Zhao *et al*., 2005; Carballeira *et al*., 2008; Zhang *et al*., 2004; Feng *et al*., 2005; Chou *et al*., 2009; Shen, 1997). The other 34 compounds were detected for the first time in the *R. molle*. GC-MS analysis enriched the chemical constituents of the *R. molle* and provided some useful information for further investigation on the medicinal chemistry and phytochemistry of *R. molle.*

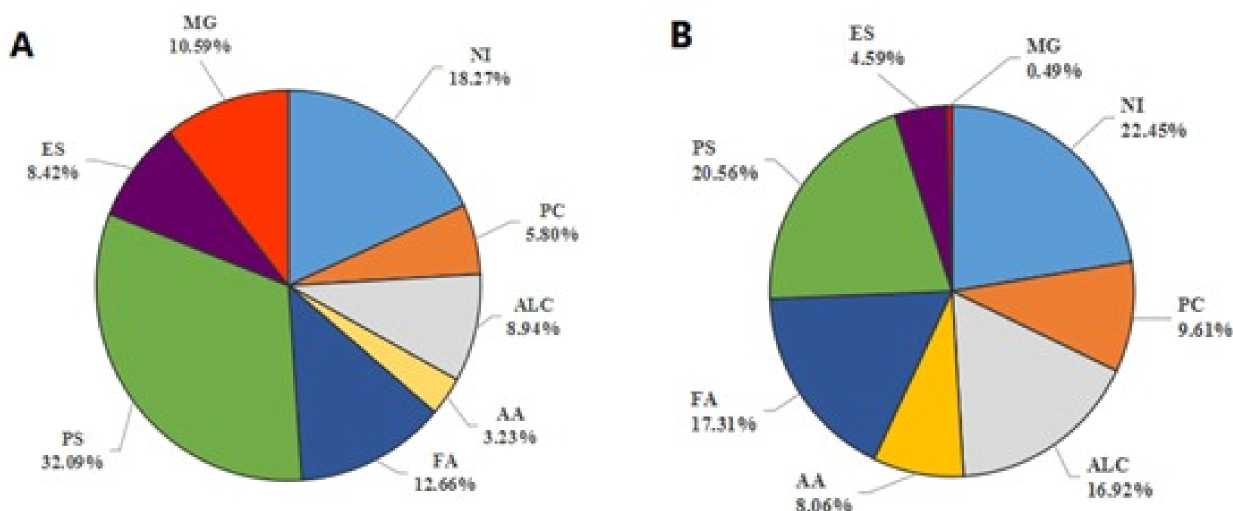

Fig. 1: Main chemical compound classes identified by GC/MS in the ethanol extracts of flowers and leaves of *R molle*. (A) The ethanol extract of *R. molle* flowers (RFE). (B) The ethanol extract of *R. molle* leaves (RLE). ALC: alcohols; AA: alkanes and alkenes; FA: fatty acids; PS: phytosterols; ES: esters; MG: minor groups; PC: phenolic compounds; NI: non-identified compounds.

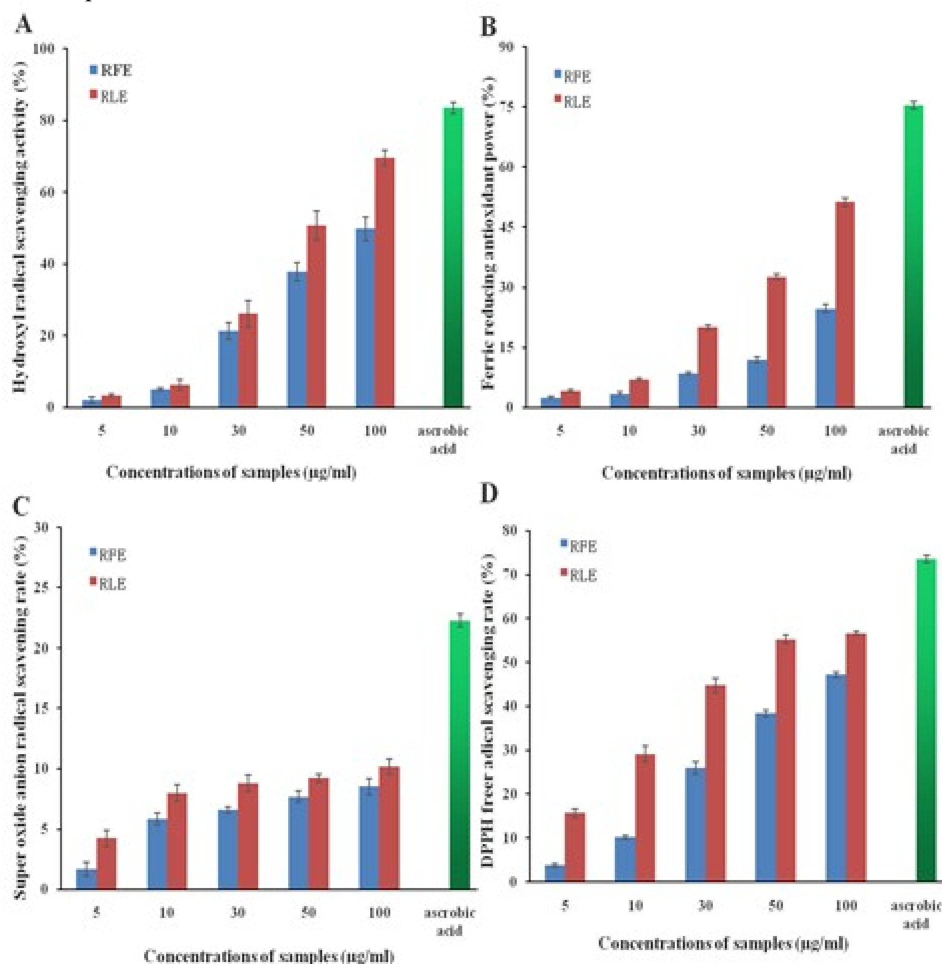

Fig. 2: Antioxidant effect of RFE and RLE. (A) Hydroxyl radical (OH^-)-scavenging capacity. (B) Ferric reducing ability. (C) Super oxide anion radical ($O_{2\cdot}^-$)-scavenging capacity. (D) DPPH scavenging capacity of the ethanol extract of the *R molle* flowers and leaves. All measurements are expressed as means ± SD of three separate determinations.

Table 1: GC-MS profile of ethanol extract of the *R. molle* flowers.

NO.	Compound	Molecular formula	Area %
Phenolic compounds			
1	δ-tocopherol	$C_{27}H_{46}O_2$	0.79
2	2,7,8-Trimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol	$C_{28}H_{48}O_2$	2.45
3	Vitamin E	$C_{29}H_{50}O_2$	2.56
Alcohols			
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.65
5	Phytol	$C_{20}H_{40}O$	1.34
6	1-Heptacosanol	$C_{27}H_{56}O$	0.57
7	17-Pentatriacontene	$C_{35}H_{70}$	2.97
8	2-Octyldodecan-1-ol	$C_{20}H_{42}O$	3.41
Alkanes and alkenes			
9	n-Tetracosane	$C_{24}H_{50}$	1.06
10	n-Triacontane	$C_{30}H_{62}$	0.64
11	Squalene	$C_{30}H_{50}$	0.56
12	n-Tetratriacontane	$C_{34}H_{70}$	0.97
Fatty acids			
13	Stigmasta-3,5-diene	$C_{29}H_{48}$	0.86
14	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.1
15	Palmitic acid	$C_{16}H_{32}O_2$	7.41
16	Cis-9,cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	3.29
Phytosterols			
17	α-linolenic acid	$C_{18}H_{30}O_2$	5.44
18	Campesterol	$C_{28}H_{48}O$	2.2
19	β-sitosterol	$C_{29}H_{50}O$	20.58
20	24b-Ethylcholest-7-en-3b-ol	$C_{29}H_{50}O$	2.53
21	Stigmast-4-en-3-one	$C_{29}H_{48}O$	1.34
Esters			
22	Phytyl acetate	$C_{22}H_{42}O_2$	1.36
23	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	0.68
24	Hexadecanoic acid	$C_{18}H_{36}O_2$	0.98
25	Linoleic acid methyl ester	$C_{19}H_{34}O_2$	0.83
26	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	0.79
27	Octadeca-9,12-dienoic acid ethyl ester	$C_{20}H_{36}O_2$	1.69
28	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	1.1
29	Palmitic acid glycerol ester	$C_{19}H_{38}O_4$	0.99
Minor groups			
30	Propionaldehyde dimethyl acetal	$C_5H_{12}O_2$	0.78
31	Methyl (Z)-Benzohydroximate	$C_8H_9NO_2$	0.67
32	1-(4-methylphenyl)-2-pyrrolidin-1-ylpentan-1-one	$C_{16}H_{23}NO$	1.77
33	3,5-dihydroxy-2-methyl-2,3-dihydropyran-4-one	$C_6H_8O_4$	1.31
34	5-hydroxymethylfurfural	$C_6H_6O_3$	5.19
35	(Z)-docos-13-enamide	$C_{22}H_{43}NO$	0.87

Our results indicated that *R. molle* has strong dose-dependent antioxidant activity in the experiment of DPPH radical scavenging capacity, FRAP assay, OH^--scavenging activity and O_2^--scavenging activity. Antioxidant activity of *R. molle* was stronger than that of some relative species, such as *Rhododendron anhwiense* Wils (Cheng *et al*., 2013) and *Rhododendron yedoense* var. Poukhanense (Lee *et al*., 2011). OH^--scavenging capacity of *R. molle* was 69.66% at 100μg/mL. In contrast, *Lycium barbarum* L. need about 14mg/mL to reach the same level (Li *et al*., 2014). The high antioxidant effect might be attributable to some phenolic compounds in ethanol extracts of *R. molle*, such as δ-Tocopherol, Phenol,2,4-bis(1,1-dimethylethyl) and Vitamin E. These compounds play a key role in human health mainly because they usually have a significant effect on free radical scavenging activities and antioxidant (Carballeira *et al*., 2008). In addition, Phytol, Squalene, α-Linolenic acid, cis-9,cis-12-Octadecadienoic acid, β-Sitosterol, Linoleic acid methyl ester, 5-Hydroxymethylfurfural and Stearic acid also have

Table 2: Phytochemical composition of ethanol extract of the *R. molle* leaves, determined by GC-MS analysis.

NO.	RT	Compound	Molecular formula	Area %
Phenolic compounds				
1	12.67	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	0.51
2	15.83	Phenol,2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	0.66
3	27.06	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl]	$C_{23}H_{32}O_2$	1.37
4	33.84	2,7,8-Trimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol	$C_{28}H_{48}O_2$	0.88
5	35.21	Vitamin E	$C_{29}H_{50}O_2$	6.19
Alcohols				
6	20.67	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	4.88
7	23.7	Phytol	$C_{20}H_{40}O$	11.61
8	34.45	17-Pentatriacontene	$C_{35}H_{70}$	0.43
Alkanes and alkenes				
9	30.9	Squalene	$C_{30}H_{50}$	3.31
10	31.59	n-Triacontane	$C_{30}H_{62}$	1.02
11	34.36	n-Tetratriacontane	$C_{34}H_{70}$	2.35
12	34.71	Stigmasta-3,5-diene	$C_{29}H_{48}$	0.69
13	38.43	n-Hexatriacontane	$C_{36}H_{74}$	0.69
Fatty acids				
14	19.34	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.61
15	21.92	Palmitic acid	$C_{16}H_{32}O_2$	7.28
16	23.94	cis-9,cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	2.64
17	24.03	α-linolenic acid	$C_{18}H_{30}O_2$	4.52
18	24.23	Stearic acid	$C_{18}H_{36}O_2$	1.26
Phytosterols				
19	37.17	Campesterol	$C_{28}H_{48}O$	0.84
20	39.28	β-sitosterol	$C_{29}H_{50}O$	16.13
21	40.16	β-amyrin	$C_{30}H_{50}O$	1.03
22	41.39	α-amyrin	$C_{30}H_{50}O$	1.79
23	43.05	Stigmast-4-en-3-one	$C_{29}H_{48}O$	0.77
Esters				
24	21.44	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	0.76
25	22.27	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	0.61
26	23.56	Linolenic acid methyl ester	$C_{19}H_{32}O_2$	0.44
27	24.32	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	0.66
28	27.78	Palmitic acid glycerol ester	$C_{19}H_{38}O_4$	0.92
29	28.43	Hydrocortisone acetate	$C_{23}H_{32}O_6$	0.73
30	38.64	Triacontyl acetate	$C_{32}H_{64}O_2$	0.47
Minor groups				
31	30.41	cis-13-Docosenoamide	$C_{22}H_{43}NO$	0.49

significant antioxidant effect (Farhoosh, 2005; Chen *et al*., 2017; Vivancos and Moreno, 2005; Fagal and Catala, 2008; Zhao *et al*., 2013; Oh *et al*., 2012). Moreover, there were 40.58% and 45.06% potent antioxidant compounds in RFE and RLE, respectively, which might be for the reason why the leaves had stronger antioxidant activity than that of the flowers.

LPS is a cell wall endotoxin produced by Gram-negative bacteria that activate macrophages to produce inflammatory mediators such as Nitric oxide (NO) (Cheng *et al*., 2014). NO is a radical associated with chronic inflammation and it is involved in many physiological and pathological processes (Joo *et al*., 2014; Hwang *et al*., 2017). For these reasons, reducing the over production of NO has become a new target for anti-inflammatory therapy (Cirino *et al*., 2006). In this study, we found that *R. molle* had a higher dose-dependent anti-inflammatory activity compared to similar extract of other plant, such as *Carthamus tinctorius* L. (Liao *et al*., 2013). The anti-inflammatory effect of *R. molle* may be due to the presence of compound(s) with potential anti-inflammatory properties, most likely β-Sitosterol, α-Linolenic acid, cis-9,cis-12-Octadecadienoic acid, 2-Methoxy-4-vinylphenol and α-Amyrins (tables 1 and 2). β-Sitosterol was the major compound in RFE and RLE and its anti-inflammatory activity was performed by inhibiting NO and TNF-α-induced phosphorylation of

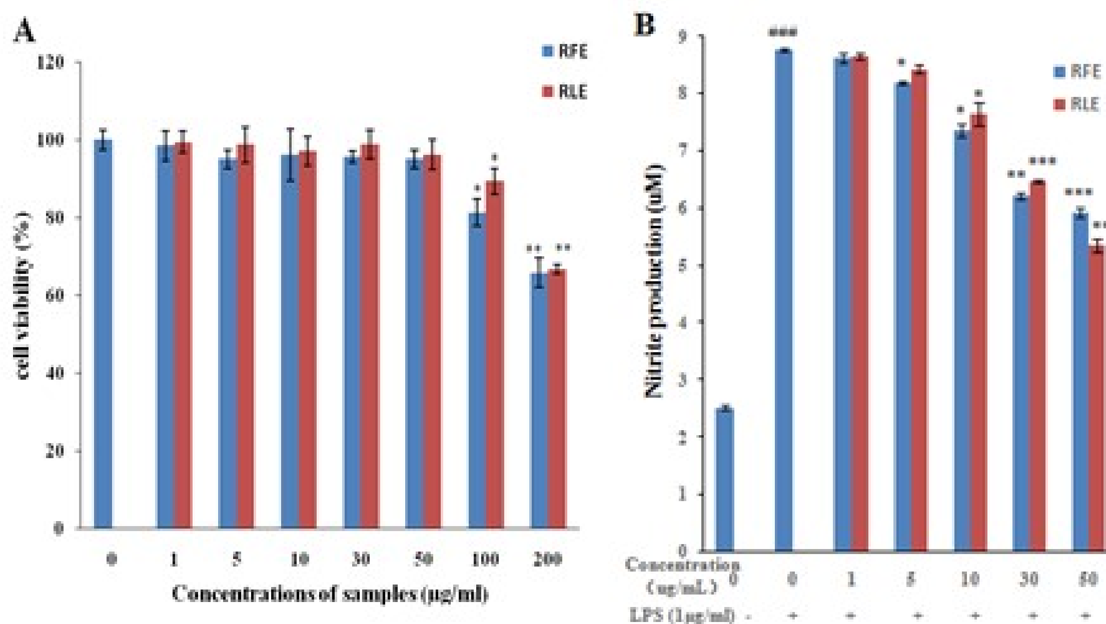

Fig. 3: Effects of RFE and RLE on LPS-induced NO production and on cell viability in RAW 264.7 cells. (A) Cells were treated with RFE and RLE (concentrations ranging from 5 to 500 μg/mL), and cell viabilities were determined by MTT assay. (B) Following pretreatment with RFE and RLE (1, 5, 10, 30, 50 μg/mL) for 2 h, the cells were treated with LPS (1 μg/ml) for 20 h, determine the nitric oxide (NO) production was based on the Griess reaction. All measurements are expressed as means ± SD of three separate determinations. Data are presented as means ± SD of three independent experiments. $^{###}$p<0.01 versus control group; *p<0.05 **p<0.01, ***p<0.001 versus LPS group.

NF-kappa B pathway (Loizou *et al*., 2010), and so do cis-9,cis-12-Octadecadienoic acid and α-Amyrin (Saiki *et al*., 2017; Medeiros *et al*., 2007). In turn, the α-Linolenic acid and 2-Methoxy-4-vinylphenol have anti-inflammatory properties through decrease the levels of TNF-α and NO (Erdinest *et al*., 2012; Jeong *et al*., 2011). Which might be why the RFE and RLE had significant NO inhibitory capacity. So, these results could provide some helpful information about the antioxidant and anti-inflammatory effect of *R. molle*. Moreover, it would also create a foundation for further study and clinical application of *R. molle*.

CONCLUSION

In this study, 43 compounds were detected in the ethanol extracts of *R. molle* by GC-MS analysis, of which there are abundant potential compounds resistant to oxidation and inflammatory syndromes. Both of RLE and RFE have strong antioxidant activity. However, the antioxidant activity of RLE was significantly higher than that of RFE. In addition, we have established the LPS stimulation RAW264.7 inflammatory reaction model, and NO was used as an index to evaluate the anti-inflammatory activity of *R. molle*. The results indicated that the RFE and RLE can significantly reduce NO on LPS-induced RAW264.7. These results would provide some useful information for further investigation on the medicinal chemistry and phytochemistry of *R. molle*.

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