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In vitro antibacterial, antioxidant, anti-inflammatory and analgesic evaluation of *Rosmarinus officinalis* L. flower extract fractions

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ABSTRACT

Rosmarinus officinalis L. (rosemary) is a common culinary spice and herbal drug, which is used for centuries all over the world. In this present study, apolar to polar fractions of *R. officinalis* flowers were evaluated for their *in vitro* antioxidant, antibacterial, cytotoxic, anti-inflammatory and analgesic activities, respectively. Phytochemical compositions of *R. officinalis* extract fractions were analyzed by GC–MS and LC–MS. The antioxidant capacity of the fractions was evaluated by using the DPPH$^{\bullet}$ and ABTS$^{\bullet}$ methods. The antibacterial potential was determined using the *in vitro* broth microdilution assay against a panel of human pathogens. The analgesic and anti-inflammatory activities were investigated measuring nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in LPS-stimulated cells, respectively. In addition, *in vitro* cytotoxicity of the extract fractions was evaluated on RAW 264.7 murine macrophage cells by using the MTT assay. The constituents of the polar fractions were identified as rosmarinic acid, luteolin, quercetin and apigenin by LC techniques, whereas the *n*-hexane fraction was analyzed by GC–MS to determine the main volatile components camphor (19.6%), 1,8-cineole (11.7%), verbenone (11.5%), borneol (10.6%), α-pinene (5.8%), and linalool (5.7%). According to the bioactivity results, the polar fraction showed the highest antioxidant activity, whereas *n*-hexane fraction was found to be most effective against *Staphylococcus aureus* (78 μg/mL). The *n*-hexane fraction (100 μg/mL) reduced the LPS-induced NO and PGE_2 production capability. In conclusion, *R. officinalis* flower *n*-hexane and ethyl acetate fractions exhibited remarkable *in vitro* antibacterial, antioxidant, anti-inflammatory and analgesic activities possibly due to their polyphenol content, to the best of our knowledge for the first time.

1. Introduction

Rosmarinus L. is a well-known member of the Lamiaceae family. The natural habitats for *Rosmarinus* species are Southern and Northern Africa, Western Asia, Anatolia and Aegean-Mediterranean parts of the world and grow well in the foothills of Malut Mountain in South Africa. *Rosmarinus* L., which is also cultivated at various sites for its culinary uses and for the production of the essential oil (Davis, 1982; Tyler et al., 1976; Mill, 1982).

Rosmarinus officinalis preparations are used mainly in the food industry as flavors, but also in fragrances, and medicines among other utilizations. It is reported that *Rosmarinus* species are used in traditional medicines for the treatment of various diseases and conditions as an antispasmodic, carminative, renal colic, antirheumatic, diuretic, cholagogue, antiepileptic, expectorant; against diabetes, dysmenorrhea, heart diseases and in relieving respiratory disorders *etc.* It has also been used for analgesic purposes against abdominal pain, stomachache, and throat ache. In addition, it was utilized as a tonic to improve memory dysfunction, especially at excessive physical or mental works. Moreover, the plant is known to be used as insecticide and herbicide among many other reported uses (Andsersen et al., 2006; Al-Sereiti et al., 1999; Bulut and Tuzlacı, 2015; Jouad et al., 2001; Afolayan and Mbaebie, 2010; Van Wyk et al., 2008).

Previous *in vivo* and *in vitro* studies showed that *R. officinalis* aerial parts have antioxidant, antimicrobial (Bozin et al., 2007), hepatoprotective (Hoefler et al., 1987; Joyeux et al., 1990), hypoglycemic–hypolipidemic (Bustanji et al., 2010; Vanithadevi and Anuradha, 2008), and anticancer activities (Kontogianni et al., 2013). Biological

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activities of *R. officinalis* extracts are mainly attributed to its phenolic constituents such as rosmarinic acid, carnosol, and carnosic acid present in the rosemary preparations (Teixeira et al., 2013; Babovic et al., 2010; Arranz et al., 2015), and α-pinene, bornylacetate, 1,8-sineole, and camphor present in the essential oils (Bozin et al., 2007; Celiktas et al., 2007; Wichtl, 2008).

The volatiles of *R. officinalis* is diverse based on the previously identified major compositions consisting of 1,8-cineole, α-pinene, borneol, camphor, bornyl acetate, and verbenone chemotypes, respectively. As a result, 13 different *Rosmarinus* essential oil chemotypes are determined, based on the relative percentages of 1,8-cineole, α-pinene, borneol, camphor, bornyl acetate, and verbenone (Celiktas et al., 2007; Satyal et al., 2017).

The aim of this present study was to evaluate the *in vitro* antibacterial, antioxidant, anti-inflammatory and analgesic activities of *R. officinalis* flower extracts. In order to reveal the flower extracts' phytochemical fingerprint and composition, the ethyl acetate and *n*-hexane fractions were analyzed by Liquid Chromatography/Mass Spectrometry (LC/MS) and Gas Chromatography/Mass Spectrometry (GC/MS), respectively.

2. Materials and methods

2.1. Materials

2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Trolox and ascorbic acid were purchased from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). All used chemicals were of analytical grade or higher if not otherwise stated.

2.2. Plant material and extraction

Rosmarinus officinalis flowers were collected during the flowering, from its natural habitat Cavusbasi Village/Beykoz, Istanbul (Turkey), in 2018. Plant material was identified by Ayse Esra Karadag and voucher specimens (specimen no. IMEF: 1056) were deposited at Herbarium of the Department of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, Istanbul, Turkey. The air-dried plant material was ground to fine powder, which macerated initially with methanol for 24 h. After filtration and evaporation (Heidolph, Germany), subfractions were prepared by liquid–liquid extraction using *n*-hexane and ethyl acetate, respectively for further analyses.

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging assay

Total antioxidant capacity was determined using DPPH• method described by Blois and co-workers (Blois, 1958). The reaction mix contained 100 μM DPPH• in methanol and *n*-hexane/ethyl acetate fractions. After 30 min, the absorbance was read at 517 nm by using a UV spectrophotometer (UV-1800, Shimadzu, Japan) at 25 ± 2 °C. The radical scavenging activity (RSA) was calculated as the percentage of radical reduction as follows:

$$\text{DPPH}^{\bullet}\text{RSA}\% = \left[\left(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test sample}}\right)/\text{Absorbance}_{\text{control}}\right] \times 100$$

2.3.2. ABTS radical scavenging assay

The antioxidant capacity of the samples was determined using the ABTS radical cation decolorization protocol described by Re et al. (1999). 2.45 mM potassium persulfate and 7 mM aqueous ABTS were reacted to produce ABTS•. The mixture was kept at 25 °C in a dark room for 16 h before use. Ethanol was added to the mixture and the absorbance was calculated at 734 nm at 25 °C. The process was performed in triplicate. Ethanol was used as negative controls and Trolox was used as a positive control as previously reported (Okur et al., 2018). The results were calculated as IC_{50}.

$$\text{ABTS}^{\bullet}\text{RSA}\% = \left[\left(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test sample}}\right)/\text{Absorbance}_{\text{control}}\right] \times 100$$

Antioxidant assay results are shown in Table 1, comparatively with standard reference substances.

Table 1
R. officinalis flower extract fractions; ABTS and DPPH radical scavenging activities.

	Ethyl acetate	*n*-hexane	Reference compounds
	$IC_{50} \pm SD$ (mg/mL)		
ABTS•	0.18 ± 0.04	1.26 ± 0.03	0.015 ± 0.001 (Trolox)
DPPH•	0.10 ± 0.03	0.94 ± 0.04	0.002 ± 0.001 (Ascorbic acid)

2.4. Antibacterial activity

The *in vitro* antibacterial activity was determined using the broth microdilution assay following the methods according to the Clinical and Laboratory Standards Institute (CLSI, 2006) to determine the minimum inhibitory concentrations (MIC) as well as the minimum bactericidal concentrations (MBC). *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 10145, and *Escherichia coli* NRLL B-3008 strains were grown in Mueller Hinton Broth (MHB, Merck, Germany) in aerobic conditions at 37 °C for 24 h. All microorganisms were adjusted to 1×10^8 CFU/mL using McFarland No: 0.5 in sterile saline (0.85%) solution.

Helicobacter pylori ATCC 43504 strain was grown for 24 h in Brucella broth (Sigma–Aldrich) containing 5% (*v*/*v*) horse blood and 10% (h/h) fetal bovine serum (FBS, Sigma–Aldrich) in an anaerobic incubator at 37 °C (5% CO_2). After incubation at 37 °C, 100 μL of 1:10 diluted and adjusted *H. pylori* (2×10^7 CFU/mL) strain, which was transferred to the microplate evaluation (EUCAST, 2011; Whitmire and Merrell, 2012). Diluted bacterial suspensions were added each well and then incubated at 37 °C for further 24 h.

Mycobacterium strain was inoculated in Middlebrook 7H11 agar (Sigma–Aldrich) and incubated in aerobic conditions at 37 °C for 4–5 days. The microorganism was transferred to the cation doped MHB and incubated for a further five days. Growing cultures were vortexed and allowed to collapse for 30 min. Diluted bacterial suspension (10^6 CFU/mL) was added to each well and incubated at 37 °C for 5 days (CLSI, 2003; Chung et al., 1995; Lee et al., 2007).

Stock solutions and serial dilutions of the test samples were prepared in dimethylsulfoxide (DMSO). The minimum non-reproductive concentration was reported as minimum inhibitory concentration (MIC, as μg/mL). A small amount of this well was transferred to the Petri dishes. The minimum concentration without bacterial growth on agar was also considered as the minimum bactericidal concentration (MBC). The MBC and MIC were calculated and reported as the mean of three repetitions compared to positive standards as shown in Table 2.

Table 2
Antibacterial activity of *R. officinalis* flower extract fractions (MICs in μg/mL).

	E. coli	*S. aureus*	*P. aeruginosa*	*E. faecalis*	*H. pylori*	*M. smegmatis*
n-hexane	>1000	78	625	156	156	>1000
Ethyl acetate	>1000	312	>1000	625	>1000	>1000
Chloramphenicol	8	8	>32	16	16	–
Tetracycline	16	0.25	>16	0.025	0.025	–
Amikacin	–	–	–	–	–	250

2.5. Anti-inflammatory and analgesic activity

2.5.1. Cell culture

The RAW 264.7 murine macrophage cell line (ATCC, USA) was grown in DMEM (10% FBS), streptomycin (10.000 μg/mL), and 1% penicillin (10.000 units/mL) at 37 °C in humidified atmosphere of 5% CO_2. Cell viability was examined by using the MTT colorimetric assay. Plated RAW 264.7 cells were treated with various concentrations of *R. officinalis* ethyl acetate and n-hexane extracts (1.5–1000 μg/mL), respectively. After 24 h, the cell medium was discarded. MTT solution (0.5 mg/mL) was added to wells and allowed to incubate for an additional 2 h at 37 °C. The cell culture medium was taken out after incubation and 100 μL of isopropanol was then added to wells for dissolving the formazan. The absorbance was determined at 570 nm wavelengths by a microplate reader (Thermo Multiskan Spectrum, Finland). The percentage of cell viability (%) was calculated as follows:

$$\text{Viability}\% = (\text{Absorbance}_{\text{treatment group}} - \text{Absorbance}_{\text{background}})/(\text{Absorbance}_{\text{control group}} - \text{Absorbance}_{\text{background}}) \times 100\%$$

The absorbance of the control group was considered as 100% as shown in Fig. 1.

2.5.2. Anti-inflammatory activity by Griess assay

Anti-inflammatory activity of *R. officinalis* n-hexane and ethyl acetate fractions was evaluated by measuring the stable nitric oxide (NO) metabolite, nitrite levels, in cell culture media with Griess reagent (Kiemer and Vollmar, 1997). RAW 264.7 cells were placed in a 48 well-plate at the density of 1×10^6/mL and incubated for 24 h at 37 °C in 5% CO_2. The cells were pre-treated with the non-toxic concentrations of ethyl acetate (1.25–10 μg/mL) and n-hexane (12.5–100 μg/mL) fractions for 2 h. After then the cells were stimulated with 1 μg/mL of LPS (lipopolysaccharide from *Escherichia coli* 0111:B4, Sigma, USA) for an additional 22 h. The 50 μL of collected culture supernatant was mixed with 50 μL Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 5% phosphoric acid and 1% sulfanilamide) and allowed to incubate at room temperature for 10 min in a dark place. The absorbance was determined using a microplate reader (Multiskan Ascent, Finland) at 540 nm wavelength. The nitrite concentration in samples was calculated by using a sodium nitrite standard curve. Indomethacin (100 μM) was used as a positive control.

2.5.3. Analgesic activity by prostaglandin E_2 levels

In the evaluation of analgesic activity, concentrations showing significant antiinflammatory activity were used. Prostaglandin E_2 (PGE_2) levels in collected cell culture supernatants were detected by using a commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kit (Abcam PGE2 ELISA Kit, UK) according to manufacturer's instructions.

2.5.4. Statistical analysis

All repeated experiments were conducted in triplicate. Statistical analysis was observed by using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA; Version 6.01). Differences between groups were determined by using one-way ANOVA following the post-hoc tests by Tukey. Group differences were considered to be significant at $p < .05$ (*), $p < .01$ (**), $p < .001$ (***).

2.6. Chemical content analyses

2.6.1. GC-FID and GC–MS analysis

GC–MS analysis (Agilent 5975 GC-MSD) was performed using an innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness). Helium was used as a carrier gas and the flow rate was 0.8 mL/min. The GC oven

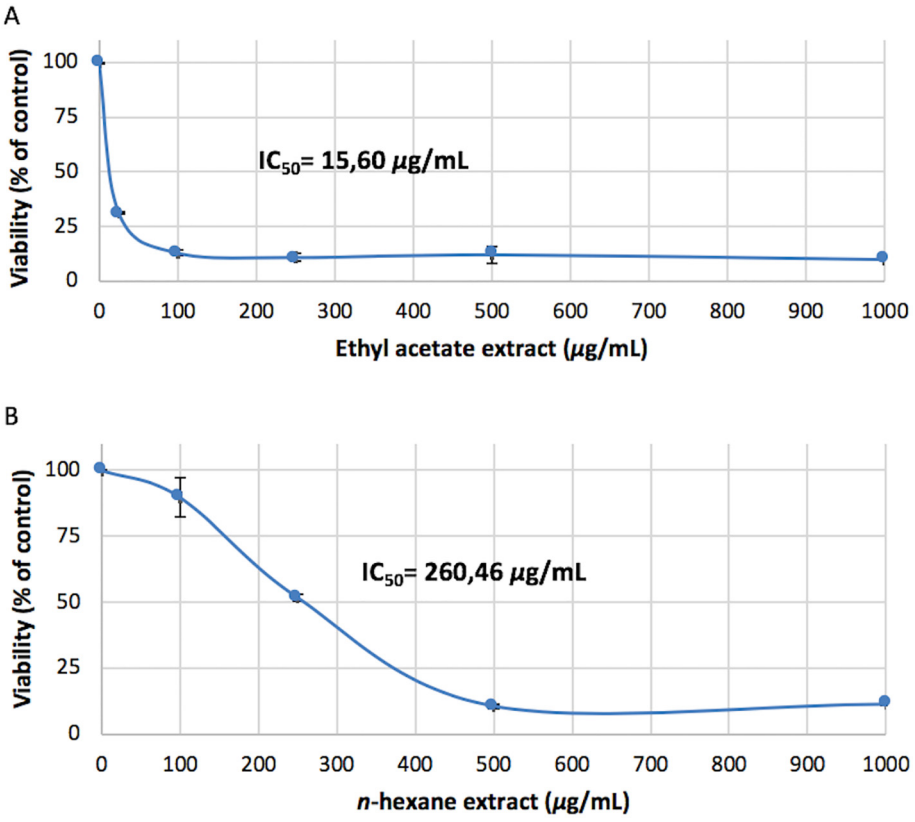

Fig. 1. Evaluation of cell viability of extracts on RAW 264.7 cells. A) Ethyl acetate fraction and B) *n*-hexane fraction. Cells were exposed with varying concentrations of ethyl acetate and *n*-hexane fractions for 24 h, and cell viability was measured by MTT assay and normalized with untreated control cells.

temperature was maintained at 10 min for 60 °C, then heated to 220 °C (4 °C/min), and it was kept for 10 min. Then it was maintained to 240 °C (1 °C/min rate). The split rate was set at 40:1. 250 °C was used as the injection temperature. The Mass Spectra were documented at 70 eV, and the mass ranges were from m/z 35 to 450.

FID temperature for GC analysis (Agilent 6890N GC) was set to 300 °C. Simultaneous auto-injection was applied using equal conditions in duplicate. Relative percentage (%) amounts of the separated compounds were determined.

Identification of the volatile components was carried out by comparing their relative retention indices (RRI) to a series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0), and in-house "Başer Library of Essential Oil Constituents" library as well as to literature (Koenig et al., 2004; McLafferty and Stauffer, 1989) was performed.

2.6.2. LC–MS analysis

Prior LC–MS analyses, ethyl acetate fraction of *R. officinalis* flowers was filtered through inert 0.22 μm membranes. Followed by the LC–MS analyses studied on a single quadrupole mass spectrometer (1200 LC, Agilent). LC–MS was run on an Agilent C18 column (4.6 × 250 mm 5 μm) and its temperature was maintained at 40 °C. The mobile phases are A: Acetonitrile:Water:Formic acid (10:89:1); B: Acetonitrile:Water:Formic acid (89:10:1). The gradient elution established in the time frame 0–35 min, B%15–100 and the flow rate was set at 0.7 mL/min. According to the method by Toplan et al. (2017), the injection volume was 20 μL. Phenolic compounds were identified by matching their retention times and mass spectra against those of the standards analyzed under the same conditions (Figs. 4 and 5).

3. Results and discussion

3.1. Antioxidant activity

Antioxidant activities of ethyl acetate and *n*-hexane fractions were measured applying the DPPH free radicals scavenging ability and ABTS radical scavenging method, by comparing with the standards ascorbic acid and trolox, respectively. Antioxidant activity was calculated by IC_{50} values, indicating the fractions concentrations scavenge 50% ABTS radical. It was observed that *R. officinalis* flower ethyl acetate fraction has a higher antioxidant capacity than *n*-hexane fraction. The results were shown in Table 1. The results show that the plant preparations are rich in antioxidant content.

3.2. Antibacterial activity

Rosmarinus officinalis flower sub-fractions antibacterial activities were evaluated according to their MBC and MIC values against various human pathogenic strains. The outcomes obtained from this study were compared with the antibacterial activities of standard antibiotics as the positive control. Table 2 shows the antibacterial activities of *R. officinalis* flowers fractions against bacterial strains.

The *n*-hexane fraction showed more inhibitory activity rather than ethyl acetate fraction on the tested microorganisms at 1000 μg/mL concentration. The bactericidal activity results showed that *n*-hexane fraction was susceptible to *S. aureus*. The MBC value was identified as 500 μg/mL for the *n*-hexane fraction against *S. aureus*, however, other tested strains exhibited no bactericidal effect.

As it is known, *S. aureus* causes infections in wounds and upper respiratory diseases such as throat infections. The results suggest that *R. officinalis n*-hexane fraction can be used as a natural antibacterial agent for the prevention of *S. aureus* infections. In addition, the traditional usage (Calvo et al., 2011) to prevent and treat throat infections also supports and is in agreement with the antibacterial findings of this present study.

In this present study, the *n*-hexane fraction showed inhibition against *H. pylori* at a concentration of 156 mg/mL. Although there is not a remarkable inhibition value in the findings, it suggests that the *R. officinalis n*-hexane fraction may support ulcer treatment. It is suggested that *R. officinalis* can be used in the treatment of a gastric ulcer when it is considered together with anti-inflammatory findings. It is understood from the previous ethnobotanical studies that *R. officinalis* is used in stomach disorders among the public (Jarić et al., 2015; Bouasla and Bouasla, 2017). In another studies, extracts and essential oils of *R. officinalis* aerial parts were studied generally for its antimicrobial activity evaluation (Santoyo et al., 2005; Celiktas et al., 2007; Okoh et al., 2010; Nascimento et al., 2000). Also, in a similar previous study, the results obtained by Moreno et al. (2006), biological activities of the leaf extracts are comparable to this present study.

3.3. Anti-inflammatory and analgesic activity

3.3.1. Cytotoxicity

Prior to anti-inflammatory activity evaluations, nitrite productions in cell cultures, non-toxic concentrations were determined for the *R. officinalis* ethyl acetate and *n*-hexane fractions, respectively. Consequently, a cytotoxicity assay was carried out on RAW 264.7 murine macrophage cells by MTT assay, after treatment with varying concentrations of ethyl acetate and *n*-hexane fractions for 24 h. As seen in Fig. 1, the ethyl acetate fraction showed a higher cytotoxic effect on macrophage cells compared to *n*-hexane fraction. The IC_{50} value of the ethyl acetate fraction was found as 15.60 μg/mL, while the *n*-hexane fraction IC_{50} value was approximately 17 times higher (260.46 μg/mL). Non-toxic concentrations of the fractions with cell viability of more than 70% were used to evaluate the anti-inflammatory and analgesic activity by measuring prostaglandin E_2 (PGE_2) and nitric oxide (NO) production in LPS-stimulated cells.

3.3.2. Nitric oxide production inhibition in LPS-stimulated raw 264.7 cells

In vitro anti-inflammatory activities of ethyl acetate and *n*-hexane fractions of *R. officinalis* flowers were assessed by observing the decrease in nitrite production levels using the Griess assay. In this present study, indomethacin, which is a well-known anti-inflammatory agent, was used as a reference compound. As seen in Fig. 2, the *n*-hexane fraction showed the capability of reducing LPS-induced nitrite production in a concentration-dependent manner. In particular, the *n*-hexane fractions, namely 50 and 100 μg/mL concentrations showed significantly higher anti-inflammatory activity ($p < .001$) on LPS-stimulated RAW 264.7 cells compared to the standard control. Moreover, the percent inhibition of nitrite at 50 and 100 μg/mL of the *n*-hexane fraction was relatively high (80%) than the reference compound, 100 μM indomethacin (50%). Also, 10 μg/mL of ethyl acetate fraction showed a significant effect on nitrite production.

3.3.3. Analgesic activity of prostaglandin E_2 levels

PGE_2 levels were detected in LPS (1 μg/mL) stimulated RAW 264.7 murine macrophage cells by using an ELISA method. As shown in Fig. 3, 10 μg/mL of the ethyl acetate fraction significantly decreased PGE_2 levels compared to LPS-induced control. 50 and 100 μg/mL of the *n*-hexane fraction also significantly suppressed the LPS stimulated PGE_2 production. Noteworthy, indomethacin reduced PGE_2 levels almost to medium control levels.

Rosmarinus officinalis preparations are used as an anti-inflammatory remedy against eczema and other illnesses in traditional medicine (Newall et al., 1996; Wichtl, 2008). According to the results obtained in this present study, it can be suggested that the *in vitro* anti-inflammatory activity data supports the traditional usage of the plant as an anti-inflammatory agent. A potential anti-inflammatory activity was observed for the *n*-hexane fraction. *R. officinalis* essential oil and the flower *n*-hexane fraction is rich in terpenes, especially 1,8-cineole, which is known as a potent anti-inflammatory mixture (Santos and

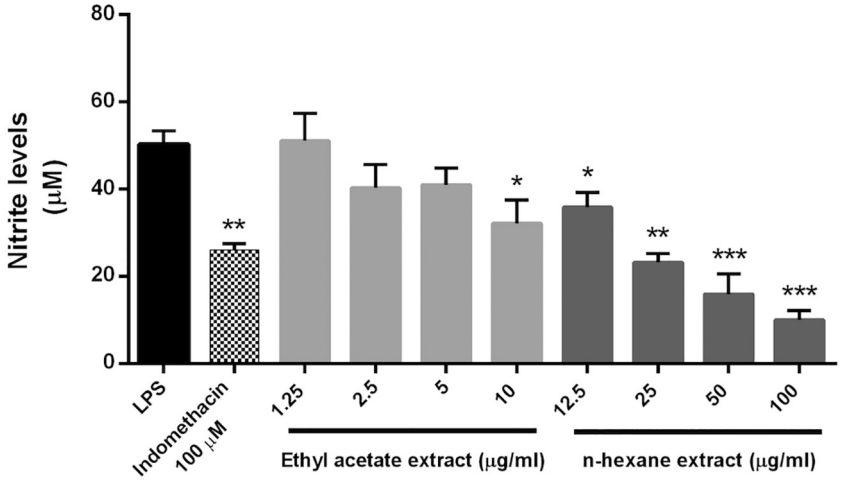

Fig. 2. *R. officinalis* ethyl acetate and *n*-hexane fractions effect on nitrite production in RAW 264.7 cells stimulated with 1 μg/mL of LPS.

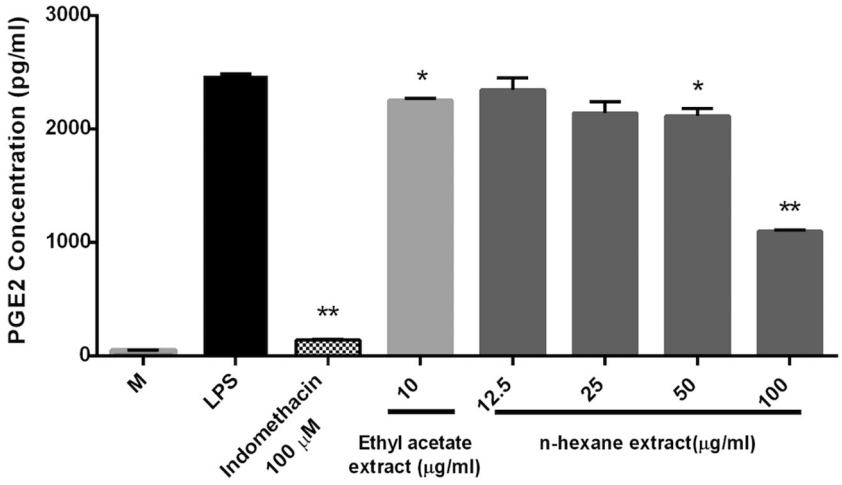

Fig. 3. Effect of *R. officinalis* ethyl acetate and *n*-hexane fractions on PGE_2 production in RAW 264.7 cells stimulated with 1 μg/mL of LPS.

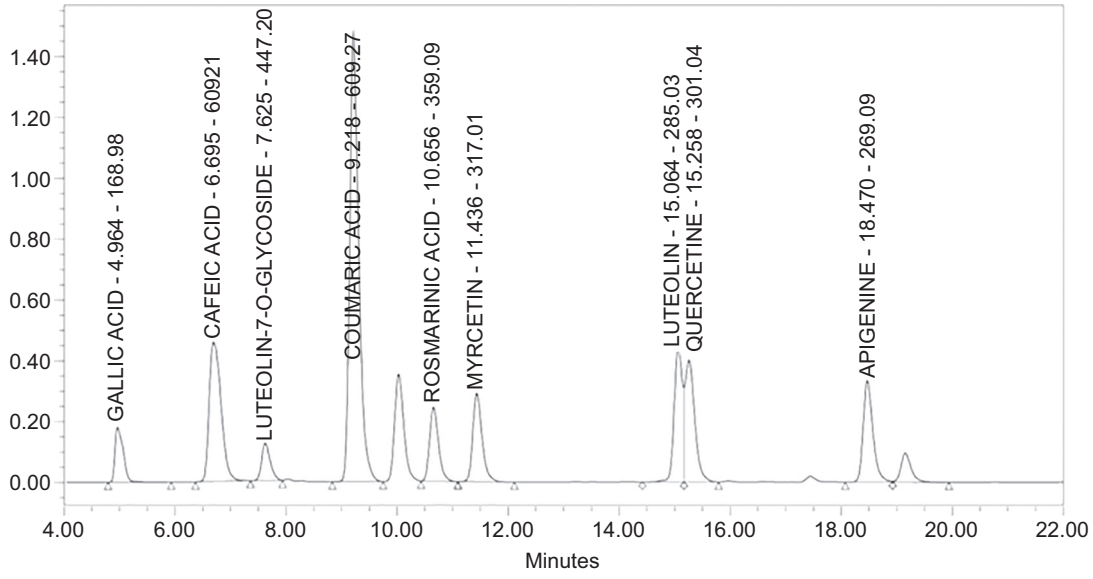

Fig. 4. LC–MS standard chromatogram. Standards: 1, Gallic acid (R.T. 4,96); 2, Luteolin-7-o-glycoside (R.T. 7,62); 3, Coumaric acid (R.T. 9,21); 4, Rosmarinic acid (R.T. 10,65); 5, Myrcetin (R.T. 11,43); 6 Luteolin (R.T. 15,05) 7, Quercetin (R.T. 15,25); 8, Apigenine (R.T. 18,47); (R.T. = Retention time).

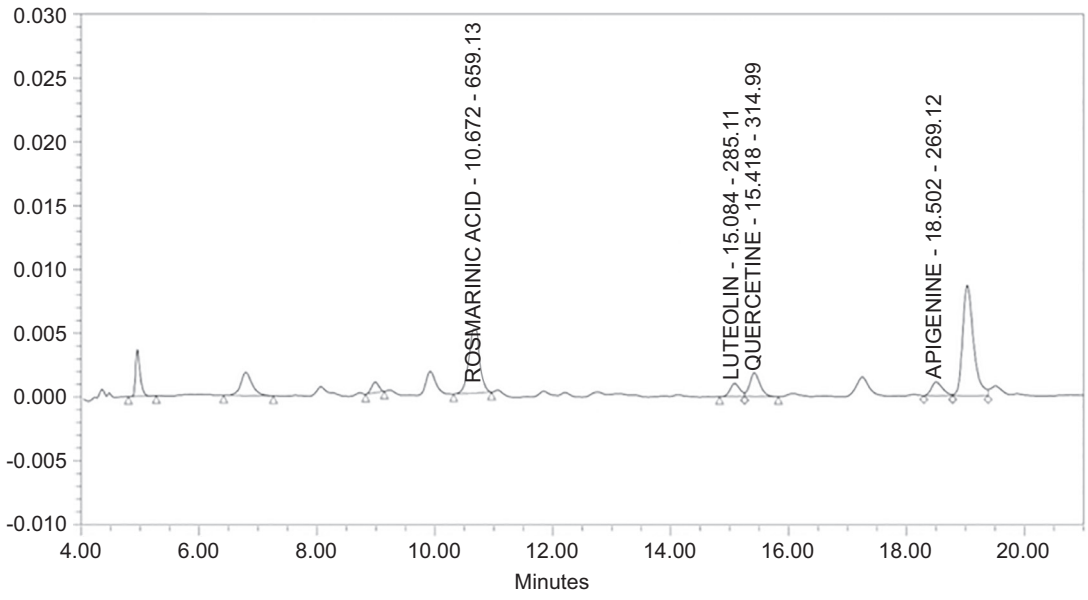

Fig. 5. *R. officinalis* ethyl acetate fractions LC–MS chromatogram.

Rao, 2000; Juergens et al., 2003). It is therefore conceivable that the anti-inflammatory activity observed in the *n*-hexane fraction may be attributed to the 1,8-cineole. The results of a previous work reported by Cheung and Tai (2007), showed that anti-inflammatory activity of the leaf extract was comparable to the activity of *R. officinalis* flower extract such as in this present study.

3.4. Phytochemical analyses

The phytochemical constituents of the fractions were analyzed using GC–MS and LC–MS. The flavonoid components of the ethyl acetate fraction of *R. officinalis* flowers were characterized as rosmarinic acid, luteolin, quercetin and apigenin (Table 3 and Fig. 5). In the present study, the high antioxidant capacity in ethyl acetate fraction can be attributed to the flavonoid compounds present. In previous reported *R. officinalis* aerial part extracts, the antioxidant activity potential, may be due to it is rich in phenols (Kontogianni et al., 2013; Erkan et al., 2008; Bozin et al., 2007). When compared to the study reported by Baño et al. (2003), *R. officinalis* flowers were investigated for its phenolic compounds and *in vitro* antioxidant activity was by different methods reported in the present study.

Rosmarinus officinalis flower *n*-hexane fraction was analyzed by using the GC–MS/GC-FID, to determine the volatile constituents. The characterized volatile components were camphor, 1,8-cineole, verbenone, borneol, α-pinene, and linalool, respectively, as shown in Table 2. Some pathogenic Gram (+) and (−) bacteria are listed in Table 4, were challenged with *R. officinalis* flower *n*-hexane and ethyl acetate fractions. Among the tested bacteria in the present study, *S. aureus* was more sensitive to the fractions, while *M. smegmatis* and *E. coli* appeared to be the most resistant. Camphor and 1,8-cineole are well-known compounds with distinct antibacterial activities (Pattnaik et al., 1997; Tzakou et al., 2001). The present *n*-hexane fraction was also found to be rich in 1,8-cineole and camphor, and thus can be concluded that these volatile compounds are associated with the observed antibacterial activity, among others.

Table 3
R. officinalis flower ethyl acetate (flavonoids).

Compounds	RT	Base peak (*m*/*z*)
Rosmarinic acid	10.672	359.13
Luteolin	15.084	285.11
Quercetine	15.418	314.99
Apigenine	18.502	269.12

Table 4
R. officinalis flower n-hexane extract (volatile components).

Compound	RRI	%	Identification method
1,8-cineole	1203	11.17	tR, MS
Camphor	1400	19.6	tR, MS
α-pinene	1032	5.8	tR, MS
Linalool	1466	5.7	MS
Borneol	1535	10.6	MS
Verbenone	1553	11.5	tR, MS

RRI: Relative retention indices calculated against *n*-alkanes, %: calculated from FID data, tr: Trace (< 0.1%), tr: identification based on the retention times, tR: of genuine standard compounds on the HP Innowax column; MS, tentatively identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

4. Conclusion

In conclusion, in the present study, it was observed that in *R. officinalis* flower *n*-hexane and ethyl acetate fractions, which exhibited antibacterial, antioxidant, anti-inflammatory and analgesic activities were remarkable. To the best of our knowledge, this is the first detailed study on polar and apolar *R. officinalis* flower extracts/fractions. The *in vitro* biological activities may be due to the polyphenolic compounds present. Based on the findings of this present study, the results of anti-inflammatory and antibacterial activity may be considered to be used *R. officinalis* in wound healing and throat infections. These results indicated that this plant material is an important natural source for future detailed evaluations. *In vivo* pharmacological studies are needed to better understand the molecular mechanisms underlying these effects.

Declaration of Competing Interest

The researchers would like to declare no conflict of interest.

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