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Original article

Chemical composition and biological activity of the essential oil from the root of *Jatropha pelargoniifolia* Courb. native to Saudi Arabia

Hanan Aati [a], Ali El-Gamal [a,b], Oliver Kayser [c,*]

[a] Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia
[b] Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, El-Mansoura 35516, Egypt
[c] TU Dortmund University, Technical Biochemistry, Emil-Figge-Strasse 66, D-44227 Dortmund, Germany

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ABSTRACT

The chemical composition of the essential oil from *Jatropha pelargoniifolia* roots was determined via GC-FID. There were 80 compounds, representing 99.99% of the total oil constituents. Among these, 77.31% were sesquiterpenes, 14.62% were fatty acids, 7.21% were other components (i.e., phenolics, hydrocarbons, etc.), and 0.85% were monoterpenes. The major compounds in the oil were γ-eudesmol (35.31%), 5-guaien-11-ol (14.43%), *epi*-cedrol (8.19%), oleic acid (5.23%), bulnesol (4.45%), α-linoleic acid (4.20%), 3,4-dimethoxycinnamic acid (3.83%), palmitic acid (2.69%), isolongifolanone (2.68%), eicosane (1.41%), and cedrol (1.14%). Oxygenated sesquiterpenes were found to represent more than 50% percent of the total oil content. Moreover, the essential oil was evaluated for anti-inflammatory, antioxidant, antipyretic, and antinociceptive activities using *in vivo* and *in vitro* models. Additionally, the antioxidant potential of the oil was evaluated using various *in vitro* antioxidant tests, including DPPH·, $ABTS^{\cdot+}$ and FRAP. At a dose of 240 μl/kg, the oil showed anti-inflammatory (59.12%), antipyretic (37.00 ± 0.11), and antinociceptive (47.58%) activities and showed significant ($p < 0.001$) effect as compared to a standard drug (phenylbutazone and indomethacin). These findings demonstrated that the essential oil of *Jatropha pelargoniifolia* root could be used as a natural source for their anti-inflammatory, antinociceptive, antipyretic, and antioxidant effects.

1. Introduction

Jatropha pelargoniifolia is a member of the Euphorbiaceae family. It is a frutescent plant of about 30 cm in height with bluish-green small petioles; there are usually 3–5 digitately-lobed ones with serrate margins covered with smooth silky hairs. Female flowers are larger in size with a larger perianth than the male flowers. Capsule is pale, straw-colored, scaly, and about 1 cm long with smooth black seeds. This plant is known locally in Saudi Arabia as "Obab" (Migahid, 1978).

Species of *Jatropha* became popular over the years to treat various diseases. The roots of some species of *Jatropha* (*J. glandulifera, J. gossypiifolia, J. multifida*) were used to cure individuals suffering from leprosy and gonorrhea (Sabandar et al., 2013). Traditionally, the sap of *J. pelargoniifolia* petiole is applied to cure ulcers and wounds in Ethiopia and Saudi Arabia (Schmelzer and Guribfakim, 2007).

Essential oils are complex mixtures of various chemical classes derived from secondary plant metabolism. Mostly, essential oils contain terpenes, including monoterpenes and sesquiterpenes, as well phenolics and some lipophilic constituents. These oils are extracted from specific plant organs (e.g., leaves, seeds, and peels) and also from the roots and rhizome. Generally, the synergistic biological activity of essential oils attributed to the presence of active constituents (Tripathi and Shukla, 2007). In fact, the essential oils were used in human being and animals and exhibited many promising biological activities such as; antimicrobial, antioxidant,

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); b.w., body weight; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GC-FID, gas chromatography-flame ionization detector; i.p., intraperitoneal; RI, retention indices; Rt, retention time; s.c, subcutaneous; TCA, trichloroacetic acid.

* Corresponding author.
E-mail address: oliver.kayser@tu-dortmund.de (O. Kayser).

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anti-inflammatory, antispasmodic, and muscle relaxing properties (Burt, 2004). Biologically active essential oils represent a potential source for alternative medicine (Tripathi and Shukla, 2007). As a consequence, essential oils are one of the promising candidates amongst natural compounds for the development of safe therapeutic agents.

Much phytochemical and biological work has been carried out on essential oils isolated from the genus *Jatropha,* such as *J. curcas*, *J. ribifolia, J. gossypifolia,* and *J. mutabilis* (Nzikou et al., 2009; Da Silva et al., 2015; Aboaba et al., 2015; Costa et al., 2014). It is worthy to note that this is the first study describing the chemical constituents and biological activities of the essential oil isolated from *J. pelargoniifolia* roots. The motivation of this study was to shed light on the composition of *J. pelargoniifolia* essential oil and to prove if some of the ethnomedical claims about the species are linked to the essential oil and its related constituents.

2. Materials and methods

2.1. Chemicals

ABTS [2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)], trichloroacetic acid (TCA), and DPPH (1,1-diphenyl-2-picrylhydrazyl) were procured from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Potassium Ferricyanide from Loba Chemie Pvt. Ltd. (Mumbai, India) and ascorbic acid was obtained from SD Fine Chem. Ltd. (Biosar, India) while indomethacin and phenylbutazone were purchased from Spimaco (Saudi Arabia). The rest of chemicals and solvents used were of analytical grade and purchased from Sigma-Aldrich (St Louis, MO, USA).

2.2. Plant material

The roots of *J. pelargoniifolia* were collected (1 kg) in September 2015 from Wadi Mojasas, in Jazan area in the south of Kingdom of Saudi. It was identified by Jacob Thomas, taxonomist of the Botany and Microbiology Department, King Saud University, College of Science. A voucher sample (number- 23064) has been placed at the Herbarium Center College of Science, King Saud University.

2.3. Essential oil prepared

The freshly cut roots (1 kg) were exposed to hydrodistillation for 6 hr. Clevenger type apparatus was used following the technique prescribed in the European Pharmacopoeia (European Pharmacopoeia, 2004). Anhydrous sodium sulfate powder was used for drying the obtained oil. The dried oil was kept on and stored in air-tight, amber colored glass vials at 4 °C for further study.

2.4. GC–MS analysis

Sample components analysis was carried out by using Gas Chromatograph System Series (Agilent 5975MS/6890) combined with a flame ionization type of detector (FID) with fused silica capillary column (DB-5; 30 m × 0.25 mm, film thickness 0.2 μm). Oven temperature was gradually increased from 40 to 280 °C at a rate of 3 °C min^{-1}. The temperature of the injection port was kept at 230 °C while the detector temperature kept constantly at 280 °C. The split ratio was (1:20). The injection volume was 1.0 μl. A C7-C21*n*-alkanes mixture was mixed with *n*-hexane and used for determination of the temperature programmed retention indices. The sample was analyzed in *n*-hexane solution. An internal standard (*n*-alkanes) was then mixed with the sample to help in the standardization of retention times, and the sample was reanalyzed. Retention indices (RI) for all components were verified. The identity of essential oil components was achieved by comparing their retention indices (RI) and mass spectra with those reported from authentic samples and/or the Wiley and NBS/NIST libraries and those published by Adams (2001). The quantitative data regarding the essential constituents were obtained by peak-area normalization using the chromatographic technique with flame ionization detection (GC-FID) operated under similar conditions to the GC–MS. Compounds with concentrations equal or greater than 0.001% were considered for quantification.

2.5. Animal testing

The experiments were performed on male Wistar rats (with six in each group (I-IV) at 180–200 g b.w., 8/10 weeks old) and Swiss albino mice (with six in each group at 25 ± 5 g b.w., 8/9 weeks old) of either sex. The required animals were provided from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Those animals were housed in large polypropylene cages in 22 ± 2 °C and gave standard pelleted nourishment and drinking water *ad libitum*. The current study was accepted by the Institutional Animal Ethical Committee of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia (approval number CPR-7569).

2.6. Determination of median lethal dose (LD_{50}) of the animals

The LD_{50} value was evaluated adapting the procedure described by Karber (1931). The minimal dose that completely killed 100% of the animal (LD_{100}) was carried out while the highest dose that failed to kill any animal was also calculated. Many doses at equal logarithmic intervals were selected in between these two doses; each dose was administrated orally in a group of six rats. The rats were then evaluated for 24 h and recorded any behavioral, neurological symptoms as well as any sign of toxicity and mortality in each group.

2.7. Determination of anti-inflammatory activity

2.7.1. Carrageenan-induced paw edema in rats

The inflammation in rat's paw was induced following the procedure described by Winter et al. (1962). Animals were divided into four groups of six animals each as follows: Group I was given an injection of 0.05 ml of 1% carrageenan solution in the right hind paw of each rat under the plantar aponeurosis. Group II and III rats were treated orally with 120 and 240 μl/kg b.w. of the oil suspended in distilled water 1 h before the carrageenan injection. Group IV (control rats) was administered standard anti-inflammatory drug (phenylbutazone, 100 mg/kg b.w., orally) 1 h before the carrageenan administration. The measurements of foot volume were carried by the displacement technique using a plethysmometer (Apelex, France) immediately 3 h after the injection of carrageenan. The inhibitory effect percentage was measured following the equation shown below:

$$\%\text{Inhibition} = (1 - a - x/b - y)$$

where 'b' is the mean paw volume of control rats after carrageenan injection and 'y' before the injection; whereas 'x' is the mean paw volume of treated rats before injection and 'a' is the mean paw volume after carrageenan injection.

2.7.2. Cotton pellet granuloma test in rats

Goldstein et al. (1967) method was applied with minor modification. 30 mg of sterilized cotton pellet was entered subcutaneous in the groin region of rats. Animals were divided into four groups of six animals each. Animals in the control group administered

normal saline. Group I and II were administered the oil orally, in a dose of 120 and 240 μl/kg b.w. once daily for four consecutive days. Phenylbutazone 100 mg/kg b.w. was given to positive control group. On the fifth day, the animals were sacrificed using ether. The removed dried cotton pellets from extraneous tissue were weight after 24 h.

2.8. Determination of antipyretic activity in mice (Brewer's yeast-induced pyrexia method)

Fever was induced in mice by s.c. injection of 20% aqueous suspension of Brewer's yeast in the back, below the nape of the neck (20 ml/kg b.w.) (Loux et al., 1972). Animals were divided into three groups of six animals each. The animals were then fasted for the duration of the experiment (approximately 1 day), with free water supply. Regular temperature measurements were performed 24 h after the yeast administration to determine the pyretic response. The oil suspension (120 and 240 μl/kg b.w.) was given 24 h after the yeast injection and the temperatures were recorded at 30, 60, and 120 min after its administration. Indomethacin (4 mg/kg b. w., administered orally) was gave to positive control group.

2.9. Antinociceptive activity

The analgesic activity was measured against chemical and thermal stimuli.

2.9.1. Chemical method

2.9.1.1. Inhibition of acetic acid-induced writhing in mice. The experiment was done according to the method approved by Siegmund et al. (1957) with minor changes by Koster et al. (1959). Animals were divided into four groups of six Swiss albino mice each. Group I was injected i.p with 0.2 ml of 3% acetic acid solution only. Group II and III were treated with the suspension of the oil mixture in doses of 120 and 240 μl/kg b.w. orally, and group IV was administered indomethacin (4 mg/kg b.w., p.o.), as a positive control, after an overnight fast. One hour after the treatment, the mice from groups II, III, and IV were injected i.p. with acetic acid solution to stimulate the distinctive writhings. The writhing numbers that occurred between 5 and 15 min after the acetic acid injection was calculated.

2.9.2. Thermal methods

2.9.2.1. Hot-plate test. The experiment was carried out in order to assess the potential of the response as designated by Eddy and Leimback (1953) with a few modifications. Hot plate temperature was kept at 56 ± 1 °C. The mice were placed in a 24-cm diameter glass cylinder on the heated surface. The time between placement and licking of the paws or jumping was recorded as response latency. Animals were divided into three groups of six animals each. Distilled water was administered orally for the control group, and indomethacin was used as positive control (4 mg/kg b.w., administered orally). The choice of mice was achieved one day before the test based on their reactivity to the experiment. The animals were evaluated at 30, 60, and 120 min after oral administration of the oil suspension (120 and 240 μl/kg b.w.) and indomethacin administration. The cut off time was 30 s.

2.9.2.2. Tail-flick test. Acute nociception was induced using tail flick apparatus using (Tail flick Apparatus Harvard), according to the method described by D'amour and Smith (1941). Briefly, each mouse was placed in a restrainer (three groups of six animals each) for 2 min before treatment; baseline reaction time was determined by focusing on an intensity-controlled beam of light on the distal one-third portion of the animal tail. The essential oil doses of 120 μl/kg and 240 μl/kg were administered intraperitonially. The post-drug reaction time was measured after 30, 60, and 120 min. Indomethacin was used as positive control (4 mg/kg b.w., administered orally).

2.10. Estimating antioxidant activity

2.10.1. DPPH(2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The antioxidant effect of the oil, based on the scavenging potency of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was measured using the technique designed by Braca et al. (2001). Different concentrations of oil were mixed with 3 ml of a 0.004% ethanol solution of DPPH. One ml methanol instead of oil was used to prepare control. The absorbance of color intensity was measured at 520 nm after 30 min and the percentage inhibition of antioxidant effect was calculated using the below formula:

$$[(A0 - A1)/A0] \times 100$$

where A0 is the absorbance of the control (DPPH solution) and A1 is the absorbance of the oil/standard.

2.10.2. ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay

The radical scavenging potency of the oil against ABTS radical cation was evaluated using the technique described by Re et al. (1999). The ABTS solution was prepared in water with a concentration of 7 mmol/L; an aqueous solution of potassium persulphate was also prepared with a concentration of 2.45 mmol/L. The two solutions were added in equal volume (1:1) and stored in dark for 6 hr. at room temperature. During that period, ABTS radical was produced. The ABTS stock solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. An aliquot of oil was mixed with 2.9 ml of diluted ABTS radical cation solution. After the reaction was incubated at 30 °C for 20 min, absorbance was measured at 734 nm. The ability of the oil to quench ABTS free radical was calculated according to the formula:

$$Scavenging(\%) = [(Ac - Aa/Ac)] \times 100$$

where Ac = absorbance of control and Aa = absorbance of the oil.

2.10.3. Ferric reducing antioxidant power (FRAP) assay

The ferric free radical scavenging power was measured based on the technique recommended by Oyaizu (1986). The reduction of ferric ion to ferrous ion is confirmed by formation of Perl's Prussian blue color where its intensity is related to the antioxidant activity. Serial dilution of the oil (20–100 μg/ml) in 1 ml of distilled water were added to 0.2 M phosphate buffer (2.5 ml, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50 °C for 20 min. 2.5 ml of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 3000 rpm for 10 min. 2.5 ml of distilled water was added to equal amount of the supernatant followed by addition of 0.5 ml of 0.1% $FeCl_3$, the absorbance was recorded at 700 nm.

2.11. Statistical analysis

Analysis of variance (ANOVA) test was used to evaluate the significance differences. Differences between the standard and treated group were compared for significance using Dunnett's test for non-paired samples (Woolson and Clarke, 2002). All the measurements expressed as mean ± standard errors of means. Microsoft Excel Version 2010 was used for data analysis.

3. Results and discussion

Hydrodistillation of *J. pelargoniifolia* roots provided a yellowish essential oil with a yield of 0.52% (v/w), depend on fresh weight of the plant. Analysis and identification by mass fragmentation and retention index revealed the presence of 80 compounds, representing 99.99% of the total oil with 77.31% of these compounds being sesquiterpenes, 14.62% fatty acids, 7.21% other components (i.e., phenolics, hydrocarbons, cyclic compounds, etc.), and 0.85% were monoterpenes. The chemical composition of the essential oil and the percentage of each component determined from GC–MS analysis is shown (Table 1). The compounds are arranged in order of their elution from DB-5 column together with their retention indices.

The major compounds in the essential oil were γ-eudesmol (35.31), 5-guaien-11-ol (14.43%), *epi*-cedrol (8.19%), oleic acid (5.23%), bulnesol (4.45%), linoleic acid (4.20%), 3,4-dimethoxycinnamic acid (3.83%), palmitic acid (2.69%), isolongifolanone (2.68%), eicosane (1.41%), and cedrol (1.40%). Oxygenated sesquiterpenes were found to be the major group of compounds that represented more than half of the oil content. It consisted almost entirely of γ-eudesmol (35.31%), 5-guaien-11-ol (14.43%), and *epi*-cedrol (8.19%). While oxygenated monoterpenes, fatty acids, and sesquiterpenes hydrocarbons covered a small percentage of the oil content only.

A review of recent literature confirmed that γ-eudesmol is identified here for the first time in the genus *Jatropha*. The essential oils of many *Jatropha* species have been studied including *J. ribifolia*, *J. gossypifolia,* and *J. mutabilis* (Da Silva et al., 2015; Aboaba et al., 2015; Okoh et al., 2016; Costa et al., 2014). The major constituents in *J. ribifolia* aerial part essential oil were identified as β-pinene, isoeugenol methyl ether, α-gurjunene, *endo*-8-hydroxycycloisolongifolene, α-pinene, and *p*-menth-1-en-8-ol, while phytol, spathulenol, *epi*-α-cadinol, caryophyllene oxide, germacerene D, and α-cadinol were the main components derived from *J. mutabilis* leaves essential oil. Meanwhile, the essential oil identified in the leaves of *J. gossypifolia* was composed mainly of phytol, octadecanal, and viridiflorol, while major components of its stem oil were phytol, α-copaene, and limonene.

The 24-h LD_{50} was approximately more than 1.2 ml/kg b.w. for the essential oil. These results showed that the essential oil of *J. pelargoniifolia* roots is safe and non-toxic because no mice died or showed any severe side effects or intoxication.

Table 2 shows the effects of oil and phenylbutazone on carrageenan-induced rat paw edema. Injection of carrageenan into the sub-plantar tissue of the right hind paw of rats in the control group led to rapid growth of edema, which peaked (1.79 ± 0.02 in paw volume) at 180 min post-phlogistic agent injection. The percent reduction in edema after oil administration was 31.68 and 55.64% inhibition observed at 120 μl/kg and 240 μl/kg, respectively. This effect was significantly different ($P < 0.001$) as compared to that produced by 100 mg/kg phenylbutazone (77.42% inhibition).

Carrageenan-induced paw edema is widely used experiment for screening the anti-inflammatory drugs including natural products (Posadas et al., 2004). We found that the administration of oil significantly decreased edema volume induced in animal's paw by carrageenan.

The inflammatory response by carrageenan hind paw in the rodent is including many stages. Firstly, after carrageenan injection, the paw volume increased due to release of internal chemical mediators such as, histamine and serotonin (Geen, 1974). Then, the increase in vascular permeability was sustained by the release of prostaglandins and nitric oxide lead to stimulate migration of leukocytes into the inflamed site (Di Rosa et al., 1971). The last stage of edema is susceptible for commonly used anti-inflammatory drug. So, that oil might prevent increased in vascular permeability (edema) and leukocyte aggregation. The exact mechanism of action of *J. pelargoniifolia* root essential oil need further investigation.

Cotton pellet-induced granuloma test was done to assess the efficacy of oil and standard anti-inflammatory drug against the proliferative phase of inflammation in which tissue degeneration and fibrosis occur.

According to these results, the anti-proliferative potency of the oil (120 μl/kg and 240 μl/kg) was calculated to be 69.66 ± 1.78 and 51.21 ± 1.46 ($P < 0.001$), respectively, while for phenylbutazone (100 mg/kg), was 41.70 ± 1.06. After cotton pellet drying, the anti-proliferative effects were calculated based on the dry weights. The inhibition of inflammation by the oil and was 39.66 ± 1.78 (23.57%) and 21.21 ± 1.46 (59.12%) ($P < 0.001$), respectively, while for phenylbutazone was 11.70 ± 1.06 (77.45%).

The cotton pellet-induced granuloma model is popular experimental technique used to evaluate the transudative, exudative, and proliferative events during chronic inflammation (Williams and Williams, 1973). Perhaps, the reduction in granuloma size revealed the ability of oil to decrease the total number of fibroblasts and extending the time need for the synthesis of mucopolysaccharide and collagen which are involved in granuloma formation (Ionac et al., 1996).

It is better to point out that sesquiterpenes and unsaturated fatty acids, which are the major constituents of the currently investigated oil and which play a significant role as anti-inflammatory compounds, are all derived from natural sources (Pereira et al., 2014). The essential oil of *J. pelargoniifolia* roots have been reported to have more than half of its constitutes made up of oxygenated sesquiterpenes (70.36%). It consists almost entirely of γ-eudesmol, 5-guaien-11-ol, *epi*-cedrol and bulnesol; these compounds have shown significant anti-inflammatory properties due to their various pharmacological activities (Seo et al., 2013). In addition, review of literature confirmed that both oleic acid (5.23%) and α-linoleic acid (4.19%) possess strong anti-inflammatory activities (Erdinest et al., 2012). On the basis of this result, it could be proposed that the anti-inflammatory potential exerted by the essential root oil may be attributable to the synergistic action of sesquiterpenes and fatty acids since they are present in high yield.

The action of oil and indomethacin on yeast-induced fever in mice is illustrated in Table 3. The s.c. injection of an aqueous suspension of brewer's yeast has significantly elevated the rectal temperature by 3.2 °C after 24 h of injection. Animals treated with the essential oil and indomethacin (positive control) showed significant reduction in animal's rectal temperature. *J. pelargoniifolia* oil showed significant suppression in rectal temperature after 30 min; the decrease observed was by 0.22 °C and 0.53 °C, i.e., from 38.35 ± 0.15 °C to 38.13 ± 0.12 °C ($p < 0.05$) and from 38.38 ± 0.10 °C to 37.85 ± 0.11 °C ($p < 0.01$), with doses of 120 μl/kg and 240 μl/kg, respectively. The antipyretic effect of oil began 30 min after its administration ($p < 0.05$ and $p < 0.01$) and the decrease in rectal temperature was continued for 2 h ($p < 0.001$) at both doses. Indomethacin also displayed a significant decrease in rectal temperature by 1.75 °C, i.e., from 38.65 ± 0.11 °C to 36.90 ± 0.08 °C, and the percentage inhibition of pyrexia was significant ($p < 0.001$).

Additionally, after 120 min, there was no important variance between the antipyretic effect of oil in comparison to indomethacin. The current study displays that *J. pelargoniifolia* root oil has antipyretic effect in mice. Various reports have verified that yeast-induced temperature elevation is a pathogenic pyrexia (Akio et al., 1988). At a higher body temperature, the thermoregulatory center in the hypothalamus start to release prostaglandins. If

Table 1
Chemical constituents of *J. pelargoniifolia* root essential oil.

#	Constituents	Rt (min.)	Conc. (%)	RI
1	Isovaleric acid	6.79	0.006	834
2	8-α-Pinenol	11.92	0.016	939
3	Phenol	12.15	0.013	965
4	Capronic acid	13.99	0.005	987
5	Eucalyptol	16.03	0.010	1033
6	*cis*-Linalool oxide	16.46	0.013	1074
7	Heptanoic acid	17.03	0.010	1083
8	Guaiacol	17.10	0.998	1086
9	*trans*-Linalool oxide	17.66	0.012	1088
10	Linalool	19.14	0.005	1098
11	2-Phenylethyl alcohol	19.54	0.004	1110
12	Fenchol	20.00	0.018	1112
13	Camphene hydrate	20.52	0.065	1148
14	1,8-Epoxy-2-*p*-Exo-Menthanol	20.53	0.004	1158
15	Borneol	20.92	0.003	1165
16	4-Terpinenol	21.06	0.527	1177
17	8-*p*-Cymenol	21.40	0.008	1183
18	Caprylic acid	21.87	0.024	1187
19	Butyl-*n*-hexanoate	22.35	0.006	1188
20	α-Terpinol	22.45	0.128	1189
21	Myrtenol	23.24	0.030	1194
22	4-Vinyl-phenol	23.53	0.108	1229
23	Bornyl acetate	25.34	0.014	1285
24	α-Cubebene	26.15	0.511	1351
25	Eugenol	28.05	0.004	1356
26	Octadecanal	28.65	0.157	1357
27	Cyclosativene	29.13	0.006	1368
28	α-Copaene	29.39	0.589	1376
29	β-Elemene	29.77	0.061	1382
30	Vanillin	30.18	0.042	1391
31	α-Cyperone	30.30	0.109	1398
32	Capric acid	30.54	0.039	1399
33	*cis*-Isoeugenol	30.77	0.104	1402
34	Caryophylladiene	31.45	0.074	1404
35	α-Gurjunene	31.88	0.030	1409
36	Caryophyllene	31.89	0.555	1418
37	α-Guaiene	32.43	0.125	1439
38	*E*-β-Farnesene	32.86	0.049	1458
39	Alloaromadendrene	33.26	0.091	1461
40	2,6-di(t-Butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	33.73	0.011	1478
41	β-Selinene	33.74	0.071	1485
42	*Epi*-Bicyclosesquiphellandrene	33.75	0.809	1490
43	Valencene	34.01	0.168	1491
44	*cis*-Methyl isoeugenyl	34.10	0.067	1492
45	α-Selinene	34.18	0.095	1494
46	β-Dihydroagarofuran	34.71	0.157	1496
47	δ-Selinene	34.72	0.028	1497
48	α-Muurolene	34.73	1.317	1499
49	δ-Guajene	34.73	1.336	1505
50	Butylated hydroxytoluene	35.50	0.032	1512
51	δ-Cadinene	35.93	0.379	1524
52	α-Calacorene	36.30	0.647	1542
53	Elemol	36.78	0.628	1549
54	Guajoxid	37.06	0.315	1595
55	Cedrol	37.48	1.140	1596
56	*Epi*-Cedrol	38.18	8.191	1611
57	*trans*-Isolongifolanone	38.29	2.679	1618
58	10-*Epi*- γ-Eudesmol	39.07	1.447	1619
59	5-Guaien-11-ol	39.91	14.429	1625
60	γ-Eudesmol	41.10	35.309	1630
61	δ-Cadinol	41.19	0.383	1645
62	4-α-Hydroxy dihydro agarofuran	41.20	0.388	1648
63	Bulnesol	41.94	4.454	1666
64	Ethyl myristate	45.40	0.065	1777
65	Cryptomeridiol	47.71	0.510	1808
66	Myristic acid	47.89	0.760	1842
67	8,11-Heptadecadienal	48.87	0.262	1855
68	Pentadecanoic acid	49.40	0.350	1902
69	Methyl hexadecanoate	50.46	0.023	1927
70	3,4-Dimethoxycinnamic acid	51.55	3.828	1949
71	Palmitic acid	54.95	2.689	1972
72	Ethyl palmitate	55.42	0.118	1993
73	Eicosane	56.26	1.411	2000
74	Geranyl linalool	56.63	0.227	2020

Table 1 (*continued*)

#	Constituents	Rt (min.)	Conc. (%)	RI
75	4-Vinylguaiacol	57.07	0.076	2156
76	α-Linoleic acid	57.86	4.196	2173
77	Oleic acid	58.17	5.232	2175
78	Stearic acid	59.43	0.732	2192
79	5,9-Farnesyl propanone	59.76	0.417	2377
80	Dehydrodiisoeugenol	60.06	0.041	2723
Oxygenated monoterpenes		–	0.849	–
Oxygenated sesquiterpenes		–	70.366	–
Sesquiterpene hydrocarbones		–	6.941	–
Fatty acids (saturated)		–	4.939	–
Fatty acids (unsaturated)		–	9.690	–
Other		–	7.205	–
Total percentages		–	99.99	–

Rt: retention time, RI: retention indices, GC–MS, gas chromatography-mass spectroscopy; component concentrations were calculated from GC-FID peak areas in the order of DB-5 column elution.

Table 2
Effect of *J. pelargoniifolia* essential oil on carrageenan-induced paw edema in Wistar rats.

Group (n = 6)	Dose (μl/kg)	Before Carrageenan	3 h after	Net	% Inhibition
Only carrageenan	0.05 ml	0.95 ± 0.03	1.79 ± 0.02	0.84 ± 0.01	–
Essential Oil	120	0.99 ± 0.03	1.58 ± 0.03***	0.57 ± 0.02***	31.68
Essential Oil	240	0.98 ± 0.03	1.35 ± 0.02***	0.37 ± 0.01***	55.64
Phenylbutazone	100 mg/kg	1.00 ± 0.02	1.19 ± 0.02***	0.19 ± 0.01***	77.42

All values represent mean ± SEM.
*** $p < 0.001$; ANOVA, followed by Dunnett's multiple comparison test.

Table 3
Effect of *J. pelargoniifolia* essential oil on yeast-induced hyperthermia in mice.

Treatment (n = 6)	Dose (μl/kg)	Normal rector temperature	Rectal temperature after yeast administration 20 ml/kg of 20%	Rectal temperature °C (Post Drug)		
				120 m	60 m	30 m
120	38.35 ± 0.15***	35.15 ± 0.07	Essential Oil	37.75 ± 0.10***	37.83 ± 0.10**	38.13 ± 0.12*
240	38.38 ± 0.10***	35.18 ± 0.09	Essential Oil	37.00 ± 0.11***	37.18 ± 0.12***	37.85 ± 0.11**
4 mg/kg	38.65 ± 0.11***	35.45 ± 0.15	Indomethacin	36.03 ± 0.12***	36.58 ± 0.14***	36.90 ± 0.08***

All values represent mean ± SEM.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$; ANOVA, followed by Dunnett's multiple comparison test.

a central CNS effect or peripheric inhibition of cyclooxygenase which is responsible for the pyretic effect, that aspect remains to be studied but was not a part of the present study.

As seen in Table 4, the mean writhes of the mouse decreased from 25.50 ± 1.17 to 18.00 ± 0.66 as the dose of the oil increased from 120 to 240 μl/kg, which compared to 4 mg/kg of indomethacin (8.50 ± 0.42; 75.0% inhibition of writhing).

The mechanism of antinociceptive is not obvious in the current study. It is expected that prevention of prostaglandins caused reduction in mice visceral pain. Thus, it seems that the oil of *J. pelargoniifolia* prevents the second stage of inflammation and causes the inhibition of prostaglandin peripherally (Deraedt et al., 1980). This comment is supported by our finding that oil effectively suppressed yeast-induced pyrexia in animal's model.

Table 4
Analgesic effect of *J. pelargoniifolia* essential oil on Acetic acid –induced writhing in mice.

Treatments (n = 6)	Dose (μl/kg)	Number of writhing in 20 min	% Inhibition
Control (Acetic acid)	0.1 ml of 20%	34.00 ± 1.98	–
Essential Oil	120	25.50 ± 1.17**	25.00
Essential Oil	240	18.00 ± 0.66***	47.58
Indomethacin	4 mg/kg	8.50 ± 0.42***	75.00

All values represent mean ± SEM.
** $p < 0.01$.
*** $p < 0.001$; ANOVA, followed by Dunnett's multiple comparison test.

The oil at the two doses showed significant analgesic action peaking at 30, 60, and 120 min at doses of 120 and 240 μl/kg (i.p.) as compared with indomethacin.

The tail flick method indicated the central analgesic effect of *J. pelargoniifolia* roots oil. The response of the tail flick is assumed to be a spinally mediated reflex, which is controlled by a supraspinal inhibitory mechanism (Vogel, 2002). The oil was produce significant analgesic activity ($p < 0.001$) compared to the positive control group.

Moreover, in the hot plate test, treatment with oil showed significant antinociceptive effect ($p < 0.001$) as compared to that in the positive control group. The oil was produced the analgesic effect via increasing the time of response to heat sensation, from 6.33 ± 0.21 to 8.66 ± 0.33 at 120 min (36.84% analgesia) with a dose of 120 μl/kg and 6.66 ± 0.33 to 11.66 ± 0.30 at 120 min (75.0% analgesia) with a dose of 240 μl/kg. Indomethacin also significantly delayed the reaction time by 120% ($p < 0.001$). The analgesic effect caused by indomethacin was significantly stronger than that induced by the oil (8.66 ± 0.33 and 11.66 ± 0.30 s at doses of 120 and 240 μl/kg, respectively, versus 14.6 ± 0.33 s after 120 min, $p < 0.001$).

Table 5
Antioxidant effect of *J. pelargoniifolia* essential oil (DPPH, ABT, and FRAP assays).

Treatments	DPPH assay			ABTS assay		FRAP assay	
	Concentration (μg/ml)	Average% scavenging	Mean ± S.D	Average% scavenging	Mean ± S.D	Average OD.	Mean ± S.D
Essential Oil	10	13.65	13.65 ± 12.01	13.65	13.65 ± 12.09	0.47	0.47 ± 0.09
Essential Oil	20	32.05	32.05 ± 1.06	43.4	43.4 ± 11.87	0.745	0.745 ± 0.02
Essential Oil	50	57.3	57.3 ± 2.12*	57.05	57.05 ± 13.05*	0.89	0.89 ± 0.02
Essential Oil	100	78.15	78.15 ± 6.57**	84.1	84.1 ± 9.33**	1.06	1.06 ± 0.02*
Ascorbic acid	100	99.69	99.69 ± 0.03**	90.5	90.5 ± 7.77**	1.48	1.475 ± 0.007**

* $p < 0.05$.
** $p < 0.001$ compared with ascorbic acid, OD. = Optical density.

In numerous studies, the pharmacological action of essential oils has been referred to the synergistic effect of active components. The oil inactive principles pharmacokinetics and bioavailability could be supported by active components. Further, it is difficult to establish a relationship between oil constituents and its activity (Tadrent et al., 2016). Perhaps, the analgesic activity of the essential oil derived from *J. pelargoniifolia* roots is mainly due to the combined effect of sesquiterpenes as suggested by Lee et al. (2002), it is maybe responsible for blocking the release of endogenous substances, which excite the pain in nociceptive pathway.

The antioxidant activity was measured by using three methods including DPPH, ABTS and FRAP. As illustrated in Table 5, the DPPH radical scavenging assay was used to assess the antioxidant activity of the essential oil, it showed a concentration-dependent antioxidant effect by $DPPH^-$ ion. As expected, ascorbic acid showed a higher potency (99.69%) of free radical scavenging at 100 μg/ml as compared to the oil at same concentrations (78.15%).

The results and mechanism of action obtained from DPPH, ABTS methods were similar to each other since their mechanism more or less similar which based on the donation of electrons or hydrogen atoms to free radical lead to its inactivation (Olajuyigbe and Afolayan, 2011). The results obtained from these two methods, regarding scavenging activity of the oil increased with the increasing concentration.

From the investigation of Table 5, we can conclude that the oil showed significant ABTS radical-scavenging property (84.10%), which was comparable to that of ascorbic acid (90.50%) at 100 μg/ml.

These results showed that the high antioxidant potential of the oil derived from *J. pelargoniifolia* roots could be attributed to the high percentage of oxygenated compounds, such as γ-eudesmol, 5-guaien-11-ol, and *epi*-cedrol (Piccaglia et al., 1993).

The result obtained from third antioxidant experiment (FRAP) method, is also based on the reducing power of the essential oil ingredients which can reduce the ferric ions (Fe^{3+}) to ferrous ion (Fe^{2+}) by electron donation. In this experiment the intensity of Perl's Prussian blue color was measured at 700 nm. The higher increase in the Prussian blue color intensity revealed strong antioxidant activity (Gordon, 1990).

As indicated in Table 5, the reducing capabilities of the root oil towards FRAP were compared to ascorbic acid as the reference standard. The oil exhibited moderate ferric reduction capability (1.06) in comparison to ascorbic acid (1.48) at the same concentration (100 μg/ml). As result, the oil showed good reducing activity with increasing concentrations as compared to the most famous antioxidant drug; ascorbic acid.

4. Conclusion

The essential oil constituents obtained from the roots of *J. pelargoniifolia* are reported here for the first time; we have also investigated selected biological activities with regard to the ethnomedicinal use of the essential oil of the roots considering that it is a native plant to Saudi Arabia (Hanan et al., 2018). It was found that the essential oil showed promising activities as anti-inflammatory, antinociceptive, antipyretic and antioxidant agent. Perhaps, these significant biological activities can be referred to the effect of its active components. The inactive components could work by influencing pharmacokinetics and bioavailability of the active compounds. Furthermore, it is difficult to create a relationship between specific oil constituent and certain biological activity due to the combined effect between its various constituents. The essential oil isolated from roots of *J. pelargoniifolia* may serve as a promising candidate for the development of safer therapeutic agents to be used for treatment of contemporary diseases, such as various skin inflammatory conditions and acute arthritis.

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2018.09.001.

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