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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Characterization of phytochemical composition and bioactivity assessment of *Pseudotrachydium kotschyi* essential oils

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Abstract

Pseudotrachydium kotschyi is a native medicinal plant used as a local medicine from Lorestan province of western Iran having numerous biological properties. After GC−MS analysis of *Pseudotrachydium kotschyi* essential oils (PKEOs), some biological activities were investigated. Additionally, TNF-α and TGF-β1 were studied in *Leishmania*-infected macrophages (in vitro) and Carrageenan-induced paw edema in rats. Finally, anti-inflammatory and wound-healing potential of PKEOs were investigated in vivo in mice. Total components identified by GC−MS contained 53 molecules, among which Z-α-trans-Bergamotol (23.25%), Durylaldehyde (16.07%), and α-Bergamotene (10.48%) existed in the highest amount in PKEOs. Antimicrobial assay of PKEOs showed maximum growth inhibition against *Listeria monocytogenes* and *Kluyveromyces marxianus* at the dose of 625 μg/ml. PKEOs exhibited a high cytotoxicity on lung cancer cell line A549 at a dose of 4 mg/ml. Anti-*Leishmania* activity of PKEOs showed concentration of 5000 μg/ml that had similar potency of glucantime at 125 μg/ml. The highest anti-inflammatory effect of PKEOs for Carrageenan-induced paw edema was observed at 100 concentrations, with paw inflammation reduced by 83.70% after using PKEO ointment. The present study reports broad-spectrum biological functions for essential oil from *P. kotschyi*. However, these biological activities, including anti-proliferative, anti-*Leishmania*, anti-inflammatory, antimicrobial and wound-healing potential, were not studied in detail. Therefore, it may be attractive for investigating the detailed mechanistic and molecular aspects of biological activities of PKEOs. Considering that the present study is the first to report the biological activity of *P. kotschyi*, further studies on their metabolites are needed to replicate the present findings.

Supplementary information The online version of this article (https://doi.org/10.1007/s00044-020-02594-5) contains supplementary material, which is available to authorized users.

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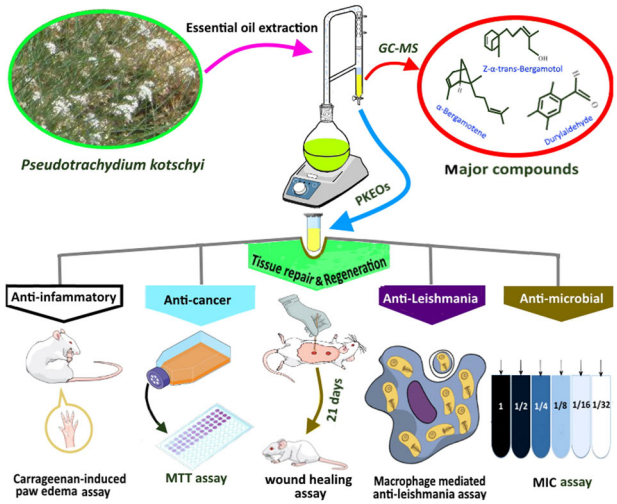
Graphical Abstract



Keywords *Pseudotrachydium kotschyi* · Anti-*Leishmania* activity · Antimicrobial activity · Anti-inflammatory · Wound-healing potential · Anticancer effect

Introduction

Medicinal plants constitute a major part of the Kingdom Plantae, which has received much attention in modern medicine due to its wide therapeutic potential. One of the most important constituents of herbs is the aromatic essential oils of medicinal plants. The constituents of the essential oils belong to different chemical families, including terpenes, aldehydes, alcohols, esters, phenols, ethers, and ketones. The essential oils are extracted from different parts of the plant with a very high diversity. Essential oils obtained from medicinal plants are reported to function as antioxidant, antimicrobial, anti-*Leishmania*, anti-inflammatory, and anticancer agents (Swamy et al. 2016).

Given the widespread use of herbs in traditional medicine for many years, their benefits to humans have been documented. Therefore, it can be expected that the bioactive compounds derived from these plants have low toxicity and are safe for human consumption (Fabricant and Farnsworth 2001). On the other hand, there may be some medicinal plants that have no scientific documentation of their therapeutic effects.

The genus *Pseudotrachydium* belongs to the family Apiaceae, obtained in 2000 by Pimenov and Kljuykov from the genus *Trachydium* (Pimenov and Kljuykov 2000). A study by Wang et al. (2016) showed the anti-inflammatory potential of *Trachydium roylei* (Wang et al. 2016). Mozaffarian also mentions in his book, *Understanding Iranian Aromatic and Medicinal Plants* (published in Persian, 2012), that various species of this genus have medicinal properties. Another report by Aziz-UL-Ikram et al. (2015) has pointed out the anti-inflammatory, antiseptic and anti-ulcer properties of the Apiaces family, in particular *T. roylei*. The climate of Iran permits the growth of a large variety of plants, including some unique endemic plants. Of particular interest is the family Apiaceae, which includes 122 endemic taxa (Mozaffarian 2007). *Pseudotrachydium kotschyi* is an endemic species of Iran and is grown in the western and central parts of Iran. *Pseudotrachydium kotschyi* (*Rumiakotschyi)* is a plant that is stacked with a height of 25–40 cm (Mozaffarian 2007). The flowers are white and the flowering season starts from early to late summer (Fig. 1). The local name of the plant used in traditional medicine is "Benjek". No previous studies of biological activity have been reported. Only one report of its classification has been published, citing its scientific name, *P. kotschyi* (*Rumiakotschyi*) (Pimenov and Kljuykov 2000). The plants of this family usually possess a characteristic pungent or aromatic smell owing to the presence of essential oil or oleoresin (Amiri and Joharchi 2016). Members of Apiaceae possess various compounds with many biological activities. Some of the main properties are apoptosis-inducing, antibacterial, hepatoprotective, vaso-relaxant, cyclooxygenase inhibitory, and antitumor activities (Sonboli et al. 2007; Wang et al. 2012).

This study is the first report of *P. kotschyi*, consisting of the biological and therapeutic properties of PKEOs, such as antimicrobial, anti-*Leishmania*, anticancer, anti-inflammatory, as well as wound-healing potentials.

Fig. 1 Morphological properties of *P. kotschy*. **a** Photograph of wild-growing plant and **b** herbarium sheet of *P. kotschyi*

Material and methods

Animal procurement and ethical considerations

Wistar male rats weighing 150−200 g were used to evaluate anti-inflammatory and wound-healing effects of the essential oil. The anti-leishmanial effects of the essential oil were studied on BALB/c mice weighing 25−30 g. Animals were purchased from the Laboratory Animal Production Center, Pasteur Institute of Iran. They were kept in a light/dark cycle with 12-h time interval, 25 ± 2 °C, humidity (55 ± 5% relative humidity), according to the standard protocols.

All animal experiments and procedures were approved by the Institutional Animal Care and Use Committee of Lorestan University of Medical Sciences (permit ID number: LUMS.REC.1395.204). The experimental protocol complied with the Guide for the Care and Use of Laboratory Animals. Therefore, all experiments were performed with minimum suffering to animals.

Plant material preparation

Flowering aerial parts of *P. kotschyi* were collected in August 2017 from Garin Mountain in the Lorestan province of western Iran; the region's altitude was ca. 2800 m above the sea level. The plant was identified by Dr. Mehrnia, an expert plant taxonomist at the Lorestan Agricultural Research and Natural Resources Center, Khorramabad, Iran and was deposited by a herbarium voucher specimen code: LUR13403.

Isolation and analysis of the essential oils

A portion (300 g) of the dried and completely ground aerial parts of *P. kotschyi* was subjected to water distillation using a Clevenger-type apparatus (British type) for 3 h. Analyses of the essential oil were performed by the application of gas chromatography with flame ionization detection (GC−FID) and gas chromatography–mass spectrometry (GC−MS). According to the property of the essential oil extract, the condition of GC or GC−MS was optimized. GC–FID analysis was conducted on an HP 6890 gas chromatograph equipped with an FID detector and an HP-5 fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 µm) (Ashrafi et al. 2019).

Antimicrobial activity

The antimicrobial activity of PKEOs was evaluated using the broth microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI) protocol (Wayne 2011). Microbial groups included three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 12600), *Bacillus cereus* (ATCC 14579), and *Listeria monocytogenes* (ATCC 13932); Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (PTCC 1609) and *Klebsiella pneumonia* (ATCC700603); and fungal species: *Candida albicans* (PTCC 5072), *Candida glabrata* (PTCC 5297) and *Kluyveromyces marxianus* (PTCC 5193). Since there was no report on the antimicrobial effect of *P. kotschyi*, antimicrobial studies of PKEOs were based on the characteristics and pathogenicity of each of the microorganisms and had no relation to its use in traditional medicine. All standard strains were obtained from the Culture Collection of Iranian Research Organization for Science and Technology (IROST). In order to determine the antibacterial activity of PKEOs through the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations, firstly, the PKEOs were dissolved in 5% dimethyl sulfoxide (DMSO) with the highest concentration (20,480 µg/ml), and then, serial twofold dilutions were made in concentrations ranging from 20 to 5120 µg/ml in Mueller-Hinton broth. Accordingly, MIC and MFC (minimum fungicidal concentration) were investigated similarly

to antibacterial tests in the Sabouraud dextrose broth and Sabouraud dextrose agar. The DMSO solution (5%) was used as a negative control. Vancomycin and Gentamycin were applied as positive controls for antibacterial assay; Fluconazole was also used as a positive control against fungal strains. To detect the growth of microorganisms, 2,3, 5-triphenyltetrazolium chloride (Sigma, T8877) was used (Mohammadzadeh et al. 2006).

Antitumor activity by cytotoxicity assay

Three cancerous cell lines, including lung carcinoma tissue A549 (ATCC CCL-185), epidermoid carcinoma in the mouth KB (ATCC CCL-17), and Mouse Breast Cancer Cell 4T1 (ATCC CRL-2539), were selected to study the cytotoxic effects of PKEOs and the normal T-cell lymphocyte was used as a control. The cytotoxicity was assessed using the MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay (Denizot and Lang 1986). The cells were plated in 96-well plates at a concentration of 1×10^5 cells/ml. Then, 10 µl of PKEOs at a concentration range of 40.0 −5000 µg/ml was added to each well. The microplate was incubated at 37 °C in 5% CO_2 for 72 h. Cisplatin (Sigma-Aldrich) was used as a positive control at a final concentration of 0.9 µM. Then, 10 µl of MTT reagent solution (5 mg/ml) was added to phenol-free RPMI 1640 and the plate was incubated for 4 h. Finally, for dissolving the reduced reagent, 100 µl of DMSO was added to each well and then absorbance of the samples was determined with an ELISA reader (Thermoplate TP-Reader) at 570 nm. The analyses were performed in triplicate and the percentage of inhibition was calculated using the following formula (de Lima et al. 2016):

$$\%\,\text{Cytotoxicity} = 1 - \frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}} \times 100,$$

$$\%\,\text{Viability} = 100 - \%\,\text{Cytotoxicity}.$$

Anti-leishmanial effects of PKEOs

Anti-leishmanial activity of PKEOs was studied against *Leishmania* major (MRHO/IR/75/ER). For this purpose, the macrophages were first obtained from peritoneal fluid of male BALB/c mice by injecting 2–5 ml of cold RPMI-1640 medium. The aspirated macrophages were washed twice, re-suspended in the RPMI 1640 medium, transferred to six-well plates with the cell number 5×10^4 cell/well and then incubated for 5 h at 37 °C in 5% CO_2. Then, the wells were washed using RPMI1640 medium to remove the suspended cells from the cultures. The adherent macrophages were kept for 24 h in the cultivation conditions and then infected with promastigote parasites at the ratio of 10:1 (parasite: macrophage). After 2 h of incubation at 37 °C, free parasites were removed from the media. After 24 h, PKEOs at the concentrations of 625, 1250, 2500, and 5000 µg/ml were added to each well. Glucantime was used as a positive control and an untreated group, only containing amastigote-infected macrophage, was considered as a negative control. In addition, amastigote-infected macrophages were studied for the secretion of TNF-α and TGF-β1 (Kheirandish et al. 2016). These cytokines were quantitated under similar conditions to the experiments mentioned above at different concentrations of PKEOs. TNF-α and TGF-β1 were measured by ELISA according to the kit instructions (ZellBio, GmbH, Germany).

Lipopolysaccharides (LPS) stimulation assay

The macrophages were isolated from male BALB/c mice as aforementioned (Kheirandish et al. 2016). They contained a density of 4×10^5 cells/ml onto 96-well plates containing 100 µl of RPMI 1640 medium. After overnight incubation, the cells were treated with a varied concentration of PKEOs (5000–625 µg/ml). They were observed after 1 h in the presence or absence of LPS (1 µg/ml) according to the experimental design (Hua et al. 2014). Dexamethasone with a concentration of 33 µM was used as a positive control. After 24 h, the supernatant was collected for cytokine measurement. Then, TNF-α as an inflammatory cytokine and TGF-β_1 as an anti-inflammatory cytokine were measured by ELISA according to the kit instructions (ZellBio, GmbH, Germany).

Carrageenan-induced paw edema in mice

Anti-inflammatory activity of PKEOs was studied using Carrageenan-induced paw edema in mice according to a method described by Gupta et al. (2015). Briefly, to induce inflammation in the paw, 0.1 ml of 1% Carrageenan was subcutaneously injected in the hind of paw. Anti-inflammatory effect of the PKEOs was studied by intranasal injection of 25, 50 and 100 mg of PKEOs per kg body weight of the mice. Tween-20-treated control and Dex-treated mice (Dex 33 µM) were used as negative and positive controls, respectively. After 48 h, blood samples were taken from the mice for measurement of TGF-β_1 and TNF-α cytokines. In addition, edema in the paw was determined as inhibition percent in treated groups compared to the negative (Tween-20-treated) and positive (Dex-treated) controls. The inhibition percent was determined by using the following formula (Gupta et al. 2015):

$$\text{Inhibition percent} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{control}}} \times 100,$$

where $(C_t - C_0)_{control}$ is the difference in the size of paw after 5 h in untreated control, and $(C_t - C_0)_{treated}$ is the difference in the size of paw after 5 h in PKEOs or Dex-treated groups.

Wound-healing study

In order to evaluate the wound-healing properties of PKEOs, 5% ointment of PKEOs consisting of 100 g Eucerin in 5 ml of PKEOs was prepared. For this purpose, 36 adult male rats aged 7 weeks and weighing 200−300 g were used. These animals were randomly divided into 12 groups for treating with PKEOs, phenytoin (positive control), and Eucerin (negative control). First, rats were wounded 1 cm in diameter. During a 21-day period, the wounds were treated daily with ointment and the diameter of the wounds was measured on days 3, 7, 14, and 21 (see Table S1 and Fig. S1 in Supplementary File).

Tissue samples were taken on days 3, 7, 14, and 21. The samples were fixed in 10% formalin. For histological studies, the sample tissues were prepared alcohol-free, digested for 30 min, clarified by m-Xylene for 1 h and embedded with paraffin blocks. The next day, the block samples were cut using a manual rotary microtome (SLEE Cut 4060). Hematoxylin and eosin staining (H&E) was used for counting the number of PMNLs (polymorphonuclear leukocytes) and number of fibroblast cells. The wound samples were taken for analyzing by light microscopy (Pradhan et al. 2009; Sayyedrostami et al. 2018).

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 6.02). Two-way ANOVA analysis was used based on Bonferonni multiple comparisons post hoc for comparing between the control and treated groups. Confidence intervals were considered 95% significant with p value < 0.05. The results were presented as mean ± SD from three replicate experiments.

Results and discussion

Chemical composition of the PKEOs

The essential oil of the aerial parts of *P. kotschyi* contains numerous compounds as seen in the GC−MS chromatogram in Fig. 2. Four strong signals show the frequent compounds present in the PKEOs. In addition, the details of chemical compositions were presented in Table 1. A total of 51 compounds were identified by GC/MS.

This study is the first to report the biomedical properties of *P. kotschyi*, although other members of the Apiaceae family have been previously studied. The results showed that the major components of PKEOs were Z-α-trans-Bergamotol (23.25%), Durylaldehyde (16.07%), and α-Bergamotene (10.48%), which are classified in three subclasses of terpenoides including oxygenated sesquiterpenes, oxygenated monoterpenes, and sesquiterpene hydrocarbons, respectively. Figure 3 shows the chemical structures of these major compounds using https://www.chemdoodle.com. The major compounds present in PKEOs have been identified to be a series of terpenoid derivatives that possibly associate with their biological activities, concentration, and type. As the literature stated, terpenoids are the most diverse class of terpenes, in which massive studies have been fulfilled about their biological activities, such as

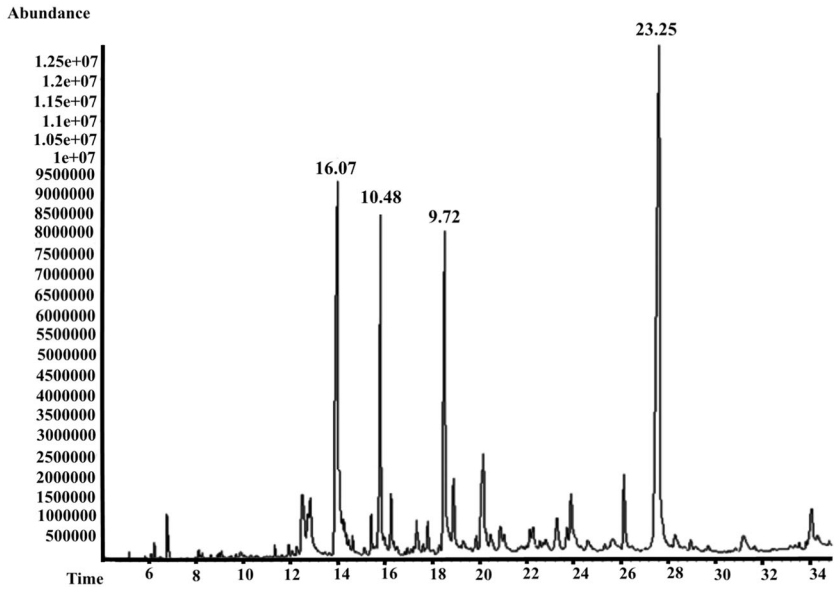


Fig. 2 GC−MS chromatogram of the PKEOs

Table 1 GC−MS output of PKEOs components with quantitative analysis and their chemical names that were identified using library spectral data (NIST and Wiley)

Retention time	Compounds	%	Retention time	Compounds	%
5.159	α-Pinene	0.07	16.258	Trans-β-Farnesene	1.34
5.522	verbenene	0.02	16.498	α-Amorphene	0.39
5.833	Sabinene	0.01	17.624	Δ-Selinene	0.30
5.908	β-Pinene	0.01	17.807	β-Bisabolene	0.99
6.096	β-Myrcene	0.04	18.299	Δ-Cadinene	0.36
6.388	α-Phellandrene	0.02	18.527	3-(3-Furyl)propanol	9.72
6.479	Δ-3-Carene	0.03	18.91	Cycloheptane, 1,3,5-tris (methylene)-	2.52
6.759	Isodurene	0.41	19.31	Germacrene B	0.87
6.822	dl-Limonene	0.34	19.613	Longifolenaldehyde	0.25
7.359	β-Phellandrene	0.01	20.162	Caryophyllene oxide	4.40
8.965	trans-Pinocarveol	0.09	20.465	Acetyltyramine	1.04
9.508	endo-Borneol	0.06	21.671	valerenol	0.26
10.571	trans-Carveol	0.07	22.002	Ledene	0.27
11.623	γ-Terpinene	0.07	22.276	Aromadendrene oxide	1.19
11.92	Nerol	0.28	22.557	γ-Gurjunene	0.45
12.263	verbenene	0.4	22.814	β-Selinene	0.77
12.492	Thymol	2.95	23.288	α-Farnesene	1.70
12.738	Carvol	1.01	23.705	β-Atlantone	0.82
12.835	Benzaldehyde, 2,4,6-trimethyl-	2.8	23.877	Z-α-trans-Bergamotol	3.10
13.641	Phenol, 2-methyl-5-(1-methylethyl)-	0.12	25.134	α--trans-sequicyclogeraniol	0.19
14.029	Durylaldehyde	16.07	25.654	Tetradecanoic acid	1.52
14.469	β-Bourbonene	0.41	26.129	Curcumene	2.33
14.624	Tetradecane	0.51	27.615	Z-α-trans-Bergamotol	23.25
14.801	β-Ethylnaphthalene	0.11	28.283	Isoaromadendrene epoxide	1.18
15.412	trans-Caryophyllene	0.68	28.946	Nonadecane	0.47
15.812	α-Bergamotene	10.48	34.056	Heptacosane	1.87
15.967	Aromadendrene	0.75			

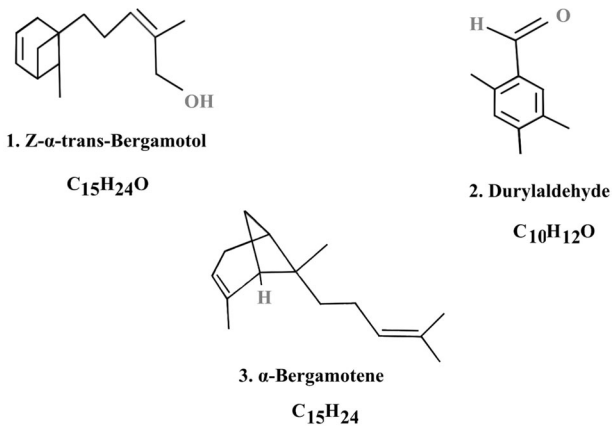


Fig. 3 Structure of the major compounds identified in PKEOs

antimicrobial, anticancer, antiparasitic, and others (Prakash 2018). In addition, many aromatic terpenoides and their derivatives are used in the pharmaceutical, food, and cosmetics industries as well as in the production of vitamins and hormones (Caputi and Aprea 2011; Lu et al. 2016). The most abundant compounds in PKEOs are bergamotene and bergamotol, which was found to belong to sesquiterpenes derivatives. As reported in other studies, sesquiterpene and their derivatives are often found in some flowering plants that have a wide range of biological activities such as antimicrobial and antitumor, anti-insecticide, antiviral, and antiprotozoal activity (Ashour et al. 2018; Bartikova et al. 2014). Durylaldehyde was characterized as a main constituent of *Eryngium foetidum* (culantro), *Foeniculum vulgare* (fennel), and *Cyclorhiza waltonii* (Fang et al. 1990; Pino et al. 1997). These compounds are synonymous with 2,4,5-trimethylbenzaldehyde, which is known in the present study as the second compound in PKEOs, belonging to oxygenated monoterpenes (Gyawali et al. 2006). Most of the monoterpenes are potent antibacterial agents against a wide range of bacterial species (Prakash 2018; Sonboli et al. 2007).

Table 2 Antimicrobial activity of PKEOs measured by MIC/MBC for bacterial species and MIC/MFC for fungi

Microorganisms	PKEOs		Antibiotics	
	MIC	MBC/MFC	MIC	MBC/MFC
			Vancomycin	
S. aureus ATCC 12600	2500	>5000	16	128
B. cereus ATCC 14579	1250	>5000	16	64
*L. monocytogenes*ATCC 13932	625	>5000	16	64
			Gentamycin	
P. aeruginosa ATCC 27853	2500	>5000	5	5
S.typhi PTCC 1609	2500	5000	5	5
K. pneumoniae ATCC 700603	2500	5000	8	128
			Fluconazole	
C. albicans PTCC 5072	2500	5000	64	128
C. glabrata PTCC 5297	2500	5000	8	32
K. marxianus PTCC 5193	625	2500	128	>128

Table 3 IC_{50} values of PKEOs and Cisplatin against the cancer cell lines and T cells

Test samples	IC_{50} values (μg/ml)			
	A549	KB	4T1	Lymphocyte T
PKEOs	965.92 ± 30	1803.62 ± 22.59	1089.4 ± 30.86	>5000
Cisplatin	5.42 ± 0.08	2.97 ± 0.029	916.66 ± 17.43	>5000

Antimicrobial activity

Antimicrobial activity of the PKEOs was studied on three Gram-negative bacteria, three Gram-positive bacteria, and three fungi as shown in Table 2. Maximum inhibitory activity on *L. monocytogenes* and *K. marxianus* was observed at a PKEO concentration of 625 μg/ml. As compared to positive controls (vancomycin, gentamycin, and fluconazole) and according to CLSI standard, it can be concluded that PKEOs had a moderate antimicrobial activity against all microorganisms. Importantly, as evidenced by other studies, antimicrobial activity of PKEOs is attributed to total sesquiterpene contents, including oxygenated sesquiterpenes and monoterpens (Elghwaji et al. 2017; Sardrood et al. 2019). As reported in the literature, the presence of known compounds, including caryophyllene, thymol, bergamotene and curcumene can be a strong reason for the antimicrobial activity of PKEOs (Dahham et al. 2015; Nascimento et al. 2011).

Cytotoxicity assays

Different concentrations of PKEOs were used to study the cytotoxic characteristics on cancer cell lines. As shown in Table 3, PKEOs had a moderate inhibitory effect on the breast cancer line (4T1); however, there was a maximum inhibition on the lung cancer cell line (A549) at a concentration of 965.92 μg/ml. The highest IC_{50} of PKEOs was against T cells. Thus, it could be concluded that PKEOs is safe at the doses <965.92 μg/ml for normal cells. The PKEOs exhibited promising anticancer activities in vitro against three types of cancer cell lines, while decreased cytotoxicity was seen in a normal T cell. In this regard, some studies have attributed the anticancer properties of the plant essential oils to their terpenoide content (Iqbal et al. 2017; Sharma et al. 2013). Several studies have demonstrated the antitumor activity of many essential oils containing monoterpenes compounds. Therefore, anticancer activity of different essential oils is attributed to their terpene content (Prakash 2018; Sharma et al. 2013; Silva et al. 2003; Sobral et al. 2014).

Anti-leishmanial activity

Anti-leishmanial activity of the PKEOs was assessed by counting promastigote-infected macrophages in vitro. First, the percentage of promastigote-infected macrophages was

determined in the presence of different concentrations of PKEOs and glucantime. As seen in Fig. 4, *Leishmania* infection and the number of amastigotes significantly decreased with the increase of PKEOs. Glucantime, a standard anti-*Leishmania* drug, was used as control. The anti-leishmanial potency of PKEOs was observed at 5000 µg/ml compared to glucantime at 125 µg/ml. The antiparasitic activity of various essential oils has been widely investigated. Some of the most effective compounds include the terpenoid molecules found in the members of

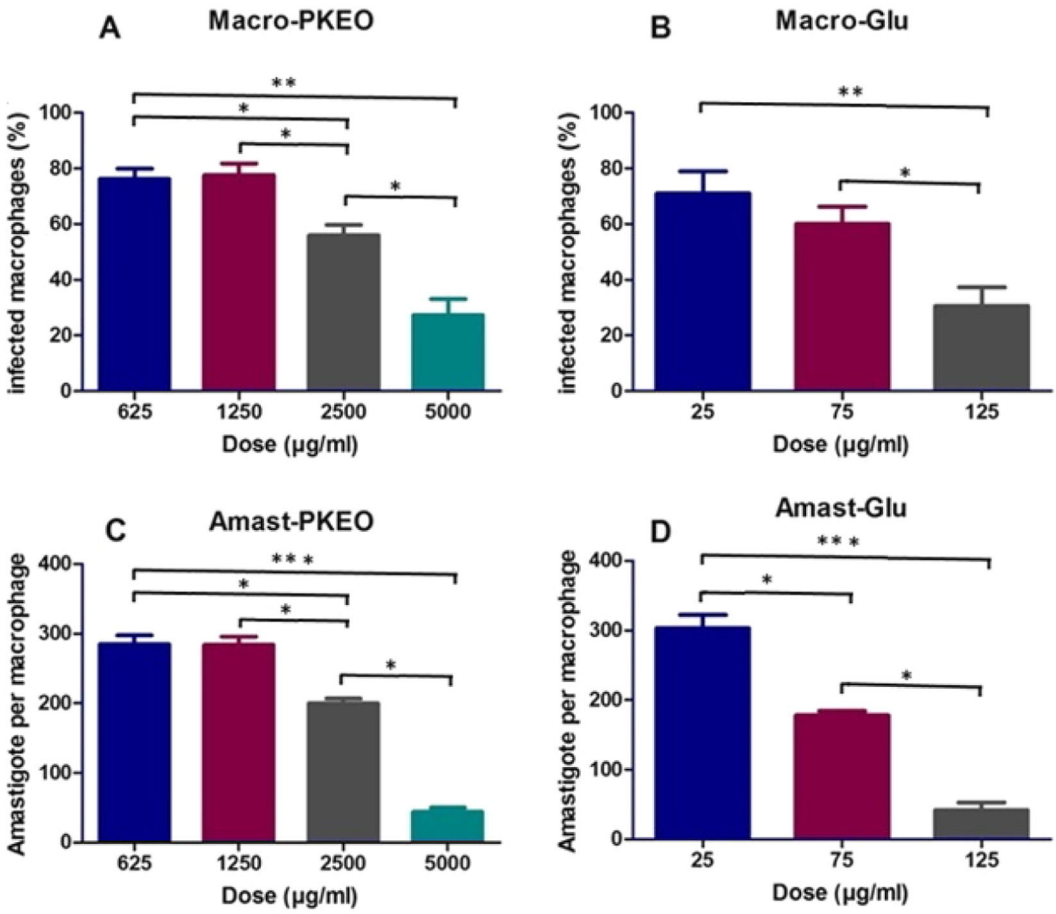


Fig. 4 Effect of PKEOs and glucantime on infectivity of promastigote in macrophages. The percent of promastigote-infected macrophages in the presence of PKEOs (**a**) or glucantime (**b**). **c**, **d** show the number of amastigotes per macrophages in the presence of PKEOs and glucantime, respectively. The values were presented as mean ± SD of triplicate experiments. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

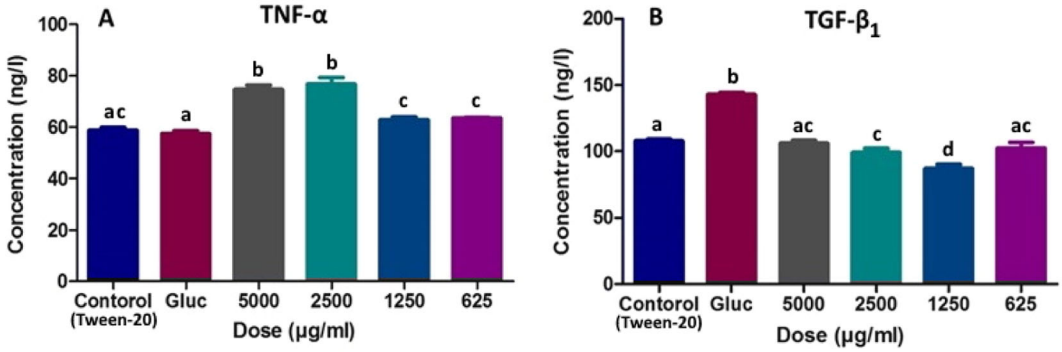


Fig. 5 **a** TNF-α and **b** TGF-β1 levels in *Leishmania*-infected macrophages. Superscript letters a, b, and c express significant differences between the experimental groups. Significant level was considered as confidence interval of 95% with p value of < 0.05

the Apiaceae family (Borg-Karlson et al. 1993). For instance, Barati et al. (2014) reported that essential oils of *Ferulaassa-foetida* from Apiaceae family had potent cytotoxicity against *Leishmania major* promastigotes (Barati et al. 2014). In this study, PKEOs were able to protect the macrophage against promastigotes infection. Although it has been claimed that the anti-inflammatory mechanism of terpenoids is independent of TNF-α expression level (Gallily et al. 2018), our data indicate that PKEOs exert anti-*Leishmania* activity through affecting TNF-α and TGF-β1 levels in the macrophage. Enhanced secretion of TNF-α and decreased TGF-β1 from *Leishmania*-infected macrophage suggest an increased activity of classical macrophages versus decreased activity of alternative macrophages, respectively. This macrophage polarization toward classical type, following PKEO treatment, is critical for killing of *Leishmania* amastigotes.

As seen in Fig. 5, two cytokines, TNF-α and TGF-β1, were determined in the *Leishmania*-infected macrophages following PKEOs treatment. The results showed the increase of TNF-α in the concentration range of 2500–5000 μg/ml. In contrast, TGF-β1 level had no significant change in the PKEOs-treated groups in comparison with controls.

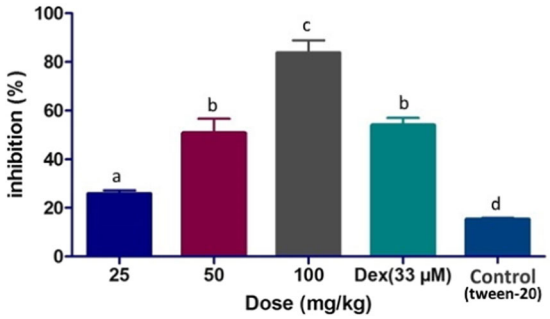


Fig. 6 Anti-inflammatory effect of PKEOs on Carrageenan-induced paws edema in mice. Dex and Tween-20 were considered as positive and negative controls, respectively. The a, b and c letters show significant differences among the experimental groups. All experiments were performed in triplicate and the data were presented as mean ± SD

Carrageenan-induced paw edema in mice

Carrageenan-induced paw edema in mice is considered a reliable approach to study inflammation. Figure 6 shows the percentage inhibition of inflammatory edema in the mice model of hind paw in the PKEOs-treated groups compared to the Dex-treated (positive control) and Tween-20-treated (negative) group. These results clearly demonstrate a significant reduction in paw thickness in the Dex- and PKEOs-treated groups as compared to the negative control group (Tween-20). The highest anti-inflammatory effect of PKEOs was observed at 100 mg/kg, which reduced 83.70%± to 11.79% of the paw inflammation after injection of Carrageenan. This value for Dex was 54.12 ± 6.57%. As compared with the Dex-treated group, the inhibition percent at 100 mg/kg group was significantly increased ($p < 0.05$). However, our results demonstrated that anti-inflammatory effects of PKEOs on the Dex-treated group compared with that group treated with 50 mg/kg group PKEOs were not significant ($p > 0.05$).

Measuring cytokine inflammation of the paw

By increasing the PKEOs concentration, the level of TNF-α decreased, while TGF-β1 showed no significant changes (Fig. 7). In this experiment, statistical comparison of the TNF-α cytokines level between the groups indicated a significant increase in the minimum concentration of PKEOs (25 mg/kg), while the other treatments indicated no differences in TNF-α serum level. There was no significant

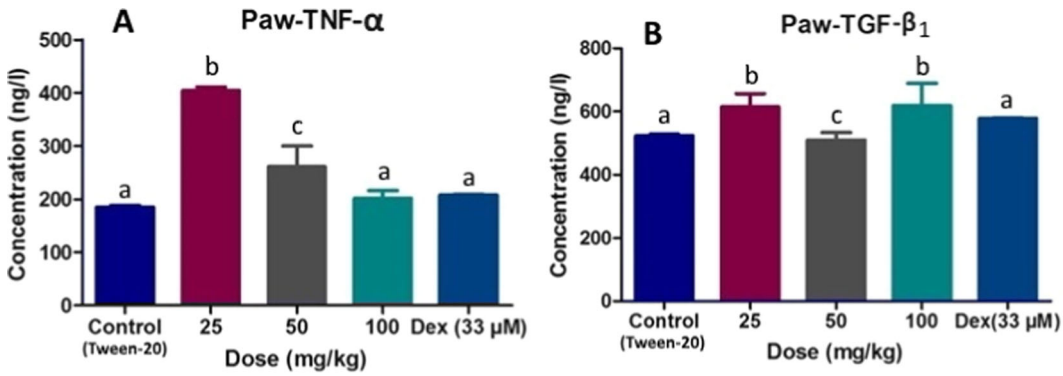


Fig. 7 Effect of PKEOs on anti-inflammatory cytokines in Carrageenan-induced paw edema in mice. **a** Serum TNF-α level in the presence of different doses of PKEOs. **b** Serum TGF-β1 cytokines level in the presence of different doses of PKEOs. The differences between groups were indicated as superscript letters a, b and c above the bars

difference between different groups of TGF-β1 cytokines between treatments.

Wound-healing study

According to the observed tissue-healing properties of aerial parts of *P. kotschyi* among resident people, we studied the wound-healing potential of PKEOs in the form of an ointment. Three important parameters indicating the healing process of the skin wound were studied. As shown in Fig. 8, the first criteria, including the restoration and formation of epidermis, was analyzed from day 3 in the treated groups, phenytoin and Eucerin. Histopathological study revealed that the healing and formation of epidermis begin from the third day in treated samples with PKEOs ointment, phenytoin and Eucerin, but the process was clearly visible from the seventh day of the treatment. After 21 days of treatment, even the scars were completely wiped out in all three groups. PKEOs resulted in improved wound-healing response in rats with complete tissue regeneration. Prior studies have found terpenoide compounds, such as Bergamotene, likely to have wound-healing potential. In this respect, Wagner et al. (2017) showed the potential of oral wound healing for the essential oil of *copaiba* containing terpenoid compounds such as trans-α-Bergamotene and β-Caryophyllene in Wistar rats. Pradhan et al. (2009) attributed the therapeutic effect of the aqueous and ethanolic extracts of *Vernonia arborea* to its sesquiterpenes structures responsible for the wound healing in rats.

As seen in Fig. 9, the results show that angiogenesis process in the treatment with the 5% PKEOs ointment occurred faster than those groups treated with phenytoin and Eucerin; however, the healing trend was declined from the 7th to 21st day.

Given that granulation is one of the indicators for wound-healing process, the granulation process equally occurred in all treatments until the seventh day (Fig. 10). The healing trend was declined in the PKEOs-treated group compared with the Eucerin and phenytoin groups.

Conclusions

The present study reports for the first time the biological activity of the essential oil from *P. kotschyi*, which exhibited dose-dependent cytotoxic effects against cancer cell lines. In addition, anti-inflammatory properties of the bioactive compounds present in PKEOs were demonstrated with significant reduction in Carrageenan-induced Paw edema induced in rats. Inflammation induced by LPS was also improved in the presence of PKEOs. According to the GC−MS results, we conclude that the major therapeutic effects of the PKEOs are associated with three components, including Z-α-trans-Bergamotol (26.77%), Durylaldehyde (15.86%), and α-Bergamotene (10.18%),

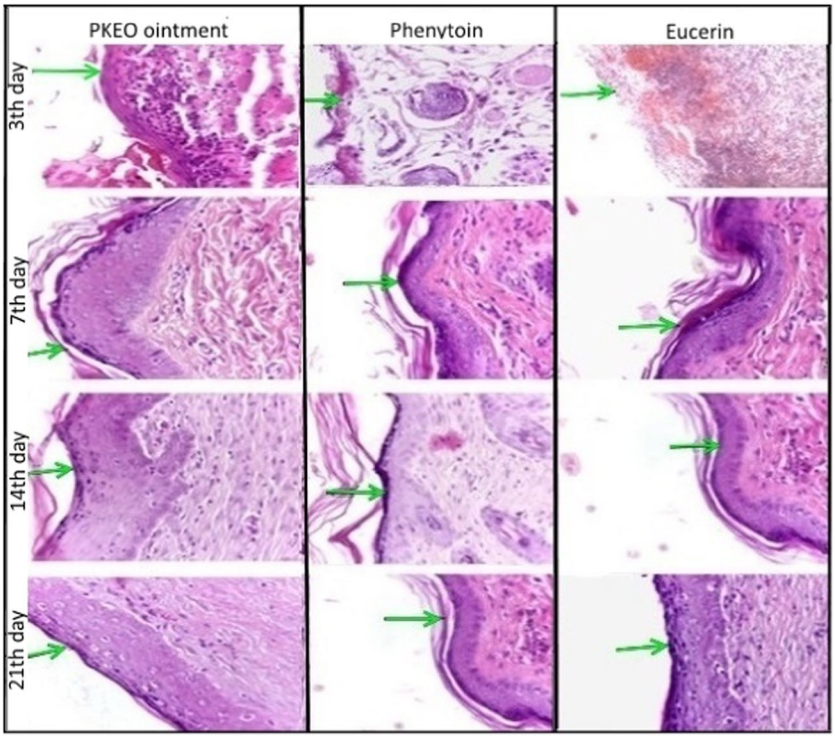


Fig. 8 Epithelization process in wound location after treatment with PKEOs, phenytoin and Eucerin. During the 21-day treatment, epithelial layer of the skin was completely generated

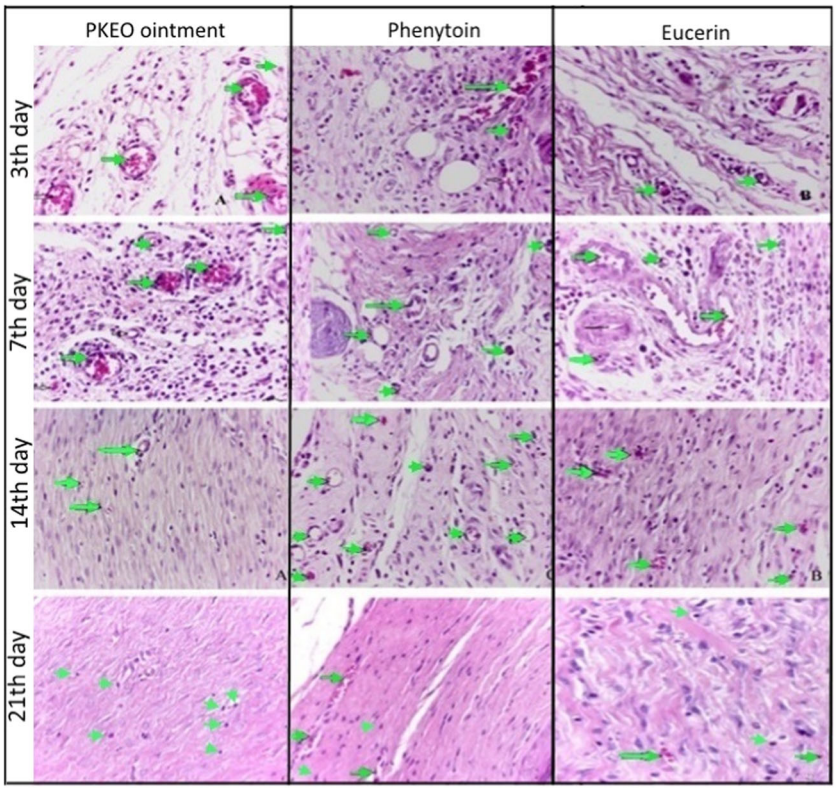


Fig. 9 Angiogenesis process in wound location after treatment with PKEOs, phenytoin and Eucerin. Angiogenesis shows the wound healing during the 21-day treatment

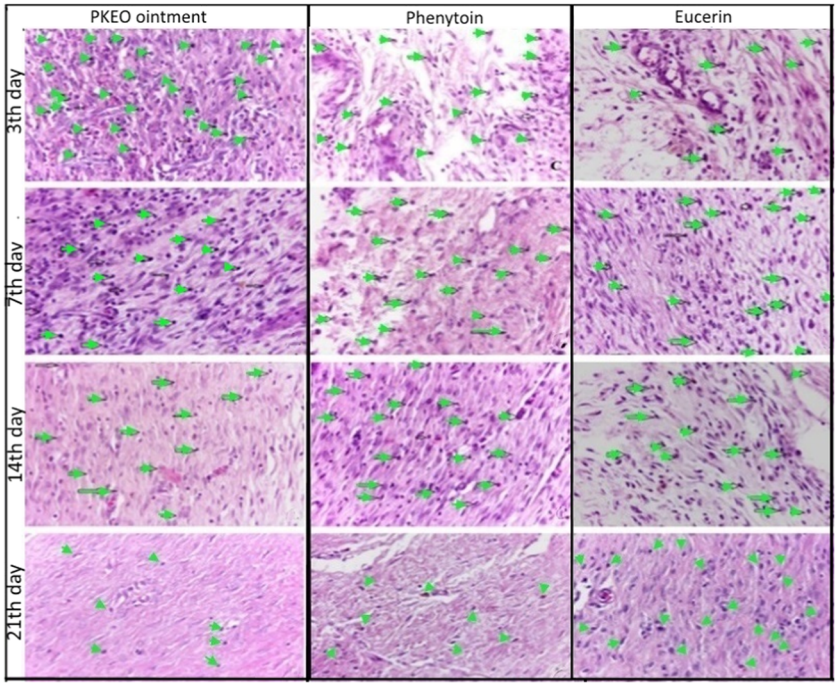


Fig. 10 Granulation of wounded tissue after treatment with PKEOs, phenytoin and Eucerin. Granulation shows the healing process of the wound during the 21-day treatment

which together constitute about 53% of total metabolites. Considering that the present study is the first to report the biological activity of *P. kotschyi*, further studies on their metabolites are needed to replicate the present findings.

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Author contributions BA designed the study as a major researcher. FB and FA contributed to animal studies, especially histology studies. MR performed essential oil extraction by Clevenger apparatus. AM was responsible for data arrangements, scientific writing, and graphical designing. FK performed anti-*leishmania* studies and cytokine assay. SV analyzed all the data statistically. PR was responsible for the plant and SS was responsible for study design, project management, and reviewing the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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