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RESEARCH ARTICLE-BIOLOGICAL SCIENCES

Chemical Composition, Antimicrobial, Antiparasitic, and Cytotoxic Activities of *Rhanterium intermedium* Pomel Leaves Essential Oil

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Abstract
This study evaluates the chemical composition, antimicrobial, antiparasitic, and cytotoxic activities of essential oil extracted from *Rhanterium intermedium* Pomel (EORI) leaves. The chemical composition of the essential oil was analyzed by GC/MS analysis. In addition, the antimicrobial capacity of EORI against five pathogenic bacteria and two yeast strains was determined by disk diffusion and broth microdilution methods. Alamar Blue® assay was used to evaluate the antiparasitic potential of EORI against epimastigotes forms of *Trypanosoma cruzi*, trophozoïtes forms of *Acanthamoeba castellanii* Neff., and promastigotes forms of *Leishmania amazonensis* and *Leishmania donovani*. The same assay was used to assess the cytotoxicity effect of oil against murine macrophage J774. A1 cell line. GC/MS results showed that the EORI comprised 22 compounds, representing 97.99% of the total composition. The main constituents of EORI were camphene (32.08%), myrcene (13.86%), α-pinene (11.29%), sabinene (5.85%), and β-pinene (5.73%). Additionally, a significant antibacterial effect was recorded against *Escherichia coli* with MIC inferior to 14.78 mg/mL. Concerning the antifungal activity, EORI showed a remarkable efficiency against the two tested *Candida albicans* strains (MIC = 14.78 mg/mL). The EORI showed promising antiparasitic activity against epimastigotes forms of *Trypanosoma cruzi* (IC_{50} = 07.21 ± 0.09 µg/mL) and low cytotoxicity levels on murine macrophages (LD_{50} = 47.91 ± 3.61 µg/mL). Overall, it was shown that our tested essential oil possesses potential antimicrobial and antiparasitic capacities; also, it is considered safe to use. Further detailed investigations are needed to ensure EORI as a novel source of alternative drugs for microbial and parasitic diseases.

Keywords *Rhanterium intermedium* · Essential oil · Antimicrobial · Antiparasitic · Cytotoxicity

1 Introduction

The problem of resistant microorganisms is growing all over the world. Fungi, Gram-negative, and Gram-positive bacteria are permanent causes of infections in hospitals, which leads to high morbidity and mortality [1, 2], and remain a grave public health problem that has caused vast social and economic difficulties [3, 4].

Parasitic diseases such as trypanosomiasis are a leading health concern in numerous African countries where about 50 million people are infected. Furthermore, leishmaniasis is also a disease that has a worldwide distribution. It is part of the group of neglected infectious diseases, affecting 98 countries with approximately 1.7 million cases global wide each year [5, 6]. Regarding, *Acanthamoeba castellanii* is a free-living protozoan belonging to the genus *Acanthamoeba*. This parasite can cause serious health risks, such as granulomatous amebic encephalitis and *Acanthamoeba* keratitis [7].

Until now, drugs used to treat these diseases have cytotoxic effects, long-term treatment periods, variable efficacy, and drug resistance. In that sense, there is a clear need to search for new, active, affordable, and safe drugs to treat a wide variety of microbial and parasitic diseases [6, 8, 9].

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Essential oils (EOs) are volatile mixture of constituents obtained from aromatic plants based on methods such as hydrodistillation [10]. EOs could represent an appropriate alternative to current drugs, given the numerous biological properties reported, including antibacterial [11], antifungal [12], trypanocidal [13], anti-*Leishmania* [14], amebicidal [15], and cytotoxic [16] activities.

By its geographical location, Algeria offers a rich and diverse flora; moreover, many aromatic plants grow spontaneously. Among them, the *Rhanterium* species belonging to the Asteraceae family are aromatic and medicinal plants. In traditional medicines, *Rhanterium* species is used to treat various microbial infections and gastrointestinal disorders [17]. Three species of *Rhanterium* grow in the northern Algerian desert, namely, *Rhanterium adpressum* Coss. & Durieu, *Rhanterium intermedium* Pomel, and *Rhanterium suaveolens* Desf. These species are found from Aïn Sefra in the west, passing by the Mzab, Laghouat, and Bou-Saâda in the middle, and arriving at Biskra in the east [18, 19].

R. intermedium Pomel is a spontaneous plant, a sub-shrub, with alternate leaves, small, entire, and toothed. Also, it has somewhat spreading and sharp scales at the top; this characteristic allows its differentiation from other *Rhanterium* species [20].

In our survey of the desert aromatic plants, we detected that only a few studies concerning essential oils of the *Rhanterium* species were done, and a lot remains to be explored. EOs of these species have been shown to process interesting biological properties such as antioxidant [21, 22], antimicrobial [23–26], and insecticidal [27]. So, in this study, it is reported, for the first time, the chemical composition and antimicrobial, antiparasitic, and cytotoxic capabilities of EO of *R. intermedium* (EORI). The specific aim of the work is focused on the Algerian EORI species hoping to validate or establish its medicinal properties.

2 Materials and Methods

2.1 Plant Material

R. intermedium Pomel was collected in March 2021 from Ghardaïa (32.313176°N and 3.835825°E) in southern Algeria. Plant material was identified by Dr. TAHRI Djilali from the Department of Biology at the University of Amar Telidji, Laghouat, Algeria, in view of lack of research, area distributions, and ethnomedicinal importance of aromatic wild plants from genus *Rhanterium*. For these reasons, the *R. intermedium* Pomel was selected in this study.

2.2 Essential Oil Extraction

Freshly collected samples were dried at room temperature, protected from light and moisture. Then, the leaves separated from the plant. The essential oil was extracted by hydrodistillation using Clevenger apparatus for 5 h, where the leaves-to-water ratio was 1:2 [28, 29]. Leaves are immersed with distilled water in a heating flask. Water vapor produced during heating, transfers volatile molecules through extractor inlet tube, condenses in the condenser, and returns to the extractor body. Next, the liquid oil separates from the hydrophilic phases due to its low density. The obtained essential oil was dried over anhydrous sodium sulfate. Until the chemical and biological tests were performed, the extracted essential oil was kept at 4 °C in clean containers wrapped in aluminum foil.

2.3 Composition Analysis Using GC–MS

The analysis of the chemical composition of EORI was carried out in the Technical Platform of Physicochemical Analysis (PTAPC-CRAPC)—Laghouat—Algeria, using a SHIMADZU GCMSQP2020 Instruments, equipped with a fused Rxi®-5MS capillary column (Phase: Crossbond® 5% diphenyl/95% dimethyl polysiloxane), its dimensions are 30 m × 0.25 mm and 0.25-μm film thickness. A volume of 0.5-μL solution prepared by 10% vol. of the sample dilution in n-hexane, was injected in split mode (80:1). Injector and detector temperatures were maintained at 250 °C and 310 °C, respectively, the column temperature was programmed at: 60 °C fixed for 3 min then increased to 310 °C with an increase increment of 2 °C/min, and then maintained at 310 °C for 10 min. The carrier gas used was helium (99.995% purity) with a flow rate of 1 mL/min. The mass spectrometer conditions were as follows: Ionization voltage 70 eV, ion source temperature 200 °C, and electron ionization mass spectra were acquired over the mass range of 45–600 m/z. The isolated compounds were identified by their respective retention indices determined in reference to a series of n-alkanes (C7-C20), and verified by a comparison of mass spectral data with those obtained using pure standards and with those reported in the literature (Adams, 2007) and eventually by comparing their mass spectra with the GC–MS spectral library (FFNSC 1.2. library).

2.4 Antimicrobial Activity

2.4.1 Test Microorganisms

The test microorganisms used in this work included one Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), four Gram -negative bacteria (*Escherichia coli*

ATCC 25922, *Klebsiella pneumoniae* ATCC 70603, *Pseudomonas aeruginosa* ATCC 27853, and *Yersinia enterocolitica* ATCC 9610), and two yeasts (*Candida albicans* ATCC 10231 and *Candida albicans* ATCC 26790). These microbial strains were selected for their marked resistance to many antimicrobial drug classes.

2.4.2 Disk Diffusion Assay

The *in vitro* evaluation of the antimicrobial activity was performed by modified disk diffusion method [30]. The microbial inoculums were prepared from overnight broth culture in physiological saline (0.8% NaCl) to obtain an optical density range from 0.08 to 01 at 625 nm, which is equivalent to 10^8 CFU/mL) Mueller–Hinton agar (for bacteria) and Sabouraud agar (for yeast strains) were prepared in Petri dishes before inoculation. Sterile disks (6 mm) were placed on inoculated agars with test microorganism, added with 10 µL of pure EORI, and diluted EORI (1:2 and 1:5 v:v of DMSO). DMSO and standard antibiotics were used as negative and positive controls, respectively. The Petri dishes were incubated at 37 °C for 24 h, and the diameter of the clear zone was measured.

2.4.3 Broth Dilution Assay

The minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC), and minimal fungicidal concentration (MFC) values of EORI were determined using the broth dilution assay according to the modified method described by [31, 32]. The sterile 96-well plates were prepared by dispensing into each well 100 µL of nutrient broth. Then, 100 µL of pure essential oil solutions were added into the first wells. After that, 100 µL from the serial dilutions were transferred into consecutive wells, the 100 µL of the last well was thrown. In addition, the microbial suspensions were added with a load of 10^8 CFU/mL, the volume being 100 µL in each well. Cefazoline and amphotericin B were used as positive controls for all bacterial and fungal tested strains, respectively. After incubation period (24 h, 37 °C), 40 µL of 0.2-mg/mL *p*-IodoNitroTetrazolium violet salt (INT) (Sigma) dissolved in 99.5% ethanol was added to each well of the plate as a colorimetric indicator. Afterwards, the plates were incubated one more time at 37 °C for 5–30 min. The color change from pink to purple indicates the microbial growth in the well. The lowest concentration showing a significant inhibitory effect was identified as MIC. The lowest concentration without any color change was identified as MBC (for bacterial strains) or MFC (for fungal strains).

2.5 Antiparasitic Activity

2.5.1 Parasites Strains

The activity of the EORI was evaluated against epimastigotes stage of *Trypanosoma cruzi* (Y strain), trophozoites stage of *Acanthamoeba castellanii* str. Neff. (ATCC 30010), and promastigotes stage of *Leishmania donovani* (MHOM/IN/90/GE1F8R) strains and *Leishmania amazonensis* (MHOM/BR/77/LTB0016).

2.5.2 Trypanocidal Activity

The trypanocidal activity of the assayed EORI was determined by the modified Alamar Blue® assay as described by [33, 34]. For the *in vitro* studies, EORI was dissolved in dimethyl sulfoxide (DMSO), and further dilutions were made in Liver Infusion Broth (LIT) medium. Epimastigotes were adapted for culture in LIT liquid medium at 26 °C. Logarithm phase cultures of epimastigotes were used for experimental purposes, and the *in vitro* susceptibility assay was performed in sterilized 96-well microtiter plates (Corning™). Parasites were added to sterilized microtiter plates with 96 wells at a concentration of 1×10^6/well. The final volume was 200 µL in each well. After addition of 10% of Alamar Blue Assay Reagent® (Biosource Europe, Nivelles, Belgium), the plates were incubated at 26 °C. After 72 h, the plates were analyzed with an EnSpire microplate reader (PerkinElmer, Massachusetts, USA) using a test wavelength of 570 nm and a reference wavelength of 630 nm. The 50% inhibitory concentration (IC_{50}) was determined by linear regression analysis with 95% confidence limits. All experiments were performed three times each in duplicate, and the mean values were also calculated.

2.5.3 Anti-Acanthamoeba Activity

The *in vitro* anti-*Acanthamoeba* activity of EORI against *Acanthamoeba castellanii* Neff. (ATCC30010) was evaluated using the modified Alamar Blue® assay as described by [35]. As first step, the *Acanthamoeba* culture was seeded in triplicate on a 96-well microtiter plates (50 µL from a stock solution of 5×10^4 cells/mL). After, the trophozoites were attached to the bottom of the well, 50 µL of serial dilutions of the EORI were added to the 96-well plate. As a negative control, we have used *Acanthamoeba castellanii* trophozoite in Peptone Yeast Glucose medium. Finally, the Alamar Blue™ was placed onto each well at 10% as a final concentration. Then, plates were incubated during 96 h at 28 °C with a soft agitation and finally analyzed in the EnSpire Multimode Plate Reader (PerkinElmer, Madrid, Spain) using emitted florescence at 570/585 nm. The 50% inhibitory concentration (IC_{50}) was determined by linear regression analysis

with 95% confidence limits. All experiments were performed three times each in duplicate, and the mean values were also calculated.

2.5.4 Leishmanicidal Activity

The leishmanicidal activity of the assayed EORI was determined by the modified Alamar Blue® assay as described by [33, 34]. The percentage of inhibition and 50% inhibitory concentration (IC_{50}) were determined by linear regression analysis with 95% confidence limits. All experiments were performed three times each in duplicate, and the mean values were also calculated.

2.6 Cytotoxicity

Cytotoxicity was assessed against murine macrophage J774. A1 cell line (the major host cells for *Leishmania* sp.) according to the method described by [36]. Macrophages were incubated with different concentrations of the tested EORI at 37 °C in a 5% CO_2 humidified incubator. Briefly, cells were seeded in duplicate with 50 µL from a stock solution of 2*10 cells/mL on a 96-well microtiter plate. J774 cells were allowed to adhere for 15 min, and 50 µL of serial dilution of the tested oil were added. The viability of the macrophages was evaluated with the Alamar Blue® assay as previously described in our laboratory. Control cells were incubated in the presence of DMSO and without drugs. Dose–response curves were plotted, and the lethal concentration reduces 50% (LC_{50}) of the J774 cell viability were obtained. The selectivity index was determined as the ratio of LC_{50} for the macrophages to IC_{50} for the protozoa. The analyses were performed in triplicate.

2.7 Statistical Analysis

All tests were performed by means of three independent replicates, and the values were determined in duplicate where appropriate. These results expressed as mean values ± standard deviation (MV ± SD). A paired two-tailed *t*-test was used for analysis of the data interpretation. Values of $p < 0.05$ were considered significant.

3 Results and Discussion

3.1 Chemical Composition

In the present study, GC/MS analysis of the EORI (yield 1.10%) revealed the presence of 22 specialized metabolites, which represent 97.99% of the total composition (Table 1). Monoterpene hydrocarbons were the main type of compounds in EORI with 84.86% of which camphene (32.08%) was the main component, followed by myrcene (13.86%), α-pinene (11.29%), sabinene (5.85%), and β-pinene (5.73%). Additionally, D-limonene, spathulenol, p-cymene, γ-eudesmol, α-terpineol, t-cadinol, α-phellandrene, and bornyl acetate have been reported at significant concentrations (1%–4.90%).

To the best of our knowledge, no studies have been published concerning the chemical composition of EORI. However, there are some reports on the chemical composition of EO from other *Rhanterium* species that can be used for comparison. Our findings differ from those reported by Mabrouka et al. and Chemsa et al., but both analyzed the EO of the Tunisian *R. suaveolens* and Algerian *R. suaveolens*, respectively. The main differences are the presence of carvacrol (12.1%), α-terpinolene (6.3%), and pinocarvone (5.6%) in Tunisian *R. suaveolens* EO, whereas in the Algerian *R. suaveolens* EO were found perillaldehyde (45.79%), caryophyllene oxide (24.82%), and β-caryophyllene (5.17%) [22, 23]. On the other hand, Salah et al. did not find different compounds in their analysis of the *R. suaveolens* flowers oil [26]. Kala et al. reported the main compounds of *R. adpressum* EO, and the main difference is the presence of myristicin (5.05%) [38]. However, Hamia et al. did not report different compounds in their study of *R. adpressum* EO [21]. Considering that we study and report herein the chemical composition of a different species, it was expected that several differences could be observed. Nonetheless, the differences in the main constituents may be attributed to several factors, including environmental factors, such as climatic and geographic conditions, and also physiological factors, such as the age of the plant and their development stage. Additionally, genetic factors influence in the chemical variability of essential oils [39, 40].

3.2 Antimicrobial Activity

The antimicrobial activity of EORI was assessed by the diameter of the inhibitory zone. The results demonstrated that when in contact with the different concentrations of EORI, the strains of bacteria and yeast showed variable sensitivity, as confirmed by the presence of inhibition zones. The data clearly indicate that the tested essential oil exhibited a significant dose-dependent antibacterial activity against most bacterial strains treated. As shown in Fig. 1, the EORI at high concentration exerted strong zone of inhibition about 30 mm ± 0.00 against *Y. enterocolitica*, but mild zone of inhibition against *S. aureus* (19 mm ± 1.00), *E. coli* (17.66 mm ± 0.58), and *K. pneumoniae* (14.66 mm ± 0.58). However, *P. aeruginosa* showed some resistance to the EORI, with inhibition zone diameters varying from 7.33 to 13.33 mm at all concentrations tested. The two yeast strains examined showed sensitivity to the EORI, with the inhibition zone varying

Table 1 Chemical profile of essential oil obtained from leaves of *R. intermedium*

No.	Compounds[a]	RI[b]	RI[c]	Relative percentage
1	α-thujene	943	924	0.30
2	α-pinene	948	932	11.29
3	Camphene	959	946	32.08
4	Sabinene	978	969	5.85
5	β-pinene	980	974	5.73
6	Myrcene	992	988	13.86
7	α-phellandrene	1003	1002	1.31
8	α-terpinene	1015	1014	0.42
9	p-cymene	1026	1020	3.54
10	D-limonene	1026	1024	4.90
11	γ-terpinene	1056	1054	0.81
12	Linalool	1099	1095	0.81
13	α-terpineol	1184	1186	2.70
14	Bornyl acetate	1283	1287	1.26
15	Germacrene D	1476	1484	0.88
16	Bicyclogermacrene	1492	1500	0.92
17	δ-cadinene	1521	1522	0.19
18	Spathulenol	1573	1577	4.82
19	t-cadinol	1635	1638	1.78
20	γ-eudesmol	1643	1630	3.21
21	α-eudesmol	1646	1652	0.76
22	α-cadinol	1648	1652	0.57
	Total identification %			97.99
	Yield % (w/w)			1.10
	Total monoterpenes%			84.86
	Monoterpene hydrocarbons %			80.09
	Oxygenated monoterpenes %			4.77
	Total sesquiterpenes %			13.13
	Sesquiterpene hydrocarbons %			1.99
	Oxygenated sesquiterpenes %			11.14

[a]Compounds are listed in order of their elution from a Rxi-5MS capillary column (30 m × 0.25 mm, 0.25-μm film thickness)
[b]RI: Retention indices relative to n-alkanes (C7-C20) on a Rxi-5MS capillary column
[c]RI: Literature retention index [37]

between 14 and 30 mm, depending on the different concentrations. Moreover, EORI was more potent against the yeast strains than the bacteria strains, especially at higher concentrations. The tested essential oil produced a good inhibition zone of about 30 mm ± 0.00 against *C. albicans* 10 and about 22.16 mm ± 0.76 against *C. albicans* 26 at pure dose.

The antimicrobial potential of EORI was also examined by using MIC assay that is presented in Table 2. The MIC ranged from 14.78 to 118.26 mg/mL tested in all the bacterial strains, while the MBC values ranged from 14.78 to 236.51 mg/mL. The EORI had promising antibacterial potential with a MIC value of less than 14.78 mg/mL against *E. coli*. A moderate antibacterial effect with a MIC of 59.13 mg/mL was showed by EORI against *Y. enterocolitica*, *P. aeruginosa*, and *K. pneumoniae*, with the exception of *S. aureus*, for which a higher concentration of the EORI was needed to inhibit the growth of this strain with a MIC value of 118.26 mg/mL. With respect to the yeasts tested, the obtained results indicated that the EORI had better antifungal effect against the two *C. albicans* strains employed where the MIC value was 14.78 mg/mL.

Antibiotic resistance is increasing in worldwide. Various clinical data displayed that *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Y. enterocolitica*, and *C. albicans* have developed a variety of resistance processes against many antibacterial and antifungal drug classes. This increasing

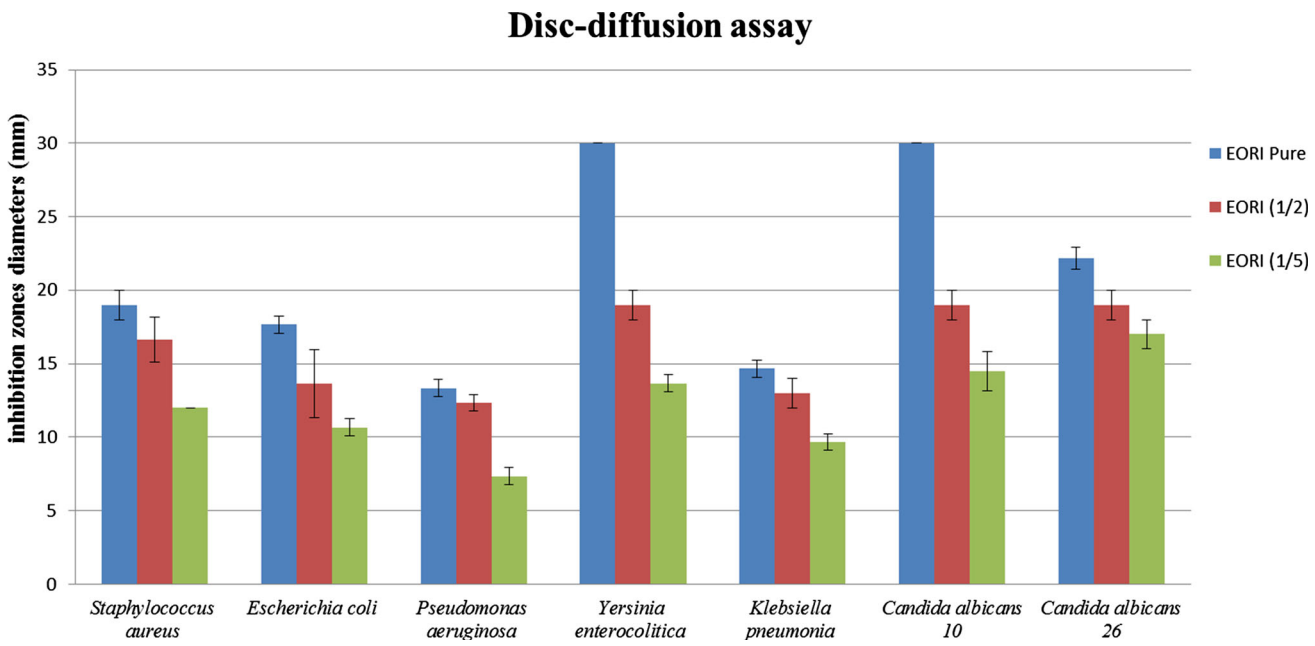

Fig. 1 Effect of essential oil of *R. intermedium* against test microorganisms using disk diffusion assay

Table 2 Inhibition zone diameter (IZD mm ± SD), minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC), and minimal fungicidal concentration (MFC) showing antimicrobial activity of EORI against human pathogenic microbial

Microbial strains	EORI					
	Inhibition zone (mm)			MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)
	Pure	1/2	1/5			
Gram-positive bacteria						
Staphylococcus aureus	19 ± 1.00	16.66 ± 1.53	12 ± 0.00	118.25	236.5	ND
Gram-negative bacteria						
Escherichia coli	17.66 ± 0.58	13.66 ± 2.31	10.66 ± 0.58	< 14.78	14.78	ND
Pseudomonas aeruginosa	13.33 ± 0.58	12.33 ± 0.58	7.33 ± 0.58	59.13	118.25	ND
Yersinia enterocolitica	30 ± 0.00	19 ± 1.00	13.66 ± 0.58	59.13	118.25	ND
Klebsiella pneumoniae	14.66 ± 0.58	13 ± 1.00	9.66 ± 0.58	59.13	236.5	ND
Yeasts						
Candida albicans 10	30 ± 0.00	19 ± 1.00	14.5 ± 1.32	14.78	ND	29.56
Candida albicans 26	22.16 ± 0.76	19 ± 1.00	17 ± 1.00	14.78	ND	29.56

ND, Not determined and *SD,* Standard deviation

resistance has created a need to develop new antimicrobial agents [41, 42]. Also, the study on the development of natural antimicrobial drugs has attracted a great deal of consideration [43]. In the current work, EORI possessed highest antibacterial activity against *E. coli* and modest efficacy against *Y. enterocolitica* and *P. aeruginosa*. In contrast, the lowest antibacterial potential was obtained for *K. pneumoniae* and *S. aureus*. In other words, EORI has significant antimicrobial effect against Gram-negative and fungal strains, while a low antimicrobial potential was detected against Gram-positive bacteria. According to these findings, the modes of the action of EO and their different selectivity against some bacteria are still poorly understood [44, 45]. It appears that their mechanisms of action are fundamentally associated with the structure of the cell wall and the membrane

penetrability of bacteria (Gram-positive and Gram-negative) [46]. Generally, the antibacterial action of the natural products is conditioned to their capacity to alter cell membrane structures, disrupt their permeability barrier, and, thus, cause a loss of control in chemiosmotic [22]. Moreover, various reports proposed that the antimicrobial potential exerted by EOs may be explained by the demolition of certain enzymatic systems, including those which engage in the production of structural constituents and cellular energy [47, 48].

To the best of our knowledge, no reports have been published on the antimicrobial properties of EORI. Nevertheless, there are few studies on the antimicrobial potential of essential oil from *Rhanterium* species for comparison. Salah et al. demonstrated an antimicrobial potential with MICs values around 11 and 230 µg/mL, using *R. suaveolens* EO against *S. aureus* and *C. albicans*, oppositely to our results [26]. In another work, Mabrouka et al. reported that the EO from *R. suaveolens* showed MICs values ranging from 37 to 150 µg/mL for *S. aureus*, *E. coli,* and *P. aeruginosa*, which differs from our data [22]. Djermane et al. showed different results to our findings; they obtained from EO of *R. adpressum* no antimicrobial activity against *P. aeruginosa* and *K. pneumoniae* [49]. Moreover, a different result was obtained from the EO of *R. epapposum* by Demirci et al. on *S. aureus* with a MIC = 1 mg/mL [27].

The differences between our findings and those previously reported by other authors may result from the complexity and variability of the active constituents in the tested essential oils. The noticeable antimicrobial potential of the EORI against some microbial strains could be related to the presence of a high quantity of bioactive molecules such as monoterpenes and oxygenated monoterpenes. Effectively, many researchers have proved the presence of a relationship between the chemical composition of the major compounds of the essential oils and the antimicrobial activity [50, 51]. The main constituents identified in the EORI, such as camphene, myrcene, α-pinene, and β-pinene, have not been tested for their antimicrobial potential in this work. However, some previous studies have shown their antimicrobial properties. In fact, a number of researchers have shown that α-pinene and β-pinene are well-known molecules with pronounced antimicrobial activity against several pathogenic bacteria such as *S. aureus*, *E. coli*, and *P. aeruginosa* and against yeast strains, especially *C. albicans* [52, 53]. Likewise, it was found that camphene and myrcene were endowed with remarkable antibacterial and antifungal properties [26, 54]. According to our findings, to those reported previously and considering the fact that EORI contained camphene (32.08%), myrcene (13.86%), α-pinene (11.29%), and β-pinene (5.73%). We suggested that the antimicrobial activity of EORI is mainly due to the presence of these main compounds. Indeed, the minor components provide more activity against microorganisms by synergetic effect with the major constituents [55]. It is worthy to note that EORI exhibited significant antimicrobial activities against some multidrug-resistant strains but further studies are required to elucidate the importance of these notable antimicrobial capabilities.

3.3 Antiparasitic Activity

Table 3 presents the results obtained on the antiparasitic activity of EORI on epimastigotes forms of *T. cruzi*, trophozoïtes forms of *A. castellanii* Neff., and promastigotes forms of *Leishmania* species (*L. amazonensis* and *L. donovani*). These results show significant differences between the values of IC_{50} of various parasites ($p < 0.05$). The EORI gave clear effects against all evaluated parasites with varying degrees of inhibition (IC_{50} values 07.21–48.31 µg/mL). This EO was strongly active when tested against *T. cruzi*. There was reduction in the viability of trypomastigote cells with the increase in the essential oil concentration, with an IC_{50} value of 07.21 µg/mL. Referring to another parasite, the EORI showed inhibitory ability against trophozoïtes forms of *A. castellanii* Neff. with IC_{50} value of 37.11 µg/mL. While, the leishmanicidal potential of EORI was observed against *L. donovani* and *L. amazonensis* at IC_{50} values equals to 38.35 µg/mL and 48.31 µg/mL, respectively.

This is the first work that will highlight the *in vitro* antiparasitic effects of EORI. It is essential to mention that the investigation of the *in vitro* trypanocidal, anti-*Acanthamoeba,* and leishmanicidal activities of EOs has been recently described [56, 57]. To the best of our knowledge, no previous studies have been documented on the antiparasitic properties of EOs from *Rhanterium* species. It is interesting to comment that *Rhanterium*, *Plectranthus*, *Pterocaulon*, and *Plucheae* are closely related genera, belonging to the same tribe *Inuleae* [58]. About anti-*Acanthamoeba* activity, our results were comparable to those of Hikal and Said-Al Ahl, essential oil from *Plectranthus amboinicus* had lethal concentration 50% on *Acanthamoeba* spp., at the concentration of 55.87 µg/mL [59]. In a study conducted by Sauter et al., the amebicidal potential of a *Pterocaulon polystachyum* essential oil against the *A. polyphaga* was demonstrated, with 100% mortality at the doses of 10 and 20 mg/mL [60]. On the other hand, García et al. reported that at the concentration (24.7 µg/mL) of the essential oil from *Pluchea carolinensis* achieved 50% of growth inhibition for *L. amazonensis* [61].

Nevertheless, the IC_{50} values of EORI were still superior to the positive controls benznidazole (0.83 µg/mL), chlorhexidine (0.92 µg/mL), and miltefosine (3.32 µg/mL and 6.48 µg/mL) used against *T. cruzi*, *A. castellanii*, and *Leishmania* species (*L. donovani* and *L. amazonensis*), respectively. These positive controls are pure compounds and constitute the most active antiparasitic drugs. In addition, all positive controls showed better activity *in vitro* than the EORI evaluated, which is a complex substance. Purification

Table 3 Antiparasitic and cytotoxic activities of essential oil of *R. intermedium*

Samples	IC_{50}[a]				LC_{50}[b] murine macrophage	SI[c]			
	Ta	Ac	Ld	La		Ta	Ac	Ld	La
EORI	07.21 ± 0.09	37.11 ± 4.11	38.35 ± 0.08	48.31 ± 2.51	47.91 ± 3.61	6.64	1.29	1.25	0.99
Benznidazole[d]	0.83 ± 0.05	–	–	–	104.1 ± 1.04	125.42	–	–	–
Chlorhexidine[d]	–	0.92 ± 0.10	–	–	78.23 ± 3.46	–	85.03	–	–
Miltefosine[d]	–	–	3.32 ± 0.27	6.48 ± 0.24	72.19 ± 3.06	–	–	21.74	11.14

Tc, *T. cruzi* epimastigotes; Ac, *A. castellanii* trophozoïtes; Ld, *L. donovani* promastigotes; and La, *L. amazonensis* promastigotes
[a]IC_{50}: Inhibitory concentration inhibits 50% of the growth of the parasite (μg/mL)
[b]LC_{50}: Lethal concentration reduces 50% of the J774 cell viability (μg/mL)
[c]SI: Selectivity index is a ratio that measures the window between cytotoxicity and antiparasitic activities by dividing the given LC_{50} value into the IC_{50} value (LC_{50}/IC_{50})
[d]Reference positive control for each parasite

of potential constituents could result in a grand increase in their antiparasitic activity [62].

The antiparasitic property of the EORI could be attributed to the action of specific molecules within the essential oil or the synergistic action between them [60]. The mode of action by which EOs reduce parasite growth is still poorly known, but the previous reports have proposed that parasite cellular changes are caused by drugs that inhibit ergosterol synthesis, or interact with ergosterol at the plasma membrane level [63, 64]. Other work indicated that the activity of EO on parasites is principally due to terpene compounds. Terpenes are responsible for the hydrophobic property of EOs, thus permitting their passage through the plasma membrane, affecting intracellular reaction pathways of the parasite [65]. Further, detailed studies are recommended to understand the possible mechanisms of the action of EORI as antiparasitic agent.

3.4 Cytotoxicity

Cytotoxicity of EORI was investigated in macrophage (J774) cells lines. The J774 macrophages were used in this work, as in several other studies [66, 67], because they are simple to culture, permissive host cell to *Leishmania* infection, and widely employed in predicting drug cytotoxicity as a physiologically closer model. Furthermore, J774.A1 cells are differentiated, which reduce costs and assay time, unlike other model cells frequently used as *Leishmania* hosts [68]. The *in vitro* assay demonstrated no significant cytotoxicity in the cells. Table 3 illustrates that EORI has a modest cytotoxic effect on the murine macrophage, since its LC_{50} of the J774 cell viability was 47.91 μg/mL. It is possible to notice that the EORI showed high selectivity for *T. cruzi* (SI: 6.64) and an average selectivity for *A. castellanii* (SI: 1.29) and *L. donovani* (SI: 1.25). However, it is possible to note that for *L. amazonensis* strains, selectivity was less than 1, as presented in Table 3.

The application of natural products in alternative therapies can be limited if their chemical composition is toxic to the organism. Therefore, analyzing the cytotoxicity of these products is essential to confirm their efficacity and safety, mainly when used as medicaments. In this context, it is possible to identify new products with great selectivity. Thus, the antiparasitic activity is more effective against parasites than against cells of host organisms [13]. In our study, results obtained in the selectivity index of EORI showed good selectivity for the epimastigotes forms of *T. cruzi*. This better selectivity index value of EORI may lead to the development of novel and more effective trypanocidal drugs.

4 Conclusion

The present work's findings could be considered the first evidence of the chemical composition, antimicrobial, antiparasitic, and cytotoxic properties of EORI. Camphene, myrcene, α-pinene, sabinene, and β-pinene are found to be the major compounds of EO of *R. intermedium* growing in Algeria. Moreover, the EORI exhibits a potent antibacterial effect against *E. coli*. Furthermore, a significant antifungal activity was recorded against the two tested *C. albicans* strains. Likewise, the oil sample showed the highest antiparasitic potential against epimastigotes forms of *T. cruzi*, while it has low cytotoxic effect on murine macrophages J774. In light of this, our results marked the medicinal potential of *Rhanterium* species and suggested that essential oil from *R. intermedium* Pomel leaves may be used in developing novel therapies to substitute antibiotics, thereby helping to limit the complex problems of conventional drugs. To fully exploit the biological potential of EORI as an alternative to conventional treatments and medications, more investigations could be conducted for other bioactive applications such as antioxidant, anti-inflammatory, antidiabetic, and anticancer activities.

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Declarations

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

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