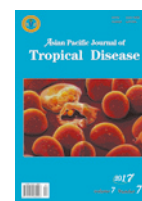


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journal homepage: <http://www.apjtdm.com>Original article <https://doi.org/10.12980/apjtd.7.2017D6-462> ©2017 by the Asian Pacific Journal of Tropical Disease. All rights reserved.Phenolic extracts of *Centaurium erythraea* with novel antiradical, antibacterial and antileishmanial activitiesAbdelhakim Bouyahya^{1,2*}, Youssef Bakri¹, Omar Belmehdi², Abdeslam Et-Touys^{1,3}, Jamal Abrini², Nadia Dakka¹¹Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, Genomic Center of Human Pathologies, Mohammed V University, Rabat, Morocco²Biology and Health Laboratory, Department of Biology, Faculty of Science, Abdelmalek Essaadi University, Tetouan, Morocco³National Reference Laboratory of Leishmaniasis, National Institute of Health, Rabat, Morocco

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ABSTRACT

Objective: To evaluate the antiradical, antibacterial and antileishmanial properties of phenolic extracts of *Centaurium erythraea* (methanol: MECE, ethanol: EECE, *n*-hexane: HECE, ethyl acetate: EACE) from north-west of Morocco.**Methods:** The antiradical activity was evaluated using DPPH scavenging assay. The antibacterial activity was tested against four reference strains (*Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*) using the well diffusion method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by microdilution assay. The antiparasitic activity was evaluated against *Leishmania major*, *Leishmania tropica* and *Leishmania infantum* using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. The levels of polyphenol and flavonoid extracts were estimated by colorimetric assay.**Results:** The HECE extract has shown a significant ability to trap the radical DPPH [$IC_{50} = (49.54 \pm 2.43) \mu\text{g/mL}$] compared to EECE, MECE and EACE. This value is significantly important compared than those of ascorbic acid [$IC_{50} = (27.20 \pm 0.17) \mu\text{g/mL}$] and Trolox [$IC_{50} = (43.72 \pm 0.31) \mu\text{g/mL}$] used as standards. The inhibitory activity of methanol, *n*-hexane and ethyl acetate extracts was especially remarkable (inhibition zone ≥ 14 mm) against all strains tested. The values of MIC and MBC have ranged from 0.25 to 8.00 mg/mL. *Pseudomonas aeruginosa* was the most resistant strains compared to all other tested bacteria, while *Staphylococcus aureus* was the most sensitive toward the tested extracts. The MTT based colorimetric assay has shown a reduced promastigotes viability on all strains tested. The best growth inhibition was observed with HECE against *Leishmania tropica* [$IC_{50} = (37.20 \pm 1.62) \mu\text{g/mL}$] and *Leishmania major* [$IC_{50} = (64.52 \pm 2.20) \mu\text{g/mL}$] compared to N-methyl Glucamine Antimoniate (Glucantime®) ($IC_{50} > 500 \mu\text{g/mL}$) used as control, after 72 h of treatment.**Conclusions:** Considering these results, *Centaurium erythraea* can be used as a source of novel antioxidant, antibacterial and antileishmanial compounds.

1. Introduction

The excessive and inappropriate use of antibiotics in human's medication to treat infectious diseases is responsible for the emergence of resistant organisms[1,2]. Indeed, several diseases have been reported as a result of bacterial infections in humans, viz. diarrhea and vomiting caused by *Bacillus cereus*[3], listeriosis

caused by *Listeria monocytogenes* (*L. monocytogenes*)[4], and oral-genital infections caused by *Candida albicans*[5]. In addition, some parasites such as *Leishmania* species [*Leishmania major* (*L. major*), *Leishmania infantum* (*L. infantum*) and *Leishmania tropica* (*L. tropica*)] cause leishmaniasis disease. The treatment of this disease has been based on synthetic antileishmanial drugs. The issue is that this treatment is expensive and causes many side effects such as toxicity[6]. In addition, leishmanial species have developed the resistance against these molecules. This situation highlights the need to seek for new molecules that could exert antileishmanial effects[6]. Furthermore, the reactive oxygen species (ROS) are a group of highly reactive molecules including the free radicals such as superoxide ion ($O_2^{\cdot-}$) and hydroxyl radical (OH) as well as the no free radicals such as the hydrogen peroxide

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(H₂O₂). In homeostasis situation, the human body produces ROS via normal aerobic respiration and during inflammatory process. This production can be affected by some aggressions as radiations, stress, pollution, alcoholism and nicotism[7]. The cells have many enzymatic systems to protect against ROS including superoxide dismutase, catalase and the selenium glutathion peroxidase. Some chemical molecules can also protect against ROS by their capacity to donate electron or hydrogen for them[8]. The oxidative modifications in the intracellular and/or cellular membrane have been defined as the result of the imbalance between ROS production and the defense mechanisms. Such alterations can lead to a high number of diseases including diabetes, cancers and cardiovascular diseases[9]. Furthermore, an increase in ROS can also cause lipids peroxidation in food during the manufacturing and storage process.

The most important sources of antioxidant, antibacterial and antiparasitic molecules are the natural bioactive products of plants such as flavonoids and phenolic compounds that have known for their several biological activities such as an antibacterial effect[10-12], suppress the production of free radicals[11,13-17], delay oxidative stress reactions and have an important role against one of the world major human diseases like cancer[18]. In the search for such natural products and modern drugs, medicinal plants play an important role[19-24].

In Morocco, the Gentianaceae family represents a great ecological and economic interest, as it includes many medicinal and aromatic species[25]. *Centaurium erythraea* (Rafin) (*C. erythraea*), known in Morocco under its vernacular name "Gosset El Haya", has been used in traditional medicine as related by some ethnopharmacological surveys for the treatment of digestive disorders, kidney diseases, as well as an antipyretic and anti-diabetic[26,27]. The extracts of *C. erythraea* possess various biological activities such as anti-hyperglycemic[28], diuretic[29], antibacterial[30], antioxidant[31] and hepatoprotective activities[32].

Many compounds of *C. erythraea* extracts have been reported to exhibit important biological activities such as secoiridoid glycoside and gentiopicoside, which have been assessed for antibacterial and free radical scavenging activities[4], and phenolic compounds isolated from the flowers of *C. erythraea* for antioxidant activity[31]. The aim of this present study was to determine the total flavonoids and phenolic contents of various extracts of *C. erythraea* and to evaluate their antibacterial, antileishmanial and antioxidant properties.

2. Materials and methods

2.1. Collection of plant material and preparation of organic extracts

C. erythraea was collected at the flowering phenological stage (May, 2015) from the north-west of Morocco (Ouezzane Province: Zoumi area). The collected parts were dried in dark at room temperature and then they were ground to obtain the powder. The obtained powder was extracted by maceration using ethyl acetate, *n*-hexane, ethanol and methanol. After 72 h of maceration, the plant extracts were filtered and then the solvents were eliminated using a rotary evaporator.

2.2. Total phenolic contents (TPC)

TPC of plant extracts was estimated by the Folin-Ciocalteu assay. The extract was diluted to the concentration of 1 mg/mL, and aliquots of 100 µL or a standard solution of gallic acid (20, 40,

60, 80 and 100 mg/L) were mixed with 500 µL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 400 µL of Na₂CO₃ (7%). After 40 min of incubation at room temperature, the absorbance was measured at 760 nm and compared to a blank sample[11]. The TPCs content expressed as gallic acid equivalent (GAE) was calculated based on the calibration curve using the following linear equation[33]:

$$y = 0.01 \times [\text{GA}] + 0.002; R^2 = 0.998$$

where *y* is absorbance, [GA] is concentration (mg GAE g⁻¹ extract) and *R*² is correlation coefficient.

2.3. Total flavonoid contents (TFC)

The TFC of plant extracts was measured by the aluminum chloride (AlCl₃) colorimetric assay[11]. 1 mL of the extract (1 mg/mL in methanol) or a standard solution of quercetin (20, 40, 60, 80 and 100 mg/mL) was mixed with 1 mL of 2% AlCl₃ in methanol. After 40 min at room temperature (23 ± 2 °C), the absorbance against blank was measured at 430 nm. Total flavonoids content expressed as quercetin equivalent (QE) was calculated based on the calibration curve using the following equation[33]:

$$y = 0.022 \times [\text{Q}] + 0.006; R^2 = 0.999$$

where *y* is absorbance; [Q] is concentration (mg QE) and *R*² is correlation coefficient.

2.4. Antiradical potential of different extracts

2.4.1. DPPH free radical-scavenging activity

Antiradical activity of *C. erythraea* extracts was evaluated using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging activity assay. Briefly, aliquots (0.2 mL) of various concentrations (62.5–1000 µg/mL) of the plant extracts samples were added to 1.8 mL of a 0.004% methanolic solution of DPPH. After 30 min of incubation at room temperature, the absorbance was measured against a blank at 517 nm[11]. The percentage (%) to scavenge DPPH radical was calculated by the following formula[34]:

$$\text{DPPH scavenging activity (AA in \%)} = \frac{(\text{Ac} - \text{At})}{\text{Ac}} \times 100$$

where Ac is the absorbance of the control (without extract), and At is the absorbance of the test (with extract).

Trolox and ascorbic acid were used as positive controls. The concentration of extract that provided 50% of DPPH inhibition (IC₅₀) was calculated from the graph plotted of inhibition capacity (AA in %) against extracts and standards concentrations using linear regression equations[11].

2.5. Antibacterial activity

2.5.1. Bacteria strains

The antibacterial activity of *C. erythraea* extracts was tested against *Escherichia coli* K12 (*E. coli*) (Laboratory of Food Microbiology, UCL, Belgium: MBLA), *Pseudomonas aeruginosa* (*P. aeruginosa*) (Institute of hygiene, Rabat, Morocco: IH), *Staphylococcus aureus* CECT 976 (*S. aureus*), and *L. monocytogenes* serovar 4b CECT 4032 (Spanish Type Culture Collection: CECT). Strains were maintained on an inclined agar medium at 4 °C. Before use, the bacteria were revived by two subcultures in an appropriate culture medium: Luria-Bertoni (LB) broth at 37 °C for 18–24 h. For the test, final inoculum concentrations were about 10⁶ CFU/mL[11].

2.5.2. Agar-well diffusion assay

For the determination the diameter of inhibition of extracts against

the tested bacteria, we have used agar-well diffusion assay as described in our previous studies[10-12]. A basal layer was prepared by Muller-Hinton agar. After the agar plates were solidified, sterile 8 mm diameter cylinders were deposited. Six mL of LB medium in superfusion containing 0.8% agar were inoculated by a fresh culture of indicator bacterial strain (the final concentration was 10^6 CFU/mL). After solidification, the wells were filled with 50 μ L of diluted extracts at 25 mg/mL. After incubation at appropriate temperature for 24 h, all plates were examined for any zone of growth inhibition, and the diameter of these zones was measured in millimeters. All the tests were performed in triplicate.

2.5.3. Minimum inhibitory concentration (MIC)

MICs were determined using the broth micro-dilution assay as described in our previous studies[11,12]. LB medium containing 0.15% (w/v) of agar was used as a stabilizer of the extract-water mixture and the resazurin was used as a bacterial growth indicator. 50 μ L of LB (0.15% agar w/v) was distributed from the 2nd to the 8th well of a 96-well polypropylene microtitre plate. A dilution of each extract was prepared in methanol, to reach a final concentration of 32 mg/mL; 100 μ L of these suspensions were added to the first test well of each microtitre line, and then 50 μ L of scalar dilution was transferred from the 2nd to the 7th well. The 8th well was considered as a growth control, because no extract was added. Then, we added 50 μ L of a bacterial suspension to each well at a final concentration of approximately 10^6 CFU/mL. The final concentration of the extract was between 16 and 0.25 mg/L. After incubation at 37 °C for 18 h, 10 μ L of resazurin was added to each well to assess bacterial growth. After further incubation at 37 °C for 2 h, the MIC was determined as the lowest concentration that prevented a change in resazurin color[11,12]. Bacterial growth was detected by reduction in blue dye resazurin to pink resorufin. A control was carried out to ensure that, at the tested concentrations, the extract did not cause a color change in the resazurin. Experiments were performed in triplicate.

2.5.4. Minimum bactericidal concentration (MBC)

MBC corresponds to the lowest concentration of the extract yielding negative subcultures after incubation at appropriate temperature for 24 h[11,12]. It was determined in broth dilution tests by sub-culturing 10 μ L from negative wells on PCA medium[11,12]. All the tests were performed in triplicate.

2.6. Antileishmanial activity

2.6.1. Culture of leishmania species

Leishmania species tested in this study were: *L. infantum* (MHOM/MA/1998/LVTA), *L. tropica* (MHOM/MA/2010/LCTIOK-4) and *L. major* (MHOM/MA/2009/LCER19-09). These three species were isolated and identified from Moroccan infected patients at the National Reference Laboratory of Leishmaniasis, National Institute of Health, Rabat-Morocco. The species were cultivated as described by Et-Touys *et al.*[35]. Briefly, parasite cultures of each *Leishmania* species were washed with phosphate buffered saline (PBS) and centrifuged at 1500 r/min for 10 min. Cells were then re-suspended in RPMI 1640 (GIBCO) supplemented with 10% of heat-inactivated fetal calf serum and 1% of penicillin-streptomycin mixture. Cultures were maintained at 23 °C.

2.6.2. Antileishmanial activity

Before evaluating the antileishmanial activity, the cellular density of each species was calculated using light microscopy. When cellular density reached a threshold concentration of 10^6 cells/mL,

L. infantum, *L. tropica* and *L. major* promastigotes were washed twice with PBS and centrifuged at 2500 r/min for 10 min. To evaluate the anti-promastigote activity, 100 μ L of parasites culture was resuspended in a 96-well tissue culture plate, in fresh culture medium according to Et-Touys *et al.*[35]. Briefly, parasites were incubated at 2.5×10^6 cells/well for 72 h at 23 °C in the presence of various concentrations of extract (μ g/mL) dissolved in 1% DMSO. DMSO was used at a final concentration never exceeding 1% which is not toxic to parasites[36,37]. Sterile PBS and 1% DMSO (vehicle) were used as negative controls. Glucantime® was used as positive control.

2.6.3. Cell viability assay

The viability of *Leishmania* species was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay as described by Essid *et al.*[37]. Briefly, 10 μ L of MTT (10 mg/mL) was added to each micro-well and incubated for 3 h at 30 °C. The reaction was stopped by addition of 100 μ L of 50% (v/v) isopropanol-10% (w/v) sodium dodecyl sulfate (SDS) mixture to each well in order to dissolve insoluble formazan formed after tetrazolium dye reduction. After 30 min of incubation at room temperature, absorbance was measured at 560 nm using an ELISA plate reader (Synergy HT, Bio-TEK). All assays were conducted in triplicate and compared to negative control (parasites) and reference drug (Glucantime). Cell viability was also evaluated by determination of the extracts concentration which inhibited half of the cell population (IC_{50}), obtained by modeling by plotting percentage of inhibition versus concentration of extract using Original Program. Inhibition percentage (I) was calculated using the following formula[35]:

$$I (\%) = 100 \times (\text{Absorbance untreated cells} - \text{Absorbance treated cells}) / \text{Absorbance untreated cells}.$$

2.7. Data analysis

The statistical analysis was performed by a one-way ANOVA analysis of variance. We considered that the difference is significant for $P \leq 0.05$. Pearson correlation tests were used to assess the relationship between phenolic contents and the IC_{50} values of the antioxidant capacity and antileishmanial activity. These tests were performed by using XLSTAT 2012 program. All experiences were carried out for three replicates and the results were expressed as mean \pm SD.

3. Results

3.1. TPCs

The *C. erythraea* organic extracts showed a good yield and their values have varied according to the used solvent. These values have ranged from (23.21 ± 0.34) to (105.54 ± 0.84) GAE mg/g extract (Table 1). The TPC of methanol extract of *C. erythraea* (MECE) and *n*-hexane extract of *C. erythraea* (HECE) were the highest compared with the TPC of ethyl acetate extract of *C. erythraea* (EACE) and ethanol extract of *C. erythraea* (EECE) ($P < 0.05$).

3.2. TFCs

The TFC was estimated by a colorimetric method using quercetin as a standard flavonoid. The concentration of TFC in the extracts was depended to the solvent of extraction. The values of TFC have ranged from (15.21 ± 0.43) to (38.43 ± 1.30) QE mg/g extract (Table 1). The

HECE showed higher ($P < 0.05$) flavonoids content compared to the other extracts except for the MECE, which contained similar levels ($P > 0.05$). On the other hand, EACE showed the lowest value of TFC ($P < 0.05$).

3.3. Antiradical activity

Figure 1 shows the DPPH radical scavenging ability of *C. erythraea* extracts. As summarized, we noted a highly significant decrease in the DPPH radical concentration ($P < 0.05$) due to the scavenging activity of each extract concentration and standards. At 30.125 $\mu\text{g/mL}$, the activity of the MECE, EECE, HECE and EACE has ranged from $(43.82 \pm 4.10\%)$ to $(11.22 \pm 2.34)\%$. The activity of the various extracts was significantly lower than that of the ascorbic acid and Trolox ($P < 0.01$). In all cases, it has been found that the ability to scavenge DPPH radical increases significantly with increasing extract concentration ($P < 0.05$). The antiradical capacity of HECE was superior to all tested samples with an IC_{50} value of (49.54 ± 2.43) $\mu\text{g/mL}$, which was near to the inhibition capacity of the positive controls Trolox [$\text{IC}_{50} = (43.72 \pm 0.31)$ $\mu\text{g/mL}$] and ascorbic acid [$\text{IC}_{50} = (27.20 \pm 0.17)$ $\mu\text{g/mL}$], followed by MECE [$\text{IC}_{50} = (58.34 \pm 2.86)$ $\mu\text{g/mL}$], EECE [$\text{IC}_{50} = (382.25 \pm 5.59)$ $\mu\text{g/mL}$] and EACE [$\text{IC}_{50} = (376.08 \pm 3.18)$ $\mu\text{g/mL}$] (Table 1).

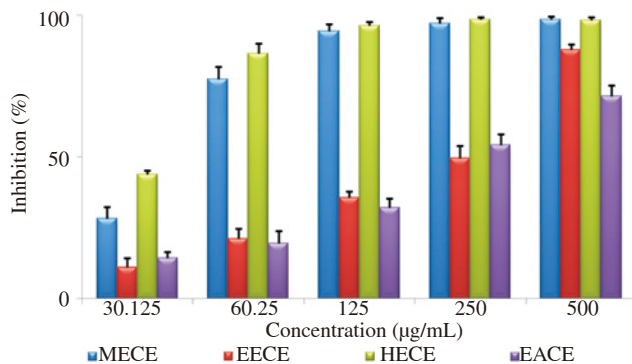


Figure 1. DPPH radical scavenging activities (%) of *C. erythraea* organic extracts (MECE, EECE, HECE and EACE).

Values are means \pm SD of three determinations. In this case, effect is increased in a concentration dependent manner; each concentration of extract induces an effect significantly different than other concentration.

Table 1

Percentage yield, TPC, TFC and antioxidant capacity ($\text{IC}_{50} \pm \text{SD}$) of *C. erythraea* extracts and antioxidant standards.

Extract	% Yield	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	Antiradical capacity IC_{50} ($\mu\text{g/mL}$)
EECE	27.47	69.42 ± 0.55	29.42 ± 1.05	382.25 ± 5.59
MECE	32.15	105.54 ± 0.84	34.27 ± 1.17	58.34 ± 2.86
EACE	26.32	23.21 ± 0.34	15.21 ± 0.43	376.08 ± 3.18
HECE	23.45	95.61 ± 0.64	38.43 ± 1.30	49.54 ± 2.43
Trolox	–	–	–	43.72 ± 0.31
Ascorbic acid	–	–	–	27.20 ± 0.17

Table 2

Antibacterial activities of *C. erythraea* extracts (MECE, EECE, HECE and EACE) and antibiotics (erythromycin and chloramphenicol) against *E. coli*, *S. aureus*, *P. aeruginosa* and *L. monocytogenes*.

Microorganisms	EECE	MECE	EACE	HECE	Erythromycin	Chloramphenicol
<i>E. coli</i>	19.00 ± 2.34	27.00 ± 1.23	29.00 ± 1.86	32.00 ± 2.42	21.00 ± 2.66	33.00 ± 0.50
<i>S. aureus</i>	24.00 ± 0.50	15.00 ± 2.30	14.00 ± 0.11	24.00 ± 1.60	26.00 ± 3.00	29.00 ± 1.75
<i>P. aeruginosa</i>	19.00 ± 0.20	NA	18.00 ± 0.10	17.00 ± 1.00	NA	21.00 ± 1.50
<i>L. monocytogenes</i>	36.00 ± 1.70	34.00 ± 1.10	43.00 ± 1.50	34.00 ± 2.30	24.00 ± 1.66	27.00 ± 2.33

Final bacterial density was around 10^6 UFC/mL. Inhibition zone diameters (mm) produced around the wells by adding 50 μL of diluted extract (25 mg/mL). All values are means of three assays. NA: Not active.

3.4. Antibacterial activity

The qualitative antibacterial effects of *C. erythraea* extracts are listed in Table 2. We have noticed that except of *P. aeruginosa* which has been resisted to the ethanol extract, all other bacterial strains were sensitive to *C. erythraea* extracts. However, the inhibition values have varied between bacterial species and from an extract to another ($P < 0.05$). With the exception of their effects against *E. coli* K12, any significant difference ($P < 0.05$) has been noted between MECE and HECE. The MIC and MBC values of *C. erythraea* extracts against tested bacteria are shown in Table 3. The MIC and MBC values have ranged from 0.25 to 4 and 0.5 to 8 mg/mL, respectively. The HECE has shown the highest antibacterial activity against all bacteria with the MIC and MBC values ranged from 0.25 to 2 and 0.5 to 8 mg/mL, respectively, but the MECE, EECE and EACE have not shown any antibacterial activity. The MIC value was equal to the MBC value of extracts against strains in some cases which indicated a bactericidal action.

Table 3

MIC (mg/mL) and MBC (mg/mL) of different extracts against the bacteria.

Extracts	<i>E. coli</i> K12		<i>S. aureus</i>		<i>L. monocytogenes</i>		<i>P. aeruginosa</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
MECE	> 8	> 8	0.25	1	4	4	2	8
HECE	8	> 8	0.5	1	2	4	0.5	2
EACE	> 8	> 8	0.5	0.5	4	4	1	> 8

Final bacterial density was around 10^6 UFC/mL.

3.5. Antileishmanial activity

The results of antileishmanial activity of *C. erythraea* extracts against *L. major*, *L. infantum* and *L. tropica* are summarized in Table 4. All *C. erythraea* extracts have inhibited the growth of *Leishmania* species tested with significant variability with Glucantime (positive control). The highest antileishmanial activity was obtained with HECE which exhibited an IC_{50} value of (37.20 ± 1.62) and (64.52 ± 2.20) $\mu\text{g/mL}$ against *L. infantum* and *L. major*, respectively. The MECE has shown a moderate antileishmanial effect with an IC_{50} value of (124.82 ± 1.75) and (126.16 ± 3.29) $\mu\text{g/mL}$ against *L. infantum* and *L. major*, respectively. Also, the IC_{50} values of EACE were (143.20 ± 3.82) and (128.30 ± 2.35) $\mu\text{g/mL}$ against *L. infantum* and *L. major*, respectively.

Table 4

Antileishmanial ($\text{IC}_{50} \pm \text{SD}$) of *C. erythraea* extracts (MECE, EECE, HECE and EACE) and positive control (Glucantime).

Extracts	<i>L. infantum</i>	<i>L. major</i>	<i>L. tropica</i>
MECE	124.82 ± 1.75	126.16 ± 3.29	247.24 ± 2.59
HECE	37.20 ± 1.62	64.52 ± 2.20	37.20 ± 1.62
EACE	143.20 ± 3.82	128.30 ± 2.35	> 500
EECE	373.18 ± 2.23	> 500	> 500
Glucantime	> 500	> 500	> 500

3.6. Relationship between phenolic content and antileishmanial and antioxidant capacity

Table 5 shows the coefficient correlation between TPC and IC₅₀ of antioxidant and antileishmanial activities on one hand and TFC and IC₅₀ of antioxidant and antileishmanial activities on the other hand. The correlation coefficients between TFC and antileishmanial capacity (IC₅₀) were $R^2 = 0.935$, $R^2 = 0.905$ and $R^2 = 0.647$ for *L. major*, *L. infantum* and *L. tropica*, respectively. While, the coefficients between TPC and antileishmanial capacity were $R^2 = 0.018$, $R^2 = 0.020$ and $R^2 = 0.553$ for *L. major*, *L. infantum* and *L. tropica*, respectively. These correlations confirm that the IC₅₀ decreases with increasing TFC, which gave a good antileishmanial activity. For antioxidant capacity (IC₅₀) it was moderately correlated with TPC ($R^2 = 0.709$) and TFC ($R^2 = 0.635$).

Table 5

Correlation coefficient between phenolic content and antileishmanial and antioxidant capacity (IC₅₀).

Components	<i>L. major</i>	<i>L. infantum</i>	<i>L. tropica</i>	Antioxidant activity
TPC	$R^2 = 0.018$	$R^2 = 0.020$	$R^2 = 0.553$	$R^2 = 0.709$
TFC	$R^2 = 0.935$	$R^2 = 0.905$	$R^2 = 0.647$	$R^2 = 0.635$

4. Discussion

The *Leishmania* and bacterial species have developed several mechanisms which allowed them to resist against numerous antimicrobial agents. This situation has led to the emergence and the re-emergence of infectious diseases. On the other hand, several studies have proved the implication of oxidative stress caused by disequilibrium in electron transfer; in some complications such as neurodegenerative, cardiovascular and cancers diseases. Several approaches have been investigated to search now molecules that have antimicrobial and antioxidant effects. Medicinal plants constitute a key candidate for screened natural compounds that possess antibacterial[12,19], antioxidant[11,13] and antileishmanial activities[35,37].

Polyphenols are compounds bio-metabolized by medicinal plants in their different parts (leaves, root, flowering, fruit, etc.) as secondary metabolites. Several studies have showed that these compounds possess numerous pharmacological properties including antibacterial, antileishmanial and antioxidant effects[38]. These properties have been correlated with a group of phenolics compounds called flavonoids that have a benzo- γ -pyrone structure[39]. These molecule groups have showed other pharmacological activities such as a mitochondrial adhesion inhibitor, an antiulcer, an anti-arthritis, an antiangiogenic and an anticancer agent[40]. Furthermore, some studies have established the correlation link between the consumption of these molecules and prevention of some diseases such as oxidative stress related diseases, chronic inflammation, immune related diseases, diabetes and cancer[41].

In our study, we have estimated firstly the total phenolic and flavonoid contents by colorimetric methods using gallic acid and quercetin as standards of polyphenols and flavonoids, respectively. It must be noted that the used extracts have presented a good amount of polyphenols and flavonoids which have varied according

to the solvent polarity. According to Tawaha *et al.*, the amount of flavonoids and polyphenols is considered very high if it's higher than 20 mg GAE/g extract[42]. Based on this study, *C. erythraea* can be considered a very important source of phenolic compounds.

The antioxidant effects were determined by DPPH scavenging assay (a stable free radical that has mostly been used for screening the ability of compounds to scavenge free radicals). This method aimed at the reduction of DPPH radical by molecules which are capable to donate hydrogen[43,44]. We have chosen this method because of its advantages to measure the antioxidant activity at ambient temperature preventing therefore the probability of thermal degradation of the molecule tested[45,46]. Our results were in consensus with other studies demonstrating the antiradical activity of *C. erythraea*[47,48]. The antiradical activity of plant extracts due to their redox potentials[49], which play a key role in neutralizing free radicals, to donate hydrogen, quench singlet oxygen and act as metal chelators[50].

The test of the antibacterial activity has revealed that all *C. erythraea* extracts were active against bacterial strains using agar well diffusion assay. While, the MICs of some extracts were showed at the highest concentration. The difference observed could be explained by the difference of the chemical contents of different extracts. Indeed, the solvents used for extraction have a large difference in their polarity and a dielectric constant specific to charge separated between different chemical compositions. The antibacterial activity of these compounds has been attributed to their hydrophobic character of phenolic content[51].

In our study, we have found that Gram-positive bacteria presented by *S. aureus* and *L. monocytogenes* were more sensitive to *C. erythraea* extracts than Gram-negative ones presented by *E. coli* and *P. aeruginosa*. This finding was in consonance with other works that have indicated the sensitivity of Gram-positive bacteria to natural products[12,52]. On the other hand, Gram-negative bacteria resist to natural antibacterial agents. Indeed, these strains possess a hydrophilic outer membrane which allowed them the penetration of hydrophobic compounds[53].

These results are important due to the fact that *L. monocytogenes serovar 4b* (pathogenic bacteria implicated in several pathology such as meningitis) has been inhibited by EECE with MBC = 1 mg/mL. In addition, HECE has inhibited the growth *S. aureus* MBLA (strain that produce beta-haemolysin and enterotoxin A which are mainly implicated in food poisoning incident). On the other hand, it must be noted that the extract showing the highest inhibition diameters are not always the ones that present the lowest MIC and MBC values. Indeed, the inhibition diameters are influenced by several factors such as solubility of extracts.

When evaluating the antileishmanial activity of the *C. erythraea* extracts, it has appeared that some of them exhibited a stronger and even greater activity than Glucantime (positive control). Moreover, in contrary to other studies[37,54], *L. major* promastigotes has appeared more sensitive than *L. infantum*. Previous studies have ascertained the antioxidant, ant-diabetic, anti-hyperglycemic and hepatoprotective activities of this medicinal plant[28,32,47,55], but only scarce data on their leishmanicidal activity are available. To our knowledge, our report is the first to deal with leishmanicidal activity of *C. erythraea* on promastigote cultures of *L. major*, *L.*

infantum and *L. tropica*. Therefore, this *Leishmania* species have been tested with a Moroccan endemic medicinal plant (*Salvia barbecana*) and hexane extract has proved IC₅₀ value which reaches 14.11 µg/mL against *L. infantum*[32].

Interestingly, we report here that the differential antileishmanial effects of these extracts were related not only to their chemical composition but also to the nature of the promastigote species and the differential antileishmanial activity of these extracts against the same promastigote species is related to the differential composition of such extracts. It's the first time that the antileishmanial properties of *C. erythraea* extracts have tested against *Leishmania* promastigote forms. Our results are important compared with others tested extracts against the same species[13,35].

The mechanism of action of these extracts can be suggested to the specific cellular targets that disrupt the cell membrane and cell lysis. The interaction with mitochondrial membrane can be suggested in this case as another targeting pathway inducing the death of parasites by apoptosis. These actions could be attributed in our study to the TFC, which showed a very height correlation with antileishmanial capacity especially against *L. major* ($R^2 = 0.935$) and *L. infantum* ($R^2 = 0.905$).

The results obtained in our study gave suggestions that the high pharmacological properties of HECE and MECE may be attributed to the major compounds present in these two extracts. This does not exclude the possibility that the other constituents may also participate in these biological properties alone or in synergetic with major compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

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