Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.07.011

Antioxidant antileishmanial cytotoxic and antimicrobial activities of a local plant *Myrtus nivellei* from Algeria Sahara

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ARTICLE INFO

Article history:
Received 2 Mar 2016
Received in revised form 6 Jun 2016
Accepted 26 Jul 2017
Available online 1 Aug 2017

Keywords:
Phenolic compounds
Flavonoids
Antioxidant activity
Antimicrobial activity
Antileishmanial activity
Myrtus nivellei

ABSTRACT

Objective: To study the phytochemical constituents and *in vitro* biological activities of hydromethanolic extract and fractions from Algerian Sahara *Myrtus nivellei (M. nivellei)* collected in Hoggar region and to identify the active fraction that can act as an alternative of commonly used antibiotics and as antileishmanial or antioxidant agents.

Methods: Phytochemical screening of *M. nivellei* aerial parts was realised according to the literature. Extract was firstly prepared by using aqueous methanol then fractionated with ethyl acetate and butanol solvents. Total phenolics, tannis and flavonoids, of the hydromethanolic extract and their fractions were determined by Folin–Ciocalteu method as gallic acid equivalents and by aluminium chloride as rutin equivalent respectively. Extract and fractions were tested for their antimicrobial and antiparasital activities against standard bacteria using agar diffusion method and two kinds of leishmania visceral and cutaneous. The antioxidant activities were realized using phosphomolybdenum, FRAP and DPPH tests.

Results: Preliminary phytochemical screening exhibited the presence of flavonoids, tannins, saponins, and alkaloids. The experimental results showed that plant extract and fractions were high in phenolic compounds and exhibited an important role as antioxidant, antimicrobial and had a moderate antileishmanial activity.

Conclusions: These observations lead us toward more studies in this field, so that we can get more benefits from our local Algerian medicinal plants.

1. Introduction

Plants produced bioactive compounds that act as protective agents against external stress and pathogenic attack [1]. The wide variety of separation techniques and analytical determination available support the possibility of finding natural molecules which in turn leads to the development of new drugs [2]. However, they can not be used for treatment, only if their effectiveness versus chemical drugs is proven [3]. Natural antioxidants when present in foods markedly delay or prevent their oxidation process [4]. These constituents may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species (ROS), which are responsible of many diseases including cancer, diabetes, shock, arthritis, and acceleration of the ageing process [5]. More than 4 000 phenolic compounds have been found in vascular plants, which prevent free radical damage to macromolecules such as proteins, carbohydrates, lipids and DNA caused by UV radiation from the sun [6]. *Myrtus nivellei (M. nivellei)* (Myrtaceae family) commonly known as Sahara myrtle, and Tefeltesst or Tafeldest in Tamahaq [7–9]. It is a shrub frequently up to 1.5 m with green leaves, opposite, lanceolated. The flowers parts are in multiple of

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Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

five with large number of stamens, petals are white and the fruits are black berries [7,10]. The Sahara myrtle is endemic to the central Sahara of Algeria [9,11]. In traditional medicine the aerial parts mainly leaves are recognised to threat intestinal diseases, fever and diabetes [8,9,11]. According to Sahki and Sahki Boutamine, this species is the only indigenous plant of Myrtaceae family in Hoggar region. To the best of our knowledge, there are only two reports about the *M. nivellei*, one on the phenolic compounds and its antioxidant activity [12], the other about the composition of *M. nivellei* essential oil and its antifungal activity [7]. As far as our literature survey could ascertain, no information was available on the antileishmanial activity of the experimental plant species given here. Therefore, the aim of this study was to investigate the antioxidant free radical scavenging potentials of the aerial parts extract and fractions using various *in vitro* models and antimicrobial antileishmanial activities for the first time.

2. Materials and methods

2.1. Plant collection

The aerial parts of *M. nivellei* were collected in September 2014 from Hoggar located in the central Sahara of Algeria and identified at the National Institute of Forest Research (in Algeria). The dried plant was stored in the dark place for further use.

2.2. Preparation of extract

The air-dried aerial parts were pulverized into powdered by using domestic blander. Fifty gram (50 g) of this powder was extracted with hydro-methanol (80%) during 3 day at room temperature. The filtrates were evaporated by a rotary evaporator (IKA-RV 05-ST) at 45 °C under reduced pressure. The extract was then filtered and collected. After evaporation the aqueous methanolic extract was resuspended in water and then fractionated with ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) solvents. The resulting fractions of EtOAc, *n*-BuOH, and water were then removed under reduced pressure and were dried in room temperature. The crude extract and fractions were stored at 4 °C in dark for further analysis [13].

2.3. Phytochemical analysis

A standard phytochemical test was adopted in this study as described according to Chew *et al.* [1], for determination of flavonoids, tannins, saponins and alkaloids…. in Sahara myrtle.

2.4. Total phenolic content

The Folin–Ciocalteu colorimetric method was used for determination of phenolic content in the extract and fractions [14]. Reaction was prepared by mixing the sample (1 mg/mL) with 5 mL freshly Folin–Ciocalteu reagent diluted ten fold, after that 4 mL (75 g/L) of sodium carbonate was added (three replicates). The mixture was shaken and allowed to stand for 30 min at room temperature for colour development. Absorbance was measured at 765 nm using the Shimadzu UV–VIS-1240 spectrophotometer. Total phenolic content is expressed as μg gallic acid equivalent per mg of plant extract.

2.5. Total flavonoid content

Total flavonoid content was determined using the method of aluminium chloride colorimetric method developed by Wang *et al.* [15]. In this assay an aliquot of diluted sample (1 mg/mL) was added to 0.5 mL of 2% $AlCl_3$ methanol solution. The absorbance of the mixture was determined at 420 nm after 1 h at room temperature in which a yellow color was developed indicated the presence of flavonoids. Total flavonoid content was calculated as μg rutin equivalent per mg of extract. All samples were analyzed in triplicates.

2.6. Determination of total tannin

Total tannin was estimated by gelatine method described in Chouhan *et al.* [16]. About 200 mg of gelatine was mixed with 2.0 mL of water and 2.0 mL of sample and allowed to stand for 15 min at 4 °C followed by vortex and resulting mixture was filtered through Whatman filter paper No. 1. One hundred and fifty μL (150 μL) of filtrate was diluted with water (up to 1.0 mL) and then non-tannin phenolics were estimated by the procedure similar to that of total phenolic content estimation. Total tannin content of *M. nivellei* was determined by subtracting non-tannin phenolic from total phenolic content.

2.7. In-vitro antioxidant assays

2.7.1. Determination of total antioxidant capacity

The antioxidant activity of *M. nivellei* hydromethanolic extract and fractions was determined by the phosphomolybdenum method [17]. About 0.4 mL of the methanolic extract (1 mg/mL) was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The total antioxidant capacity was expressed as μg equivalent to ascorbic acid per mg of extract. The tubes were capped and incubated in water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the green phosphomolybdenum complex was measured at 695 nm. In the case of the blank, 0.4 mL of methanol was used in place of sample. The antioxidant activity was determined using a standard curve with ascorbic acid solutions as the standard. The mean of three readings was used and the reducing capacity of the extracts was expressed as μg of ascorbic acid equivalents per mg of extract.

2.7.2. Ferric reducing antioxidant power (FRAP assay)

The principle of this method is based on the reduction of a ferric 2,4,6-Tris(2 pyridyl) 1,3,5-triazine (Fe^{+}_{3}-TPTZ) to ferrous, coloured form (Fe^{+}_{2}-TPTZ) in the presence of extract. 2.7 mL of FRAP reagent was prepared according to Maksimovic *et al.* Of 0.3 M acetate buffer (pH 3.6), 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively and 0.3 mL of extract (0.1 mg/mL). The results are expressed as ascorbic acid equivalents per μg of plant extract [18].

2.7.3. DPPH method

The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical [19]. About 100 μL of standard or extract and fractions were added to 2 mL of DPPH in

methanol solution (100 μM) in a test tube. After incubation at 37 °C for 30 min, the absorbance of each solution was determined at 517 nm using spectrophotometer. IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

2.8. Antimicrobial activity

2.8.1. Microbial strains

The antimicrobial activity of hydromethanolic extract and the fractions were individually tested against pathogen microorganisms, including *Listeria monocytogenes (L. monocytogenes)* ATCC 19195, *Escherichia coli* ATCC 25922. *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 14759 and *Staphylococcus aureus* ATCC 25923. Bacterial strains were cultured overnight at 37 °C in Mueller Hinton.

2.8.2. Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activity of the extract and fractions [20]. Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cells per mL) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 20 μL of hydromethanolic extract and fractions (50 mg/mL), so 1 mg/disc and placed on the inoculated plates. These plates, after remaining at ambient temperature for 30 min, were incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimetres.

2.8.3. Determination of minimum inhibitory concentration

Minimum inhibitory concentration was determined as the method developed by Taye and collaborator [21]. All tests were performed in Mueller Hinton Broth. Bacteria were suspended in MHB to give a final density of 5×10^5 cfu/mL. Geometric dilutions, ranging from 1 000 to 7.81 μg/mL of extract and fractions, were prepared and discs were impregnated as showed in Section 2.8.2. Petri plates were incubated under normal atmospheric conditions, at 37 °C for 24 h and the minimum concentration that inhibited growth was considered as MIC value of the fraction.

2.9. Antiparasital activity

2.9.1. Parasite culture

Two strains of *Leishmania* were tested: *Leishmania major (L. major)* (LCO3) and *Leishmania infantum* (*L. infantum*) (LV20). They were maintained at 27 °C by sub-passages in RPMI-1640 medium (Gibco) containing 100 μg of streptomycin/mL and 100 U penicillin/mL supplemented with 10% of fetal bovine serum (FBS).

2.9.2. Antileishmanial activity

Antiparasitic activity was investigated on leishmanial promastigotes culture in 96-well plates. Culture of *L. major* and *L. infantum* promastigotes (2×10^5 parasite/mL) were added to each well. Two-fold serial dilutions of extract and fractions were tested at final concentration ranging from 7.81 to 1 mg/mL. Plates were then incubated at 27 °C for 72 h [22]. Revelation of parasite viability was assessed by the addition of 10 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (SIGMA, St. Louis, MO, USA) at 10 mg/mL to each well. After 4 h of incubation, the medium was removed and formazan crystals were dissolved by the addition of 100 μL of DMSO. Absorbance was determined using an ELISA plate reader (Bio-TEK) at 560 nm. Amphotericin B was used as positive control for all tests. Median inhibitory concentration (IC_{50}) was obtained by fitting a sigmoidal Emax model [22] to a dose–response regression (GraphPad Prism 5). Three replicates were performed and the average was calculated from the IC_{50} values obtained for each separate experiment.

2.9.3. Cytotoxicity assay and selectivity index

Cytotoxicity was evaluated in murine macrophagic cells (Raw 264.7). Macrophages culture was maintained in RPMI-1640 medium supplemented with 10% FBS, in the presence of antibacterial and antifungal solution (Gibco). Macrophages viability was controlled by microscopically counting after staining with 0.1% Trypan blue solution. Macrophages were initially dispatched on a 96 well culture plate at a concentration of 10^5 cells/well then allowed to adhere overnight at 37 °C under 5% CO_2. The medium was then replaced by a fresh one containing 100 μL of extract. After 72 h of incubation at 37 °C, viability was estimated by the MTT technique [22]. The selectivity index (SI) is a parameter defining the balance between cytotoxicity and biological activity of the compound, corresponding to the highest active concentration with no toxicity and expressed as the ratio IC_{50} macrophage/IC_{50} parasite. A SI value higher than 4 was considered effective for parasites and safe for macrophage [23].

3. Results

3.1. Phytochemical screening

Phytochemical screening of Sahara myrtle by simple chemical tests showed presence of polyphenols, alkaloids, flavonoids, terpenoids and saponins. The yields of aqueous methanolic extract, ethyl acetate fraction, butanolic fraction and water fraction were 21.16%, 1.16%, 1.89%, 8.28%, respectively.

3.2. Phenolic content

Total phenolic, flavonoid and total tannin content of *Myrtus* hydromethanolic extract and fractions were presented in Table 1. The total flavonoid content was assayed by aluminium colorimetric assay and total tannin with Folin Ciocalteu reagent but after precipitation of tannin with gelatine. The data showed in Table 1 indicated that the highest flavonoid and tannin content 344.22 μg rutin equivalent/mg and 310.64 μg gallic acid equivalent/mg dry extract respectively were observed in butanolic fraction and the lowest content 22.42 μg rutin equivalent/mg and 55.45 μg gallic acid equivalent/mg dry extract respectively were observed in the water fraction.

3.3. Total antioxidant activity

Finding of this study on total antioxidant of extract and fractions (Table 2) revealed high antioxidant capacities of butanolic and ethyl acetate fractions, (598.60 ± 25.45) and (490.27 ± 17.71) μg is as equivalent to ascorbic acid/mg of extract, respectively. Extract with high concentration demonstrated increase of the total

Table 1
Yield and total phenols, flavonoids and tannins of hydromethanolic extract and fractions from *M. nivellei*.

Extract and fractions	Yield (%)	Phenolic content	Flavonoid content	No tannin content	Tannin content
Hydromethanolic extract	21.16	222.98 ± 4.70	116.36 ± 6.06	100.92 ± 4.85	123.05 ± 4.85
Ethyl acetate fraction	1.16	308.4 ± 7.40	142.76 ± 3.26	62.30 ± 13.09	246.09 ± 13.09
Butanolic fraction	1.89	414.96 ± 2.40	344.22 ± 15.61	103.42 ± 6.62	310.64 ± 6.62
H_2O fraction	8.28	128.03 ± 1.87	22.42 ± 6.92	72.57 ± 6.62	55.45 ± 6.22

Phenolic and total tannins contents values were determined as µg equivalent to gallic acid/mg of extract; Flavonoids contents were determined as µg equivalent rutin/mg of extract.

Table 2
Antioxidant activities of *M. nivellei* aqueous methanol extract and fractions.

Extract and fractions	PM	FRAP	DPPH assay IC_{50}
Hydromethanolic extract	364.58 ± 29.38	551.43 ± 9.42	6.65 ± 0.15
Ethyl acetate fraction	490.27 ± 17.71	710.28 ± 10.27	04.97 ± 0.08
Butanolic fraction	598.60 ± 25.45	938.68 ± 27.16	05.42 ± 0.10
H_2O fraction	210.41 ± 13.66	253.90 ± 11.20	16.33 ± 0.06
Ascorbic acid	–	–	5.86 ± 0.02

PM and FRAP values were determined as equivalent to ascorbic acid µg/mg of extract; DPPH assay IC_{50} values were determined as µg/mL.

Table 3
Antibacterial activity of *M. nivellei* hydromethanolic extract and fractions using disc diffusion method.

Microorganisms	Extract and fractions			
	Hydromethanolic extract	Ethyl acetate	Butanolic fraction	Aqueous fraction
Bacillus cereus	13 (250)	12 (125)	14	11
Escherichia coli	10	12	12 (125)	10
Pseudomonas aeruginosa	NA	NA	NA	NA
Listeria monocytogenes	NA	18 (31.5)	18 (31.5)	20 (62.5)
Staphylococcus aureus	NA	NA	NA	NA

Bacterial growth inhibition zones in Muller Hinton Broth disc diffusion method (mm); CMI: minimal inhibition concentration (µg/mL); NA: not active.

antioxidant capacity. No report was published on the total antioxidant activity of Sahara myrtle extract and fractions.

3.4. FRAP

The data in Table 2 indicated that *M. nivellei* extract and fractions showed reducing power activity. The reaction was determined by forming the intense blue color complex; a higher absorbance value indicates a stronger reducing power of the samples. This one was showed in the following order: butanolic fraction > ethyl acetate fraction > aqueous methanolic extract > water fraction.

3.5. Radical-scavenging activity by DPPH assay

In current research, three different methods, phosphomolybdenum, FRAP and radical-scavenging by DPPH assay were used to measure the antioxidant activities of Sahara Myrtle hydromethanolic extract and fractions. The IC_{50} values were found 4.97 µg/mL, 5.42 µg/mL, 6.65 µg/mL and 16.33 µg/mL in ethyl acetate, butanolic fractions, hydrometholic extract and water fraction, respectively. IC_{50} of ethyl acetate fraction 4.97 µg/mL was less than standard 5.86 µg/mL, and this may be due to the high chelating capacities of this extract.

3.6. Antimicrobial activity

The data pertaining to the antimicrobial potential of aqueous alcohol extract and fractions against three Gram positive bacteria and two Gram negative bacteria is presented in Table 3. All tested sample showed activity against bacteria but to a varying level especially on *L. monocytogenes*, however, this bacteria was resistant to hydromethanolic extract. The most important results were observed in butanolic fraction which exhibited activity on two kinds of bacteria.

3.7. Antileishmanial and cytotoxicity activity

Aqueous methanol extract and fractions of *M. nivellei* aerial parts exerted no antileishmanial activity when tested against promastigotes of *L. major* and *L. infantum* to the exception of ethyl acetate fraction. This one exerted a moderate activity with IC_{50} values of (224.10 ± 2.32) µg/mL and (190.43 ± 1.65) µg/mL, respectively. The fraction has been shown negligible cytotoxicity on macrophagic cells (Table 4). This is the first report on antiparasital activity of Sahara myrtle from Algeria.

Table 4
Antileishmanial activity of hydromethanolic extract and fractions of *M. nivellei*.

Extract and fractions	IC_{50}		LC_{50}	SI	
	L. infantum	*L. major*		*L. infantum*	*L. major*
Hydromethanolic extract	NA	NA	–	–	–
Ethyl acetate	190.43 ± 1.65	224.1 ± 2.32	785.34 ± 3.98	4.12	3.50
Butanolic fraction	NA	NA	–	–	–
Aqueous fraction	NA	NA	–	–	–
Positif control Amphotericin B	0.22 ± 0.09	0.80 ± 0.18	9.23 ± 0.13	11.53	41.95

IC_{50}: Inhibitor concentration 50 (µg/mL). LC_{50}: Lethal concentration 50 (µg/mL). SI: Selectivity index. SD: Standard deviation. NA: Not active.

4. Discussion

Extensive progress in scientific research has been focused on medicinal plants and their extracts with antioxidant and antimicrobial proprieties in recent years. The proprieties are commonly postulated to play an important role in preventing diseases caused by oxidative stress, such as cancer, coronary arteriosclerosis, and the ageing processes [14,24]. The present study aimed to develop the phytochemical screening antioxidant and antimicrobial activities from an endemic species *M. nivellei* from Hoggar. Flavonoids, alkaloids, saponins, tannins and terpenoids were determined in this species and the total phenolic levels were based on their chemical reducing capacity relative to gallic acid. Aerial parts extract and fractions showed a high phenolic content from (414.96 ± 2.40) to (128.03 ± 1.87) μg GAE/mg of extract in butanolic and water fraction respectively; however, high sugar levels may react appreciably with Folin–Ciocalteu method in overestimation of the total phenolic content [25]. This result suggests that butanolic fraction might be the part that is rich in phenolic compounds and that butanol is suitable to extract phenolic compound from *Myrtus* species. Our results are not comparable to the value reported in the literature for the extract from leaves of *M. nivellei* which indicated that the ethyl acetate fraction demonstrated the highest values in phenolic and flavonoid contents (521.22 ± 6.16) mg GAE/g of freeze dried extract, (74.15 ± 2.73) mg catechin equivalent/g of freeze dried extract respectively [12] and in common myrtle the content of ethyl acetate extract was (435.37 ± 3.15) mg GAE/g DW and (130.75 ± 2.86) mg QE/g DW for phenolic and flavonoid [26]. This can be attributed to extraction solvents, which have dramatic influence on phytochemicals [13] and differences in sources and chemical nature of the compounds present within the extracts [27]. Polyphenols are the major group of compounds that contribute to the antioxidant properties [1]. The antioxidant activity of polyphenols is due to the reactivity of phenol moiety (hydroxyl group on aromatic ring) [27,28]. Polyphenols have the ability to scavenge free radicals via hydrogen donation or electron donation. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) complex with a maximal absorption at 695. The assay being simple and independent of other antioxidant measurements was commonly employed, and its application was extended to plant polyphenols [28]. Ethyl acetate and butanolic fractions exhibited significant antioxidant activity which can be related to their highly phenolics content.

In the first time FRAP assay has been used for determining plasma blood reducing power described by Benzie and Strain [29]; after that the assay has also been developed and used for measuring of antioxidants in plants [28]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity; higher absorption indicates a higher ferric reducing power [29]. The antioxidant activities of phenolic compounds were different due to their different structures; therefore, there is a wide degree of variation between different phenolic compounds in their effectiveness as antioxidant [30]. Butanolic and ethyl acetate fractions were good electron donors by converting Fe^{3+} to Fe^{2+}.

The reaction of plant extract and fractions with a deep violet stable radical DPPH is based on the conversion of it to the decolourised radical α, α–diphenyl-β- picrylhydrazine due to their antioxidant property [4]. This assay is widely employed and preferred for the measurement of antioxidant activity, because of its imperviousness to the side and enzymatic reactions [31]. The discolouration degree indicates the scavenging potentials of the sample antioxidant. In this study, the extract and fractions were able to decolourise DPPH, and the free radical scavenging potentials of the extract and fractions of *M. nivellei* were found to be in the order of butanol fraction > ethyl acetate fraction > aqueous methanol extract > water fraction.

Oxidative stress and free radicals are involved in pathophysiology of diarrhoea either through a direct or indirect induction such as intestinal membrane damage caused by lipid peroxidation [32]. Therefore, antioxidants play beneficial roles in human health and cure/prevent ailment such as inflammatory disorders, cancer and diabetes which occur due to the deregulation of these free radicals generation in the cells [31]. These results can explain in partly the use of this plant in folkloric medicine of Touarg and local people of Tamanrasset mainly to threat diabetes and pathological disorders such as diarrhoea [9].

The antibacterial activity of aqueous methanol extract and fractions were carried out in the target of founding of new sources of antibacterial agents. Most of the extract and factions showed an antibacterial activity against the human pathogens such as *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, L. monocytogenes*. Butanolic fraction was the active one, which indicates the active principle on bacteria growth were extracted by butanol more than other solvent; this fraction was summarized an intense phenolic content like tannins that are very good antimicrobial agent [33] and specially flavonoids are known to be effective antimicrobial agent against a wide array of microorganisms [34]. The gram positive *L. monocytogenes* was the most inhibited bacteria by most of plant fractions. Gram negative bacteria are frequently reported to have developed multi-drug resistance to many of the antibiotics [35].

In this study, plant extract and fractions from *M. nivellei* have been evaluated for their potential efficacy against *Leishmania* parasites using promastigotes of *L. major* and *L. infantum*. The antileishmanial effect had shown that the ethyl acetate fractions exhibited an IC_{50} value > 100 μg/mL, this activity was less with a relative weak selectivity as well (SI = 3.5) and (SI = 4.12) [22] than that of positive control amphotericin with IC_{50} = (0.22 ± 0.09) μg/mL and (0.80 ± 0.18) μg/mL on *L. major* and *L. infantum* respectively. In previous investigation this genus has not showed any mortality in the mice in dose up to 1 000 mg/kg [37] and no toxicity has been mentioned by nomads in our recent ethnobotanical study [9]. There is no experimental data available about a possible antileishmanial activity of preparations from *M. nivellei*. These results with the nonexistent or relatively modest anti-leishmanial effects in cultured promastigotes might be attributed to solvent preparation of bioactive compounds or there is no synergistic effect between phytochemical constituents in extract or fraction, as could be confirmed by the study of Duarte *et al.* in 2016 which demonstrated that fraction from *Zingiber officinalis* Roscoe water extract was more active against *in vitro* promastigotes and *in vivo* amastigotes of *Leishmania amazonensis* in comparison with the extract [36].

This report envisages the antioxidant, antimicrobial, antiparasital and cytotoxic activities of *M. nivellei* hydroalcoholic extract and fractions for the first time. Hence the species could be a good source of antioxidant phenolics. And can be used as

an antibiotic. Further studies are warranted for the isolation and identification of individual phenolic compounds and also *in vivo* studies are needed for better understanding their mechanism of action and formulate best alternative herbal preparations as antioxidant antimicrobial and antileishmanial agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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