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Correlation of radical-scavenging capacity and amoebicidal activity of *Matricaria recutita* L. (Asteraceae)

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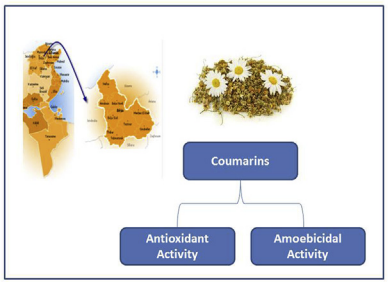
HIGHLIGHTS

- The evaluation of the antioxidant and anti-*Acanthamoeba* activity of chamomile extracts.
- Bio-guided fractionation was developed in order to identify and isolate the molecules responsible for the observed effects.
- Our results suggest coumarins from chamomile as a novel source of anti-*Acanthamoeba* compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Some Acanthamoeba strains are able to cause Granulomatous Amoebic Encephalitis (GAE) and *Acanthamoeba* keratitis (AK) worldwide because of their pathogenicity. The treatment of *Acanthamoeba* infections is complicated due to the existence of a highly resistant cyst stage in their life cycle. Therefore, the elucidation of novel sources of anti-*Acanthamoeba* agents is an urgent need. In the present study, an evaluation of the antioxidant and anti-*Acanthamoeba* activity of compounds in flower extracts of Tunisian chamomile (*Matricaria recutita* L.) was carried out. Chamomile methanol extract was the most active showing an IC_{50} of 66.235 ± 0.390 μg/ml, low toxicity levels when checked in murine macrophage toxicity model and presented also antioxidant properties. Moreover, a bio-guided fractionation of this extract was developed and led to the identification of a mixture of coumarins as the most active fraction. These results suggest a novel source of anti-*Acanthamoeba* compounds for the development of novel therapeutic agents against *Acanthamoeba* infections.

1. Introduction

Acanthamoeba is a widely distributed genus worldwide and it has been isolated from many environments such as water, soil, dust and many others (Lorenzo-Morales et al., 2015). To date, molecular

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classification of *Acanthamoeba* genus established 20 genotypes: T1-T20 that are based on the rRNA sequence of the isolates (Booton et al., 2005; Corsaro et al., 2015; Magnet et al., 2014; Nuprasert et al., 2010; Qvarnstrom et al., 2013). Of the 20 genotypes, T4 is the most abundant in the environment and includes many pathogenic strains that have been associated with lethal encephalitis and *Acanthamoeba* keratitis (AK) (Lorenzo-Morales et al.,. 2013; Siddiqui et al., 2012). Current therapeutic approaches against these infections are affected by drug resistance, variable efficacy between strains, toxic side effects and long course treatments. Therefore, there is a need to identify novel sources of drugs that are active against *Acanthamoeba*. Recently, agents with plant origins have been widely studied as a source of novel antiprotozoal drugs including *Acanthamoeba* (Derda and Hadaś, 2014; Lorenzo-Morales et al., 2015; Sifaoui et al., 2014).

Chamomile, (*Matricaria recutita* L.) has been included for centuries in the pharmacopoeia of several countries including Tunisia (Hajaji et al., 2017; Jabri et al., 2016; Sebai et al., 2014). Previous phytochemical screenings of this plant have discovered a high content of antioxidant and anti-inflammatory active molecules including phenolic compounds (McKay and Blumberg, 2006; Sebai et al., 2015). Many of these compounds are responsible for antimicrobial, larvicidal, anti-cancer and anti-inflammatory effects of chamomile reported previously (Miraj and Alesaeidi, 2016; Sakkas et al., 2016). Compounds showing these activities have been found to include coumarins, flavonoids, phenolic acids, and fatty acids among others (Morales-Yuste et al., 2010; Miraj and Alesaeidi, 2016; Sebai et al., 2015).

No previous studies have been carried out to evaluate the activity of this plant and its extracts against *Acanthamoeba*. Therefore, the aim of this study was to evaluate the antioxidant and anti-*Acanthamoeba* activities of flower extracts of Tunisian chamomile (*Matricaria recutita* L.), and to explore a possible correlation between these activities. After an initial screening of extracts, a bio-guided fractionation of the most active fraction was carried out to identify and isolate the major molecules responsible for these activities.

2. Materials and methods

2.1. Plant material

Chamomile flowers were collected in March 2013 from the region of Amdoun, Beja governorate (North-West of Tunisia, alt. 448 m; 36° 81′ N; 9° 05′E). Collected flowers were separated and thoroughly rinsed in running tap water and air dried for a period of 14 days. Finally, they were ground to a fine powder using a mill and stored at 4 °C until subsequent experiments were carried out.

2.2. Extract preparation

Chamomile extracts were prepared by maceration of 100 g of powdered plant material in 500 ml of extraction solvent (chloroform (99%), n-hexane (95%), water and methanol (99.9%). After 24 h of agitation at room temperature (20–25 °C, 3 × 500 ml) in the dark, the collected extracts were filtered using Whatman No 1 paper for three times. The solvents were removed by a rotary vacuum evaporator at 40 °C and finally the samples were weighed and stored at 4 °C until used.

The essential oil of *Matricaria recutita* L dried flowers was extracted by hydrodistillation using a Clevenger type apparatus. The volatile distillate was dried over anhydrous sodium sulfate and stored at 4 °C in a dark and sealed container prior to analysis.

2.3. Bioassay guided fractionation of chamomile

Matricaria recutita L. essential oil, aqueous and organic extracts were initially tested for their antioxidant and anti-*Acanthamoeba* activities using *Acanthamoeba castellanii* Neff (ATCC 30010), a type strain from the American Type Culture Collection (ATCC) was used in this study. The fractionation of the active extract was guided by the anti-amoebic activity.

Initially, 5 g of methanolic extract were diluted in water. The aqueous solution was fractionated by liquid-liquid bipartition using 450 ml of organic solvents (hexane, chloroform and ethyl acetate successively). The obtained organic phases were evaporated and weighed. The water phases were collected and lyophilized. The most active fractions were subjected to silica or Sephadex column and subanalysed by TLC and NMR (Fig. 1).

^{1}H (600 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Bruker Avance 600 spectrometers; the chemical shifts are given in δ (ppm) with residual $CDCl_3$ (δ_H 7.26, δ_C 77.0) as internal reference and coupling constants in Hz; experiments were carried out with the pulse sequences given by Bruker. Silica gel 60 (particle size 15–40 and 63–200 μm, Macherey-Nagel) and Sephadex LH-20 (Pharmacia Biotech) were used for column chromatography, while silica gel 60 F_{254} (Macherey-Nagel) were used for analytical or preparative thin layer chromatography (TLC). Centrifugal preparative TLC was performed using a Chromatotron (Harrison Research Inc. model 7924T) on 4 mm or 1 mm silica gel 60 PF_{254} disks with flow rate 2–4 mL min^{-1}. The spots were visualized by UV light and heating silica gel plates sprayed with H_2O-H_2SO_4-$C_2H_4O_2$ (1:4:20). All solvents used were analytical grade from Panreac.

2.4. Antioxidant activities

2.4.1. $ABTS^{+}$ method

This assay is based on decoloration that occurs when the radical cation $ABTS^{+}$ is reduced to ABTS (2,20-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). The radical was generated by reaction of a 14 mM solution of ABTS in water with 4.9 mM potassium persulphate (1:1). The mixture was then diluted with ethanol until an absorbance of 0.70 ± 0.02 at 734 nm. A volume of 20 μl of each sample solution at different concentrations was added to 180 μl of ABTS solution (Siddhuraju, 2006). The radical-scavenging activity is calculated as the inhibition percentage using the equation:

$$\%\text{inhibition} = ((A_C - A_S)/A_C)*100$$

A_C is the ABTS + solution absorbance at 734 nm and A_S is the sample absorbance at 734 nm.

2.4.2. DPPH· method

The DPPH assay (diphenyl-1-picrylhydrazyl) was performed according to the method of Brand-Williams et al. (1995). Briefly, 20 μL of various concentrations of extract or fractions were added to 180 ml of 6.10^{-5} mM methanol solution of DPPH and incubated at 25 °C during 60 min. DPPH radical scavenging activity (RSA), expressed as a percentage, was estimated using the same formula used in ABTS test.

2.4.3. FRAP method

The Ferric-reducing antioxidant power FRAP assay method measures the ability of antioxidants to reduce ferric-2,4,6- tripyridyl-s-triazine (Fe3+-TPTZ) to a ferrous form (Fe2+), which absorb at 593 nm. Briefly, a mixture of 0.3 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ dissolved in 40 mmol/L hydrochloric acid, and 20 mmol/L ferric chloride (10:1:1 v:v:v) was prepared. 3.75 μl of

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