Mentha suaveolens from Morocco: Phytochemical screening and in vitro antioxidant activity for aqueous extracts

Accepted 8th June, 2018

ABSTRACT

The aim of this work is the study of the aqueous extracts of Moroccan Mentha suaveolens obtained using different extraction modes: maceration, infusion and refluxing. It was carried out in a preliminary phytochemical screening and used to determine the amount of total phenolic and flavonoid contents and also investigate the in vitro antioxidant activity. To evaluate this activity, three different methods were used: 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH•), phosphomolybdate method and Ferric Reducing Antioxidant Power (FRAP method). The results were expressed as mg of Ascorbic acid (AA) and Butylated hydroxyanisole (BHA) equivalents per 100 g dry matter (d.m.), the quantity of AA or BHA required to produce the same antioxidant activity as the extract in 100 g of sample (AA Equivalent Antioxidant Capacity (AEAC) or BHA Equivalent Antioxidant Capacity (BEAC). According to the AEAC and BEAC values of the aqueous extracts of M. suaveolens in the different methods, E3 (extract 3) showed highest antioxidant capacity, followed by E2 and E1. The AA and BHA contribution respectively to AEAC and BEAC varied greatly among extracts, from 354.57 to 444.53 mg/100 g d.m. with maceration to 739.25 to 855.85 mg/100 g d.m with refluxing using FRAP assay. The statistical analysis showed very significant results (p <0.002) for all the tests.

Keywords: Antioxidant activity, Mentha suaveolens, aqueous extracts, phenolic compounds, refluxing.

INTRODUCTION

The Moroccan aromatic and medicinal flora is remarkable for its richness, diversity and socio-economic value (M., 2009). Morocco, as many countries in the world, has an old tradition and important know-how in traditional pharmacopeia. Local inhabitants have made use of this diverse flora since prehistoric times. Plants are used traditionally as food, fodder and medicines for people and livestock including dyes etc. Taking only medicinal uses into account, about 570 species, distributed over 98 families and 486 genera, representing about 15% of the total Moroccan flora have been recorded (BRAHMS, 2017). This work fits into the ethnopharmacological researches, in support of the traditional use of herbal medicines. The aim was to study the aqueous extracts of the plant Mentha suaveolens from Morocco obtained using different extraction modes: maceration, infusion and refluxing. A preliminary phytochemical screening was carried out with measure of the amounts of total phenolic and flavonoid contents, and then evaluation of antioxidant activity with DPPH assay mostly used and two other methods FRAP and phosphomolybdate assays. Aqueous extracts have been preferred to organic extracts or essential oils, because this is the form used by default in the Moroccan population, especially in traditional medicine.

Mentha suaveolens commonly called "Apple mint" is an aromatic herb with a fruity, spearmint flavor. Table 1 summarizes the commons names and uses in Morocco of M. suaveolens according to traditional pharmacopeia by
Table 1: Common names and uses of Mentha suaveolens in Morocco by region (Bellakhdar, 1997).

<table>
<thead>
<tr>
<th>Common name</th>
<th>Region</th>
<th>Traditional medicinal use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marseta, timersit</td>
<td>Mid-range Atlas mountains, Meknes, Rabat</td>
<td>1) Dried leaf powder as a laxative (Meknes). 2) In the Moorish bath, we sit on clumps of plants on a hot brick for treating colds, hemorrhoids and pains in the lower abdomen (Talembot) and 3) Fresh crushed plant as healing (Rif, BeniTouzin).</td>
</tr>
<tr>
<td>Timijja</td>
<td>Imlil</td>
<td></td>
</tr>
<tr>
<td>Mšištró na’na’lemzewwaf</td>
<td>North of Morocco</td>
<td></td>
</tr>
<tr>
<td>Tifergalí</td>
<td>Talembot, Western Rif mountains</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Modes of extraction.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Mode</th>
<th>Yield (%)</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₁</td>
<td>Maceration</td>
<td>5</td>
<td>The mixture was allowed to stand at room temperature for 24 h in the dark.</td>
</tr>
<tr>
<td>E₂</td>
<td>Infusion</td>
<td>5</td>
<td>The mixture was prepared with boiling distilled water, and then allowed to stand at room temperature for 24 h in the dark.</td>
</tr>
<tr>
<td>E₃</td>
<td>Refluxing</td>
<td>6</td>
<td>The powdered plant material was mixed with distilled water in a round bottom flask and refluxed for about 30 min at 100 to 110°C. Then the mixture was allowed to stand at room temperature up to 24 h in the dark.</td>
</tr>
</tbody>
</table>

Bellakhdar. It is mostly used for treating the common cold, but can also have other applications in different regions.

MATERIALS AND METHODS

Plant material

The plant M. suaveolens was collected from the region of Temara, Morocco. The fresh leaves were washed under running tap water and shade dried at room temperature.

Preparation of the extracts

Dried leaves were ground using a domestic blender and 30 g of this material was extracted using 200 ml of distilled water following the three modes of extraction (Table 2). The aqueous extracts were obtained by filtering the mixture through Whatman No.1 filter paper and the dry extracts obtained using rotary evaporator. The dried plant extracts were then redissolved in distilled water to get the solution of 1 mg/ml or more (2.5 mg/ml for DPPH assay) for each extract which was diluted to the target concentrations and used for different analysis without further treatment.

Phytochemical screening

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the various aqueous extracts of leaves of M. suaveolens using standard methods. The methods of positive reactions were reported hereafter (Table 3).

Determination of total phenolic content

Total phenolic content was determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton and Rossi (1965).

Folin-Ciocalteu colorimetric assay is based on a chemical reduction of the reagent, a mixture of phosphotungstic and phosphomolybdic acids that are reduced by the oxidation of phenolic compounds in a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 760 nm and are proportional to the total phenolic concentration (Amorim, 2008).

Briefly, 0.50 ml of the diluted sample was reacted with 2.5 ml of Folin-Ciocalteu reagent 0.2 mol/L for 4 min, and then 2 ml saturated sodium carbonate solution 7.5% (w/v) was added into the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The total phenolic contents were expressed as mg Gallic Acid Equivalents per gram (mg GAE/g) dry weight of herbal material using standard Gallic acid calibration curve.

Determination of flavonoids

The aluminum chloride colorimetric method was followed for estimation of flavonoids as described by Marinova(2005). The aluminum ion (Al³⁺) is reacted with
the flavonoids in the sample to form the stable flavonoid-Al\textsuperscript{3+} complex, which has a yellow colour and intensity proportional to the flavonoid concentration. To the aliquot of 1.5 ml was added 1.5 ml of AlCl\textsubscript{3} reagent (2%). After 30 min of incubation the absorbance was recorded at 430 nm against a blank. The flavonoid contents were calculated using a standard calibration curve prepared from quercetin. The amount of flavonoids was expressed as mg of quercetin per gram (mg QE/g) of dry matter (Bahorun, 1996).

### Measurement of antioxidant activity

**DPPH• assay**

To evaluate the anti-oxidative activity of specific compounds or extracts, the latter are allowed to react with a stable radical, 2,2-Diphenyl-picrylhydrazyl (DPPH) in a methanol solution. In its radical form, DPPH* absorbs at 515 nm, but upon reduction by an antioxidant (AH) the absorption disappears (Brand-Williams, 1995) (Figure 1).

The DPPH radical scavenging capacity was measured according to the method described by (Popovici, 2009) with few modifications. 100 μl of each sample at different concentrations (0.5 to 2.5 mg/ml) was added to 3.9 ml of DPPH (25 μg/ml) solution in methanol. Test tubes were vortexed and incubated for 30 min at room temperature in the dark; the absorbance values were determined at 515 nm against a blank in a RayLeigh UV 1800 spectrophotometer. The inhibition percentage of DPPH radical (1%) was assessed by the formula:

\[
\text{Inhibition percentage} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

DPPH• solution (3.9 ml) plus distilled water (100 μl) was used as a negative control. Also, AA and BHA solutions at different concentrations (10 to 160 μg/ml) were used as positive control. The concentration of extract that could scavenge 50% of the DPPH radicals (IC\textsubscript{50}) was thereafter calculated.

**Phosphomolybdate assay**

It is a spectroscopic method for the quantitative determination of total antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample compounds and subsequent formation of a green phosphate Mo (V) complex at acidic pH (Javdan, 2012; Alam, 2013). An aliquot of 0.3 ml of sample solution was combined in a vial with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. Thereafter, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of AA and BHA.

**Ferric reducing antioxidant power (FRAP) assay**

FRAP assay depends upon the ferric ferricyanide (Fe(III)-CN) complex to the ferrous ferricyanide (Fe(II)-CN) by a reductant. Fe(II)-CN has an intensive blue colour and can be monitored at 700 nm. The reducing power of the individual plant extract was determined according to the method described by Oyaizu (1986).

1 ml of each sample at different concentrations (200 to
Table 4: Positive reactions of phytochemical screening of aqueous extracts of leaves of Mentha suaveolens from Morocco.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Methods</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>Ferric Chloride Test</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkaline Reagent Test</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Lead acetate Test</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>Froth Test</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Salkowski’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Libermann Burchard’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+++: highly present; + +: moderately present; +: present.

1000 µg/ml) was mixed with 2.5 ml phosphate buffer 0.2 M (pH 6.6) and 2.5 ml of 1% potassium ferricyanide K₃Fe(CN)₆. The mixture was then incubated at 50°C for 20 min. After incubation, 10% of 2.5 ml of trichloroacetic acid was added to the mixture. Thereafter, 25 ml of each solution was diluted with 2.5 ml distilled water and 0.1% of 0.5 ml ferric chloride. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The results were expressed as mg of AA and BHA equivalents per 100 g of dry matter.

Statistical analysis

The results of all experiments performed were expressed as Mean ± SD (Standard Deviation) of three determinations, the test of significance was applied wherever necessary and values obtained as p<0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

Table 4 shows the secondary metabolites found in these aqueous extracts using standard protocols for qualitative phytochemical screening. In general, it is observed that water is a solvent which can extract interesting phytochemicals from medicinal plants, in particular, the phenols and flavonoids which have been assayed hereafter. Note, we could extract the same compounds either with maceration, infusion or refluxing. Nevertheless, extraction with refluxing can make extracts more concentrated, which was confirmed during the determination of total phenolic and flavonoid contents.

Total phenolic and flavonoid contents

Total phenolic and flavonoids contents of the aqueous extracts were measured using respectively the Folin-Ciocalteu and the aluminum chloride colorimetric methods (Table 5). Phenolic compounds including flavonoids are plants secondary metabolites (Fattahi, 2014). They are considered as a major group of compounds that contribute to the antioxidant activities of botanical materials due to their scavenging ability on free radicals owing to their hydroxyl groups (Balasundram, 2005; Djeridane, 2006). Besides, the phenolic compounds possess multiple biological properties such as anti-tumor, anti-mutagenic and anti-bacterial properties; these activities might be related to their antioxidant activity (Guanghou, 2002).

Measurement of antioxidant activity

There are several methods to determine the antioxidant capacity of plant extracts. However, the chemical complexity of extracts could lead to scattered results obtained from different techniques, depending on the test employed. Therefore, an approach with multiple assays in the screening work is highly advisable (Prior, 2005).

Among these methods, three, DPPH, Phosphomolybdate and FRAP assays were used as common and useful methods to evaluate the antioxidant capacity of extracts. All the extracts tested showed an antioxidant activity and the extract obtained by refluxing (E₃) demonstrated a significantly greater antioxidant activity than the other two, with the various methods tested.

The model of scavenging the stable DPPH radical is a widely-used method to evaluate antioxidant activities in a relatively short time as compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Baumann, 1979).

According to this assay, all the extracts exhibited a noticeable concentration-dependent antiradical effect (Figure 1) but differed in their inhibiting activities. The IC₅₀(DPPH⁺) values of the extracts decreased in the following order: E₁>E₂>E₃. They were 8.62 ± 2.08; 2.60 ±
0.03 and 1.41 ± 0.02 mg/ml, respectively.

In fact, a lower value of IC_{50} indicates a higher antioxidant activity of a compound and vice versa. Hence, it can be seen that, for the same extract, it is possible to obtain an activity up to 6 times higher from simple maceration to refluxing. This is explained by its higher concentration of phenolic compounds (Figure 2). However, the activity of aqueous extracts remains far lower than that of the synthetic antioxidants AA (0.13 ± 0.01 mg/ml) and BHA (0.09 ± 0.00 mg/ml), also determined in parallel experiments.

Concerning phosphomolybdate and FRAP assays, the antioxidant activity was confirmed for all extracts and the higher activity was also observed for E3 with an equivalent antioxidant activity exceeding the 700 mg/100 g d.m (Figure 4).

The statistical analysis with One-way ANOVA showed a significant difference between the three extracts for the whole assays with very low p-values:

**Conclusion**

Plant-derived antioxidants, especially the phenolic compounds have gained considerable importance due to their potential health benefits. The antioxidant capacity of the plant extract largely depends on both the composition of the extract and the test system. It can be influenced by a large number of factors and cannot be fully evaluated by one single method. It is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (Frankel, 2000).

In this investigation, water was used as solvent to extract the hydrophilic antioxidants present in the plants. Indeed, in traditional medicine or culinary practices in Morocco, water that is mainly used for herbal preparations. The results of our study showed that a boiling of 30 min during refluxing makes the aqueous extracts two to three times richer in natural active ingredients and thus, more effective in terms of antioxidant activity.

**REFERENCES**


BRAHMS (20117). Flora of Morocco. from BRAHMS http://herbaria.plants.ox.ac.uk/bol/floraofmorocco


