Evaluation of Antioxidant Activity And Secondary Metabolites of Menthapiperita L Under Effect of Acetylsalicylic Acid And Methyl Jasmonate

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ABSTRACT: This study investigated the effect of salicylic acid (SA) and methyl jasmonate (MJ) on secondary metabolites as well as antioxidant activities in Menthapiperita L. We used 2 kinds of treatment including (SA) and (MJ) at concentrations of (50, 100 µm). Treatments carried out on 7 weeks plants. At first plants were treated with (SA) 3 days then 3 days with (MJ). In order to measure content of phenolic and flavonoid compound, we used spectrophotometric method. Finally antioxidant activity of the extract was measured in different concentrations using free radical 2, 2-diphenyl-1 PykrylHydrazyl (DPPH). Data Analyzed with SPSS version 16 and one-way ANOVA. Experiment was carried out based on completely randomized design with six replication. Results of variance analysis showed that radical scavenging of Menthapiperita L cultivare significantly decreased by treated (SA,MJ) and reduced antioxidant activities levels compared to control treated with alcohol (P<0.01). Also, treatments (SA,MJ) caused increase in phenolic content. The result of this treatment showed these treatments stimulated amount of essential oil, total phenolic content but it doesent have positive effect on flavonoids of Menthapiperita L.

KEYWORD: MENTHA PIPERITA, METHYL JASMONATE, ACETYLSALICYLIC ACID, ANTIOXIDANT ACTIVITY, PHENOLIC COMPOUNDS, FLAVONOIDS

INTRODUCTION

Methyl jasmonate and their esters (methyl jasmonate) are considered as a new group of plant growth regulators, derived from linolenic acid. Methyl jasmonate hasa variety of biological effects similar to other plant hormones, including accelerating aging and falling leaves, twists closing of stomata to prevent root growth and seed germination of dormant seeds [16,2]. Jasmonate as a key messenger in the induction compounds leads to the accumulation of secondary metabolites [6].

Antioxidants are used to slow the oxidation of essential nutrients [11]. Phenolic and flavonoid compounds in plants having antioxidant potential and the use of medicinal plants would be readily available as a resource for food industry [1].

Salicylic acid as a chemical stimulus to be treated outside of plants to enhance resistance and antioxidant activity as well as positive role of salicylic acid on the growth of wheat plants under salt stress[9]. Salicylic acid has a favorable effect on the amount of yield and yield components of pea. Menthapiperita L. has antioxidant and antibacterial properties and is very essential as a food preservative [15].

The purpose of this study is the use of acetylsalicylic acid and methyl jasmonate as chemical elicitor compound to stimulate secondary metabolites compounds production and antioxidant activity of Mentha piperita

MATERIAL AND METHOD

Menthapiperita L were gained from the Faculty of Pharmacy, Tehran University and fresh rhizome cultivated in the pots with a diameter of 12cm. Soil steamed with phosphate to prevent the growth of fungi and weeds. The pots were divided into four categories and each one in six replicates. Control line, Control treated with alcohol, treatment 1 that includes a plant for the concentration of 50 µM Salicylic acid for 3 days and methyljasmonate for 3 days later and treatment 2 also includes plant a concentration of 100 µM Salicylic acid and methyljasmonate. Therefore, we had control groups with similar plants in terms of the number and condition as control group and control were taken with alcohol. All the conditions of light, temperature, watering
daily were the same respectively. Green house condition with 14 hours light and 10 hours darkness was provided. The temperature was measured with a thermometer to be 22±1°C. The treatment carried out on 7 weeks samples with spraying shoots up to the soil surface. Treatment carried out every morning for six days. Finally in the morning of the sixth day, the aboveground plant parts including stems and leaves were collected in all groups. The collected plant materials were washed with running tap water to remove surface contaminants and finally air dried, then ground into fine powder using an electric blender and were stored in air tight containers until use.

**Extraction of samples**

Powdered plant materials (100g each) were soaked in solvent methanol for a week. The extracts were then filtered through Whatman No.1. filter paper and the solvent was evaporated using rotatory evaporator. These crude extracts were stored in sealed containers at room temperature until use. Measurement and extraction of essential oils:

50 g of powder of each group To steam distillation by Clevenger apparatus, we measured amount of essential oil and methanol was used as solvent. Perculation Method of methanol and 50 g of powdered plant extracts were obtained and then placed in the refrigerator in a plastic sealed container.

**Chemicals**

Ascorbic acid, gallic acid, 1, 1-Diphenyl-2-picyrylhydrazyl (DPPH), ferric thiocyanate, nitric oxide, aluminium chloride and sodium nitrite rutin from purchased Qualigens. Folin-Ciocalteau’s phenol reagent and sodium carbonate were from Merck chemical supplies (Damstadt, Germany). All the other chemicals used including the solvents, were of analytical grade purchased from SD fine and Qualigens manufacturing Ltd.

**Determination of total phenolic content**

The total phenolic content (TPC) of the crude extracts of shoot were determined using the method of Singleton et al.(1999) with slight modifications. To 0.5 ml of test sample, 1.5 ml (1:10 v/v diluted with distilled water) Folin-Ciocalteau reagent was added and allowed to stand for 5 min at 22ºC. After 5 min, 2.0ml of 7.5% of sodium carbonate was added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation development of blue colour was observed. Finally absorbance of blue colour in different samples were measured at 725nm using colorimeter. The phenolic cotent was calculated as gallic acid equivalents GAE/g on the basis of standard curve of gallic acid. The results were expressed as Gallic acid equivalents (GAE)/g of the plant material. All the determinations were carried out six times.

**Determination of total flavonoid content**

The total flavonoid content(TFC) of different shoot was determined using the aluminium chloride assay through colorimetry. An aliquot (0.5 ml) of extracts were taken in different test tubes then 2ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% NaNO2, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminiumtrichloride (10% AlCl3) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and volume was made upto the 5ml with distilled water. After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm using a colorimeter. Distilled water was used as blank. The TFC was expressed in mg of catechin equivalents (CE) per gram of extract. All the determinations were carried out six times. Ordoñez A AL 2006.

**Determination of antioxidant activity**

DPPH, (Sigma-Aldrich, Germany; M.W. 394.32) as a free radical. 1 mg/ml solution of plant extract in methanol was prepared. 6 x 10-5 mol/L DPPH in methanol was prepared. 0.1 ml of plant extract was added to 3.9 ml of DPPH solution. The decrease in absorbance at 515nm was recorded at 1 min interval upto 15 min or until the reaction reached a plateau. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control. Ascorbic acid (Merck; M.W. 176.13) was used as standard. The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula: % DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100.

**Statistical analysis**

The results were recorded after repeating the experiments six times. The experimental results were expressed as mean ± standard error (SE) of (6n) measurements. The statistical analysis of the data were carried out using student's t-test and the results were considered significant when p<0.01
RESULTS

A. Antioxidant Activity Assay

Table 1. IC50 levels of different samples with produced essential oil

<table>
<thead>
<tr>
<th>mgGE/gdry sample</th>
<th>Mean absorption ± st error</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.6*</td>
<td>.3405±.00985*</td>
<td>control</td>
</tr>
<tr>
<td>7.9*</td>
<td>.2952±.00552*</td>
<td>Control with alcohol</td>
</tr>
<tr>
<td>18*</td>
<td>.4037±.00982*</td>
<td>50µm methyl jasmonate and Salicylic acid</td>
</tr>
<tr>
<td>24*</td>
<td>.4737±.00592*</td>
<td>100 µm methyl jasmonate and Salicylic acid</td>
</tr>
</tbody>
</table>

Table 2. Phenolic compounds in the extracts p <0.01 * Examples of absorption mean ± st error, mgGAE / gdry sample

<table>
<thead>
<tr>
<th>Efficiency of essential oil</th>
<th>IC50</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%*</td>
<td>138.22</td>
<td>Control</td>
</tr>
<tr>
<td>0.5%*</td>
<td>105.72</td>
<td>Control with alcohol</td>
</tr>
<tr>
<td>0.36%*</td>
<td>144.97</td>
<td>50µm methyl jasmonate and Salicylic acid</td>
</tr>
<tr>
<td>0.7% *</td>
<td>125.93</td>
<td>100 µm methyl jasmonate and Salicylic acid</td>
</tr>
</tbody>
</table>

IC50 increased in both treatments compared with control treated with alcohol which shows decrease in the antioxidant activity of both treatment. On the other hand amount of essential oil rised significantly in plants treated with 100 µm of salicylic acid and methyl jasmonate, while the amount of essential oil decreased with treatment of 50 µm.

Table 3. Flavonoid content of extracts with p <0.01 *

<table>
<thead>
<tr>
<th>mgGAE/gdry sample</th>
<th>Mean absorption ±st error</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>.2355±.01172*</td>
<td>control</td>
</tr>
<tr>
<td>300</td>
<td>.2754±.00777*</td>
<td>Control with alcohol</td>
</tr>
<tr>
<td>305</td>
<td>.2822±.00524*</td>
<td>50µm methyl jasmonate and Salicylic acid</td>
</tr>
<tr>
<td>265</td>
<td>.2595±.00735*</td>
<td>100 µm methyl jasmonate and Salicylic acid</td>
</tr>
</tbody>
</table>

The total phenolic content in accordance with Table 2 in comparison with the increase in treated water has increased by more than 50 mM to 100 micromoles of treatment and this increase was significant according to Table 2. So acetylsalicylic acid and methyl jasmonate treatment 2 (100µm) has created the highest total phenolic content.

The amount of flavonoid

The content of flavonoids increased in both treatments compared to control, but in comparison with control treated with alcohol, we observed rise in the amount of flavonoids in 50µm of treatments. In 100µm of treatments there was a decrease in flavonoids content compared with control treated with alcohol(Table 3). Generally in the higher concentration of treatments antioxidant activity and phenolic and flavonoid content increased more than lower concentration compared to the control treated with alcohol.

DISCUSSION

Essential Oils and extracts can be used as a source for the preparation of medicinal plants as antioxidants and preservatives in food, pharmaceutical and cosmetic products. Due to higher safety in general, natural ingredients such as these compounds can be replaced by other chemical agents. The purpose of this study was to evaluate the effect of acetylsalicylic acid and methyl jasmonate both together on secondary metabolites present in Mentha Piperita. Antioxidant activity is directly related to the amount of flavonoid and phenolic compounds across all plants [15]. Rosemary extract has been proven that Antioxidant activity and phenolic content of these plants has a direct connection [12]. Induced oxidative stress affected by methyl jasmonate and acetylsalicylic acid have also been reported [5,7].
These two elicitors can simultaneously enhance the antioxidant activity and phenolic content in Mentha piperita. Compared with control, but further studies is needed.

REFERENCES


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