BIOCHEMICAL COMPOSITION AND IN-VITRO THROMBOLYTIC ACTIVITY OF MELISSA OFFICINALIS GROWING NATURALLY IN KURDISTAN REGION\IRAQ

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ABSTRACT

The present study was aimed to evaluate different parts of Melissa officinalis plant from biochemical composition and thrombolytic activity.

Methods: Leaf and stem part of Melissa officinalis growing naturally were assessed for total phenolic, total flavonoid and total tannin contents. Both parts were examined for the invitro thrombolytic activity.

Results: Significant greater amount of total phenolic (27.882 ± 0.544 mcg GAE/g DW), total flavonoid (256.708 ± 1.447 mcg QE/g DW) and total tannin contents (424.266 ± 1.026 mcg GAE/g DW) were expressed by the leaf part (p < 0.01) in comparison to the stem part. A significant thrombolytic activity were expressed by both parts in comparison to the control negative (distilled water) (p value < 0.0001) at all tested concentration rage. Greatest thrombolytic activity were detected at high concentration (1000 mcg/ml) for both parts. Generally the thrombolytic activity exhibited by the leaf part was higher than that of stem part.

Conclusion: From the results of study we concluded that leaf part of Melissa officinalis was richest in phytochemical constituents with moderate thrombolytic activity.

Key Words: Melissa officinalis leaf, Melissa officinalis stem, Thrombolytic activity

INTRODUCTION

Hemostasis failure of circulatory system causes thrombus formation consequently blockage of circulatory vascular system leading to series complications such as acute myocardial or cerebral infarction, at times causing death. Allopathic thrombolytic agents used are altepase, streptokinase, anistreplase, urokinase and tissue plasminogen activator (TPA) which are characterized by series side effects such as bleeding, intracranial haemorrhage, severe anaphylactic shock, and lacks specificity [1,2].

Different medicinal uses have been reported for plants from epidemiological researches and studies in addition to their usage in folk medicine. Thrombolytic activity were proven for some foods experimentally, having ability of clot lysis. Significant observations were recorded for some herbs exhibiting thrombolytic effect [3].

Melissa officinalis, family Lamiaceae is perennial aromatic herb, widely used for food and cosmetics. Different medicinal uses have been reported for the plant such as anti-spasmodic, anti-diabetic, antioxidant, anti-inflammatory, antibacterial and antiviral [4-10]. Variant phytochemical constituents were recorded in Melissa officinalis plant such flavonoid, tannin, phenolic compounds [11,12]. Melissa leaf was the preferred part of plant as medicinal part described by pharmacopeia, often whole herb were used as herbal remedy in some places [13,14].

Lemon balm locally known as “Trinj” is commonly used herb by Kurdish community either for culinary or medicinal purposes. The present study was aimed to find the chemical composition and thrombolytic activity of different parts of Melissa officinalis growing naturally in Erbil city, Kurdistan Region\Iraq.

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MATERIALS AND METHODS:

Plant collection:
Atrial parts of Melissa officinalis were collected in mountain places (Shaqlawa) of Erbil city, Kurdistan Region\Iraq at March 2015, have been identified by Department of Pharmacognosy, College of Pharmacy \ Hawler Medical University. Stem and leaf parts of plant were dried in shade. Dried plant part materials were kept in closed container under 21-23 ° C.

Chemical composition:
Plant materials have been evaluated for quantitative chemical compositions by estimation of total phenolic, total flavonoid and total tannin contents of both parts separately.

Estimation of total phenolic contents:
Total phenolic compounds have been estimated according to the Folin-Ciocalteu method with slight modifications [15]. One ml of extract prepared from (0.5g) crude drug was mixed with (9 ml) of distilled water. One ml of Folin-Ciocalteu reagent was added to diluted extract and allowed to stand at room temperature for 5 minute, then (10ml) of (7%) sodium carbonate were added. The volume have been adjusted to (25ml) and incubated at room temperature for 90 minutes. The absorbance level was measured at 750nm using UV visible spectrophotometer. Total phenolic content was estimated from calibration curve obtained from measuring the absorbance of standard concentration of gallic acid [20, 40, 60, 80, 100 mcg/ml in distilled water]. The results were expressed as mcg gallic acid equivalent (GAE) \ g of dry weight (DW).

Estimation of total flavonoid:
Total flavonoid was measured by the aluminium chloride colorimetric method [16]. Aliquot of (1 ml) of extract prepared from (1g) was added to (10 ml) volumetric flask containing (4 ml) of distilled water. Then (0.3 ml) NaNO2 5% was added to the flask, after 5 min (0.3 ml) AlCl3 solution (10%) was also added. At 6th min, (2 ml) NaOH (1 M) was added and the total volume was made up to (10 ml) with distilled water. The solution was shaken and the absorbance level was measured versus prepared reagent blank at 510 nm. The total flavonoid content was estimated from calibration curve obtained from measuring absorbance of standard concentration of quercetine [20, 40, 60, 80 and 100 mcg/ml in distilled water]. The results were expressed as mcg quercetine equivalents (QE) \ g dry weight (DW).

Estimation of total tannin content:
Total tannin content have been estimated according to the Folin-Ciocalteu method described by Tamilselvi et al, 2012 [17] with slight modifications. (0.1 ml) of the plant mate-rial extract prepared from (0.5g) crude drug was added with (7.5 ml) of distilled water and (0.5 ml) of Folin-Ciocalteu reagent, to the mixture add (1ml) of (35%) sodium carbonate solution. The volume completed to (10ml) with distilled water. The mixture was shaken and incubated at room temperature for 30 min and absorbance was measured at 725 nm.

Total tannin content were measured from calibration curve obtained from measuring absorbance of standard concentration gallic acid [20, 40, 60, 80, 100 mcg/ml prepared in distilled water]. Total tannin content were expressed as mcg gallic acid equivalent (GAE) \ g dry weight (DW).

In-vitro thrombolytic assay:

Plant extract preparation:
Powdered plant parts [leaf (MOL) and stem (MOS) separately] introduce for aqueous ethanolic (80%) extraction using ultrasonic assisted extractor as described by Alupuli et al, 2009 [18]. Extracts were concentrated and dried under vacuum using rotary vapour machine. The dried extracts were reconstituted using distilled water at a concentration of 10mg/ml serving as stock solution, then a serial dilution extracts were prepared with concentrations of [200, 400, 600, 800 and 1000 mcg/ml], the solutions were kept overnight. Later the insoluble material were removed through filtration, the clear solution have been evaluated for thrombolytic activity [19,20].

Streptokinase (SK) solution preparation:
Lyophilized Streptokinase vials of 150000 L.U commercially available in pharmacies (Abbott). One vial content were reconstituted using sterile distilled water, mixed thoroughly. This suspension was used as a standard stock from which 100μL (30,000 L.U) was used for in-vitro thrombolytic activity evaluation [19,20].

Blood sampling:
Five ml blood sample were drawn from health human volunteers (n=5), without recent history of anticoagulant and contraceptive therapy [at least 7-10 days duration]. (0.5 ml) of blood sample were transferred in a sterile aseptic condition to ten previously weighed properly labelled eppendorf tubes [19,20].

Thrombolytic assay:
Thrombolytic assay were carried out according to the described method by Sweta et al, 2007 and Daginawala et al, 2006 [19, 20]. Each properly labelled filled eppendorf tube were incubated at 37 °C for 45 min. for clot formation. After clot formation the serum was withdrawn without disturbing the clot using syringe. Each tube was weighed again to obtain the clot weight according to the following equation:
weight of clot = weight of clot filled tube - weight of empty tube

To each eppendorf tube add (0.1 ml) of each concentration of extracts of different parts of *Melissa officinalis* separately, further more incubate the tubes for 90 min at 37 °C. After incubation the supernatant fluid released from the clot lysis were removed using syringe without disturbing the clot and the tubes were re weighed. Streptokinase (SK) drug and distilled water were used as control positive and control negative respectively. The deviation in the weight of clot between two periods of incubation were expressed as percentage of clot lysis according to the following equation:

\[
\% \text{ clot lysis} = \left( \frac{\text{weight of released clot \ before lysis}}{\text{weight clot before lysis}} \right) \times 100
\]

Statistical analysis:

All experiments were carried out in triplicate, the results were expressed as mean ± standard deviation (SD). Comparison between means were performed using one tail unpaired t-test method using Graph pad prism 6 program considering p value < 0.001 statistically significant.

RESULTS

Chemical composition

Chemical composition comprising in estimation total phenolic, total flavonoid and total tannin contents of both parts (leaf and stem) of *Melissa officinalis* plant from standard curves obtained from gallic acid and querstine (figure.1, figure.2 and figure.3), respectively. A significant chemical constituents have been detected in leaf part in comparison to the stem part of the plant (p value < 0.0001) (Table.1.).

Thrombolytic activity:

*Melissa officinalis* leaf and stem parts have been evaluated for thrombolytic activity, which are exhibited a linear relation-ship with concentration increment. Significant activity exhibited by both parts (p value < 0.0001) in comparison to the control negative (distilled water). Highest activity were expressed by the leaf part at concentration (1000 mcg/ml) (Table.2) and (figure.4).

DISCUSSION

Chemical composition

The chemical composition of leaf and stem parts of *Melissa officinalis* have been estimated inform of total phenolic, total flavonoid and total tannin contents.

M. officinalis leaves exhibited a significant amount of total phenolic contents (p value <0.001) (Table.1) which have been estimated from standard curve equation of gallic acid (figure.1) in comparison to the stem part. Other biochemical compositions of the leaf part comprised in total flavonoid and total tannin contents were showed significant values (p value <0.001) in correspondence to the stem part they have been estimated from standard equation curve of querstine (figure.2) and gallic acid (figure.3), respectively. The high phytochemical contents of leaf part confirm it is activity and medicinal value of the part [13, 21].

CONCLUSION

In attempt to evaluate different parts of *Melissa officinalis* plant growing naturally in Erbil city, we conclude that the leaf part of plant was rich in phytochemicals and both parts exhibit significant thrombolytic activity which may open a venue for production of new thrombolytic drugs from plant source. Further studies recommended for in-vivo thrombo-lytic activity of the plant.

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REFERENCES

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Table 1: Total phenol contents, total flavonoid contents and total tannin contents of leaf and stem parts of Melissa officinalis:

<table>
<thead>
<tr>
<th>Age growth period</th>
<th>Total phenol* (mcg GAE/g DW)</th>
<th>Total flavonoid* (mcg QE/g DW)</th>
<th>Total tannin* (mcg GAE/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>27.882 ± 0.544a</td>
<td>256.708 ± 1.447a</td>
<td>424.266 ± 1.026a</td>
</tr>
<tr>
<td>Stem</td>
<td>9.494 ± 0.535</td>
<td>67.412 ± 0.761</td>
<td>95.066 ± 0.551</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Data are means ±SD, (n=3).
statistically express higher value.

Figure 1: Calibration curve of Gallic acid for total phenol estimation
Mutalib: Biochemical composition and in-vitro thrombolytic activity of melissa officinalis growing naturally in kurdistan region\iraq

**Figure 2:** Calibration curve of Querstine for total flavonoid estimation

**Figure 3:** Calibration curve of Gallic acid for tannin content estimation

**Figure 4:** Clot lysis percentage of *Melisa officinalis* leaf and stem parts at different concentration in correspondence to the control positive streptokinase (SK) and control negative distilled water (DW)

**Table 2:** Thrombolytic activity of different parts of clot lysis percentage of Melisa officinalis at different concentrations:

<table>
<thead>
<tr>
<th>Tested Herb\Drug*</th>
<th>Clot lysis % (Mean ± SD)**</th>
<th>t-test value</th>
<th>P value in comparison to control negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOL1</td>
<td>27.257 ± 0.935</td>
<td>31.0314</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOL2</td>
<td>33.295 ± 0.519</td>
<td>35.8823</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOL3</td>
<td>40.839 ± 0.469</td>
<td>45.7784</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOL4</td>
<td>42.156 ± 0.841</td>
<td>48.1226</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOL5</td>
<td>44.773 ± 1.945</td>
<td>53.4302</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOS1</td>
<td>25.872 ± 1.089</td>
<td>33.0183</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOS2</td>
<td>25.293 ± 0.546</td>
<td>41.6823</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOS3</td>
<td>36.150 ± 0.835</td>
<td>52.5066</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOS4</td>
<td>37.873 ± 0.521</td>
<td>54.3973</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MOS5</td>
<td>41.482 ± 0.948</td>
<td>58.1523</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>72.164 ± 1.945</td>
<td>33.0183</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Distilled water</td>
<td>4.245 ± 0.526</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Herb concentration, MOL1 & MOS1 = 200 mcg/ml, MOL2 & MOS2 = 400 mcg/ml, MOL3 & MOS3 = 600 mcg/ml, MOL4 & MOS4 = 800 mcg/ml, MOL5 & MOS5 = 1000 mcg/ml

** n = 3