

# Original Article



## The effect of Chitosan on antioxidant activity and some secondary metabolites of *Mentha piperita* L.

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Sima Salimgandomi<sup>1\*</sup>, Azita Shabrangi<sup>1</sup>

<sup>1</sup>Department of Medicinal Chemistry, Islamic Azad University-Pharmaceutical Sciences Branch (IAUPS), Tehran, Iran.

### Abstract

Plants are almost reach sources of phenolic compounds such as flavonoids which are the most important natural antioxidants. Antioxidant compounds are essential for protecting human body against oxidative stress. However use elicitors could increase the antioxidant activity of plant. The purpose of this study was to evaluate the effects of Chitosan on the content of phenolic and flavonoid compounds and also antioxidant activity *Mentha piperita* L. In this study, firstly the *Mentha piperita* L. were grown up for 6-week period at greenhouse conditions and then were treated with 50-100  $\mu$ m of chitosan, then total phenolic and flavonoid contents were determined using spectrophotometry and finally antioxidant activities of extracts were evaluated with 2,2-diphenyl-1-picryl hydrazyl (DPPH) method and the results were analyzed with Excel software and Variance analysis testing method with SPSS software. The results showed the content of phenolic compounds in the methanol extract based on sample mg of Gallic acid /g for control with water, 50 $\mu$ m and 100 $\mu$ m treatments respectively were 146.8,233.1,339.1. Meanwhile the total volume of flavonoid content of the methanol extract per mg of Rutin/g respectively were 9.88,12.11,14.06 and concentration of the said extracts respectively were 196.3,147.7,128.62. In regard to the above results it can be concluded that due to having phenolic and flavonoid contents, *Mentha piperita* L showed that antioxidant activity could be stimulated upon Chitosan treatment moreover, antioxidant activity increased by increasing Chitosan treatment content. Therefore, this method can be used to increase antioxidant effect of plant as a natural antioxidant and all the phenolic and flavonoid contents.

**Keywords:** *Mentha piperita* L., Antioxidant, Chitosan, Phenolic content, Flavonoid content.

\*Corresponding author: Sima Salimgandomi, Islamic Azad University-Pharmaceutical Sciences Branch (IAUPS), Tehran, Iran.

Tel: 0380925650

Email address: s.sima1368@yahoo.com, sepehr\_shabrangi@yahoo.com

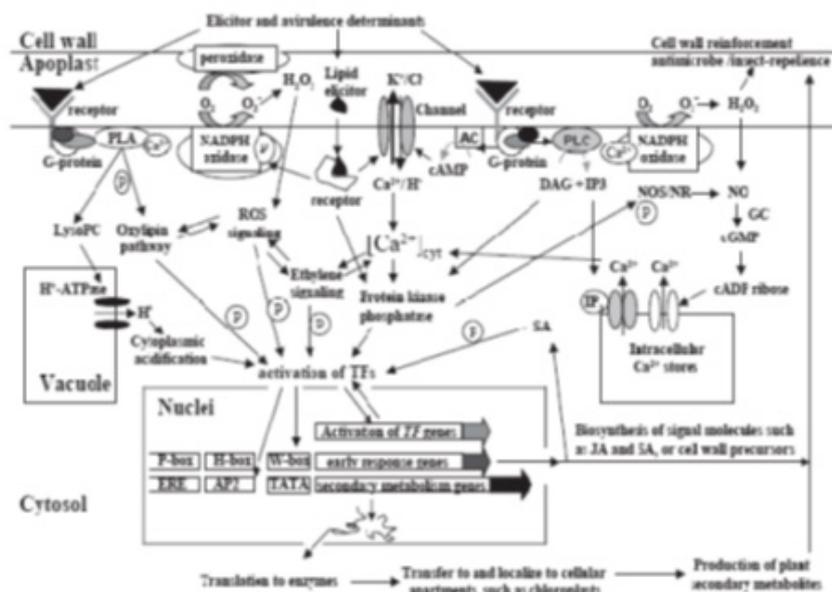
## Introduction

Peppermint *Mentha piperita* L. belongs to the split of flowering plants, Order of Tublifloral family Lamiaceae, dark Lamiaceae, mint genus, such a hybrid is a cross between the species *Mentha spicata* and *Mentha aquatica* been achieved (Foster, 1996). Peppermint used as carminative, analgesic and lifter dyspepsia Peppermint stomach stimulates the secretion of sap and reduces bloating and diarrhea in addition relieves stimulation and digestive secretion. It was a good bile laxatives, sedatives, windbreaker and stomach tonic, also, if you use it as incense against flu, bronchitis, inflammation of the larynx and has weak antiseptic effect. The use of peppermint for painful menstruation, morning sickness, and recommended use of the new plant was crushed and its use to improve headache have been reported (Fetter, 1983; Singh, 2011).

Elicitors are compounds with biological or abiotic origin that leads to the biosynthesis and through the induction of immune responses leading to the biosynthesis and accumulation of secondary metabolites (Zhao, 2005).

Generally, the use of elicitors in studies of plant metabolites biotech pursues two main objectives. 1: Obtain information about the biosynthetic

pathways leading to the formation and regulation of secondary metabolites. 2: Production of secondary metabolites for commercial applications (Roberts & Shuler 1997). The widespread use of fungal elicitor in orders to produce secondary metabolites in plants used and is effective in stimulating the production of secondary metabolites (Namdeo, 2007; Ionkova, 2007). In nature, plants respond to attack by pathogens, insects, herbivores, and other biotic and abiotic stress by activating defense mechanisms including the induction of secondary metabolites such as phytoalexins and hypersensitivity responses and structural barriers, such as the deposition of lignin in the cell wall (Vasconsuelo & Boland 2007). Elicitors may activate new genes that enzyme and various biosynthetic routes of secondary metabolites are set up and the formation (Zhang et al. 2006; Howlett, 2006). Starting in plant defense responses induced a network of signal transduction that starts with molecular recognition of elicitor by receptors. The most important compounds that induce a large number of genes related to defense are regulators of jasmonate, ethylene and salicylic acid (Furden et al. 2005). When the plants are affected by elicitor rapid biochemical response occurs as follows (figure1).



AC: AMP cyclase; DAG, diacylglycerol; GC: GMP cyclase; IP3: Inositol-1,4,5-trisphosphate; JA: jasmonic acid; NOS: nitric oxide synthase; NR: Nitrate reductase; PLA: phospholipase A; PLC: phospholipase C; PLD, phospholipase D; ROS, reactive oxygen species; SA: salicylic acid.

Figure 1: Signal transduction pathway signaling in plant defense responses

- Elicitor binding to plasma membrane receptors
- Output current induction  $K^+$  and  $Cl^-$
- Rapid changes in the pattern of protein phosphorylation, activation
- Protein kinase, mitogen-activated protein kinase, (MAPK) and G protein
- Synthesis of phospholipases A, C and (PLA, PLC, PLD) Send to secondary production, such as Inositol -1, 4, 5 triphosphate (IP3) Diacyl -glycerol (DAG) that regulator of  $Ca^{2+}$  intracellular signal transduction pathways and Nitric oxide.
- Cytoplasmic acidification caused by inactivation  $H^+$ -ATPase, PH reduction and increased polarity of the membrane outside the cell
- Activation of NADPH responsible for the production of reactive oxygen Cytoskeletal reorganization
- Production of reactive oxygen species such as superoxide and peroxide Hydrogen
- Production proteins associated with pathogens, pathogenesis-related (PR), In order to release Polysaccharides of the cell,( Internal elicitor), rich Glycoprotein of Hydroxyproline and proteinase inhibitor
- Cell death at the site of infection (hypersensitive response)-
- Structural changes in the cell wall (wall lignin cell)-
- Produce jasmonate and salicylic acid as messengers secondary-
- Activate defense genes such as phenyl ala-

nine ammonia-lyase (PLA), glutathione S-transferase(GST), Chalcone synthases (CHS)

- Production of defensive molecules Such as tannins and phytoalexins
- Systemic acquired resistance (SAR). (Zhao J, Davis LC, Verpoorte R ,2005).

Chitosan is a Cationic polysaccharide that is obtained through the process of deacetylation of chitin alkaline. There are three groups of active chitosan contains an amino group and two hydroxyl groups in C-3 and C-2 and C-6 (figure2). These groups can be obtained by causing changes in various derivatives of chitosan that have many applications (Shahidi et al. 1999; Maghsoodi, 2003). In fact, chitosan is a polymer combination of glucosamine and N-acetyl glucosamine, which are connected by bonds of 1 and 4 glycoside. It has no environmental hazard because no effect on the mammalian toxicity and the environment and on the other hand has a high frequency in nature. Chitin and chitosan highly regarded because the benefits. For a lot of applications that covers a wide range of industries .Chitin polymer applications in the food industry can be cited food preservation and prevent microbial spoilage of Production of films and coatings with biodegradability, recycling waste from food processing, water treatment and transparency in the process and juices. Chitosan can be achieved from the shells of crustaceans, insects, many fungi, algae and yeasts (Shahidi et al. 1999; No & Meyers1995; Bhattacharya, 2010).

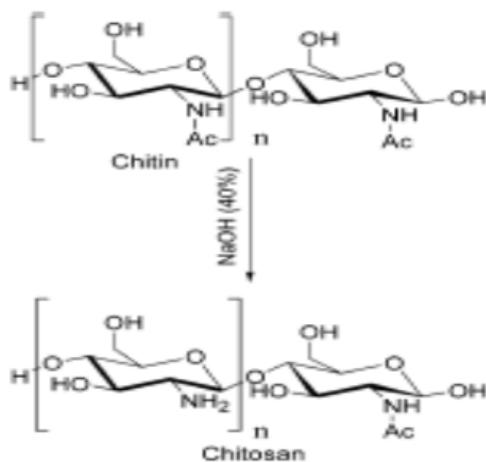


Figure 2: chemical structure of chitin and chitosan

Despite the existence of various antioxidants in plasma, immune system alone can not eliminate free radicals in the body that is why it needs to provide antioxidants from external sources it is therefore essential need for potent antioxidants with less toxicity and better efficiency (Shariatifar et al. 2012). Antioxidants are natural and synthetic origin the use of synthetic and synthetic antioxidants is limited because of the deleterious effects. Plants having phenolic compounds and flavonoids have antioxidant potential (Sharifi, 2012, Singh, 2011). The aim of this study is that using chitosan as a biological elicitor increase the production of secondary metabolites and antioxidant activity.

## Materials and Methods

All materials tested in this study were purchased from Sigma and Merck, Germany.

### Research Methodology

peppermint *Mentha piperita* L. were prepared of Khuzestan Fadak Research Center, and fresh rhizomes were planted with a diameter of 12 cm in the soil phosphate .studied for 6 weeks at room temperature  $23 \pm 1$  ° C and photoperiod were maintained for 16 hours . Then spray the shoots and the soil surface with chitosan at 50 and 100  $\mu$ M concentrations in both groups were treated for a week. In addition to these two groups, a control group as well as water was considered. Finally, the aboveground plant parts are arranged in three separate groups and were dried and then powdered and ready on the day of extraction were extracted.

### Extraction

By adding ethanol to the samples in the hopper decantation, extraction operation was carried out by percolation and dried extracts were stored in a closed container in the refrigerator

temperature of +4.

### Determine the content of phenolic

Total phenolic content of the plant was measured using the Folin-ciocalteu reagent, so that each ml of the extract concentration of 0.01 grams per 100 ml, 5 ml of Folin reagent, 4 mL of 7.5% sodium carbonate was added. After one hour at room temperature and dark place absorption at a wavelength of 765 nm by spectrophotometer and measured 3 times. Gallic acid as standard for calibration curve .total phenolic content in mg gallic acid per gr of extract was measured (Chang et al. 2002).

### Determine the flavonoid content

Flavonoid content using a method Pharmacopoeia (1989) Rutin as a reference compound used to determine, aluminum chloride colorimetric method was used with a standard method for measuring the total flavonoid. One ml of the extract solution with a concentration of 0.01 to 1 mL of 2% solution of aluminum chloride was mixed in a test tube. After an hour, the absorption of the sample taken from the test tube was read at a wavelength of 415 nm against blank at ambient temperature. The routine used as a standard for calibration curve. According mg rutin per gram of extract the flavonoid was measured. The experiments were performed at 3, and the average was reported (Ordoñez et al. 2006).

### Evaluation of antioxidant activity

Evaluation of antioxidant activity using free radical scavenging 2,2-diphenyl-1-Pyryl Hydrazyl (DPPH) were measured. Purple DPPH in a specific wavelength decreased by antioxidants and it is yellow. The ability of the hydrogen atoms or electrons in various combinations with discolorations purple DPPH solution in methanol is examined. So that 1 ml of different concentrations of plant extract was mixed with 3 ml of 0.004% of DPPH. After half an hour then kept in the dark at room temperature, the absorbance at 517 nm was read

against the blank. And free radical scavenging rate was calculated by the following formula (Ames, 2005; Burits & Bucar 2000).

$$I\% = \left( \frac{A_{blank} - A_{sample}}{A_{blank}} \right) \times 100$$

In this formula A Blank is the optical density of the negative control all ingredients except extracts. A sample is represents the optical density of different concentrations of the extracts. Then the concentration of the extract to inhibit 50% of free radicals has been calculated. The higher this number is smaller percent free radical scavenging more and the antioxidant power increases. Repeat in 3 separate experiments were performed.

## Results

Analyze the results to determine the mean and standard deviation charts is used Excel software. Also of significance of differences of one-way analysis of variance (ANOVA) was used in SPSS. Radical-scavenging and antioxidant activity was measured using DPPH free radical scavenging, with increasing concentrations of chitosan treatment decreased the percentage of free radical scavenging. In other words, antioxidant and free radical scavenging increased. The amount of phenolic and flavonoid content was measured and indicated that the content of phenolic and flavonoid compounds with increasing concentration of chitosan. Results are presented in tables.

**Table1: Extent of IC<sub>50</sub> extracts**

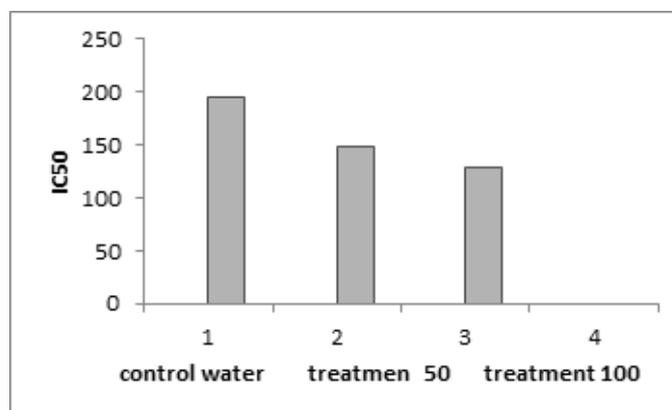
Sample extract	water control	50 μM	100μM
IC <sub>50</sub>	196.3μg/ml	147.7μg/ml	128.6μg/ml

**Table 2: The phenolic compound in the extracts**

Sample extract	water control	50 μM	100μM
Mg Gallic acid / gr sample	146.8	233.1	339.1

**Table 3: The flavonoid compounds in the extracts**

Sample extract	water control	50 μM	100μM
Mg Rutin/ gr sample	9.8	12.11	14.06



**Chart 1: Comparison of IC<sub>50</sub> in the extracts**

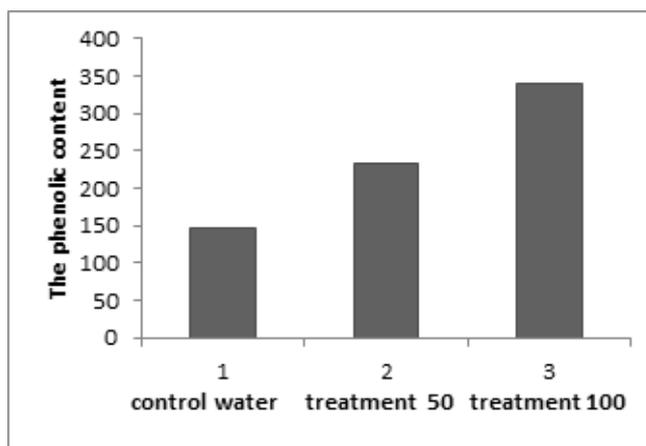


Chart 2: Comparison of the content of phenolic in extracts

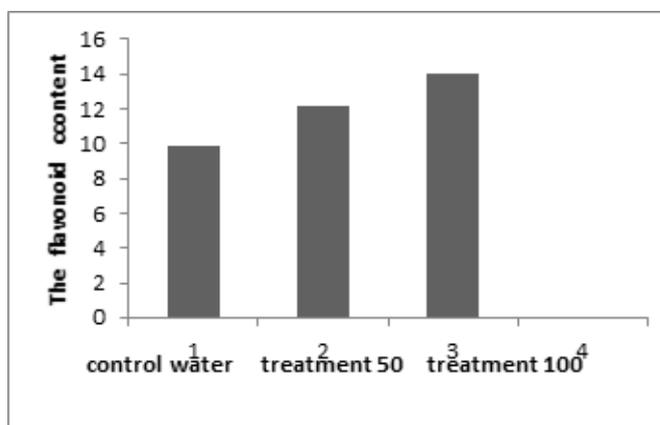


Chart 3: Comparison of the content of flavonoids in extracts

## Discussion

In this study, we used chitosan as biological elicitor, with the aim of increase the amount of phenolic and flavonoid content or in other words, secondary metabolites, and increase the antioxidant activity of peppermint. Chitosan sprays significantly increased growth of young orchid plants (Chandrkrachang, 2002). Limpanavech et al. 2003, studied the effects of concentration, degree of deacetylation, and polymerisation of chitosan at deflasking, on growth and development of *Dendrobium SoniaJo 'Eiskul'*, the major cut flower orchid of Thailand (Pornpeanpakdee et al. 2006). Chitosan-treated orchid plants (*Dendrobium Sensational 'Purple'*) had more flower shoots and yields tended to be higher on extra grade

compared to the control plants (Chandrkrachang et al. 2005). That it was closer to our study, we have seen, the growth of peppermint increased with chitosan foliar spray. Although the amount has not been thoroughly considered and measured, but this increase was visible. According to research about peppermint a rich source of phenolic and flavonoids compounds and according to studies peppermint extract shows good antioxidant activity, is the effect of absorbing oxygen free radicals that most or the radical species (Solecka, 1997). The effect was seen in samples in this study, the content of phenolic and flavonoids compounds in the measurement samples and their increase after treatment with elicitor chitosan

as a foliar spray, amount absorption, in total phenolic and flavonoid content increased, that is directly related to the amount of phenol and flavonoids compounds. Decreased absorption of antioxidant activity that is represents the increase Radical-scavenging and neutralizing and a significant increase in antioxidant power with increasing concentrations of chitosan. The use of chitosan foliar spray, Significant increase are in secondary metabolites, especially phenolic and flavonoids compounds that perhaps destroyed the combination of hydrogen peroxide and free radicals and increased antioxidant activity in this way. Chitosan has anti-fungal and anti-microbial properties and thus may protect plant against bacterial and fungal pathogens, that this is a positive side to improve total phenolic and flavonoid content and secondary plant compounds. Generally in this study it was found that the use of chitosan as a foliar spray as an elicitor increased phenolic compounds and flavonoids and antioxidant power, that is consistent with chitosan properties and previous research.

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