Characterization of Peppermint Plant Extract and Antimicrobial Activity

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Abstract. The use of plant extracts in the medical and pharmacological fields have been increased in the last years. The plants have shown to possess materials with important features that can be used as a treatment in multiple health risks such as inflammation, cardiovascular disorders, bacteria and fungus. Peppermint was used extensively as medical herbs and have given a remarkable results. We aimed to prepare water and alcoholic (methanol) extract of peppermint leaves and characterize the presence of some phytochemical compounds qualitatively, along with some trace elements (copper, zinc and selenium). The antimicrobial activity of peppermint extract was investigated against two Gram negative e*scherichia coli* as well as *klebsiella* sp, also two Gram positive bacterial strains *Staphylococcus epidermidis* as well as *Staphylococcus aureus*, as well as one fungi (*Candida albicans*). We have obtained the presence of flavonoids, polyphenolic compounds, steroids, carbohydrate, and protein contents in the extract of peppermint. Copper, zinc and selenium levels were detected in good amount in the extract. Furthermore, both water and methanol extract of peppermint have shown good antimicrobial activity against the experimented strains. Nevertheless, the extraction by methanol have shown more powerful activity than water against all strains.

Keywords: Peppermint, mentha piperita, antibacterial, phytochemicals.

1. Introduction

Plants have long been seen as a possible source of medicine, and have been utilized to treat a variety of maladies in primitive form. A range of bioactive chemicals found in various regions of plants has rekindled interest in producing an alternative medicine. The conventional medicinal herbs system has been utilized internationally since olden days, and as a result, a large body of literature on the antibacterial activity of many plant species is accessible [1].

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In the culinary, beverage, cosmetic, health, and tobacco industries, peppermint (Mentha Piperita) oil is frequently utilized [2-5]. Menthone, menthofuran, and menthol are the main components of peppermint oil. Peppermint oil has antioxidant effects [6, 7], antibacterial activity [8,] and is one of the most important ingredients in certain over-the-counter irritable bowel syndrome medicines in Europe [9]. Although both the extract and the leaves are listed as biological additions, only the extract is said to be employed. Peppermint Water is a flavoring ingredient or scent component, according to [10]. The volatile oil content of peppermint leaves ranges from 0.5 to 4%. Furthermore, polyphenolic components such as rosmarinic acid, flavone and flavanone glycosides, and both flavone and flavanone glycosides make up much to 21.7 percent of this extensively used herbal medication [11].
Peppermint oil has antibacterial efficacy against Aspergillus niger, Rhizopus solani, and Alternaria alternative [12], as well as Pseudomonas syringe, Xanthamonas campestris, E. coli, Pseudomonas aeruginosa, and Salmonella typhimurium [13]. Peppermint leaf juice had stronger antibacterial activity than peppermint stem juice against Gram negative bacteria (Saeed and Tariq, 2005). Peppermint oil had stronger antibacterial activity than S. aureus against Candida albicans and E. coli [14]. Because of the reports on the importance features of peppermint extract, we have designed this study to characterize the extract of peppermint leaf extract for phytochemicals identification, some trace elements presence such as copper, selenium, and zinc. Furthermore, we have investigated the antimicrobial effect of peppermint extract on four different types of microbes.

2. Materials and Methods

2.1. Preparation of peppermint extract

Peppermint were purchased from the local market in Kut – Wasit – Iraq. The leaf of peppermint plants were washed to remove the dust and any other pollutants, then it was minced. A Weight of 1 g of the minced peppermint were added to 50mL of deionized water or methanol (Merk, Germany). The water or methanol mixtures of peppermint were shaked by using a waterbath with shaker under mild heat (50-55 °C) for 1h. Then, the beakers were allowed to stand at room temperature to cool down, then it was filtered, and the solution was stored for analyses.

2.2. Trace elements analyses

The determination of copper (Cu), Zinc (Zn), and Selenium (Se) were performed by using atomic absorption spectroscopy (AAS) approach. The analyses required a pre-step, in which 10mL of the water extract were mixed with 10 drops of HNO_3 (Merck, Germany) in a flash and covered with a glass sealer. The trace elements were measured by using Shimadzu AA-670, Flame AAS [15].

2.3. Phytochemical qualitative analyses

The chemical components of the produced fruit extract were detected using several assays according to the standard AOAC (1990) procedure [16]. Glycosides, alkaloids, saponins, phenolic substances, tannins, flavonoids, proteins, and steroids were among them.

2.4. Antimicrobial activity

Both methanol and water extracts of peppermint were examined against two Gram negative bacterial strains e*scherichia coli* as well as *klebsiella* sp, also two Gram positive bacterial strains *Staphylococcus epidermidis* also *Staphylococcus aureus*, as well as one fungi (*Candida albicans*). In Petri dishes, well diffusion method was used. Two wells in the agar

medium were made in a radius of 1mm, and 15µL of each extract were added to the corresponded wells. The plates were incubated at 37 °C for one day, and the inhibition zones were determined.

3. Results and Discussion

3.1. Characterization of the plant extract

Table 1 contains the results of qualitative analyses of phytochemicals in peppermint extract. The peppermint extract has shown the presence of carbohydrates, this was in agreement with Petkova *et al.* (2017) who have reported the presence of fructose and sucrose in the extract of peppermint [17]. Phenolic compounds and flavonoids were observed in the extract of peppermint leaves. This content of peppermint results in its strong antioxidant behaviors. This was in agreement with several studies in different countries [18-21]. Proteins, steroid, alkaloids were positively obtained in the extract of peppermint leaves, and this was in agreement with Sujana *et al.* (2013) [22]. On the other hand, saponins were not detected in the extract.

Table 1: Qualitative phytochemical outcomes.

Test	Reagent	Color	Result
Carbohydrates	Molish test	Violet ring	Positive
	Benedict test	Orange	Positive
Proteins	Biuret test	Purple blue	Positive
Steroids	Liebermann	Yellow	Positive
	Burchard test	Yellow	Positive
Phenolic compounds	Ferric chloride test	Green	Positive
Tannins and Flavonoids	Lead acetate	Light yellow	Positive
Alkaloids	Mayer's reagent	White	Positive
	Wagner reagent	Brown	Positive
Saponins	Fast stirring	Dense foam for long time	Negative

Table 2 contains the concentrations of Cu, Zn and Se that obtained by using a flame AAS on peppermint sample. The sample has shown to consist of 1.16 µg/L of Cu, 69.36 µg/L of Zn and 1.26 µg/L Se. The results were in agreement with a previous studies [23, 24]. Zn and Se are introduced in two antioxidant enzymes [25], this high content of these two trace elements would result in the enhancement of the plant's antioxidant capacity.

Table 2: Trace elements in peppermint extract.

Element	Concentration (µg/L)
Cu	1.16421
Zn	69.3580
Se	1.2576

3.2. In vitro antimicrobial

Table 3 contains the inhibition zones created by the presence of water or methanol extract of peppermint leaves. The extract solution of peppermint has shown to have an antimicrobial

effect against all of the strains that have been used in this study. The observations has indicated that methanol extract of peppermint exhibited a more powerful antimicrobial effect than the water extract solution. This data were close to the antimicrobial activity of peppermint extract from a previous studies [1, 14, 22, 26-28]. This effect of the peppermint extract could attributed to the high content of medicinal phytochemical such as flavonoids and phenolic compounds whose have an anti-inflammatory characteristics [29].

Table 3: Inhibition zones of water and methanol extracts of peppermints.

Type of microbe	Water extract	Methanol extract
E. coli	4.0	4.5
klebsiella sp.	4.2	5.1
S. epidermidis	3.6	3.8
S. aureus	4.3	5.0
Candida albicans	2.5	3.1

4. Conclusions

The extraction of peppermint leaves have shown the presence of multiple important and medicinal phytochemicals such as the flavonoids and phenolic compounds. Furthermore, it has shown a good percentage of the essential trace elements Cu, Zn, and Se. These components of the peppermint extract would result in enhancement the properties of medical application. The antimicrobial examination of the peppermint extract revealed a good inhibition zones against the five experimented strains.

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