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Estimation of Total Phenolic Compounds, Essential Oils and Antibacterial Activity of Tannins and Glycosides Extracted from Rosemary (*Rosemarinus officinalis* L.) and Wild Mint (*Mentha longifolia* L.).

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Abstract

The recent study was conducted to evaluate total phenolic compounds and essential oils obtained from rosemary (*Rosemarinus officinalis* L.) and wild mint (*Mentha longifolia* L.) purchased from the local market in Baghdad, Iraq. Tannins and glycosides were extracted from them to study their antibacterial activity against *Bacillus cereus* and *Escherichia coli*. It was found that the content of total phenolic compounds of wild mint extract (43.684 mg/ml) was slightly higher than that of rosemary extract (36.584 mg/ml), whereas the oil contents were 2.33 and 2.41%, respectively. The results also showed that the contents of tannins and glycosides of rosemary (14.1 and 13.09%, respectively) were higher than that of wild mint (6.35 and 3.64, respectively), but the tannins and glycosides extracted from wild mint were more efficient in inhibiting the growth of Bacillus cereus as compared with that extracted from rosemary. Tannins and glycosides extracted from the two plants had no effect on growth of gram-negative bacterium. No antibacterial activity was observed when a combination of tannins from the two plants was used. The same observation was recorded for glycosides.

Keywords: rosemary, wild mint, tannins, glycosides, antibacterial activity

INTRODUCTION

Rosemary (*Rosemarinus officinalis* L.) is evergreen perennial herb belonging to the family Lamiaceae. It is known in its uses in different medical and industrial fields, as well as its use in food as a flavor [1]. In the medical field, this plant was used as a protective agent for the liver [2], against microorganisms [3], against inflammation [4], hyperglycemia [5], blood clotting [6] and cancer [7, 8]. It was used industrially in the production of perfumes and cosmetics, and ready-to-use extracts were prepared from this plant [9] It was also included in the food industry because it has a distinctive flavor and antioxidant capacity []10]. This medicinal herb with the volatile oils produced from it represent one of the desired flavors in the preparation of food, and is also considered as a source of high-phenolic compounds, which are highly effective against both Gram negative and positive bacteria , and among these compounds are the derivatives of the caffaic acid, especially rosemaric acid which is very effective [11].
Wild mint (*Mentha longifolia* L.) has been widely grown in the Mediterranean regions, Europe, Australia and North Africa (12). This herb has a distinct flavor, and several of its parts have been used in traditional medicines ([13]. Many authors (14) proved the antimicrobial activity that is distinct various wild mint extracts. Others [15] previously mentioned the possibility of using different parts of this plant in different areas such as treatment of abdominal pain, headache and antispasmodic. Furthermore, it has been found to have sedative and useful effects for the nervous system [16].
Tannins and glycosides are important active ingredients in medicinal plants because they have therapeutic effects in many conditions. Tannins are a group of compounds with complex chemical compositions derived from carbohydrates, which are amorphous polyphenols. They are soluble in water forming an acidic emulsion with a clenching taste. These substances can deposit proteins and alkaloids from their solutions. Their molecular weights are between 500 and 3000 Dalton. Tannins hurt

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nutrition due to their ability to bind to vitamins and minerals and prevent or reduce their absorption [18]. Glycosides are compounds that result from the binding of a special type of organic matter resulting from metabolism with one or more simple sugars. Glycosides are widely different between plants due to differences in both glycon and aglycon parts of glycosides. These substances have many uses in the treatment of urinary tract infections, headaches, fever, and high blood pressure [19].

Due to the importance of medicinal plants in therapeutic aspects and the increasing dependence on natural alternatives in the treatment of some cases, the current study was conducted on two medicinal, namely rosemary and wild mint to determine their contents of total phenols, tannins, glycosides and volatile oils. Antagonism test was conducted to study the effect of tannins and glycosides on two types of bacteria, one positive and the other negative.

MATERIALS AND METHODS

Collection and preparation of tissue specimens

Samples of rosemary and wild mint were obtained from more than one shop in the local market (Shorja , Baghdad). The samples were cleaned and ground using a laboratory mill and passed from a fine sieve to obtain homogeneous powder. The samples were placed in opaque sealed containers and kept in a cool place for testing.

Total phenols extracted

Total phenols were extracted in the manner described by [20] with some modifications. The Fulin Ciacalteau reagent was used in the estimation process, and the absorbance was measured on 750 nm wavelength in the UV110 spectrophotometer (Shimadzu, Japan). The concentrations were determined from a standard curve prepared from suitable concentrations of gallic acid.

The volatile oils were extracted using a Clavinger distillation device according to the procedure described in (10). The tannins were extracted and estimated using the method described by [21]. Forty-grams samples were extracted with distilled water and re-extracted using 80% methanol and acetone 70%. The extracts were concentrated using rotary evaporator and dried in an electric oven at a temperature of 60 m. The glycosides were extracted according to [(22] and were gravimetrically estimated after drying the glycosidic extracts using the rotary evaporator.

The antimicrobial activity test of both tannins and glycosides extracted from rosemary and wild mint was done separately using Well method described by [23]. A type of Gram-positive bacteria (Bacillus sp.) and a type of Gram-negative bacteria (Escherichia coli) isolated in a nutritional and immunological study [24].

RESULTS AND DISCUSSION

Figure 1 shows the phenolic content of rosemary and wild mint. It is clear that the extract of the wild mint is higher in total phenols compared with the rosemary extract (43,684 vs. 36,584 mg/ml). Numerous studies [25] were pointed to the contents of genus Mentha from total phenols. It was found [14] that the wild mint oil contained 4,5 g / 100 g total phenols calculated based on gallic acid, which is higher than recorded in the current study. This may be due to differences among varieties and the methods used in extraction. In a previous study, the percentage of total phenols in the rosemary extract was 31.86%, expressed as tannic acid and based on the dry weight of the extract. Another study [27] claimed that rosemary contained total phenols with a concentration of 30.2 mg / g, confirming that this plant is an important source of phenolic compounds since it contains many types of them. Many phenolic compounds were characterized in the extract of the rosemary and the compound so-called Isocarnosol forms a high proportion of them [28]. A team of researchers (1] obtained an extract of rosemary leaves containing different phenolic compounds, most notably di- and triterpenes, which proved to be effective as antioxidants and inhibitors of bacterial growth.

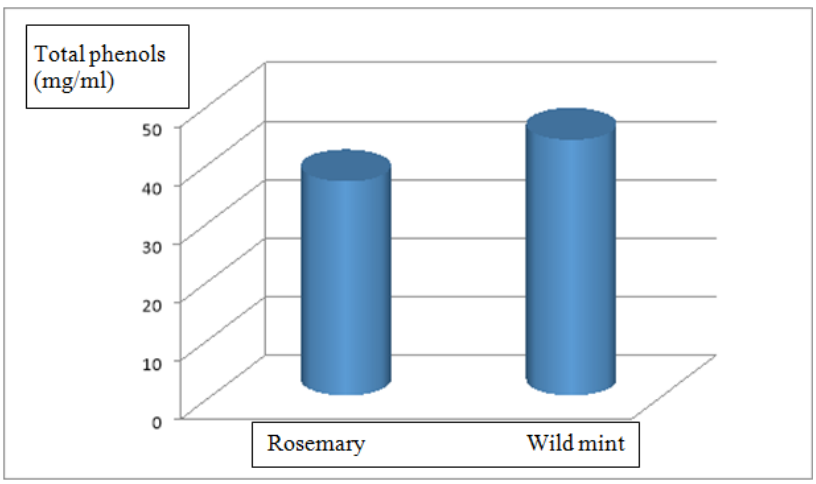

Figure 1. Concentrations of total phenols (mg/ml) in plant extracts of rosemary and wild mint

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Table 1 shows the percentages of tannins, glycosides and volatile oils in the rosemary and wild mint extracts. It is clear that rosemary is significantly higher in the ratio of tannins and glycosides (14.10 and 13.09%, respectively) compared with the wild mint (6,35 and 3.64%, respectively), while the ratio of volatile oils was almost similar between the two species (2.33% in the rosemary and 2.41% in the wild mint).

The proportion of tannins in several species of plants was found to be between 3.77% in buckwheat and 17.2% in peanut. When comparing the results of this study with the mentioned range, the rosemary can be considered as one of the tannin-rich plants. Some studies (30) indicate the high content of tannins in the rosemary extract, and some researchers (31) found that the solvent system used for extraction significantly affects the proportion of extracted tannins. For example, the ratio of extraction was 26% using distilled water, and it was 41 % when using the mixture 75% water + 25% acetone.

The results of this study indicate the high content of the glycosides in the rosemary plant, and this result reinforces the previous observations (32) about the possession of this plant different types of glycosides, which represent the important part of the active substances such as epinegene glycosides and lutoline glycosides. However, The percentage of glycosides is lower than that recorded in the rosemary plant. Studies (33) emphasize the importance of the plant's glycosides as an additional source of energy along with the important physiological roles it plays.

Table 1. Percentage of some active ingredients in the rosemary and fenugreek plants (%)

Component	Rosemary	Wild Mint
tannins	10.14	6.35
glycosides	13.09	3.64
essential oils	2.33	2.41

The results of the percentages of volatile oils extracted from the rosemary varied widely, as it was found (1) that the percentage of volatile oils in the leaves of rosemary plant was 0.93%, while (34) found that it was 4.83%. These differences may be due to the variations in genetic ability and susceptibility that varies according to species and environmental conditions. Furthermore the extraction method has a significant effect on the ratio of oil extracted. It was found (35) that the percentage of oil extracted from one of the varieties was estimated to be 1.3%, and this percentage was low compared to other studies (36). The authors suggested thatthis variation clearly due to the differences between species and environmental conditions during the growing season (37), as well as the effect of the extraction method.

Table 2 shows the results of the inhibitory effect of tannins and glycicides obtained from rosemary and wild mint against Gram-positive bacteria, as these extracts did not show a biological effect on the Gram-negative bacteria. The table shows that active compounds in the wild mint were more efficient in inhibiting the growth of *Bacillus sp*. The diameters of the inhibition zone were 27 and 25 mm when the first dilution of the wild mint and rosemary tannins was used, respectively. The superiority was apparent when the second dilution was used, with the diameter of the inhibition zone were 25 and 20 mm, respectively, although the rosemary was superior in its content to the tannins (Table 2). This can be attributed to the type of tannins found in the extract, not to its quantity. The wild mint contains a range of phenolic compounds with a variety of effects (38).

The glycosides obtained from the rosemary and the wild mint showed high efficacy in inhibiting the growth of *Bacillus* sp. Glycosides of wild mint were more effective in this respect. The diameters of the inhibition zone (picture1) were 28 and 25 mm for the first and second dilution respectively, while these values were 22 and 20 mm for rosemary extract used in the two dilutions, respectively. It was mentioned (39) the types of glycosides and basic synthetic pathways of them and referred to the distinctive characteristic of having multiple biological activities. Some authors (40) noted that these compounds are highly effective against many types of bacteria and indicated the possibility of the introduction of these compounds in food products as well as in pharmaceutical applications.

There is no observed inhibitory effect on the two types of bacteria when using a combination of plant-derived tannins, and the same condition applies to the glycosides. This may indicate undesirable inter-compounds interferences that may have eliminated the biological efficacy of the combinations. The probability of occurrence or absence of such harmful interference is possible, especially with the presence of many compounds with effective side groups, and the use of the extracts of tannin and glycoside in raw form and not pure.

Table 2. Antibacterial activity of tannins and glycosides extracted from rosemary and wild mint towards *Bacillus* sp

Plant	Concentration	The diameter of Inhibition Zone (mm)	
		Tannins	glycosides
Rosemary	1	25	22
	2	20	20
Wild Mint	1	27	28
	2	25	25

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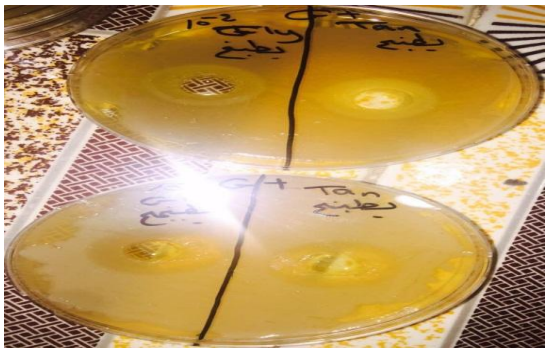

Picture1. Zones of inhibition caused by some active components of the extracts of the plants under study

The current study indicates that tannins and glycosides obtained from rosemary and wild mint can be used to treat the pathogenic conditions caused by the rod-shaped Gram-positive bacteria belonging to the genus Bacillus. It is not useful to use these herb extracts to treat the pathogenic conditions caused by Gram-negative bacteria, especially *Escherichia coli*, as tannins and glycosides did not inhibit their growth. The simultaneous use of these two types of treatment may be of no benefit for the low efficacy of both the recorded tannin and glycoside mixture. The antagonism between active compounds prepared from the most specific plant type requires detailed studies to find and try to address the causes.

CONCLUSION

Rosemary and wild mint extracts are characterized by high contents of tannins and glycosides. These compounds have antibacterial activities against some Gram-positive and negative bacteria. They must be used separately because of the probable interaction between them.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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