

Extraction, identification and antimicrobial activity of phenolic compounds associated with HPLC in extracts of *Teucrium polium* wild plant

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Abstract

Teucrium polium plants have been used in traditional medicine for treatment of different diseases. The aim of this study was to investigate phenolic contents by high-performance liquid chromatography (HPLC) and the antimicrobial activity of *Teucrium polium* extracts against six types of pathogenic bacteria (three Gram-positive and three Gram-negative). The HPLC analysis showed that extracts consist of phenolic compounds (Catechol, Coumarin and Cinnamic acid). Cultures were treated with extracts in separate treatments; both herbal extracts significantly inhibited the growth of all six types of bacteria in 200 and 100 mg/ml concentrations comparing with the very similar inhibition of two antibiotics as controls (Amikacin and Gentamycin). The lowest concentrations (50, 25 and 12.5) showed lower inhibition comparing with the extracts with high concentrations for all six types of bacteria.

Keywords: HPLC, *Teucrium polium*, Catechol, Coumarin, Cinnamic acid, Amikacin, Gentamycin.

INTRODUCTION

Thousand years ago, people had already known about the usage of natural source as medicinal agents, nowadays, with rapid development of science and technology, there are a lot of modern

drugs with less side effects which were derived from natural source. Every year, abundant new compounds were isolated from traditional medicine or herbal. This also indicates that the isolated compounds from herbal plants play a very important role in pharmaceutical industry; however, a lot of herbal plants still have not been explored for their phytochemical constituents (Hostettmann K. *et al.* 1998; Balandrin M. F. *et al.* 1985).

Teucrium polium L. (felty germander) is a wild growing, flowering species; it is a perennial, aromatic plant, 20–50 cm high, with green-grayish leaves and white to light pink flowers, that occurs from June to August and is found abundantly in Southwestern Asia, Europe (Mediterranean region), and North Africa (Djabou *et al.*, 2012). Traditionally, *T. polium* has been used for the treatment of different diseases in humans such as gastrointestinal disorders (kidney and liver diseases, abdominal and intestinal pain), inflammation, eczema, urinary tract inflammation, diabetes, and rheumatic diseases (Abu-Irmaileh and Afifi, 2003; Everest and Ozturk, 2005). This plant is mainly used in traditional medicine to improve mental performance (Perry *et al.*, 1996). Numerous *in vivo* and *in vitro* studies have confirmed different biological activities of *T. polium* such as anti-inflammatory and anti-rheumatoid (Tariq *et al.*, 1989), antimicrobial (Balmekki *et al.*, 2013), anti-hypertensive (Suleiman *et al.*, 1988) and hypoglycaemic properties (Kasabri *et al.*, 2011). Moreover, many studies reported that different extracts of *T. polium* exhibit significant free radical scavenging activity, hydroxyl radical scavenging, and antioxidant activity *in vitro* (Kadifkova-Panovska *et al.*, 2005).

The biological activity of this plant, including its antioxidant activity as well, depends on its phenolic compositions. Phenolic compounds are one of the largest groups of secondary plant constituents. In addition the aromatic benzene ring system, phenols may bear other substituent especially methyl groups. Simple phenols consist of aromatic ring in which a hydrogen is replaced by hydroxyl group (Waterman, P. and Mole, S., 1994; Bruneton, J., 1995). Phenolic compounds occupy a specific place among biologically active substances of plant origin. As secondary metabolites, they are synthesized by almost any plant (Zaprometov, M.N. and Fenol'nye S., 1993).

MATERIAL AND METHODS

Plant material collections

In May 2019, aerial parts of the selected species of flowering plant *Teucrium polium* were collected from the natural population in the territory of Mosul. The taxonomic authenticity of the samples was conducted at the college of education for pure sciences, University of Mosul. They were dried under ambient laboratory conditions, sealed and stored until needed for the experiment.

Preparation of plant extract

Dried powdered plants material (32g) was successively extracted by mixing with 800 ml solvent for 24 h at room temperature. Each of the homogenates was filtrated and the residue was re-extracted for complete exhaustion. Each filtrate was concentrated to dryness by evaporated under vacuum and re-dissolved in respective solvents, ethanol, ethyl acetate and n-hexane.

Separation of phenols

Different sizes of columns used for the separation of phenols associated with silica gel mesh size 60-120 and by using different percentages of solvents to make many fractions. This was followed by using TLC technique to separate all phenols which detected in this study.

High-performance liquid chromatography (HPLC) analysis of phenols

The extracts were analyzed by HPLC system (Shimadzu, Kyoto, Japan) Separation was achieved on a Luna C18 (250×4.6 mm I.D., 5 mm) (Phenomenex, Torrance, CA, USA) column at 30 °C, with 1.0 ml/min flow rate. The chromatography data were processed using LC Solution computer software (Shimadzu).

Antimicrobial activity

Six types of pathogenic bacteria (three Gram-positive and three Gram-negative) from college of education for pure sciences, University of Mosul were used for antimicrobial activity of the plant extracts,

Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Escherichia coli, Proteus vulgaris and Salmonella typhimurium.

RESULTS AND DISCUSSION

The results of the study are presented in Figures 1-5 and Tables 1 and 2.

HPLC identification

A chromatogram of isolated Catechol is shown in Figure 1 and the chromatogram of control Catechol is shown in Figure 2.

Figure 1: Catechol identification by HPLC in the extract of *Teucrium polium*.

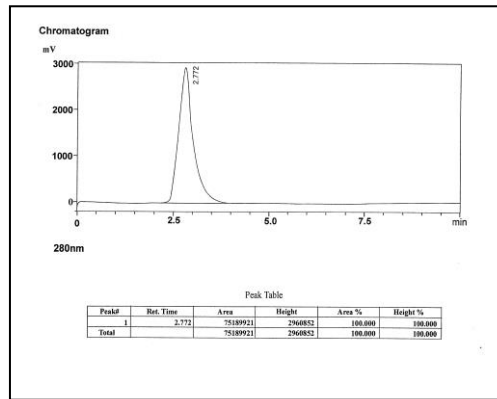
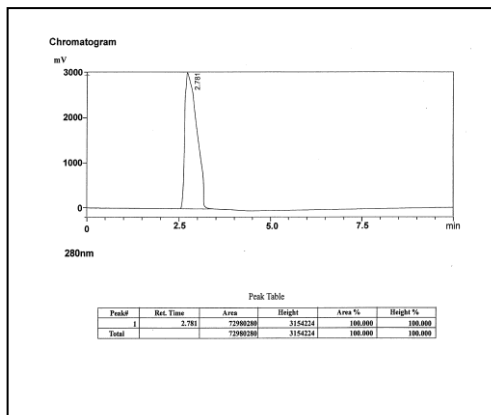


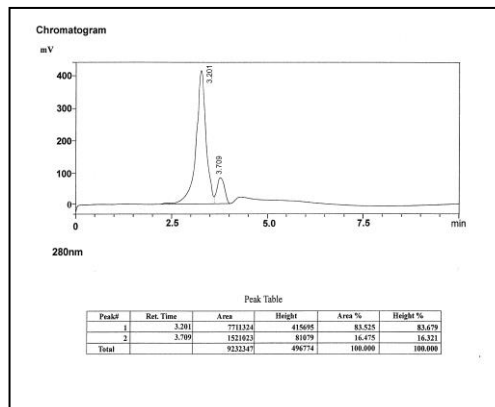
Figure 2: HPLC chromatogram of control Catechol.



The chromatograms in both Figure 1 and 2 are largely identical and refer to the identification of the first phenolic compound of extracts of *T. polium* which is Catechol. The Reproducible peak shapes were obtained under optimum conditions.

The chromatograms of extracts of *T. polium* are presented in Figure 3 shows the polyphenolic compounds Coumarin are Cinnamic acid.

Figure 3: Coumarin and Cinnamic acid identification by HPLC in the extract of *T. polium*.



The chromatogram of controls Coumarin and Cinnamic acid are shown in Figure 4 and 5.

Figure 4: HPLC chromatogram of control Coumarin.

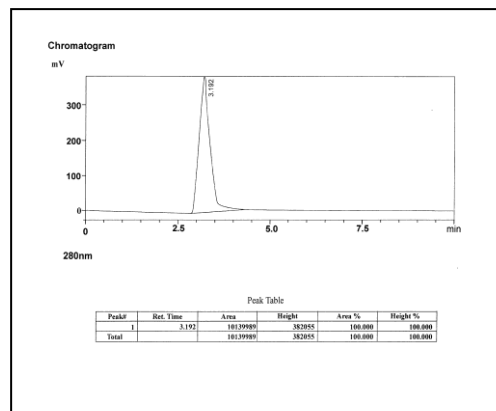
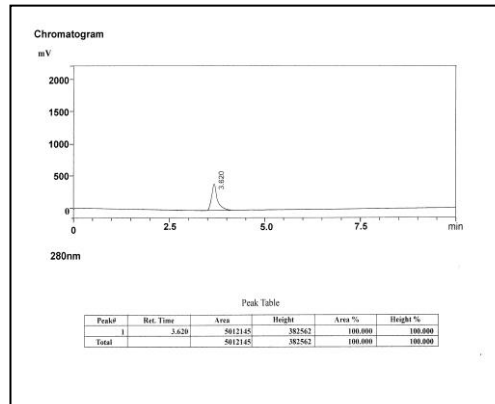


Figure 5: HPLC chromatogram of Cinnamic acid.



The chromatograms in Figure 3 are largely identical with chromatograms of controls of Coumarin and Cinnamic acid in Figure 4 and 5 which refers to the identification of phenolic compounds in extracts of *T. polium* which are Coumarin and Cinnamic acid.

Antimicrobial activity

The results of the antimicrobial activity of *Teucrium polium* extracts against six types of pathogenic bacteria (three Gram-positive and three Gram-negative) are presented in Tables 1 and 2.

Table 1: Aantimicrobial activity of Catechol isolated from *Teucrium polium* extract.

Bacteria mg/ml	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhimurium</i>
200	24	23	26	20	22	22
100	20	21	23	17	19	15
50	17	18	20	15	18	12
25	15	16	17	13	16	10
12.5	10	13	15	8	11	8
Antibiotics*						
Amikacin	25	22	24	24	25	23
Gentamycin	27	23	25	25	24	26

*Disc diameter 6 mm.

Table 2: Aantimicrobial activity of Coumarin and Cinnamic acid isolated from *Teucrium polium* extract.

Bacteria mg/ml	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhimurium</i>
200	27	28	25	26	28	22
100	24	26	24	23	27	19
50	22	20	20	19	23	16
25	18	18	17	16	20	13
12.5	13	12	10	14	18	9
Antibiotics*						
Amikacin	25	22	24	24	25	23
Gentamycin	27	23	25	25	24	26

*Disc diameter 6 mm.

Both results in Tables 1 and 2 shows significantly inhibition of the growth of all six types of bacteria in 200 and 100 mg/ml concentrations comparing with the very similar inhibition of two antibiotics as controls (Amikacin and Gentamycin). The lowest concentrations (50, 25 and 12.5) showed lower inhibition comparing with the extracts with high concentrations for all six types of bacteria. The present results showed that all the extracts possess good antimicrobial activity against selected test pathogenic bacteria. These results explain that certain plants showed potential antimicrobial activity against these bacteria which can be used as very good treatment for many diseases. Overall, these extracts showed appreciable activity against selected test bacteria and that justify their use in our traditional system of medicine to cure various diseases.

CONCLUSION

Results presented antimicrobial activity of these extracts against selected bacteria. The results are supported the traditional use of *Teucrium polium* for the treatment of bacterial infections.

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