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"Teucrium polium" Extracts Jordanian Ja'adeh

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Abstract: This research is intended to record the possible variations in the constituents, chemical composition and antimicrobial activity extracts of cyclohexane, dichloromethane and ethanol extracts of *Teucrium polium* (Jordanian Jeadaha). That collected on locality Almojub Valley (southern deserts of Jordan). Cyclohexane extracts of *T. polium* possessed high activity against *Bacillus subtilis* (MIC = 65 μg/mL Dichloromethane extract was more effective against *Bacillus subtilis* (MIC = 32.5 μg/mL). The ethanol extract of *T. polium* possessed no activity against *Bacillus subtilis*. Herb extracts of *T. Polium* have shown weak antibacterial activity on *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aurous.* The antioxidant activities of the extracts were evaluated using 2 different tests: the thiocyanate method and scavenging of the 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical. The cyclohexane, dichloromethane and ethanol extracts had antioxidant activities. The flavonoid contents of all of the extracts were also determined. The amounts of total flavonoids were higher in dichloromethane and ethanol extracts. The extracts, were subjected to chromatographic methods for the isolation of the active compounds. The structures of isolated compounds were characterized by UV, IR, and ^{13}C NMR, spectroscopic methods as cirsilineol (1), luteolin-7-O-rutinoside (2), luteolin-7-O-glucoside (3), hesperetin-7-O-rutinoside (4), apigenin (5) diosmetin (6), Luteolin-7-sambubioside (7), Diosmetin 7-O-rutinoside, (8) and Nicotiflorin (9) (Fig. 1). These isolated compounds were also tested for their antioxidant and DPPH radical scavenging activities. Flavonoids showed potent antioxidant and DPPH radical scavenging activities. The highest antioxidant activity was shown by luteolin-7-O-glucoside (Djilas *et al*., 2006). This study found that the total phenolic content of the extracts ranged from 50.0 mg/g to 170.0 mg GA/g. The present results therefore offer a scientific basis for traditional use of *Teucrium polium* for the treatment of bacterial and fungal infections.

Keywords: Antibacterial activity, extracts, flavonoids, phenolic content, phenols, spectroscopic methods, *Teucrium polium*

INTRODUCTION

Teucrium polium (Jordanian Ja'adeh) is a dwarf shrub plant which grows wild in Mediterranean countries. The genus *Teucrium* (Germander) belongs to the family *Polium*, (El-Shazly and Hussein, 2004). In the flora of Jordan, genus *Teucrium* has been divided into seven sections with 49 species. They are mostly perennial herbs, shrubs or subshrubs, the species of this genus are widespread on all continents of the world, and a very large number of species are present in the Mediterranean. *T. polium* is consumed by many Jordanians and other people in Mediterranean countries for the treatment of several ailments (Beghalia *et al*., 2008). The previous studies have shown the presence of flavonoids (Harborne *et al*., 1986), and furanoid diterpenes in the aerial parts.

A large number of known medicinal species belonging to the genus *Teucrium* are used in folk medicine and pharmacy (Chang *et al*., 2006). The species of the genus *Teucrium* are very rich in phenolic compounds with very strong biological activity (Acar and Goldstein, 1996). The most popular species of this genus are T. *chamaedrys*, T. *montanum* and T. *polium*, used in treatment of digestive and respiratory disorders, abscesses, gout and conjunctivitis, in the stimulation of fat and cellulite decomposition, and possess anti-inflammatory, ant oxidative, antimicrobial, ant diabetic and antihelmintic effects. However, their most significant therapeutic effect was the elimination of some problems in the digestive tract (Darabpour *et al*., 2010) Recent studies have shown that T. *polium* extract inhibited cell invasion and motility of human prostate cancer cells through different molecular pathways (Kandouz *et al*., 2010), and the essential oils from T. *flavum*, *Teucrium montbretii* sp., *heliotropiifolium*, T. *polium* sp., *capitatum* and *Teucrium brevifolium* were cytotoxic against CACO-2, C32 and COR-123 human tumors cell lines (Beghalia *et al*., 2008).

The purpose of this study was to evaluate *Teucrium polium* as new potential natural sources of effective antimicrobial and antioxidant agent (Tatjana *et al*., 2011).

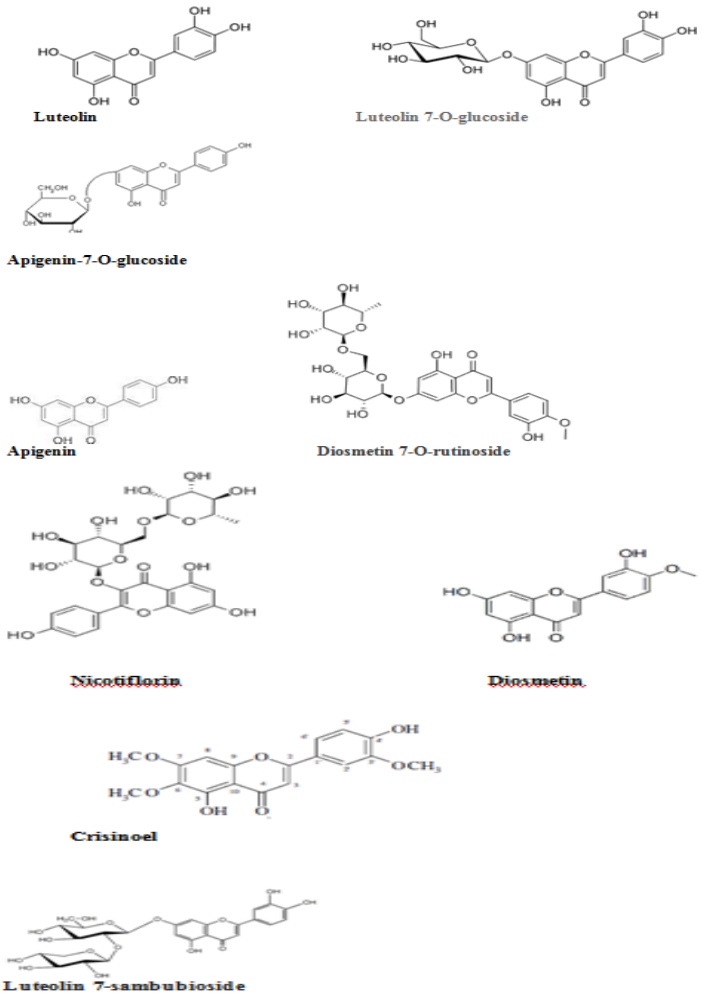

Fig. 1: Structure of compounds isolated

MATERIALS AND METHODS

Plant material: The aerial parts of T. *polium* were collected on locality Almojub Valley (southern deserts of Jordan) in September 2011, during the period of full flowering, on salty humid meadows ass. (Faculty of Pharmacy, University of Jordan) identified the plant species.

Extraction: The air-dried, powdered aerial parts (40 g) were extracted with cyclohexane (2×400 mL) during three days (two times, successively). After filtration, plant material was dried and extracted with dichloromethane and ethanol (2×400 mL) using the same procedure. The solvent was evaporated under low pressure and dried to obtain of cyclohexane extracts (0.39 g), dichloromethane extracts (0.81 g) and ethanol extracts (2.94 g) Table 1.

High Performance Liquid Chromatography (HPLC) analysis: High Performance Liquid Chromatography (HPLC) separation was performed using a Agilent 1100 series system equipped with a G-1312A binary pump, a G-1328B injector (20 µL loop) and G1315B DAD detector. The column used was a ZORBAX Eclipse XDB-C18 (250 nm) and operated at a temperature of 25°C. A gradient elution was performed with solvent A (H_2O and H_3PO_4, pH = 2.8) and B (solvent A: acetonitrile) as follows: 10-25% B (5 min), 25% B isocratic (10 min), 25-30% B (5 min), 30-50% B (5 min), 50-70% B (5 min), 70-10% B (5 min) at a flow rate of 0.8 mL/min. The injection volume was 20 µL. The present compounds were determinate on the bases of their retention times and UV spectra, (Fig. 2, 3 and 4.)

Table 1: Total phenolic contents in the extracts in terms of gallic acid equivalent (mg of GA/g of extract)

Plant species	Total phenolic content
Cyclohexane extract	50.50±1.26
Dichloromethane extract	169.06±0.75
Ethanol extract	170.62±1.05

The amount of total phenolics in extracts was determined according to Folin-Clocalteu procedure.

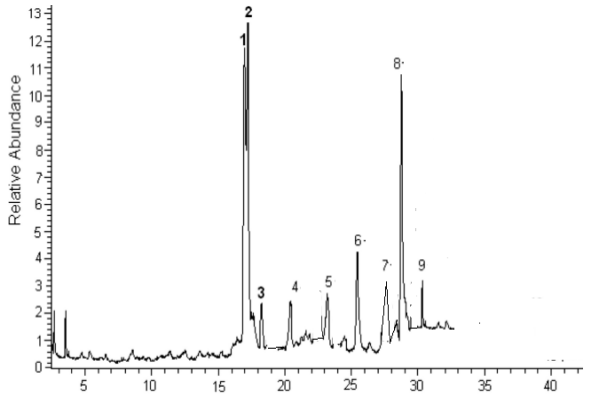

Fig. 2: HPLC-UV profile of ethanol extract, detection at 280 nm cirsilineol (1), luteolin-7-O-rutinoside (2), luteolin-7-O-glucoside (3), hesperetin-7-O-rutinoside (4), apigenin (5), diosmetin (6), luteolin-7-sambubioside (7), diosmetin 7-O-rutinoside (8) and nicotiflorin (9)

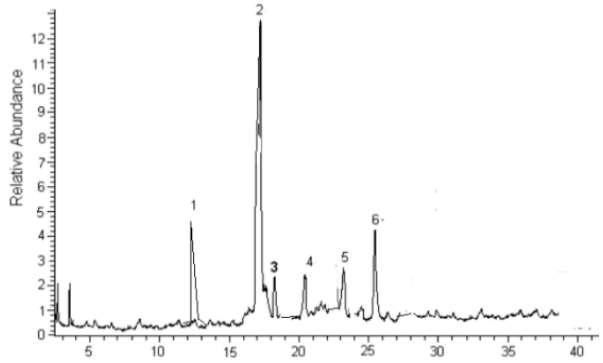

Fig. 3: HPLC-UV profile of cyclohexane extract, detection at 280 nm luteolin (1), luteolin-7-O-rutinoside (2), luteolin-7-O-glucoside (3), apigenin (4), diosmetin (5) and diosmetin-7-O-glycoside (6)

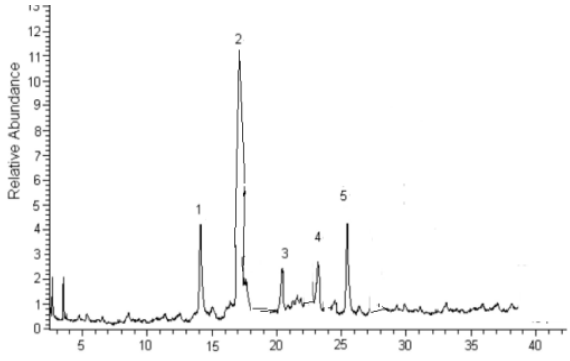

Fig. 4: HPLC-UV profile of dichloromethane extract, detection at 280 nm luteolin (1), luteolin-7-glucosides (2), diosmetin-7-rutinoside (3), luteolin-7-rutinoside (4) and diosmetin-7-O-glycoside (5)

The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in mg/g.dry material Table 2.

Stability and shelf life studied of the extract: Ethanolic T. *Polium* extracts from the freshly prepared were transferred in 1.5 mL eppendorf tubes. These tubes were distributed in 3 groups to store at -20 and 4ºC and at room temperature. The tubes were retracted at 15, 30, 60, 90 and 120 days for analysis and dissociation of glycosides bond and. In addition, we performed stability studies on ethanolic T. *Polium* extracts, as well as cyclohexane, and dichloromethane extracts at different pH, light exposure and long term storage.

Antimicrobial activity: Antibacterial and antifungal activities of the cyclohexane, dichloromethane and Goldstein (1996) on selected Gram-positive and Gram-negative bacteria and fungi: S. *aureus* (ATCC 25923), S. *epidermidis* (ATCC 12228), M. *luteus* (ATCC 10240), E. *faecalis* (ATCC 29212), B. *subtilis* (ATCC 6633BB), B. *cereus* (ATCC11778), E. *coli* (ATCC

Table 2: Percentages of compounds detected in T. *polium* collected from different solvents extract using GC-MS analysis

		Extraction%		
Ret. time	Isolated compound	Cyclohexane	Dichloromethane	Ethanol
16.5	Cirsilineol	0	0	12
17.0	Luteolin	8	12	0
18	Luteolin-7-O-rutinoside	20	23	10
18.5	Luteolin-7-O-glucoside	33	30	20
19.0	Hesperetin-7-O-rutinoside	0	0	5
20	Apigenin	15	0	1
23	Diosmetin	6	13	14.1
25.5	Luteolin-7-sambubioside	0	0	7
29	Diosmetin 7-O-rutinoside	21.8	21	30
30	Nicotiflorin	0	0	0.90

Table 3: Antimicrobial activity of T. *polium* extracts (ug/mL)

Microorganism	Cyclohexane extract	Dichloromethane extract	Ethanol extract
S. aureus (ATCC 25923)	500	125	125
S. epidermidis (ATCC 12228)	500	65	500
M. luteus (ATCC 10240)	250	250	125
E. faecalis (ATCC 29212)	125	500	125
B. subtilis (ATCC 6633BB)	65	32.5	125
P. aeruginosa (ATCC 27853)	125	125	500
K. pneumoniae (NCIMB 9111)	500	125	125

25922), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (NCIMB 9111) and *C. albicans* (ATCC 10259) Table 3

RESULTS

The analysis of HPLC chromatograms by comparison of the retention times and UV Spectra of selected picks with standard substances have shown the presence of luteolin, apigenin, diosmetin, luteolin-7-O-glucoside, luteolin-7-O-rutinoside and diosmetin-7-O-glycoside in the cyclohexane extract (Fig. 1). Harborne *et al*. (1986) reported Previously the free flavones aglycones located externally on the stems and leaves (cirsiliol, cirsilineol, 5-hydroxy-6, 7, 3', 4'-tetrametoksiflavone), the flavonoid aglycones after the hydrolysis of ethanol extract (apigenin, luteolin and diosmetin), and glycosides of apigenin and luteolin (apigenin- and luteolin-7-O-glucoside, luteolin-7-Orutinoside and luteolin-7-sambubioside) in the ethanol extract of *T. scordium* sp., scordioides from Spain. Our results are completely in accordance with previous researches because the distribution of methoxy flavones was dominant in *Teucrium* sp., while diosmetin was found especially in species of section Scordium. Also, dichloromethane extracts, luteolin and apigenin-7-glucosides were found in almost all tested *Teucrium* sp., diosmetin-7-rutinoside in some species of section Scordium and Polium. Luteolin-7-rutinoside was very frequent in all *Teucrium* sp., Vicenin-2 has not been found in the tested ethanol extract of *T. Polium*, which was also the characteristic of section Scordium. Higher concentration was observed cyclohexane extract in *T. polium* Differences in life form are in accordance with the obtained concentrations of phenolic compounds.

CONCLUSION

According to the results of investigations, *Teucrium* can be considered as a rich natural source of polyphenolic compounds (Fig. 2). Our in vitro data indicated the inhibition of extracts of many microorganisms among the investigated extracts (Table 3), and could be considered as suitable candidates for further studies to find the effective anticancer components. Plant extracts can also act as antioxidants due to lower levels of nitrites, lower superoxide anion production and higher percentage of viable cells 72 h after treatment. Natural products from plants of this genus, as important medicines for a number of digestive diseases and disorders, represent the potential natural resources of effective substances in the treatment of digestive tract cancer.

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REFERENCES

Acar, J.F. and F.W. Goldstein, 1996. Disc Susceptibility Test. In: Lo-Rian, V. (Ed.), Antibiotics in Laboratory Medicine. 4th Edn., Williams & Wilkins, Baltimore, London, pp: 1-52.

Beghalia, M., S. Ghalem, H. Allali, A. Belouatek and A. Marouf, 2008. Inhibition of calcium oxalate monohydrate crystal growth using Algerian medicinal plants. J. Med. Plants Res., 2(3): 066-070.

Chang, F.R., T.L. Hwang, Y.L. Yang, C.L. Li, C.C. Wu, H.H. Issa, W.B. Hsieh and Y.C. Wu, 2006. Anti-Inflammatory and Cytotoxic diterpenes from formosan *Polyalthia longifolia* var. *pendula*. Planta Med., 72: 1344-1347.

Darabpour, E., H. Motamedi and S.M. Seyyed Nejad, 2010. Antimicrobial properties of *teucrium polium* against some clinical pathogens. Asian Paci. J. Tropic. Med., 3(2): 124-127.

Djilas, S.M., S.L. Markov, D.D. Cvetković, J.M. Čanadanović-Brunet, G.S. Ćetković and V.T. Tumbas, 2006. Antimicrobial and free radical scavenging activities of *Teucrium montanum*. Fitoterapia, 77: 401-403.

El-Shazly, A.M. and K.T. Hussein, 2004. Chemical analysis and biological activities of the essential oil of *Teucrium* leucocladum Boiss. (Lamiaceae). Bio. Sys. Ecol., 32: 665-674.

Harborne, J.B., F.A. Tomas-Barberan, C.A. Williams and M.I. Gil, 1986. A chemotaxonomic study of flavonoids from European *Teucrium* species. Phytochem, 25: 2811-2816.

Kandouz, M., A. Alachkar, L. Zhang, H. Dekhil, F. Chehna, A. Yasmeen and A.E. Al Moustafa, 2010. *Teucrium polium* plant extract inhibits cell invasion and motility of human prostate cancer cells via the restoration of the E-cadherin/catenin complex. J. Ethnopharmacol., 129: 410-415.

Tatjana, K., M. Marina, T. Aleksandra, L. Branislava *et al*., 2011. Cytotoxicity and antimicrobial activity of *Teucrium scordium* L. (Lamiaceae) extracts. Afri. J. Microbiol. Res., 5(18): 2692-2696.