Qualitative and Quantitative Analysis of *Teucrium polium* L. and the Relation with Antibacterial Activity

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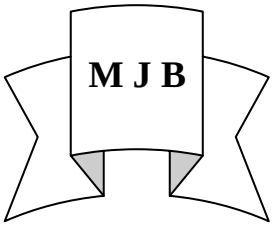

Abstract

The research was involve preparation two aqueous extracts (hot and cold) and ethanolic extract (70%) from Iraqi Al-ja´ada plant (*Teucrium polium*) which cultivated in Alsodoor/Diayla city, for qualitative and quantitative study of active components. This study provided that hot extract contained higher amount of active component (such as flavonoids) than another extracts. This is may be due to the role of heat in extraction some of active components which cannot extracted by cold water nor by alcohol. The biological activity (antimicrobial) of these extracts was studied with *E.coli*, *staphylococcus* and *streptococcus*, in comparison with some antibiotics such as Gentimycine, Tetracycline and Chloramphenicol. This study proved that biological effect of these extracts on the bacteria used, may be due to present the active compounds.

الخلاصة

يتضمن هذا البحث تحضير ثلاث مستخلصات مائية (مستخلص حار ومستخلص بارد ومستخلص كحولي) لنبتت الجعدة العراقية (*Teucrium polium*) المزروعة في منطقة الصدور/ديالى لدراسة المكونات الفعالة فيها كميا ونوعيا. أثبتت هذه الدراسة إن المستخلص الحار يحتوي على كميات اكبر من المكونات الفعالة (مثل الفلافونيدات) مقارنتةً بالمستخلصات الأخرى وربما يعود السبب إلى دور الحرارة في استخلاص بعض المكونات الفعالة التي لايمكن استخلاصها بالماء البارد أو بالكحول. ودراسة الفعالية الحياتية (المضاد للجراثيم) لهذه المستخلصات مع E.coli و staphylus و strptococus مقارنةً ببعض المضادات الحيوية مثل الجنتامايسين والتتراسايكلين و الكلورامفنيكول، و أثبتت الدراسة التأثير الأحيائي لهذه المستخلصات على الجراثيم المستخدمة في هذه التجربة.

Introduction

Teucrium polium L Labiate (Ja'adah in Arabic) is a widely grows in Iraq-Alsodoor/Diyala, the plant classified by Mrs. Hanan Ahmed in Babylon university/science college/Biology department . Some of species of *Teucrium* are used for medical aspect [1], various pathological purpose such as antidiabetic, anti-infammatory, anti-ulcer, hypotensive, antispasmodic, anorexic and antipyretic agents [2]. Most of these effects have been related to the various compounds such as volatile oil, Flavonoids and terpenoids components [3].

The *lamiace* family contain a strong antioxidant activity. This antioxidant is maintenance of healthy and protection from diseases such as coronary heart disease and cancer [4]. Antioxidant compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation steps of oxidative chain reactions [5]. The antioxidant acts as scavenger by absorbing and neutralizing free radicals, or by quenching singlet and triplet oxygen, or by decomposition

peroxides, these lead to form redox system and that may be associated with different type of diseases. The antioxidative effect is mainly due to phenolic components, such as Flavonoids, phenolic acids and phenolic diterpenes. [6].

The most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more. The phytochemicals can classified according to metabolic function into sugars, amino acids, proteins and chlorophyll while the second group consist of alkaloids, terpenoids and phenolic compounds and may be more such as flavonoids and tannins [7].

The biological activity of plant has been discovered by evolution of pharmacological plant data, because of these plants may be offered by the local population immediately as therapeutic products. Several plants have properties of ability to resist the microbial (make as antimicrobial), from these properties can lead to make extensive use of synthetic drugs [8] , especially in medication drugs that cause increase side effects in the body, therefore at sometimes, the toxic effects which produced by the administration of drugs are much more a serious problem than that of the disease itself, but the plant extract is not make on target directly, but prevent the mechanism lead to cause different disease, therefore the plant extracts are more safety [8].

Aim of the Study

The present work is based on the study of the effect of different types of *T.polium* extract (hot extract, cold extract, and ethanolic extract) or natural products which found in these extracts on three different types of bacteria by various concentrations of each extract, and to explain plants significance in treatment as drug for different diseases.

Material and Methods

Collection of plant samples:

The plant materials are brought from Diyala markets.

Collection and processing of plant samples:

The leaves of the plants are properly washed in tap water and then rinsed in distilled water. The rinsed leaves are dried in an oven at a temperature of 35-40°C for 3 days. The dried leaves of each plant are pulverized, using a sterile electric blender, to obtain a powered form. The powdered form of these plants is stored in airtight glass containers, protected from sunlight until required for analysis.

Preparation of the extracts:

The extraction was performed by macerating 500 g in 1.5 L of ethanol (70% v/v) for one week with occasional stirring. The macerated mixture was filtered by filter paper and evaporated at 40°C up to one third of initial volume. Remaining solvent was completely evaporated at 40°C, using a hot air oven and kept in desicator for two days. The yield (10% w/w) of the powdered plant material was collected dried and stored at 5°C in air tight container without light exposure. In the same vein, part of the pulverized sample was extracted with water only to made cold extract and with hot extract but the extraction at 50ºC, to compare the phytochemical constituents of hot and cold extracts with ethanolic extract, (the yield of cold extract is 12%, and for hot extract is 15% w/w). [9]

Phytochemical analysis

Chemical tests are conducted on the aqueous and ethanolic extracts powdered form of the plant sample using standard methods. [10]

Qualitative analysis on photochemical constituents:

Test for Flavonoids

A portion of crude powder was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration. [10]

Test for Saponins

0.5 g of crude powder was shaken with water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of saponins. [10]

Test for Tannins

0.5 g of the crude powder was stirred with 10 ml of distilled water. This was filtered and 0.1% ferric chloride reagent was added to the filtrate, a blue-black colouration was indicate for the presence of tannin. [10]

Test for Anthraquinones

0.5 g of crude powder was shaken with 10 ml of benzene and was filtered, 0.5 ml of 10 % ammonia solution was added to the filtrate and the mixture was shaken well. The presence of the violet color in the layer phase indicate the presence of the anthraquinones. [10]

Test for Alkaloids

0.5 g of crude powder was defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 min with 5 ml of aqueous HCl on a boiling water bath. The resulting mixture was centrifuged for 10 min at 3000 rpm. 1 ml of the filtrate was treated with few drops of Mayer's reagent and a second 1 ml with Dragendroff's reagent and turbidity was observed. [10]

Test for Phlobatannins

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl) to observe the deposition of red precipitate. [10]

Test for Terpenoids

5 ml of aqueous extract of each plant sample is mixed with 2 ml of CHCl3 in a test tube. 3 ml of concentrated H2SO4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present. [10]

Test for glycosides

2 g of the sample was mixed with 30 ml of distilled water and it was heated for 5 min on a water bath, filtered and used as follows: five ml of the filtrate was added to 0.2 ml of Fehling solution A and Fehling solution B until it turns alkaline and heated in a water bath for 2 min. A lightish blue colouration was observed (instead of brick red precipitate) which indicates the absence of glycosides. [11]

Qualitative analysis on photochemical constituents:

Flavonoids determination

10 g of each plant crude powder was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 m). The filtrate was later transferred into a crucible and evaporated into dryness and weighed to a constant weight.

Saponins determination

20 g of crude taken from each plant were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55 ºC. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ºC. The concentrate was transferred into 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60 ml of n-butanol was added. The combined n-butanol extracts were

washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to constant weight and the saponin content was calculated. [10]

Alkaloids determination

For the determination of total alkaloid content of plant and extracts, the reference method chosen was the measurement of chelidonine content according to the German Pharmacopoeia [12].

The activity of extracts against bacteria:

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/liter) Beef 2g,

casein acid hydrolysate 17.5g, starch 1.5 g and agar 17g; pH 7.4 ± 0.2.

About 38g of MH agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.3 ± 0.2, sterilized by autoclaving at 121ºC for 15 minutes at 15 psi pressure and was used for sensitivity tests. [8]

Cup-Plate Method

The cup-plate agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions (108-109) colony – forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 10mm in diameter, were cut using a sterile cork borer No.4 and the agar discs were removed. Cups were filled with 0.1ml of extracts, Gentamycin and saline using microliter-pipette and allowed to diffuse at room temperature for two hours. The plates were incubated in the upright position at 37ºC for 18 hours. After incubation the diameter of the results and growth inhibition zones were measured, and the mean values were recorded. [8]

Results and Discussion

The results of present study consider the *Teucrium polium* as plants have strong antioxidant properties as it has several active compounds shown in the Table (1).

Table 1 the qualitative analysis of *Teucrium polium* extracts.

Extraction test	Cold extract	Hot extract	Ethanolic extract
Alkaloids	-ve	-ve	-ve
saponines	+ve	+ve	+ve
Tannins	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve
phlobatannins	-ve	-ve	-ve
Anthroquinones	-ve	-ve	-ve
Amino acids or primary and secondary amine	+ve	+ve	+ve

Table (1), show that the hot, ethanolic, and cold extracts of *Teucrium polium* contain on the same active compounds such as saponines, tannins, flavonoids, terpenoids, glycosides, and amino groups. The absence of the alkaloids, phlobatanins, and anthroquinones, are an evidence on the detection about this active compounds neither dependent

on the nature of extraction method nor on the nature of the solvent.

Table 2 the quantitative analysis of *Teucrium polium* extracts.

Extracts test	**The percentage of products %**		
	Saponins	flavonoids	Alkaloids
Hot extract	4.45	9.33	_
Ethanolic extract	3.67	7.46	_
Cold extract	4.29	6.32	_

The results which showed in Table (2) explain the quantitative analysis of *Teucrium polium* about presentation of flavonoids ,and saponines, the larger amount of aqueous hot extract compared with ethanolic (70%) and aqueous cold extracts. This evidence indicate the important role of the heat in extraction process, because of the heat lead to increase speed and efficiency of extraction. However, the ethanolic extract given values higher than cold extract, because of the photochemical compounds have organic properties and this lead to extraction with higher quantities by using the ethanol as extraction solvent.

Table 3 the inhibition zone of *Teucrium polium* on some types of bacteria. where (-) = no activity, EC, *Escherichi coli*; STR, *Strptococus*; STA, *Staphylus.*

Test extracts	**Concentration mg/ml**	**Diameter of inhibition zone (cm)**		
		EC	**STR**	**STA**
Hot extract	50	0.6	0.7	0.6
	100	0.7	1	0.8
	200	1	1.4	1.2
	500	1.6	1.9	1.6
Ethanolic extract	50	-	0.9	0.6
	100	0.7	1	0.9
	200	0.7	1.2	1.3
	500	0.8	1.4	1.6
Cold extract	50	-	0.6	-
	100	-	0.8	0.6
	200	0.5	0.9	0.7
	500	0.9	1.1	0.9
Antibiotic	**Concentration/mg**			
Tetracycline	30	1.4	1.3	1.1
Gentamycine	10	1	1.6	1.4
Chloramphinicol	30	0.7	1	1.2

The Table (3) showed the higher ability of hot extract of inhibition on the growth of bacteria compared with cold and ethanolic extracts. Also Table (3) show that, the ethanolic extract is the best than cold extract by inhibition ability. This mean every extract contain on the active components and

ability of this components to kill the microbial. From this results, we found that aqueous hot extract is better than cold aqueous and alcoholic extracts due to increasing of active component solubility with temperature.

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