Extraction, Identification and Antimicrobial Activity of Some Phenolic Acids As Antioxidants in Teucrium polium Plant

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(K . J . Pharm . Sci)

(Received May 2011 , Accepted Nov. 2011)

Abstract

A direct solvent extraction was used for the extraction of aerial parts of local medicinal plant *Teucrium polium* using methanol ,ethanol, iso-propanol, butanol, chloroform, acetone , ethyl acetate, and hexane. The total phenolic contents of extracts was determined using Folin-Ciocalteu method , the results shows that the highest extraction was obtained by methanol which was found to be equal to 100.144 mg/ g of dry plant. HPLC was employed to identification phenolic acid content especially Gallic acid in *Teucrium polium* extract with each solvent, The results showed that the highest extraction was obtained equals to 48.07% by methanol followed by acetone and ethanol, also the results shows that the n- hexane give the lowest extraction 4.27%, also butanol with iso-propanol extract contain the large number of phenolic acid contents. The accuracy and precision of this method were determined by preparing laboratory samples of Gallic acid , the results show absolute error ranging from ±0.3 to ± 1.5 , relative errors ranging from ±0.375 to 1.87 % , the standard deviation equal to 0.48 and the relative standard deviation did not exceed ±0.478 %,the standard deviation of the mean was 0.215. The antimicrobial activity of methanolic extracts of local medicinal plant *Teucrium polium* life samples from Dehok and Al-Kut were examined for their antimicrobial potentials against selected test bacteria and fungi, *Aspergillus Niger* , *Aspergillus parasticus* , *Staphylococcus aurous positive*, *Staphylococcus aurous negative*, *Streptococcus* , *Escherichia coli* and *Cladosporium*. The present results showed that all the extracts posses good antimicrobial activity against selected test bacteria and fungus. The present results therefore offer a scientific basis for traditional use of *Teucrium polium* for the treatment of bacterial and fungal infections.

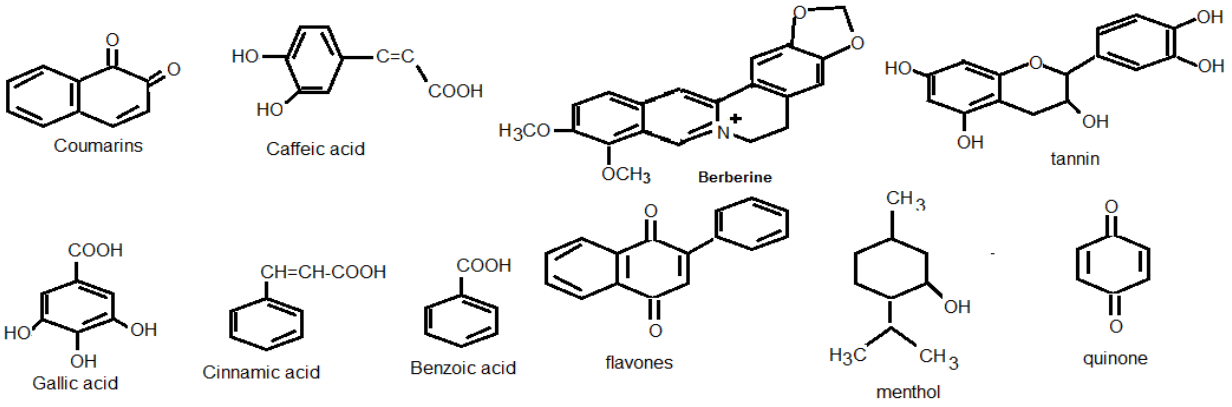
Key words : Teucrium polium, Solvent extraction , Total Phenolic Content , Biological activity

الخلاصة

استخدم الاستخلاص ألمذيبي المباشر لاستخلاص الأجزاء الهوائية من النبات المحلي الجعدة باستخدام المذيبات العضوية الميثانول , الايثانول , الايزوبروبانول , البيوتانول ,الأسيتون ,خلات الاثيل , الكلوروفورم و الهكسان .تم قياس المحتوى الفينولي الكلي باستعمال طريقة Folin-Ciocalteu وأظهرت النتائج أن الميثانول أعطى أعلى محتوى فينولي كلي مقداره 100.144 ملغم /غم من النبات الجاف . تم استخدام جهاز كروماتوغرافيا السائل عالي الأداء HPLC لتشخيص الحوامض الفينولية و خاصة حامض الكاليك في المذيبات العضوية المستخدمة وأظهرت النتائج أن الميثانول هو المذيب الأفضل للاستخلاص وبنسبة مئوية بلغت 48.07 % وأن الهكسان هو المذيب الأقل استخلاصا وبنسبة مئوية وبلغت 4.27 % , وان مستخلص البيوتانول مع الايزوبروبانول تحتوي على العدد الأكبر من المركبات الفينولية. تم إيجاد قيم الدقة والتوافق بهذه الطريقة من خلال تحضير عينات مختبريه لحامض الكاليك وبإجراء المعالجات الإحصائية أظهرت النتائج وجود خطأ مطلق يتراوح مقداره بين ± 0.3 إلى± 1.5 وخطا نسبي مئوي يتراوح مقداره بين 0.375 إلى 1.87 % ومقدار الانحراف المعياري مساو إلى 0.48 والانحراف المعياري النسبي مساو إلى ±0.478 % , والانحراف المعياري للمعدل مساو إلى 0.215 . تم اختبار الفعالية البيولوجية لمستخلصات الميثانول لعينات حياتية لنبات الجعدة المحلي من محافظتي الكوت و دهوك وملاحظة قدرة وإمكانية هذه المكونات على تثبيط وقتل أنواع مختارة من البكتيريا والفطريات *Staphylococcus* , *Staphylococcus aurous positive* , *Aspergillus parasticus* , *Aspergillus Niger* *aurous negative*, *Streptococcus* , *Escherichia coli* و *Cladosporium* . أظهرت النتائج قدرة وفعالية جيدة لهذه المستخلصات تجاه هذه البكتيريا والفطريات وإمكانية استعمال هذه المكونات تقليديا لمعالجة العدوى والإصابة لطيف واسع من البكتيريا والفطريات.

Introduction

Finding healing power in plant is an ancient idea. Numerous studies have been carried out in different parts of the globe to extract plant products for screening antimicrobial activity[1]. Herbal medicines are one of the important cultural and traditional part of the people. Today, most of the world population depend on herbal medicines for their health care needs [2]. Plants often contain wide variety of antioxidant molecules such as phenolic compound, phenolic acids, flavonoids, quinines, caffeic acid, coumarins, menthol, alkaloid (berberine) and tannins [3]. These natural antioxidants are distributed in different part of the plant such as wood, stems, pods, leaves, fruit, roots, flowers, pollen and seeds [4]. Antioxidants phenoilc compounds may function as ferminators of free radical chains or chelators of redox.-active metal ions that are capable of catalyzing lipid pre oxidation [5] . Recently, there has been a considerable interest in finding natural antioxidants to replace synthetic ones. A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts [6]. The antioxidants activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[7].



Prakash G. et al. [8] study the antimicrobial properties of extract Dioscorea pentaphylla plant consumed as food against three bacterial and five fungi strains using different solvents as extractants. Previous Epidemiological studies have shown that the intake of natural antioxidants is associated with reduced risk of cancer, cardiovascular disease, diabetes and other disease associated with aging[9]. Ethanol extracts of certain Indian medicinal plants were examined for their antimicrobial potentials against selected bacteria and fungi[10]. Hofling J.F.et.al. [11] study the potential activity of extracts from six selected plants against ten Candida species using methanol and dichloromethane as extractants. Prevalence of antibiotic-resistant strains of bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control the bacterial diseases[12]. Khanavi M. et.al. [13] study the comparison antioxidant activities of date extracts and total phenols using methanol-water (50:50), DMSO, water-:methanol-acetone- formic acid (20:20:40:0.1) as extractants.

The main objectives of present study were to determine total phenolic compounds of the methanolic extracts of Iraqi local samples of *Teucrium polium* by using Folin-Ciocalteau method as well as their biological activity that inhibit micro-organisms, especially that cases wound infection using *Aspergillus Niger* , *Aspergillus parasticus* , *Staphylococcus aurous positive*, *Staphylococcus aurous negative*, *Streptococcus* , *Escherichia coli* and *Cladosporium*.

Materials & Methods:

Apparatus:

1- Uv-Vis Shimadzu-1650, double beam spectrophotometer with 1cm quartz cells.
2- HPLC, Shimadzu equipped with ODSC-18, 25cm x 4.6mm i.d column.
3- Vortex.

Chemicals:

Absolute ethanol , methanol, iso-propanol, n-butanol, acetone, chloroform, ethyl acetate and n-hexane were purchased from Fluka and BDH , Gallic acid and Folin-Ciocalteus phenol reagent were purchased from Sigma- Aldrich. All other reagents were of analytical grade.

Collection of plant samples:

The medicinal plants used for the experiment was identified according to various literatures, and including other pertinent taxonomic literature .The part of the *Teucrium polium* plant used is the leaves. samples were collected from Shekh saad- Al-Kut and Dehok during May and June of 2010 respectively. plants were washed thoroughly and chopped into small pieces shade dried and grinded into powdered form. Clean and dry separating funnel was taken.

Preparation of Plant Test Extracts:

Dried powdered plants material (32g) were 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, methanol, ethanol ,iso-propanol, n-butanol, acetone, chloroform, ethyl acetate and n-hexane.

Separation and Identification of Phenolic Acids Using HPLC:

Gallic acid has been identification by using HPLC equipped with ODSC-18, (25cm x 4.6mm i.d) column and methanol as mobile phase at 0.5 ml/min flow-rate using UV-detector which set at 254nm.

Calibration graph:

A standard calibration graph for Gallic acid in the concentration range 50 to 500 ppm were prepared and used to determine the concentration of Gallic acid. Using the method of Least Squares[14], the regression equation [Y= Xb ±a , where b is the slope = 0.001, a is the intercept = 0.0035, X is the concentration, Y is the absorbance] was utilized for the calculation of unknown Gallic acid concentration in samples. The validity of the regression equation was tested by analyzing laboratory sample preparation. Beers law is valid within the concentration ranges of samples calculated.

Total Phenols Contents :

The total polyphenol contents of plants extracts was estimated by the Folin-Ciocalteus phenol reagent according to Hagerman et.al[15]. 0.1ml of diluted extract was transferred into test tubes and their volume up to 8 ml with distilled water. After addition of 0.5 ml Folin-Ciocalteus phenol reagent and 1.5 ml of 20% aqueous sodium carbonate solution. Tubes were vortexes and the absorbance of blue colored mixture was determined at 765nm after 30 min. against a blank solution containing 0.1ml solvent instead of the tested sample. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in milligrams per gram of dry matter of sample, using standard curve generated with different concentrations of Gallic acid[16].

Sources of Biological Test Organism :

Bacteria and fungi pure culture of all test organism namely: *Aspergillus Niger* , *Aspergillus parasticus* , *Staphylococcus aurous positive*, *Staphylococcus aurous negative*, *Streptococcus* , *Escherichia coli* and *Cladosporium* were all obtained through the microbiology laboratory in Directorate of Hazard Materials / Ministry of Science and Technology.

Culture of test microbes:

For the cultivation of bacteria and fungi, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5g Peptone, 3g

beef extract and 3g NaCl in 1L distilled water and sterilized at 120° C for 30min. Agar test plates were prepared by pouring approximately 17 ml of NAM into the sterile Petri dishes (10 mm diameter) under aseptic conditions. A saline solution was prepared by mixing 0.8% NaCl in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 25°C for 72 h . To prepare the test plates in bacteria and fungi, 20 ml of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy ,and the growth inhibition of bacteria, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant [17, 18].

Results & Discussion

3-1 : **Analysis:**

A standard calibration graph for Gallic acid (Fig.1) in the concentration range 50 to 500 ppm were used to determine the concentration of Gallic acid. In order to examine the accuracy and precision of the analysis method, Table-1, explain the accuracy of the method, the results show absolute error ranging from ±0.3 to 1.5 and relative Error not exceeding 1.87% . Table-2 , explain the precision of the method , show standard deviation equals to 0.48 and the standard deviation of the mean equals to 0.215 and the relative standard deviation not exceeding ± 0.478% .

3-2: **Total Phenolic compounds** :

It is important to choose the solvent which give high extraction , Fig.2, show the total phenolic contents with different solvents.This study established that the methanol extracts were significantly higher in phenolic contents than other solvents , the total phenolic contents in methanol was equal to 100.144 mg/g, and n-hexane have the lowest total phenolic contents quantity extract which equal to 3.946mg/g . This results attribute that the polar compound affinity to dissolved in polar solvents, hence the polarity of phenolic compound and methanol is the same [1, 19].

HPLC was employed to Separation and identification phenolic acids contents especially Gallic acid in *Teucrium polium* extract with each solvent used (Figs.3-11),(Table-3). The results show that the methanol was the best solvent for extraction which gave 48.07% and n-hexane have the lowest extraction 4.27%, and n-butanol and iso-propanol extracts contain the large number of phenolic compounds. The contents No. 2 have retention time equal to 2.978 min. which represent Gallic acid and No. 8 was found in all plant extracts samples, also the extraction efficiency of polar solvent was much more than other solvents, this result was matched other works for *Teucrium mountain* plant [20].

3-3: **Antimicrobial activity** :

The antimicrobial activity of methanolic extracts of local medicinal plant *Teucrium polium* life samples from Dehok and Al-Kut were examined for their antimicrobial potentials against selected test bacteria and fungi, *Aspergillus Niger* , *Aspergillus parasticus* , *Staphylococcus aurous positive*, *Staphylococcus aurous negative*, *Streptococcus* , *Escherichia coli* and *Cladosporium*. The present results showed that all the extracts posses good antimicrobial activity against selected test bacteria and fungus, Table-4. These results explain that certain plants showed potential antimicrobial activity against *Staphylococcus aurous negative* can be used as very good treatment for acne[10]. Overall, these methanolic extracts showed appreciable activity against selected test bacteria and fungi and hence, it justify their use in our traditional system of medicine to cure various diseases. While screening of methanolic extract of *Teucrium polium* for Al-Kut life sample showed that the given test extracts have maximum activity against *Cladosporium* strain which gave 72.9%, and *Aspergillus Niger* strain followed by *Aspergillus parasticus* which equal 61% and minimum against *Staphylococcus aurous*

positive and *negative* strain. While screening of methanolic extract of *Teucrium polium* for Dehok life sample showed that the given test extracts have maximum activity against *Aspergillus Niger* strain which gave 62% and minimum against *Aspergillus parasticus*,*Staphylococcus aurous positive* and *negative* strain. These results shows that the plant chemical content affected with weather and geographic agents like temperature, humidity, soil type etc. [19]. The present results therefore offer a scientific basis for traditional use of *Teucrium polium* for the treatment of bacterial and fungal infections.

Conclusions

The extraction efficiency increase with polarity increasing of the solvents ,hence the highest extraction done with methanol and the lowest extraction with non polar solvent hexane. Results presented antimicrobial activity of these extracts against selected bacteria and fungi. The results are supported the traditional use of *Teucrium polium* for the treatment of bacterial and fungal infections.

Acknowledgment

The authors are very grateful to professor Ali A-Mosawi /college of Science/ Baghdad University/ Biology department for many helpful in identification and authenticated *Teucrium polium* plant samples.

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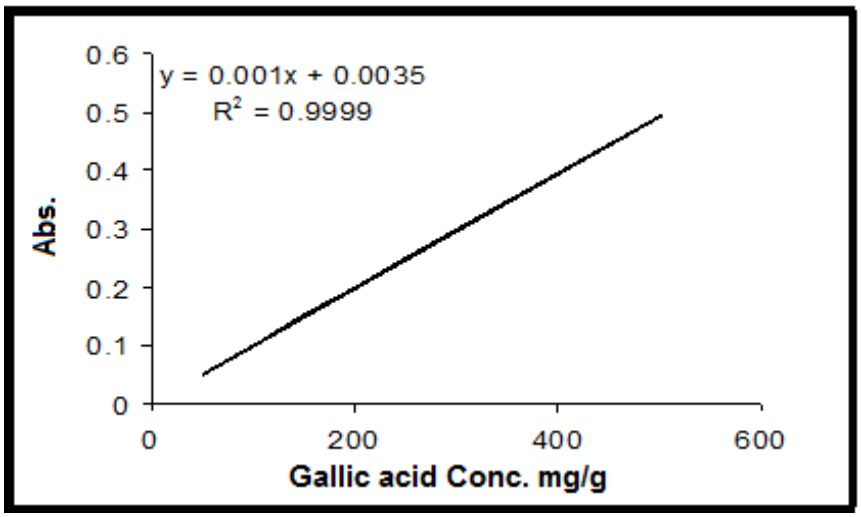

Fig.–1: Standard calibration graph for Gallic acid

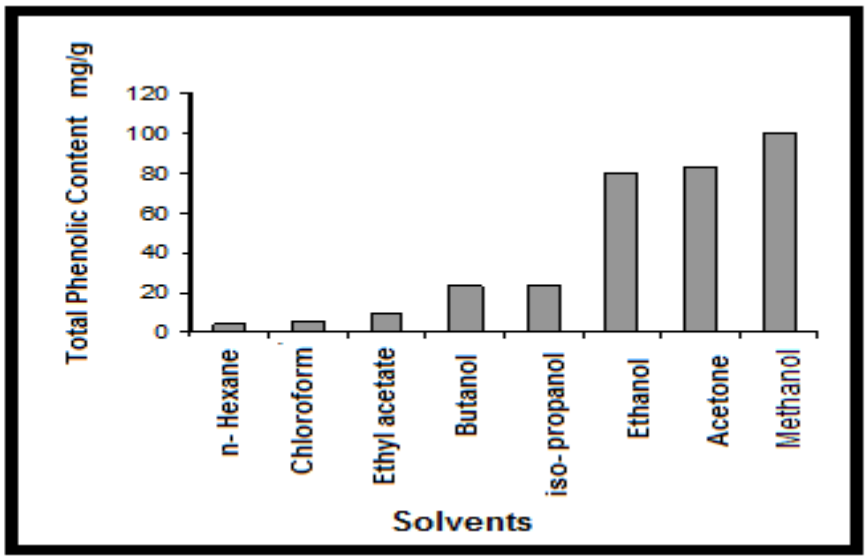

Fig.2: Effect of different solvents on Total Phenolic Contents

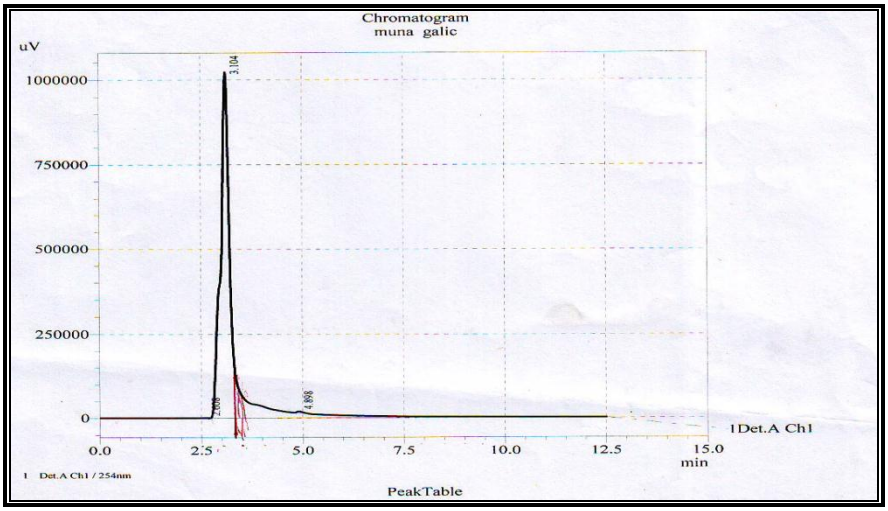

Fig.3: HPLC chromatogram of standard Gallic acid

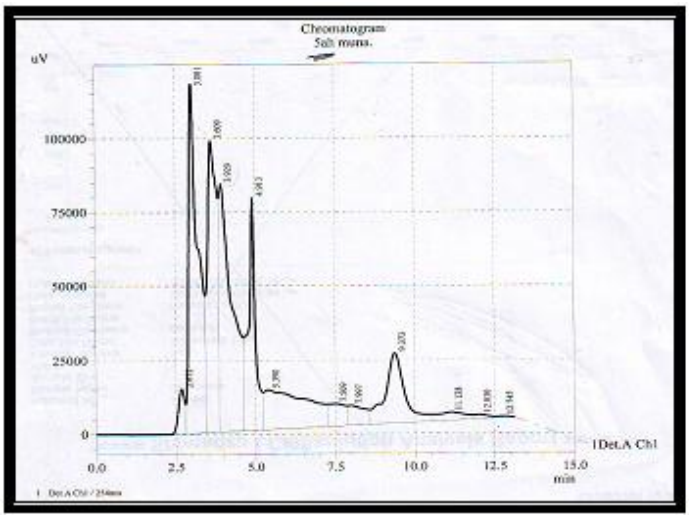

Fig.-4: HPLC chromatogram of plant extract using methanol

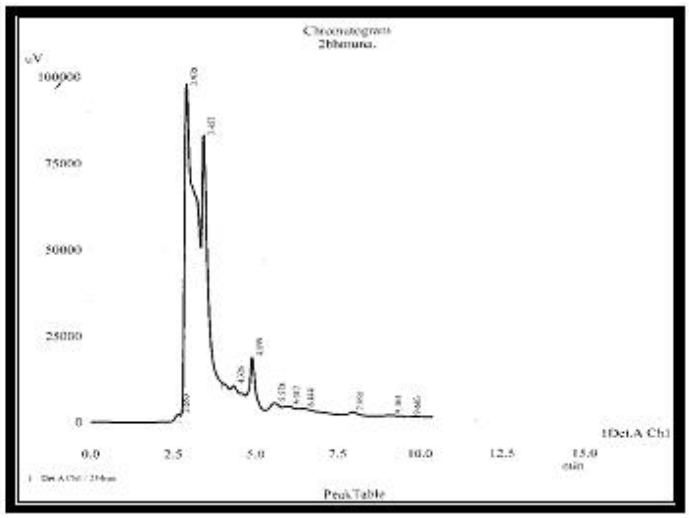

Fig.-5: HPLC chromatogram of plant extract using ethanol

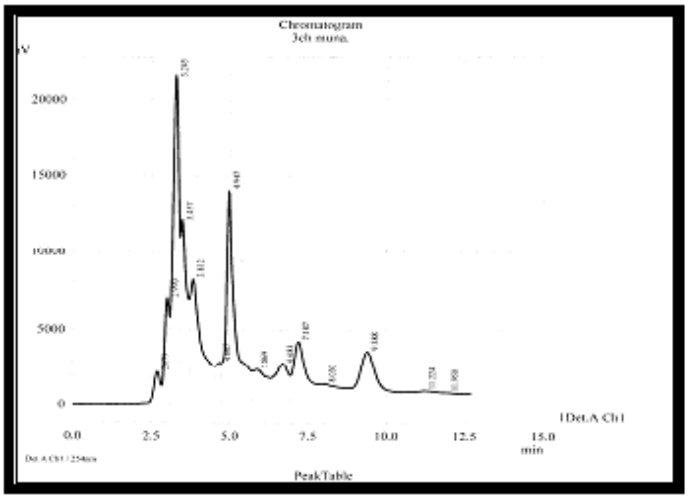

Fig.-6: HPLC chromatogram of plant extract using n-butanol

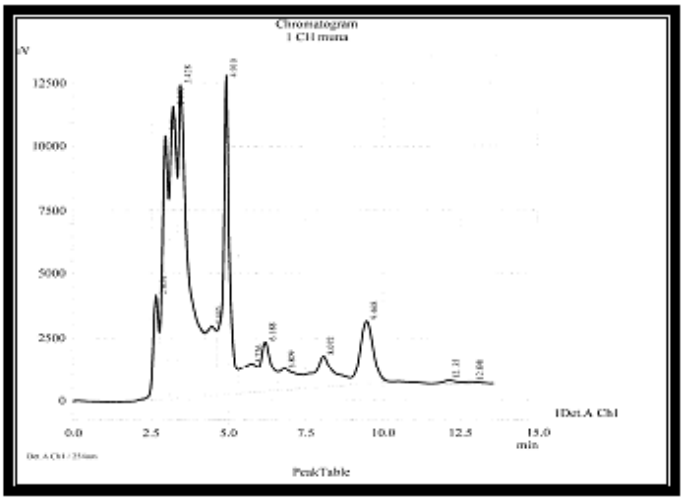

Fig.-7: HPLC chromatogram of plant extract using propanol

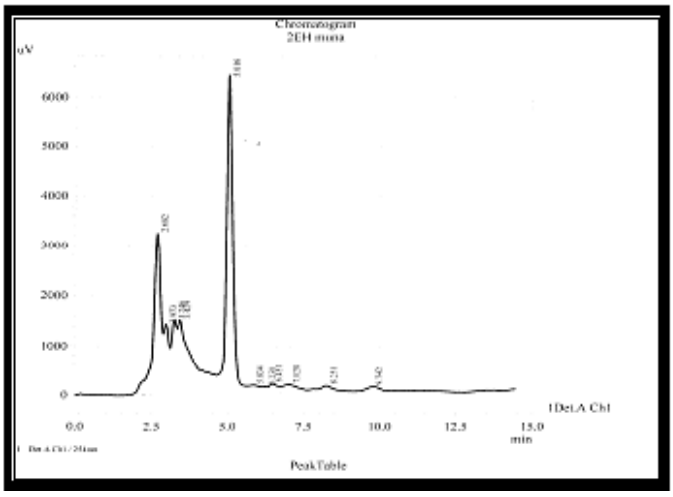

Fig.-8: HPLC chromatogram of plant extract using ethyl acetate

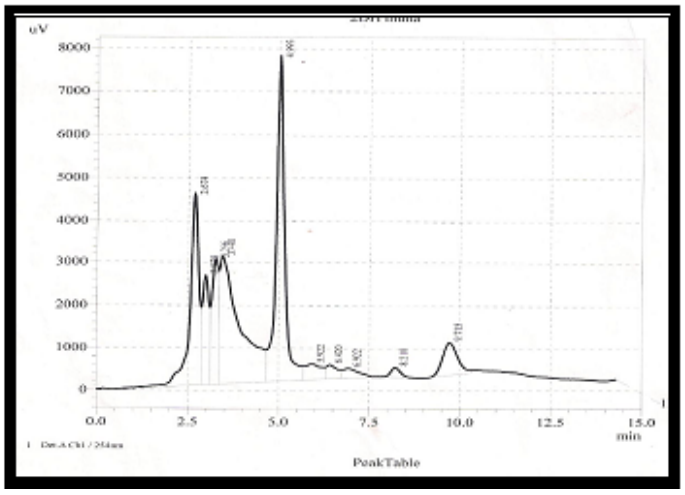

Fig.-9: HPLC chromatogram of plant extract using acetone

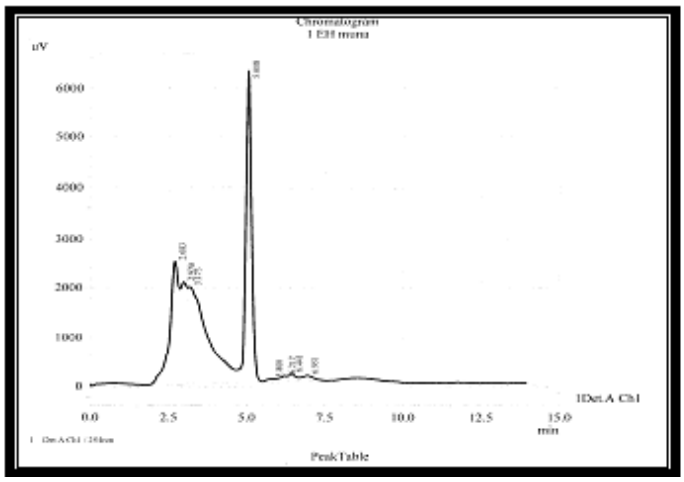

Fig.-10: HPLC chromatogram of plant extract using n-hexane

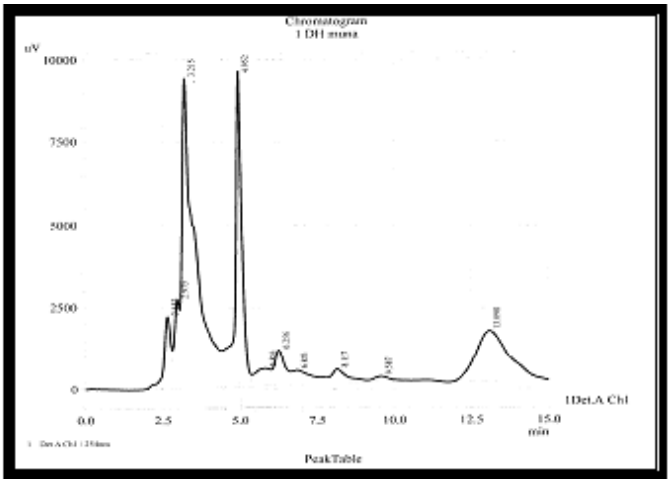

Fig.-11: HPLC chromatogram of plant extract using chloroform

Table-1 : The Accuracy of the Method

Sample No.	Abs.	Conc.mg/g	Av. Conc.	Absolute Error	Relative Error %
1	0.0780	81.5	80.42	-1.5	1.87
2	0.0760	79.5	80.42	0.5	0.62
3	0.0774	80.9	80.42	-0.9	1.12
4	0.0762	79.7	80.42	0.3	0.37
5	0.0770	80.5	80.42	-0.5	0.62

Table-2 : The Precision of the Method

Sample No.	Abs.	Conc. mg/g	Av. Conc. mg/g	S.d	R.S.D %	S.d of the Mean
1	0.1033	99.820	100.44	0.48	0.478	0.215
2	0.1046	101.15				
3	0.1041	100.562				
4	0.1038	100.324				
5	0.1038	100.364				

Table-3: Retention time and %Extraction of different contents(peaks) in different solvents

% Extraction								Retention time, min.	Total Peaks No.
Hexane	Chloroform	Ethyl acetate	Acetone	butanol	iso-propanol	ethanol	methanol		
19.993	22.171	5.083	17.474	-	4.557	-	-	2.764	1
11.608	6.084	4.274	7.381	4.960	11.236	48.070	21.755	2.978	2
33.516	7.052	39.784	8.215	18.550	14.750	-	-	3.256	3
-	20.836	-	26.927	10.895	25.269	29.079	-	3.440	4
-	-	-	-	-	-	-	17.629	3.609	5
-	-	-	-	14.569	-	-	21.420	3.929	6
-	-	-	-	-	5.225	5.119	-	4.326	7
31.391	36.265	21.831	28.029	18.427	15.938	6.159	10.693	4.993	8
-	-	-	-	-	-	-	11.264	5.390	9
-	-	-	-	7.558	-	-	-	7.187	10
-	-	-	5.139	7.779	6.159	-	8.934	9.373	11
-	-	-	-	-	-	-	-	9.715	12
-	-	16.733	-	-	-	-	-	13.098	13

Table-4: Antimicrobial , Antifungal Efficacy in term of %Inhibition and activity index of Teucrium polium extract against selected test bacteria and fungi .

Bacterial and Fungal Strains	Inhibition % (Al-Kut)	Inhibition % (Dehok)
Cladosporium	72.9	43
Aspergillus Niger	67.4	62
Aspergillus parasticus	61.0	34
Staphylococcus aurous positive	46	43
Staphylococcus aurous Negative	44	41
Escherichia coli	55	58
Streptococcus	45	43

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