Journal of Research in Biology

An International Scientific Research Journal

ISSN No: Print: 2231 –6280; Online: 2231- 6299

Original Research

Phytochemical screening and antibacterial study of two medicinal plants *Teucrium capitatium* L and *Silene vulgaris* as a part of ethnobotanical study of the region of El Hajeb (central Morocco)

Authors:
Jalila EL AMRI[1], Khalid EL BADAOUI[1], Touria ZAIR[2], Hayate BOUHARB[1], Said CHAKIR[1] and Taj el molk ALAOUI[1]

Institution:
1. Laboratoire de l'Environnement et de la santé, Faculté des Sciences, Université Moulay Ismail, BP 11201 Zitoune , Meknès, Maroc.

2. Laboratoire de Chimie des molécules bioactives et de l'Environnement, Faculté des Sciences, Université Moulay Ismail, BP 11201 Zitoune, Meknes, Maroc

Corresponding author:
Jalila EL AMRI

ABSTRACT:

Objective: This study was performed to screen phytochemical and antibacterial activity of two different plants *Teucrium capitatium* L and *Silene vulgaris*, which were chosen after an ethnobotanical study to determine the close relationship between plant species and describe the different types of conditions affecting the population

Methods: A phytochemical screening was performed for the detection of alkaloids, carbohydrates, flavonoids, phenolic compounds, resin, saponins, steroids, tannins, terpenoids, proteins, cardiac glycosides, reducing sugars and proteins. Antibacterial activity was performed against *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Pseudomonas putida*, *Pseudomonas arueginosa*.

Results: Ethnobotanical study revealed that the disease dermatitis and gastrointestinal infection tract are most common in the study area In addition, the results showed that the two plants are used for both diseases. These two selected plants were screened for the presence of different chemical components ; the plant *Teucrium capitatium* L showed a highly significant inhibitory effect against *Staphylococcus aureus* ori S and ori R (gram +), while the plant *Silene vulgaris* has no anti-microbial activity.

Conclusion: *Teucrium capitatium* L may act as an anti-microbial agent. The results are promising and encouraging because there is a strong co-relation between: active compounds / antibacterial activity.

Keywords:
Teucrium capitatium L, ethnobotany phytochemical, activity, antibacterial

Web Address:
http://jresearchbiology.com/documents/RA0496.pdf

Article Citation:
Jalila EL AMRI, Khalid EL BADAOUI, Touria ZAIR, Hayate BOUHARB, Said CHAKIR and Taj el molk ALAOUI.
Phytochemical screening and antibacterial study of two medicinal plants *Teucrium capitatium* L and *Silene vulgaris* as a part of ethnobotanical study of the region of El Hajeb (central Morocco)
Journal of Research in Biology (2015) 5(4): 1720-1725

Dates:
Received: 09 Dec 2015 **Accepted:** 20 Feb 2015 **Published:** 16 May 2015

Journal of Research in Biology

INTRODUCTION

Medicinal plants are a precious heritage for humanity and especially for the majority of poor communities in the developing countries who depend on it for their primary health care and their sustainance. (Salhi *et al.,* 2010)

Skin diseases are considered as a set of pathologies whose most visible symptoms occur in organs including skin, mucous membrane and skin appendages (Mozouloua *et al.,* 2011).

An ethnobotanical study was conducted in the area of El Hajeb; according to the survey conducted, the results stastistical allowed us to understand the close relationship between plant species and describe the different types of pathologies affecting their population.

MATERIALS AND METHODS:

Figure 11 shows the number of species found in the study area that deals with a given disease.

Diseases of skin diseases are most common in the study area, 18 species treat these diseases, most of them are healing, the most represented are primarily the species *Plantago psyllium* with a percentage of 25.17%, followed by the species *Corrigiola telephifolia* 14.42%, *Euphorbia helioscopa* 13.13%, *Rosmarinus officinalis* 13.10%, *Nerium oleander* 12.15%, *Solanum sodomeum* L 8%, *Teucrium capitatum* L 7.33% and *Silene vulgaris* 6.7%. (Fig 2) For diseases of the digestive tract 14 species are used, 13 species for the nervous system, 5 species for respiratory system and 4 species for urogenital system specific ailments.

From Figure 3, it is found that 15 species treat diseases of the gastrointestinal tract. The most represented are mainly *Thymus ciliatus*, *Euphorbia helioscopa*, *Origanum compactum* 10% each, *Solanum sodomeum* L, *Silene vulgaris, Teucrium capitatum* L, *Arbutus ajuga iva unedo*, *Helosciadium nodiflorum* 7% each, *Crum carvi* L and *Pimpinella anisum* L 6% and atlast *Ziziphus lotus* 4% (EL Amri *et al.,* 2011)

According to Figures 2 and 3, we observed that the two plants are used for both dermatitis and diseases of the gastrointestinal tract

Protocol for phytochemical screening:

Teucrium capitatium L and *S. vulgaris* plant powders were prepared for testing phytochemical characterization using conventional reagents. Different tests are based on the color reaction and precipitation of reactions specific or general characteristics. (Bruneton and Jean, 1991)

Alkaloids:

10 g of dried vegetable powder was introduced in a 250 ml Erlenmeyer flask, and 50 ml of H_2SO_4 at 10% was added. After stirring, it was let to soak for 24 hours at room temperature and then filtered through a filter paper. The filtrate was made to 50 ml with distilled water.

Characterization

In two test tubes, 1 ml of the filtrate and 5 drops of reagent were added. Mayer's reagent was added in the first tube and 5 drops of Dragendorff reagent in the second tube. If a precipitate appears, the presence of alkaloids is confirmed by their extraction.

Polyphenolic substances

5 g of powder was added in a 100 ml of boiling water taken in an Erlenmeyer flask (250 ml). After infusion for 15 minutes, the filtrate was added to 100 ml of the distilled water.

Tannins

In a test tube, 5 ml of 5% infusion was introduced, 1 ml of aqueous solution and 1% $FeCl_3$ was also added. If tannin is present, then it develops a greenish or blue-black color.

Catechin tannins

To 5 ml of 5% infused solution, 5 ml of concentrated HCl was added. The whole was boiled for 15 min and then filtered through a filter paper. In the presence of catechol tannins, it forms a red precipitate soluble in iso-amyl alcohol.

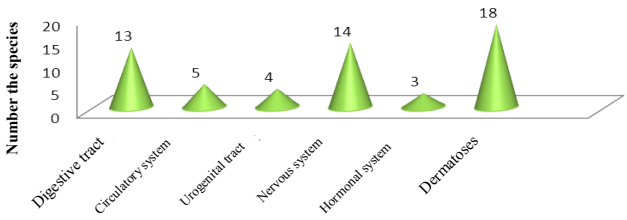

Fig 1. The number of the species in relation with the pathologies found in EL Hajeb

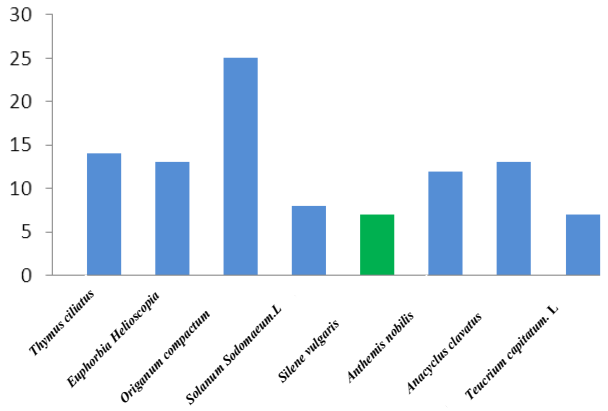

Fig 2. The different species that are used for the treatment of dermatoses

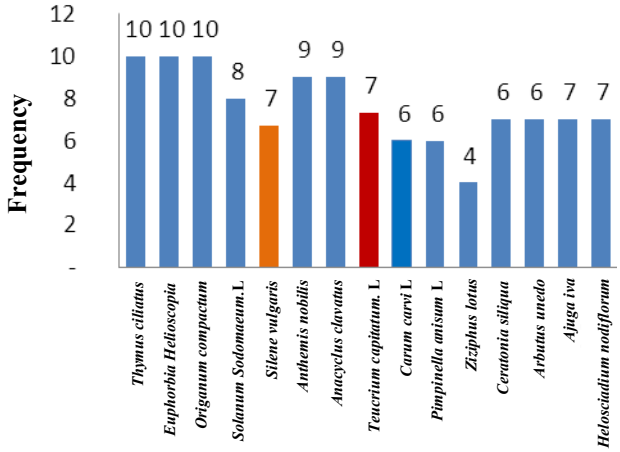

Fig 3. The different species that are used for the treatment for diseases of the digestive tract

Tannins gallic: Reaction Stiasny

30 ml of infused solution at 5% was added to 15 ml of Stiasny reagent (10 ml of 40% formalin and 5 ml of concentrated HCl), and then it was heated in a water bath at 90°C for 15 mn. After filtration, the filtrate was saturated with 5 g of pulverized sodium acetate. Then 1 ml of 1% $FeCl_3$ was added drop wise. Obtaining a precipitate shows the presence of gallic tannins.

Filter and saturate 10 ml of the filtrate of sodium acetate. Then few drops of 1% $FeCl_3$ was added. The development of a blue-black color indicates the presence of gallic tannins not precipitated by the reagent Stains.

Flavonoids

At 5% infusion a lighter or darker color, 5ml del'acide H_2SO_4 10% and a base (NH_4OH) were added. If the color is accentuated by acidification, then it turns blue-violet in the basic medium, this allows to conclude the presence of anthocyanins.

Reaction to cyanidin:

5 ml of 5% infused solution was introduced in a test tube, and 5 ml of hydrochloric alcohol (95% ethanol, distilled water, concentrated HCl in equal parts by volume) was added; then some magnesium turnings and 1 ml of iso-amyl alcohol was added to it.

The appearance of an orange-pink color (flavones) or purplish pink (flavonones) or red (flavonols, flavononols) gathered in the iso -amyl alcohol supernatant indicates the presence of a free flavonoid (aglucones).

Leucoanthocyanes

The reaction to cyanidin was performed without adding magnesium chips and heated in a water bath for 15 minutes. In the presence of leucoanthocyanes, it developed a cherry red color or purple. The presence of catechols give a red-brown color.

Anthracene derivatives:

Free anthraquinones

1 g of powder, was added to 10 ml of chloroform and heated for 3 min in a water bath. The filtrate was heated and supplemented to 10 ml. 1 ml of chloroform extract was obtained, and added with 1 ml of NH_4OH was diluted and agitated. The more or less red color indicates the presence of free anthraquinones.

Anthraquinone combined:

O -hétérosides:

From the residue of the drug exhausted with

chloroform, we have prepared a hydrolyzate which was added to 10 ml of water, 1 ml of concentrated HCl and the test tube was kept in a water bath for 15 minutes. 5 ml of the hydrolyzate are stirred with the 5 ml of chloroform. The organic phase, was added to 1 ml of diluted NH_4OH and the presence of anthraquinone was revealed by more or less dark red color.

The reaction may be further enhanced by adding 5 ml of the hydrolyzate and 3 to 4 drops of $FeCl_3$ at 10% concentration, and stirred with 5 ml of chloroform. At the chloroform phase, 1 ml of diluted NH_4OH was added and shaked. In the presence of oxidation products such as anthranols or anthrones, the red color emerges as more intense than before.

The C- glycosides:

The chloroform test was repeated which was stored in 10 ml of water and then 1 ml of 10% $FeCl_3$ was added. After boiling in a water bath for 30 min, and stirred with 5 ml of chloroform, the chloroform phase was appeared and 1 ml of dilute NH_4OH was added. A more or less intense red color indicates the presence of C -hétérosides aglucones.

Sterols and triterpenes

The sample to be tested was obtained from 1 g of plant powder and 20 ml of ether left macerated for 24 hours, then filtered and made up to 20 ml with ether. After evaporated to dryness 10ml of evaporated soluted and 1 ml of chloroform was mixed. The solution obtained is divided into two test tubes, and then 1 to 2 ml of concentrated H_2SO_4 was added to the bottom of one of the tubes, the other is used as a control. When the two liquids contact each other, there is a formation of brownish purple or red ring; the supernatant became green or violet reveals the presence of sterols and triterpenes.

Saponosides

100 ml of distilled water in a 250 ml Erlenmeyer flask was boiled and added to 1g of powder and kept simmering for 15 minutes. After filtration, the filtrate was adjusted to 100 ml by the addition of distilled water. In a series of 10 test tubes numbered from 1 to 10, we divided successively 1,2, … .10 ml of the decoction prepared in 1% and adjusted the volume in each tube to 10 ml with distilled water. Then, each tube was shaken in the longitudinal direction for 15 seconds at a rate of 2 stirs per second. After being allowed to stand for 15 minutes, height of the foam in each tube was measure. The tube in which the height of the foam is 1 cm indicates the foam index:

Foam index = 1000 / Tube Number

Reducing compounds

We introduced 5 ml of 10% aqueous decoction in a100 ml beaker and evaporated to dryness in a water bath. To the residue 1 ml of Fehling's reagent was added. Obtaining a brick-red precipitate indicates the presence of reducing compounds.

Oses and holosides

We introduced 5 ml of 10% decoction in a 100 ml beaker and evaporated to the dry water bath. To the residue 2-3 drops of concentrated H_2SO_4 was added. After 5 minutes, we ajouté3 4 drops of ethanol saturated with thymol. The development of a red color indicates the presence of monosaccharides and holosides.

Mucilage

We have 1 ml of 10% decoction in a test tube and added 5ml of absolute ethanol. After ten minutes, a flaky precipitate was obtained by mixing, indicated the presence of mucilages

The method of dilutions

Dilution method of preparing a series of Mueller-Hinton broth tubes containing essential oil concentrations ranging from 0.25 mg/ ml to 20 mg/ ml and inoculated it with a population of test organism .

Measurement of the activity:

In liquid dilution macrométhode is used to determine the parameters of the inhibition of bacterial growth (MIC, MBC), active extracts.

The results were presented in Table 1:

Name of the compounds	Test name	Plant 1	Plant 2
Alkaloids	Mayer Dragendorff	+	+
Tannins	Diluted solution of ferric chloride	+	+
Catechin tannins	Concentrated HCl	+	+
Gallic tannins	reaction Stiasny	+	
Flavonoids:			
Anthocyanins	H_2SO_4 / NH_4OH	+	+
Flavones and flavonoids free (Genine)	Reaction to cyanidin	+	-
Leucoanthocyanes	Reaction to cyanidin without Mg	-	-
Sterol and Triterpenes	Chloroform / acetic anhydride / H_2SO_4	+	-
Reducing compounds	Fehling reagent	-	-
Oses and holosides	H_2SO_4 / ethanol / thymol	-	-
Cyanogenic glycosides	Toluene	-	-
Anthraquinone free	Chloroform / NH_4OH	+	-
Anthraquinone combined:			
O - glycosides	HCL / NH_4OH	-	-
C - glycosides	$FeCl_3$ / NH_4OH		
Saponosides	Foam Index *	-	+

Antimicrobial effect by the method of direct contact:

The results of antimicrobial effect is given in the following table:

Table 2: Antimicrobial capacity according to the direct contact method

Dilutions	0	1/2	1/4	1/8	1/16	1/32	1/50	1/64	1/80	1/100
IU / ml (ET / Water)	SM	1000	333.3	142.86	66.66	32.26	20.41	15.87	12.66	10.10
***Staphylococcus aureus meti* S**	_	_	_	_	_	_	_	_ +	+	++
***Staphylococcus aureus meti* R**	_	_ _	_	_ +	_ +	_ +	++	++	++	++

Minimum inhibitory concentration (MIC) is carried out by successive dilutions 1/2, 1/4, 1/8, 1/16, 1/32, 1/50, 1/64, 1/80, 1/128 (Oussou *et al.,* 2003). Due to the immiscibility of ET in the water and therefore to the culture medium, the emulsification was carried out with a 0.2% agar solution to foster the germ contact/ compound. (Oussou *et al.,* 2003)

Minimum bactericidal concentration (MBC):

The nutrient agar poured into petri plates is streaked with 100µl of the contents of tubes having a concentration greater than or equal to CMI (≥ CMI) in the series of previous dilution. WCD is determined after incubation for 24 hours at 37°C. This is the lowest concentration that completely inhibits the growth.

The antibacterial effect was found bactericidal or bacteriostatic versus the ratio: CMB / CMI .In fact, if CMB / MIC = 1-2, the effect is bactericidal and if CMB / MIC = 4 to 16, the effect is bacteriostatic ((Berche *et al.,* 1991)

RESULTS :

Phytochemical screening of the plant *Teucrium capitatium and Silene vulgaris* L *:*

Preliminary phytochemical examination of various extracts of the plant *Teucrium capitatium* L and *Silene vulgaris* indicates the presence of sterols, steroids, alkaloids, tannins, flavonoids

The results in the table 1, the largest area of inhibition was observed in the case of *Staphylococcus*

aureus Métis. This species is more sensitive to this oil. For other strains tested, gram neg have not showed zones of inhibition, Our results are in agreement with those of Zaika (1988) who commonly recognized that Gram-negative bacteria are more resistant to essential oils that appears precisely in our study.

For the activity coefficient, we found that the ori S strain is close to 1 worth 0.73 while the value of the strain ori R has only 0.05.

For the dilution method to determine the value of MIC and MBC, there is a lack of growth in all strains at the stock solution and diluted between 1/2 and 1/50 for the ori strain S while the *Staphylococcus aureus* meti R is resistant with an MIC of 333.3 IU / ml. MICs for *Staphylococcus aureus* strains ori S are between 20, 41 and 333.3 .mu.l / ml.

The nature of the oil business is done on gram-positive bacteria. It is bactericidal against *Staphylococcus aureus* ori S and bacteriostatic for *Staphylococci aureus* ori R.

The essential oil of *Teucrium capitatium* L is active on all the strains tested. Comparatively speaking, the activity of the essential oil of *Teucrium capitatium* L by both methods (Aromatogram and direct contact) are close to that of the essential oil of *Thymus vulgaris* thymol vis-à-vis the strains of *Staphylococcus aureus* . (EL Amri J *et al.,* 2014)

CONCLUSION:

According to the ethnobotanical study, the two plants were chosen for their biological activity and their phytochemical screening; The study of the plant *Teucrium capitaium* L showed the presence of an interesting biological activity on *Staphylococcus aureus* ori S and ori R; phytochemical screening has allowed an initial characterization of active compounds. The results are promising and encouraging because there is a strong co-relation between active compounds and antibacterial activity.

RÉFÉRENCES :

Salhi S, Fadli M, Zidane L & Douira A, 2010. Études floristique et ethnobotanique des plantes médicinales de la ville de Kénitra (Maroc). Lazaroa 31(9): 133-146.

Mozouloua D, Apema AKR et Nguengue JP. 2011. Étude préliminaire des plantes médicinales à effets antidermatosiques utilisées en pharmacopée à Bangui. vol 2 URSAD. 3,5 et 6p.

EL Amri J, EL Badaoui K, Zair T, Bouharb H, Chakir S and Alaoui T. 2014. Ethnobotanical study of medicinal plants in the region Elhajeb (central Morocco)4 (8)1568-1580.

Bruneton, Jean. 1991. Pharmacognosie, Phytochimie et Plantes médicinales, 3 ème Edition, Technique & Documentation, Paris, France.

Oussou KR, Kanko C, Guessend N, Yolou S, Koukoua G, Dosso M, N'guessan YT, Figueredo G and Chalchat JC. 2003. Activités antibactériennes des huiles essentielles de trois plantes de Côte d'Ivoire. C.R. Chimie. 7 (10-11) 1081-1086.

Berche P, Gaillard JL and Simonet M. 1991. Les bactéries des infections humaines. Editeur: Flammarion, Médecine & Sciences P 650 .

EL Amri J, EL Badaoui K, Zair T, Bouharb H, Chakir S and Alaoui T. 2014. Etude del activité antibactérienne des huiles essentielles *Teucrium capitatium* L et del extraitde *Siléne vulgaris* sur différentes souches testeer.