PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS FOR FIVE PLANT SPECIES IN IRAQI KURDISTAN REGION

Pshtiwan Adel Masum and Peshawa Shafiq Osw
Chemistry Department, College of Science, Salahaddin University, Erbil, Iraq.
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ABSTRACT:

Iraqi Kurdistan region is well known for its rich traditional medicinal plants. The present study is a preliminary qualitative phytochemical screening, using ultrasonic technique for extraction through three solvents; petroleum ether, ethyl acetate and aqueous ethanol. The studied plants included *Malabaila secacula* (root), *Muscari longipes* (bulb), *Crepis sahendi* (root), *Nepeta trachonitica* (aerial part) and *Daphne mucronata* (aerial part). All studied plants were rich sources for flavonoids and carbohydrates, inversely; alkaloids were absent in both ethyl acetate and aqueous ethanolic extracts, while glycosides, phenolics, tannins, saponins, amino acids and proteins showed various results in the previous two extracts. In addition, phytosterol constituents were present in petroleum ether extracts from all the studied plants except for bulb of *Muscari longipes*. Phytochemicals diversity suggested that *Nepeta trachonitica* is the best relative to the other studied species, this will be helpful for further phytochemists and pharmacologists investigations.

KEYWORDS: *Phytochemical screening, ultrasonic, plant extracts, bioactive constituents.*

INTRODUCTION:

Natural products have served as an important source of drugs since ancient times, and about half of the useful drugs today are derived from natural sources. Since the beginning of social life organization, mankind has been on a quest to fight diseases and improve the quality of life (Wetzel *et al.*, 2010).

Kurdistan region of Iraqi is considered as a rich area for the medicinal herbs. People of this region have been used plants in many ways, as food, spices, perfumes and drugs. *Daphne mucronata* Royle is a globally known medicinal plant; it belongs to the Thymelaeaceae family **(Figure 1)**. This plant grow in open rocky high mountain slopes and valleys, screes above the tree line, in damp places on the margin of the oak forest, giving flowers and fruits during May-August. It is also distributed in Turkey, Transcaucasia, Iran, West Pakistan, Afghanistan, Kashmir and North West India (Townsend and Evan, 1980). It has been traditionally used in the treatment of skin disorders and cancer (Katayoun *et al.*, 2003). Locally it is known as Teru, and traditionally used as anti-hemorrhoids. Various chemical compounds were isolated form *D. mucronata* such as cinnamic acid, two coumarin derivatives, some steroids and flavonoids (Muhammad *et al.*, 2009).

Nepeta trachonitica Post (Labiatae) is a perennial plant locally known as Pungi Kewe **(Figure 1)**. It is Perennial plant which distributed in Kurdistan region and Syrian Desert (Djebel druze). The stem is about 35-110 cm, flowering at May-June on Rocky slopes (Davis, 1982). Phytochemically, only one investigation recorded, in which sixty seven components from the aerial parts were isolated, spathulenol was the major constituent (Tümen *et al.*, 1999).

Malabaila secacula Boiss (Apiaceae) named as Gezari Kewe in Kirkuk governorate **(Figure 1)**. It is Perennial plant species, distributed in Kurdistan region, West Syria, North West Iran.; Stems are about 15-75 cm. Flowering is in May-July (Davis, 1972).

Muscari longipes Boiss is belong to the Asparagaceae family **(Figure 1)**. Its niches are the mountain on rocky slopes, deep soil pockets on eroded sandstone ridges near lower limit of oak forest and in fields. The flowering time is between March-April and fruits appeared from May-June. In Iraq the plant commonly is distributed in the lower forest zone, steppe region and the North West sector of the desert region, and globally in Central and Mediterranean Europe, Syria, Palestine, Jordan, Egypt, Turkey, Iran, North Africa (Townsend and Evan, 1985).

Crepis sahendi Boiss is Perennial plant, 17-45 cm. This one is locally called Mam Miran, and traditionally used for abdomen ache. Mainly it is distributed in Kurdistan region, Transcaucasia, North West Iran, and Turkey (Davis, 1975) **(Figure 1)**.

In Kurdistan region, no attempt had been done on the studied plants, while globally there

is only one investigation recorded on *N. trachonitica*, and no data of chemical or medicinal investigations are available on the last three plants. Therefore the present study aimed to explore the presence of various phytochemicals in three different extracts for the studied plant species to evaluate their therapeutic values.

MATERIALS AND METHODS:

Plant materials:

The fresh whole plants *Muscari longipes* Boiss, and *Malabaila secacula* Boiss were collected during April 2014 from Seamansur village, Kirkuk governorate, Iraqi Kurdistan region. While *Crepis sahendi* Boiss, *Nepeta trachonitica* and *Daphne mucronata* were collected in Hallgurd Mountain, Erbil governorate during July 2012. The collected plant species were classified and identified in ESUH (Education Salahaddin University Herbarium) by Professor Dr. A. H. Al-Khayat and Dr. A. Shukur. The appropriate part for each studied plants used as shown in Table 1.

The plant materials were collected, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in a plastic bottle in a dark place at room temperature until the time of use.

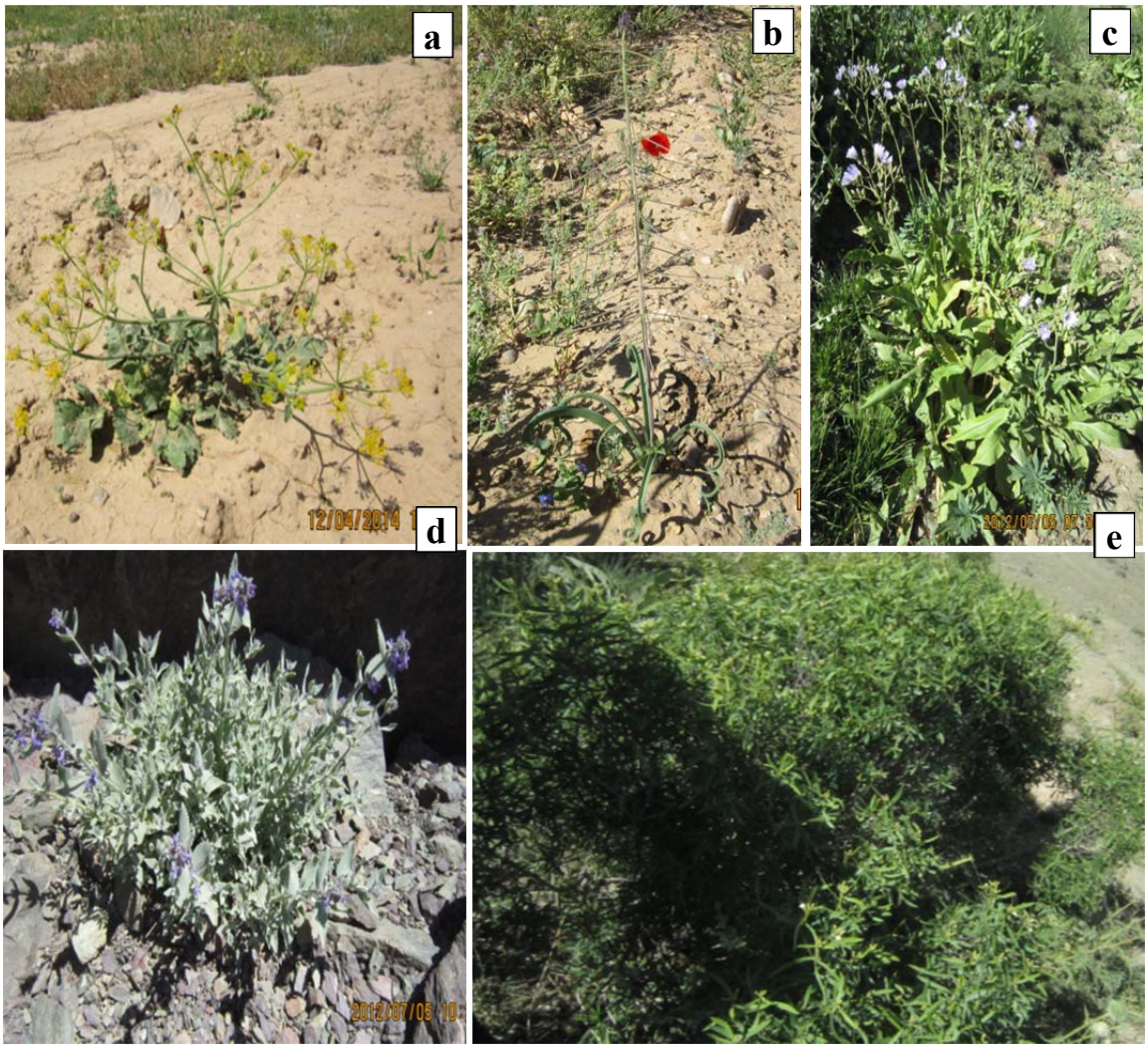

Figure (1): The plants applied used in the current investigation with presenting their parts: a. *Malabaila secacula, b. Muscari longipes*, c. *Crepis sahendi, d. Nepeta trachonitica and e. Daphne mucronata*.

Extraction of plant materials:

Twenty grams of shade dried powder of each plant were sonicated using ultrasonic bath (Telsonic Power Cleaning-25, Switzerland) firstly with 400 ml petroleum ether (100 x 4 times) for 2 hours, then filtered (Whatman no. 41) and dried. The residues were sonicated with 400 ml ethyl acetate (100 x 4 times) for 2 hours, filtered and dried. Finally the residue were further sonicated with 400 ml 80% ethanol (100 x 4 times) for 2 hours, and then filtered and dried. All solvents were removed using rotary evaporator (Buchi rotavapor R-114, Switzerland) and the crude extracts were dried at room temperature in steady air-current and stored in dark bottle at 4 °C until use.

Preliminary phytochemical tests:

The extracts were tested for the presence of bioactive constituents by using following standard methods (Palanisamy *et al.,* 2011; Yadav and Munin, 2011; Satheesh *et al.,* 2012; Dipali and Vilas, 2013; Muhammad *et al.,* 2012; Anees and Seemi, 2008 and Solomon *et al.,* 2013):

Detection of Carbohydrates:

A bout 0.5 g of various extracts were separately dissolved in 20 ml distilled water and filtered. The filtrate was subjected to various test for detecting the presence of carbohydrates (Molisch, Benedict, Barfoed, Bial, Seliwanoff and Iodine tests) and glycosides.

Detection of Glycosides:

Keller-kilani test was performed via mixing of crude extract with 2 ml of glacial acetic acid containing 1-2 drops of 2% $FeCl_3$ solution. The mixture was then poured into another test tube containing 2 ml of Conc. H_2SO_4. The appearance of reddish-brown color in the lower layer and bluish-green color in the upper layer indicated the presences of glycosides.

Detection of Phenolic compounds:

Ferric chloride test was used by taking small quantities of various extracts separately in water. Few drops of 5% $FeCl_3$ solution were added to 1 ml of each extracts. The appearance of a green-blue or deep blue (black) colour indicates the presence of phenolic compounds.

Detection of Flavonoids:

Alkaline reagent test was applied by treating crude extract with 2% NaOH solution, an intense yellow color was formed which turned colorless on addition of few drops of dilute HCl, which indicated the presence of flavonoids.

Detection of Tannins:

A bout 0.1 g of the various extracts was taken separately in 5 ml water and test for the presence of tannins, which was carried out with the following reagents.

Braymer's test was used by treating 2 ml of extracts with 10% alcoholic $FeCl_3$ solution, the formation of blue or greenish colour solution indicates the presence of tannins.

Lead acetate test was used by adding 1 ml of 10% $Pb(CH_3COO)_2$ solution to 1 ml of each extract, the appearance of white precipitate indicate the presence of tannins.

Detection of Alkaloids:

Dragendorff's test was performed through warming about 0.2 g of each extracts with 2% H_2SO_4 for 2 min., then filtered and a few drops of Dragendorff's reagent were added. Orange-red ppt. indicated the presences of alkaloids.

Detection of Phytosterols:

A bout 0.1 g of various extracts was dissolved in 5 ml of chloroform separately. Then the chloroform solution was subjected to the following tests:

Salkowski's test was used by adding 1 ml of above prepared chloroform solution with 1 ml of Conc. H_2SO_4, gently shaked and allowed to stand. Formation of pale red-pink in chloroform layer and deep red in acid layer indicates the presence of triterpenes.

Libermann-Burchard test was applied by treating the above prepared chloroform solution with five drops of acetic anhydride, mixed well, followed by adding 1 drop of Conc. H_2SO_4. A pale orange-green colour appeared indicates the presence of steroids.

Detection of Proteins and Free Amino acids:

A bout 0.5 g of various extracts was dissolved in 10 ml of water and then they were subjected to Biuret and Ninhydrin tests.

Detection of Saponins:

Foam test was used by adding of 2 ml of the extract to 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

RESULTS:

The phytochemical constituents from the studied plants were extracted using different solvents depending on increasing polarity including petroleum ether, ethyl acetate and 80% ethanol **(Table 1)**. Accordingly, *N. trachonitica* recorded the highest yield percentage in petroleum ether extract, while in ethyl acetate and 80% ethanol extracts *M. longipes* recorded the highest yields.

Table (1): The percentage yields of different extracts for the studied plants.

Plant Name	Used Part	Percentage yield		
		Petroleum ether (40-60 °C)	Ethyl acetate	%80 Ethanol
Malabaila secacula Boiss	Root	0.65	9.95	14.35
Muscari longipes Boiss	Bulb	0.60	21.70	26.70
Crepis sahendi Boiss	Root	1.35	18.95	7.00
Nepeta trachonitica Post	Aerial	1.95	10.35	7.85
Daphne mucronata Royle	Aerial	1.25	15.65	7.80

According to Salkowski test, phytosterols was present in petroleum ether extracts of all studied plants except *M. longipes*, while according to Libermann-Burchard test, only *N. trachonitica* and *D. mucronata* gave positive result for the presence of phytosterols **(Table 2)**. All tested plants in the current study were considered as rich sources for flavonoids and carbohydrates, whereas, alkaloids were absent. Glycosides, phenolics, tannins, saponins, amino acids and proteins were showed different results in both ethyl acetate and aqueous ethanolic extracts **(Table 3)**. From ethyl acetate extract, the glycosides, proteins and amino acids were found in *M. secacula*, *M. longipes* and *N. trachonitica* plants, while the phenolics were found in *C. sahendi*, *N. trachonitica* and *D. mucronata* plants. However, tannins were present in all studied plants except *M. secacula* and *M. longipes*, while saponins were found only in *M. secacula* and *N. trachonitica*. The result for glycosides, phenolics and tannins in aqueous ethanolic extract were the same as ethyl acetate extract; while, proteins and amino acids were present in all studied plants except in *C. sahendi*. Saponins were found only in *M. longipes*, *N. trachonitica* and *D. mucronata* plants.

Table (2): Phytosterol tests for the studied plants.

Phytochemical Constituents	Chemical Tests	Petroleum ether Extract				
		Ms	*Ml*	*Cs*	*Nt*	*Dm*
Phytosterols	Salkowski	+	-	+	+	+
	Libermann-Burchard	-	-	-	+	+

'+' presence; '-' absence, Ms= Malabaila secacula, Ml= Muscari longipes, Cs = Crepis sahendi, NT = Nepeta trachonitica, Dm= Daphne mucronata.

Table (3): Preliminary phytochemical screening of two extracts for the studied plants.

Phytochemical Constituents	Chemical Tests	Ethyl acetate Extract					80% Ethanol Extract				
		Ms	***Ml***	***Cs***	***Nt***	***Dm***	***Ms***	***Ml***	***Cs***	***Nt***	***Dm***
Carbohydrates	Molisch	+	+	+	+	+	+	+	+	+	+
	Benedict	+	+	+	+	+	+	+	+	+	+
	Barfoed	+	+	+	+	+	+	+	+	+	+
	Bial	-	-	-	-	-	-	-	-	-	-
	Seliwanoff	+	+	+	-	+	+	+	+	-	+
	Iodine	-	-	-	-	-	-	-	-	-	-
Glycosides	keller-kilani	+	+	-	+	-	+	+	-	+	-
Pheolics	Ferric chloride	-	-	+	+	+	-	-	+	+	+
Flavonoids	Alkaline reagent	+	+	+	+	+	+	+	+	+	+
Tannins	Braymer	-	-	+	+	+	-	-	+	+	+
	Lead acetate	-	-	+	+	+	-	-	+	+	+
Alkaloids	Dragendorff's	-	-	-	-	-	-	-	-	-	-
Proteins & Amino acids	Ninhydrin	+	+	-	+	-	+	+	-	+	+
	Biuret	+	+	-	+	-	+	+	-	+	+
Saponins	Foam	-	+	-	+	-	-	+	-	+	+

'+' presence; '-' absence, Ms= Malabaila secacula, Ml= Muscari longipes, Cs= Crepis sahendi, Nt= Nepeta trachonitica, Dm= Daphne mucronata.

DISCUSSION:

The phytochemical analysis of the present results indicated that all plants in the current investigation contain several bioactive substances such as phytosterol, flavonoid and phenolic components. These metabolite compounds are known to exhibit medicinal as well as physiological activities (Sofowra, 1993).

This result also give a special indication of medicinal importance because of the presence of flavonoids, which are beneficial for human health due to a large range of biological activities such as anti-mutagenic, immune-stimulating, anti-inflammatory, arteriosclerosis inhibiting effects, anti-oxidant or free radical scavengers (Muhammad *et al.,* 2012)

C. sahendi, *N. trachonitica* and *D. mucronata* contain phenolic compounds and tannins. Phenolic compounds posses' biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-oxidant, anti-inflammations, anti-atherosclerosis, cardiovascular protections and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Singh *et al.,* 2007; Xiuzhen *et al.,* 2007 and Brown and Rice-Evans, 1998). Tannins are polyphenolic compounds which considered as primary anti-oxidant or free radical scavengers and have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Chung *et al.,* 1998).

Glycosides have been found in *M. secacula*, *M. longipes* and *N. trachonitica,* which are usually cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia (Brian *et al.,* 1985).

M. longipes, *N. trachonitica* and *D. mucronata* contain saponins, which are other type bioactive chemical constituents which are involved in plant disease resistance because of their antimicrobial activity (Anyasor *et al.,* 2010).

Steroids have been found in all the studied plants except *M. longipes,* which have anti-bacterial properties and they are very important compounds especially due to their relationship

with compounds such as sex hormones (Raquel *et al.,* 2007 and Okwu *et al.,* 2001).

CONCLUSION:

The results of the present study revealed the importance of the studied plants to be used in folk medicine, because they are rich sources for many biologically active compounds such as flavonoids, glycosides, steroids, tannins and saponins.

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لێکۆڵینەوەیەکی کیمیایی سەرەتایی بۆ دەرهێنراوی جیاوازی پێنج جۆرە ڕووەکی هەرێمی کوردستانی عێراق

پوختە:
هەرێمی کوردستانی عێراق بەوە ناسراوە کە دەوڵەمەندە بەرووەکی پزیشکی میللی. ئەم توێژینەوەیە بریتییە لە لێکۆڵینەوەیەکی کیمیایی سەرەتایی جۆری بە بەکارهێنانی تەکنیکی شەپۆلەکانی سەروو دەنگ بۆ دەرهێنانی ئاوێتە کیمیایی یەکان لەڕێگەی سێ توێنەری جیاوازەوە: ئیسەری پیتزۆڵی، سرکەی ئەسیلی و ئیسانۆلی ئاوی. ئەو رووەکانەی کە لێکۆلینەوەیان لەسەر ئەنجام دراوە بریتتین لە *Malabaila secacula* (ڕەگ)، *Muscari longipes* (سەلك)، *Crepis sahendi* (ڕەگ)، *Nepeta trachonitica* (بەشی سەر زەوی) و *Daphne mucronata* (بەشی سەر زەوی). هەموو رووەکە بەکارهاتووەکان لەم توێژینەوەیەدا سەرچاوەیەکی دەوڵەمەندن بۆ ئاوێتە فلاڤۆنۆیدی و کاربۆهایدراتەکان، بەپێچەوانەوە هیچ جۆرە ئەلکەلۆیدێکیان تێدا نی یە لە هەردوو دەرهێنراوی سرکەی ئەسیلی و ئیسانۆلی ئاوی. هەریەکە لە ئاوێتەکانی گلایکۆسید، فینۆڵ، تانین، سابۆنین، ترشی ئەمینی و پرۆتین بە ڕێژەی جیاواز هەن لە ناو هەردوو دەرهێنراوی پێشوو. لەلایەکی تریەوە پێکهاتە فایتۆستیرۆیدەکان لە دەرهێنراوی ئیسەری پیتزۆڵی هەموو ڕووەکەکاندا هەن جگە لە سەلکی رووەکی *Muscari longipes*. هەمەجۆری پێکهاتەکیمیایەکانی ڕووەکی *Nepeta trachonitica* وایکردووە کە باشترین بێت بە بەراورد بە ڕووەکەکانی تری ئەم توێژینەوەیە، ئەمەش ڕێخۆشکەرە بۆ توێژینەوەی پتری کیمیایی و دەرمانسازی.

دراسة كيميائية اولية لمستخلصات مختلفة لخمسة انوع من النباتات المتواجدة في اقليم كوردستان العراق

الخلاصة:

اشتهر اقليم كوردستان العراق بنباتات الطبية المحلية. هذة الدراسة هي دراسة كيميائية اولية نوعية، استخدم فيها جهاز الامواج الفوق الصوتية لغرض الاستخلاص من خلال ثلاث مذيبات مختلفة: الايثر البترولي، خلات الاثيل و الايثانول المائي. يتضمن النباتات المدروسة *Malabaila secacula* (الجذر)، *Muscari longipes* (البصلة)، *Crepis sahendi* (الجذر)، *Nepeta trachonitica* (الجزء الهوائي) و *Daphne mucronata* (الجزء الهوائي). ان جميع النباتات المدروسة غنية بالفلافونويدات و الكاربوهيدرات على عكس القلويدات التي لم يتواجد فيها في المستخلصين خلات الاثيل و الايثانول المائي، في حين ان جليكوسيدات، الفينولات، التانىن، السابونىن، الاحماض الامينية والبروتينات قد اظهرت نتائج مختلفة وذلك في نفس المستخلصين المذكورين. بالاضافة الى ذلك فقد تواجدت المكونات الفيتوستيرولية في مستخلص الايثر البترولي لجميع النباتات المدروسة عدا بصلة النبتة *Muscari longipes*. ان تنوع المركبات الكيميائية لنبتة *Nepeta trachonitica* يشير الى انها الافضل من بين النباتات المدروسة، وهذا ما يفسح المجال للمزيد من الدراسات الكيميائية و الصيدلانية.