PRELIMINARY PHYTOCHEMICAL SCREENING OF *IRIS PERSICA* L. (FLOWERS, LEAVES, BULBS AND RHIZOMES) COLLECTED IN KURDISTAN REGION-IRAQ.

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ABSTRACT:

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. This paper reports the first investigation of phytochemical constituents present in the methanolic extracts of flowers, leaves, bulbs and rhizomes of *Iris persica* L. (Iridaceae), collected in Korek Mountain (Rawanduz) in the Kurdistan Region-Iraq, which is used by local people for the treatment of wound inflammation and tumor. The phytochemical analysis was performed to detect the presence of flavonoids, polyphenols, terpenoids, protiens and reducing sugar in all extracts of *Iris persica*. While tannins and saponins were found in bulb and rhizome extracts only, alkaloids, steroids, aminoacids and anthraquinons were found to be absent in all extracts.

***KEYWORDS*:** *Iris persica, Phytochemical Constituents, Qualitative Analysis.*

INTRODUCTION:

Medicinal plants are a rich source of numerous pharmacologically active molecules. Scientists are currently focusing on the phytochemicals to treat numerous ailments affecting the mankind (Rajesh, et al., 2013). The genus *Iris* belongs to the family Iridaceae, which comprises over 300 species. The phytochemical screening and chemical investigations of various species of *Iris* have resulted in the isolation of variety of secondary metabolites. Approximately more than two hundred compounds have been reported from the genus *Iris* which includes flavonoids, isoflavonoids and their glycosides, benzoquinones triterpenoids and stilbenes glycosides (Guo-Yong, et al., 2013). *Iris* species have an immense medicinal importance and are used in the treatment of cancer, inflammation, bacterial and viral infections. The compounds isolated from these species were reported to have piscicidal, anti-neoplastic, antioxidant, antitumor, anti-plasmodial, molluscicidal, and anti-tuberculosis properties, in addition to protein kinase C activation activity (Sabrin, et al., 2012) and (Wirginia, et al. 2013). *Iris* species have been used as ornamental plants in vegetative landscape of the parks and gardens in many countries since ancient times because of very beautiful and colorful flowers (Nezahat, et al., 2011). Herbal remedies in Iraqi Kurdistan can be classified into 133 different uses; for the most part they are of medicinal type, but some are also of cosmetic and ritual relevance (Mati, and De Boer, 2011). The presense of *Iris persica* L., have been reported in Iraq (Townsend, and Guest, 1985) and in the Kurdistan region (Kaššák, 2012) especially in Halgurd mountain (Choman district) and Korek mountain (Rawanduz district), which is commonly employed in the Kurdish traditional medicine for the treatment of wound inflammations and tumor. These applications are reported by the traditional herbal healers, locally called Baytars, that are highly recognized as experts in herbal medicinal uses. To this aim, the plant was collected in Korek mountain and the phytochemical investigation was performed. To the best of our knowledge, this is the first report on the qualitative phytochemical screening of the flowers, leaves, bulbs and rhizomes of *Iris persica*.

EXPERIMENTAL:

1. PLANT MATERIAL:

Iris persica L. (flowers, leaves, bulbs and rhizomes) was collected in April 2014 from Korek Mountain (Rawanduz) in the Kurdistan region (IRAQ). The plant was identified by two botanists Prof. Dr. A. H. Al-khayyat and Dr. Abdullah Sh. Sardar at the Biology Department, College of Education, Salahaddin University-Erbil/Iraq. A voucher specimen (No. 7229) was deposited at Education Salahaddin University Herbarium (ESUH), Kurdistan. The plant raw materials (flowers, leaves, bulbs and rhizomes)

were shade dried at room temperature (20-25^{o}C). After drying, the plant parts were grounded in to fine powder using a laboratory grinding mill, to provide homogeneous powder for the analysis. Powdered materials were stored in bottles in a dark room temperature and then used.

2. EXTRACTION WITH METHANOL:

The defatted flowers, leaves, bulbs and rhizomes (each 200g) were extracted with (500 mL) of methanol using ultrasonic bath for (20min) then macerated for (3hrs) with continuous stirring at room temperature. The procedure repeated three times for each part separately. Then the mixtures were filtered and the solvent was removed under "vacuum" using rotary evaporator affording a crude methanol extracts (Raphael I.). The percentage yields of different crude extracts are reported in Table 1.

Table (1): Extraction yields of *Iris persica* flowers, leaves, bulbs and rhizomes.

Plant parts	Solvent	Extraction residue (g)	Percentage yield (w/w)
Flowers	Hexane	1.87	0.93
	Methanol	31.33	15.66
Leaves	Hexane	1.17	0.58
	Methanol	26.47	13.23
Bulbs	Hexane	2.16	1.08
	Methanol	37.28	18.64
Rhizomes	Hexane	1.89	0.94
	Methanol	51.16	25.58

3. IDENTIFICATION TESTS:

Qualitative phytochemical analysis of the methanolic extract of *Iris persica* were carried out using standard procedures to assess the different types of phytochemical constituents present in the flowers, leaves, bulbs and rhizomes of *Iris persica* using different chemical tests. Screenings were carried out for flavonoids, polyphenols, tannins, alkaloids, terpenoids, saponins, protiens, amino acids, steroids, anthraquinnones and reducing sugar (Sawant, and Godghate, 2013, Mohammad, and Arun, 2009, Saxena, and Sahu, 2012, Minakshi, and Sushma, 2006, Amin Mir, 2013).

3.1. TEST FOR FLAVONOIDS:

Five methods were used to test for flavonoids. (a) Alkaline reagent test: Extract was treated with (5 mL) 10 % NaOH solution, formation of intense yellow colour indicates presence of Flavonoid. (b) Diluted ammonia (5 mL) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 mL) was then added. A yellow discolouration that on standing indicated the presence of flavonoids. (c) A few drops of 1% aluminium solution was added to a portion of the filtrate. A yellow colouration indicated the presence of flavonoids (Saxena, and Sahu, 2012). (d) A portion of the extract 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. A yellow colouration indicated the presence of flavonoids. (e) A small piece of magnesium ribbon was added to the alcohol solution of the extract followed by dropwise addition of concentrated hydrochloric acid. The colour changing from red-crimson indicates flavonols, crimson to magenta indicates flavonones and green blue indicates the test is positive (Sawant, and Godghate, 2013).

3.2. TEST FOR PHENOLIC COMPOUNDS:

Two methods were used to test for Phenolic Compounds: (a) lead acetate test: The extract (50 mg) were dissolved in distilled water and to this; 3 mL of 10% lead acetate solution was added. Formation of a bulky white precipitate indicated the presence of phenolic compounds. (b) $FeCl_3$ test: A small quantity of extract was diluted with

water and tested with Dilute $FeCl_3$ solution (5%), intense blue, green colour indicated the presence of phenolic compounds (Mohammad, and Arun, 2009).

3.3. TEST FOR TANNINS:

Gelatin test: 50 mg of extract dissolved in 5 mL of distilled water and to this; 2 mL of a 1% solution of gelatin containing 10% sodium chloride was added. The formation of white precipitates indicated the presence of phenolic compounds (Sawant, and Godghate, 2013).

3.4. TEST FOR ALKALOIDS:

Dragendroff's test: 2 drops of Dragendroff's reagent were added to 1 mL of the extract. The development of a creamy precipitate was indicative of the presence of alkaloids (Saxena, and Sahu, 2012).

3.5. TEST FOR TERPENOIDS:

The chloroform (2 mL) was added to 0.5 g of the extract. Concentrated H_2SO_4 (3 mL) was carefully added to form a layer, and the solution was observed for a reddish brown discolouration at the interface, which indicated the presence of terpenoids (Amin Mir, 2013).

3.6. TEST FOR SAPONINS:

5 mL extract was mixed with 20 mL of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponin (Sawant, and Godghate, 2013).

3.7. TEST FOR REDUCING SUGARS:

Fehling's test: The methanol extract (0.5 g in 5 mL of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction (a purple ring at the junction of two liquids) (Minakshi, and Sushma, 2006).

Note: Fehling's A is aqueous solution of copper (II) sulphate, which is deep blue. Fehling's B is a colorless solution of aqueous potassium sodium tartrate (also known as Rochelle salt) made in a strong alkali, commonly with sodium hydroxide.

3.8. TEST FOR ANTHRAQUINONES:

The extract (0.5g) was boiled with 10 mL of sulphuric acid (H_2SO_4) and filtered while it was hot. The filtrate was shaken in 5 mL of chloroform. The chloroform layer was pipetted into another test tube, and 1 mL of diluted ammonia was added. The resulting solution was observed for colour changes (Mohammad, and Arun, 2009).

3.9. TEST FOR PROTEINS

To 3 mL of extracts add 3% NaOH and few drops of 1% CuSO4. The solution turns from blue to violet (purple) or to pink, indicates the presence of protein (Amin Mir, 2013).

3.10. TEST FOR AMINO ACIDS

To 5 mL of extract add few drops of 40% NaOH and 10% lead acetate boiled the solution; formation of black precipitate indicate the presence of amino acid (Minakshi, and Sushma, 2006).

3.11. TEST FOR STEROIDS

The extract (1 mL) was dissolved in 10 mL of chloroform and equal volume of concentrated H_2SO_4 acid was added from the side of test tube .The upper layer turns red and H_2SO4 layer showed yellow with green fluorescence .This indicates the presence of steroid (Mohammad, and Arun, 2009).

RESULT AND DISCUSSION:

The present study carried out on the *Iris persica* revealed the presence of medicinal active constituents. The phytochemical active compounds of *Iris persica* were qualitatively analyzed for flowers, leaves bulbs and rhizomes separately and the results are presented in Table 2. The preliminary phytochemical screening of methanol extracts indicated the presence of flavonoids, polyphenols, tannins, trpenoids, saponins, protiens and reducing sugar.

Table (2): Qualitative phytochemical analysis of the methanol extract of *Iris persica* (flowers, leaves, bulbs and rhizomes).

Phytochemical constituents		Extracts			
		Flowers	Leaves	Bulbs	Rhizomes
Flavonoid:	Alkaline reagent test	+	+	+	+
	Ammonia test	+	+	-	+
	$AlCl_3$ test	+	+	+	+
	Ethylacetate test	+	+	+	-
	Shinoda test	+	+	+	+
Polyphenol:	Lead Acetate test	+	+	+	+
	$FeCl_3$ test	+	+	+	+
Tannin:	Gelatin test	-	-	+	+
Alkaloid:	Dragen droff's test	-	-	-	-
Terpenoid		+	+	+	+
Saponin:	Foam test	-	-	+	+
Protein		+	+	+	+
Amino acid		-	-	-	-
Steroid		-	-	-	-
Anthraquinone:	Borntrager's test	-	-	-	-
Reducing sugar:	Fehling's test	+	+	+	+

Key: + = Present, - = Absent

From phytochemical screening, we observed that the methanolic extracts of (flower, leaf, bulb and rhizome) parts gave a positive result with the Alkaline reagent test, $ALCl_3$ test and the Shinoda test, which indicated the presence of flavonoids in all extracts. The Dragendorff's reagent failed to show the presence of alkaloids in all extracts. The Gelatin test would confirm the presence of tannin in the methanolic extracts of bulbs and rhizomes. Based on the general test for terpenes, indicates the presence of terpenes in all extracts (Table 2). The borntragers test for anthraquinons gave negative results in all extracts. Test for saponins gave positive results with the methanolic extract of bulbs and rhizomes only. Lead acetate test and $FeCl_3$ test gave positive results, which indicates the presence of polyphenols in all extracts. The test of protein, gave positive results in all extracts. The test for amino acids and steroids gave negative results in all extracts.

All these facts support the usefulness of *I. persica* in folklore remedies and may be the reason these plants are used for the treatment of same diseases.

CONCLUSIONS:

This is the first report on the phytochemical screening of the flowers, leaves, bulbs and rhizomes of *Iris persica* L. growing from Kurdistan region-Iraq. Phytochemical analysis revealed the presence of flavonoids, polyphenols, tannins, terpenoids, saponins, protiens and reducing sugar in *Iris persica*. Further chemical analysis on the composition of *I. persica* methanol extract is necessary to isolate and identify bioactive compounds.

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جیاکرنەوەی سەرەتایی فایتۆکیمیایی ڕووەکی سەوسەن (گوڵەکان، گەڵاکان، ڕەگ و رایزۆمەکان) کۆکراوە لە هەرێمی کوردستانی عێراق

پوختە

سیفەتەچارەسەریەکانی ڕووەکە پزیشکیەکان بەزۆری دەگەڕێتەوە بۆ بوونی زۆر جۆری ماددەی نوێکەرەوە ناسەرەکیەکان (secondary metabolites) .

ئەم نامەیە توێژینەوەیەکە دەربارەی یەکەم لێکۆلینەوەی پێکهاتە کیمیاییە ڕووەکیەکان کە لە پوختەکراوەکانی میسانۆلدا هەن، لە گوڵەکان، گەڵاکان، ڕەگەکان (سەلك) و ڕایزۆمەکانی ڕووەکی سەوسەن کە لە چیای کۆرەك/ڕەواندوز/هەرێمی کوردستانی عیراق کۆکراوەتەوە. ئەم ڕووەکە لەلایەن خەڵکی ناوچەکە بە کاردێت بۆ چارەسەری هەوکردنی برین و شێرپەنجە.

شیکردنەوەکانی فایتۆکیمیایی ئەنجام دراوە بۆ پشکنین دەربارەی بوونی فلاڤەنۆیدەکان، پۆلی فینۆلەکان، تێرپینۆیدەکان، پرۆتینەکان و شەکری لێکەرەوە لە هەموو پوختەکراوەکانی ڕووەکی سەوسەن. هەرچەندە تانینەکا ن و سابوونیەکان تەنها لە ناو ڕەگەکان و ڕایزۆمەکاندا هەبوون، ئەلکەلۆیدەکان و ستیرۆیدەکان و ئەنسراکینۆنەکان لە هەموو پوختەکراوەکاندا نەبوون.

الفصل الاولى الفايتوكيميائي لنبات سوسن (الازهار، الاوراق، الجزور و الرايزومات) جمع فى اقليم كوردستان العراق.

الخلاصة:

ان الصفات العلاجية للنباتات طبية شيء محتمل بسبب وجود انواع مختلفة من المواد الأيضية الثانوية. وكاعتبر هذا البحث اول تحقيق عن مقومات الكيمياء النباتية الموجود في كل من(الزهور، الأوراق، البصيلات و الجذور) المستخلصة بالميثانول لنبتة سوسن الذي جمع في جبل كورك في كوردستان العراق. حيث يستخدم من قبل السكان المحليين كعلاج للجروح المتهبية والأورام.

تم إجراء تحليلات كيمياء النباتية للكشف عن وجود كل من (فلافونويدات، بوليفينولات، تيربينويدات، بروتينات وسكريات المختزلة) في جميع مستخلصات نبات سوسن. وقد وجد ايضا التنينات والصابونيات في كل من مستخلص البصيلات و الجذور فقط ولكن لم يتواجد كل من القلويدات، السموم المنشطة ، الاحماض الأمينية و المواد المهلكة للمواشي في جميع المستخلصات.