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Preliminary Phytochemical and GC-MS analysis of chemical constituents of Iraqi *Plantago lanceoleta* L.

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Abstract:

Plantago lanceoleta L. is one of medicinally important plant that officially registered in British pharmacopoeia. One of their well-known common name is ribwort, which is rich in many phytochemical compounds. The goal of this study is the qualitative evaluation of the Iraqi plant by identifying its chemical constituents. The plant was authenticated, collected, and immediately subjected to a stream of air under room temperature for rapid drying. Each part of the plant extracted separately with 50% ethanol and tested for presence of iridoid glycosides, tannins, flavonoids, terpenoids, alkaloids and saponins. While the other part of phytochemical screening of Iraqi *P. lanceoleta* L. in this work is the chromatographic fingerprint analysis of n-hexane extract of the plant leaves by gas chromatography-mass spectrometry (GC-MS). The result obtained show that Iraqi *P. lanceoleta* L. is rich in iridoid glycosides and tannins in each part of the plant, while the chromatogram of GC-MS show that the Iraqi plant contains high amount of hydrocarbons, fatty acids, steroids, terpenoids and other constituents.

Key words: *Plantago lanceoleta* L., preliminary tests, GC-MS, n-hexane extract

فحوصات أوليه كيميائيه لنبات لسان الحمل ألسهمي وتحليل مكوناته بواسطة جهاز كروماتوكرافيا الغاز المرتبطه بمطياف الكتله (GC-MS)

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الخلاصة:

لسان الحمل السهمي هي أحد النباتات الطبية الهامة المسجلة رسمياً في دستور الأدوية البريطانية. واحد من الاسماء الشائعه المعروفه لهذا النبات هو لسان الحمل السناني ، وهو غني بالعديد من المركبات الكيميائية النباتية. الهدف من هذه الدراسة هو التقييم النوعي للنبات العراقي من خلال تحديد مكوناته الكيميائية. تمت المصادقة على النبات وجمعه وتقسيمه إلى أربعة أجزاء (الأوراق والسيقان والبذور والجذور) وتعرض على الفور إلى تيار من الهواء تحت درجة حرارة الغرفة للتجفيف السريع. كل جزء من النبات استخلص بشكل منفصل بأستعمال 50% من الإيثانول وتم اختباره لوجود الجليكوسيدات الأريدويديه ، التانينات ، الفلافونويد ،التربينات ، القلويدات والصابونين. في حين أن الجزء الآخر من الفحص الكيميائي في هذه الدراسه تم بأستعمال جهاز الكروماتوغرافيا الغازية المرتبط بجهاز مطياف الكتله لمستخلص الهكسان لأوراق النبات العراقي. تظهر النتائج التي تم الحصول عليها أن النبات العراقي غني بلكلايكوسايدات ألأريدويديه و العفصيات في كل جزء من اجزاء النبات. أما نتائج الكروماتوغرافيا الغازيه اظهرت ان النبات يحتوي على كميات كبيره من الهايدروكاربونات، الأحماض الدهنيه، التربينات و مركبات أخرى.

الكلمات المفتاحية : لسان الحمل السهمي، أختبارات كيميائيه أوليه، GC-MS ، مستخلص الهكسان.

Introduction:

Plantago lanceoleta L. (family: Plantaginaceae) is an official plant that registered in British pharmacopoeia. It is considered as a drug rich in different chemical constituents mainly mucilages, iridoid glycosides and phenylethanoids [1]. It has many common names including ribwort and narrow leaf plantain [2]. Ribwort is originally native to Europe; temperate areas of Asia and it is become cultivated in all temperate regions of the world [3]. P. lanceolata L. leaves characterized by erect, straight leaves that sheltered with soft minute hairs, they reach up to about 17 inches long and taper at the base into a slender petiole, (Fig.1a). While the flowers are set as condensed spikes on the top of the stalks, (Fig.1b). Each flower is composed of a small corolla, four sepals and two stamens [4].

Figure (1): (A) Iraqi P. lanceoleta L. grown on the sidewalk. (B) a flower spike

Many phytochemical studies show that *P. lanceoleta* L. is rich in numerous active constituents including mucilages, ribwort plantain contains high amount of mucilages (~6%)[5], tannins (more than 5%)(6),phenylpropanoids[7]with verbascoside as a major constituent[8], also the ribwort contains iridoid glycosides as one of major secondary metabolites[9] mainly aucubin and catalpol; it is also contains other constituents; (e.g. flavonoids[10] , coumarins[11] , volatile compounds[12] , and saponins[1]) as minor secondary metabolites.

The medicinal uses of ribwort include: internal uses for catarrh of the respiratory tract, inflammations of the oropharyngeal mucosa [10]. Further studies show that ribwort seeds were also commonly used as a natural laxative, due to their high content of fibers [13]; It is also having external uses for skin inflammations, wound healing and also used as rubefacient [14,15].

Many researches of GC-MS analysis demonstrates that *P. lanceoleta* L. is rich in long chains hydrocarbons, esters, aldehydes and other important compounds[12]; such as phytol, which is acyclic oxidated diterpene, a component of chlorophyll and is used as a precursor in manufacturing the synthetic forms of vitamin K_1 as well as vitamin E[16]; phthalic acid is also detected, which is used in the anhydride form for production of other chemicals[17]; some of other interested compounds are Alpha-bisabolene which is an oxidated sesquiterpene that found in the essential oils of many plants that acts as intermediate in the biosynthesis of many natural compounds[18].

Materials and method

A-Plant collection: The plant widespread in many gardens and sidewalks in Baghdad, it was collected in June 2017 from the garden of Mustansirya University/Pharmacy Department, then the plant was rapidly cleaned and dried at room temperature under a stream of air. After drying, the plant was divided into four parts (leaves, stalks, seeds and roots), then each part was powdered to be prepared for the extraction process.

B-Extraction:

1. Extractions for preliminary phytochemical analysis

1.1 Iridoid glycosides

The Powdered plant (0.5gm) was added in a test tube containing 5 ml of 1% of aqueous HCl solution. After 4 hours one drop of the macerate was added to a test tube containing 2 ml of Trim-Hill reagent (1 mL of 0.2% of $CuSO_4.5H_2O$ aqueous solution + 10 mL of acetic acid + 0.5 mL of conc. HCl). Presence of aucubin, asperulin and monoterpene give blue color (Trim-Hill test) [19].

1.2 Tannins

The Powdered plant (0.5 gm) subjected to heating with DW (30 mL) in a small beaker. After that the extract filtered and freshly prepared solution of 0.1% $FeCl_3$ was added to a test tube containing 5 mL of the filtered and diluted extract ($FeCl_3$ test) [20].

1.3 Test for saponins

From the above filtered extract 4 ml was added in a test tube which diluted with water (4mL) and with vigorous shacking a stable forth must be formed to indicate the saponins presence (forth forming test) [20].

1.4 Test for flavonoids

To the diluted ethanolic extract (4 mL) of *P. lanceoleta* L. a freshly prepared alcoholic KOH (2 mL) was added. Observation of yellow color indicates flavonoids presence [21].

1.5 Test for alkaloids

A few drops of diluted HCl was added to 4 ml of ethanolic extract (4 mL) of the plant. After that, the dragendorff reagent (2 mL) was added; appearance of orange precipitate indicates the alkaloids presence [21].

1.6 Test for steroids

Powdered plant material (100mg) extracted by chloroform (2mL), then conc. H_2SO_4 (1mL) was added to the filtrate dropwise; formation of brown-red color in the lower layer indicates presence of steroids (Salkowski test) [21].

1.7 Test for terpenoid

An aqueous extract of *P. lanceoleta* L. (5mL) was mixed with chloroform (2mL), then conc. H_2SO_4 (2 mL) was added drop by drop on the wall of the test tube that contains the aqueous extract. The formation of interface between aqueous and chloroform layers with red to brown color indicates terpenoids presence [22].

2. Extraction for GC-MS analysis

The leaves powder (20 gm) extracted with n-hexane (350 mL) by soxhlet apparatus. The extraction takes about 10 hours, after that n-hexane extract filtered and concentrated by rotary vacuum evaporator to a small volume.

C-GC-MS analytical conditions

The chromatograph used was Shimadzu GC-2010 plus in Iraqi ministry of sciences and technology; the temperature of the injector and interface were maintained at 280oC; the column type used was DB-5 (25m * 0.2 mm, 0.25µm thickness), its temperature was programed at 60^{o}C for 4 min, then increased to 150^{o}C with a hold period of 4 min then raised to 250^{o}C; this column is nonpolar and cross-linked that can be rinsed and tolerate high temperature; it is consist from 5% phenyl-95% dimethyl polysiloxane above which helium moved with flow rate of 1.35 ml/min, carrying the constituents of the

sample from the injector along the column to the detector; which is quadrupole-kind detector using a 70 eV potential for ionization by electron impact (EI).

Result and discussion

1.Result of preliminary phytochemical tests

These tests for the plant were necessary for predicting the plant nature and for detecting different plant components. The results shown that the Iraqi *P. lanceoleta* L. contains all the above constituents making this plant suitable for medicinal uses [23]; the tests done for each part of the plant alone as observed in the (Table-1).

Table (1): Preliminary tests for different parts of Iraqi *P. lanceoleta* L. extract

	Leaves	Stalks	Seeds	Roots
Trim-hill test for iridoid glycosids	+++	++	+	+
$FeCl_3$ test for Tannins	++	+	+	+
alcoholic KOH for flavonoids	+	+	+	-
Dragendorff test for alkaloids	-	-	-	-
Salkowski test for steroids	+	+	+	-
Froth-forming test for saponins	+	+	-	-
Chemical test for terpenoids	+	+	+	-

2.Result of GC-MS analysis

GC-MS is one of most important instruments that used for the analysis of sample with volatile constituents because it combines both the chromatographic technique for efficient separation of sample constituents and mass spectroscopy that identifies the compounds according to their mass to charge ratio (m/z). Definitely, many peaks were detected in chromatogram of the extracted leaves of ribwort. Figure-2 shows the total Ion Chromatogram (TIC) of the GC-MS analysis of Iraqi P. lanceoleta L.

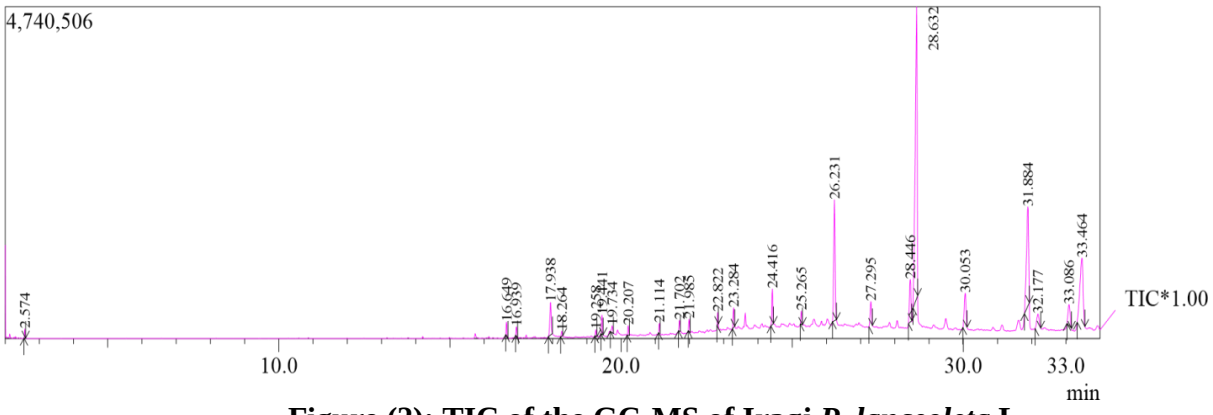
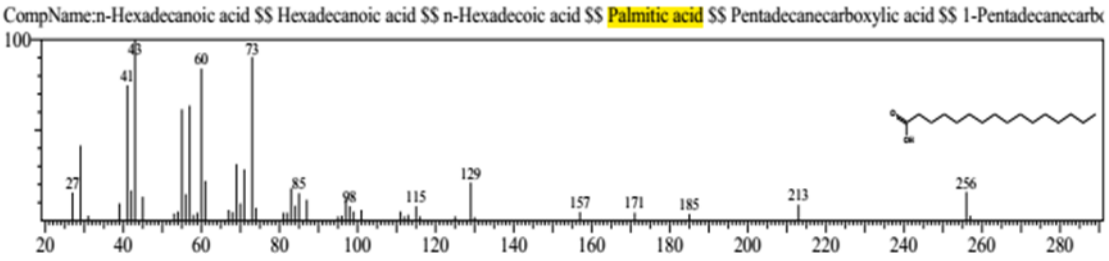


Figure (2): TIC of the GC-MS of Iraqi *P. lanceoleta* L

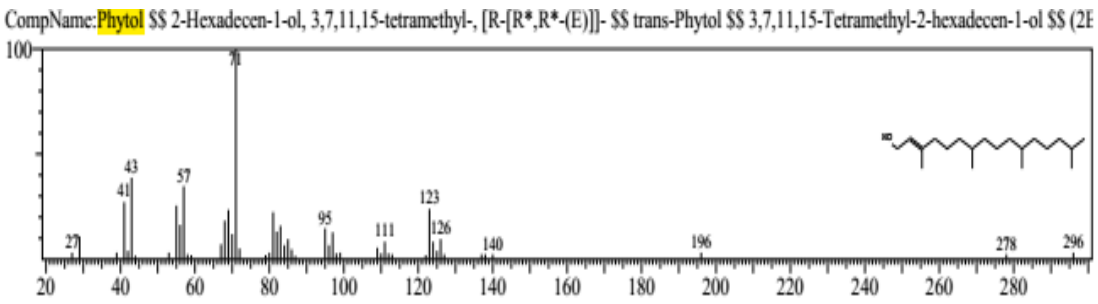
The extract was rich in long chain hydrocarbon (alkanes and alkenes) and alcohols, ketones, aldehydes, fatty acids, terpenes, sterols and another compound as shown in (Table-2). n-Hexane extract of *P. lanceoleta* L. leaves were rich in fatty acids as observed in the GC-MS chromatogram, some of these fatty acids were palmitic acid, myristic acid and stearic acid that obtained from GC-MS data, as observed in (Fig. 3).

(Table-2): important phytochemicals detected by GC-MS analysis of Iraqi *P. lanceoleta* L.

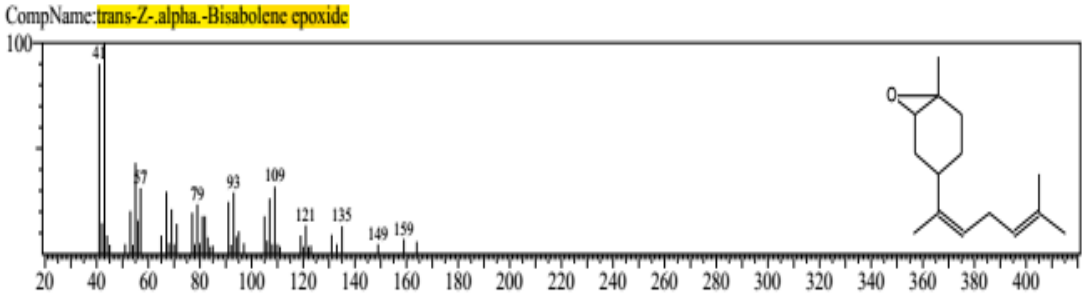
Compounds	Types	Retention times	Area%
Hexahydropseudoionone	Apocarotenoid	16.6	0.68
Diheptylphthalate	Ester of aromatic dicarboxylic acid	16.9	0.48
Palmitic acid	Fatty acid	17.9	0.48
Phytol	Oxidated diterpene	19.4	1.08
13-docosenoic acid	Fatty acid	19.7	0.48
Ditridecylphthalate	Ester of aromatic dicarboxylic acid	23.2	1.16
Hexahydrofarnesyl acetone	Sesquiterpenoid	28.4	4.03
Stigmasterol methyl ether	Sterol	32.1	2.15
Stearyl aldehyde	Aldehyde	33.0	2.67
Apha-bisabolene epoxide	Oxidated sesquiterpene	33.4	13.2



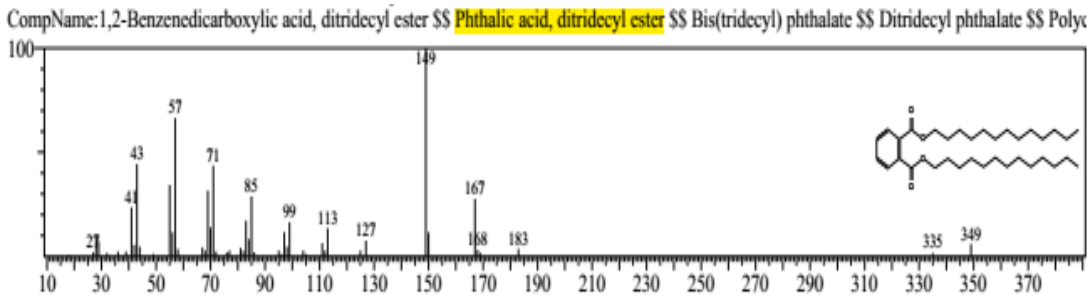
(a)



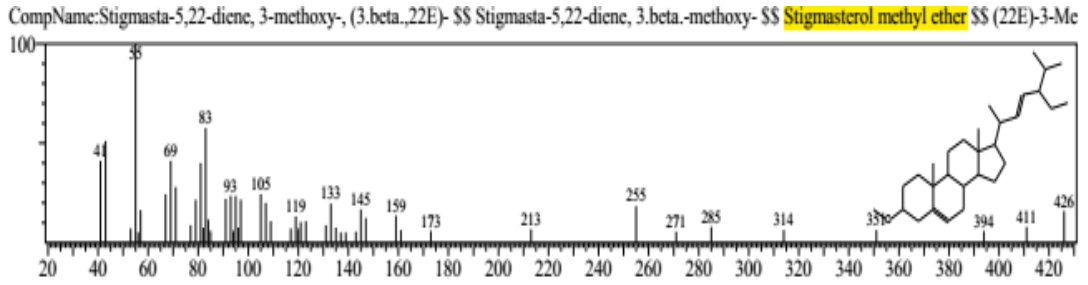
(b)



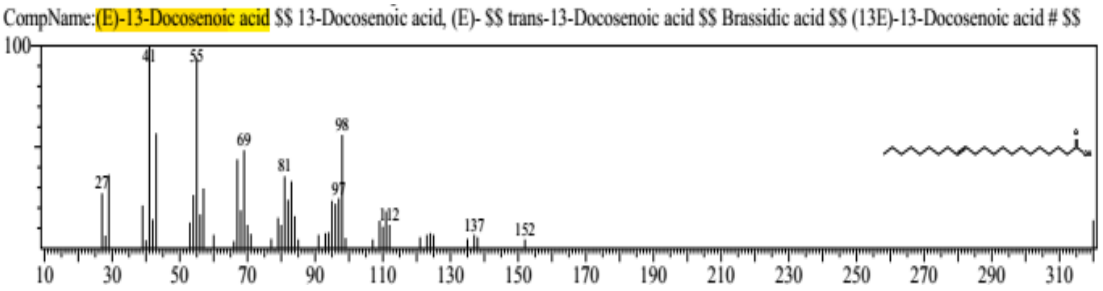
(c)



(d)



(e)



(f)

Figure(3): Mass fragmentation for some of important chemical compounds in the n-hexane extract of Iraqi *P. lanceoleta* L.

Conclusion

In this work, each part (leaves, stalks, seeds, roots) of Iraqi P. lanceoleta L. gave a positive result in the Trim-Hill test with intense blue colour in the case of leaves, which give an indication of higher concentration of iridoid glycosides in the leaves than other parts, which make this plant as rich source for iridoid glycosides[19]. Also this plant is rich in tannins in each parts as observed from the phytochemical screening tests. The

aliphatic long chain hydrocarbons was the most abundant form in the GC-MS analysis of n-hexane extract as observed in the literature[24]. Fatty acids, methyl esters of fatty acids and high amount of ketones, aldehydes and aliphatic alcohols are detected. N-hexane extract of Iraqi ribwort leaves was also contains oxidated terpenes represented by diterpenes and sesequeterpenes. Furthermore, some of steroidal compounds are also detected in the GC-MS of Iraqi P. lanceoleta, the most important is stigmasterol which is not detected by GC-MS in other researchs[24], which is used as a precursor appropriate for manufacturing the semisynthetic progesterone[25-27], it is also play a role in the vitamin D3 biosynthesis[28].

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