



Determinate Secondary Metabolites Groups of Water Citrus limon Seeds Extract

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تحديد المستقلبات الثانوية للمستخلص المائي لبذور الليمون

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Abstract

The citrus fruits are one of the most widely used plants by humans, whether as a food source or even as a source of medicine, especially lemon, as it one of the most widespread citrus fruits in different countries of the word based on the fact that all parts of lemon fruit contain substances that have great important to human health .The aim of this research is concentrated on *Citrus limon* fruit specifically the seeds through preparing an plant extract for seeds using distilled water solvent in { 25⁰,90⁰} temperature by using soxhlet. Where work was done we have two extracts of *Citrus limon* seeds {cold water seeds extract ,hot water seeds extract} in order to determinate secondary metabolites groups like {flavonoids , alkaloids , etc.}. The hot water seeds extract show presence and percentage of the secondary metabolites groups and this groups are {alkaloids 5.3 mg / g, flavonoids 4.2 mg / g, saponin 3.9 mg / g}, while the cold water seeds extract show presence and percentage of the secondary metabolites groups and this groups are {alkaloids 4.8 mg / g ,flavonoids 3.5 mg / g, saponin 2.8 mg / g}. The hot water seeds extract superiority in percentage of the secondary organic compounds than the cold water seeds extract.**Keywords:** *Citrus limon* , soxhlet, alkaloids , flavonoids , saponin

الخلاصة

تعد الحمضيات من اكثر النباتات استخداما من قبل الانسان سواء كان كمصدر غذاء او حتى استعمال الحمضيات كمصدر للدواء وخاصة الليمون كونه يعد من اكثر الحمضيات انتشارا في بلدان العالم المختلفة استنادا لما تمتلكه ثمرة الليمون بكل اجزاءها من مواد ذات اهمية كبيرة لصحة الانسان. ان الهدف من هذا البحث هو التركيز على نبات الليمون وتحديد بذور الليمون , حيث تم تحضير المستخلص النباتي للبذور من خلال استخدام الماء المقطر كمذيب في درجتي حرارة وهي (٢٥⁰, ٩٠⁰) من خلال استخدام جهاز السكسوليت. عند انتهاء العمل تم الحصول على مستخلصين لبذور نبات الليمون وهذان المستخلصان هما (مستخلص الماء البارد لبذور الليمون , مستخلص الماء الساخن لبذور الليمون) من اجل تحديد مجاميع المستقلبات الثانوية مثل (الفلافونيدات , القلويدات , الخ). مستخلص الماء الحار اظهر وجود ونسبة مجاميع المستقلبات الثانوية وهذه المجاميع هي (القلويدات ٥.٣ ملغم / غم , الفلافونيدات ٤.٢ ملغم/غم , الصابونيات ٣.٩ ملغم / غم) بينما مستخلص الماء البارد لبذور الليمون اظهر وجود ونسبة مجاميع المستقلبات الثانوية وهذه المجاميع هي (القلويدات ٤.٨ ملغم / غم , الفلافونيدات ٣.٥ ملغم/غم, الصابونيات ٢.٨ ملغم / غم). ان مستخلص الماء الساخن لبذور الليمون تفوق بنسب وجود مجاميع المستقلبات الثانوية على مستخلص الماء البارد للبذور .

Introduction

The secondary metabolites are organic compounds that are not direct interference in the normal growth, reproduction or development of plants. Conversely the primary metabolites ,which are very important in in plant essential processes ,and this process include basic cellular function like production {protein ,lipids and carbohydrates}.The secondary metabolites play main role in different processes for plants ,and this process are {antimicrobial ,antifungal and defense mechanisms against the pathogens organisms}.Common examples of secondary metabolites include{terpenoids , alkaloids , phenolics}(Khan et al ,2025).The plant extract are composed of extracted matter obtain from starting materials of botanical origin,)they result from dissolving soluble plant constituents in extraction solvents and separating them from undisclosed plant materials like water

solvent, the major benefit of this process is concentration of active ingredients (Monagas et al,2022).Solvent extraction is most frequently used technique for isolation of secondary metabolites groups, the plant extract dependent on the nature of extraction solvent like water, and type of extractor like soxhlet,soxhlet is the most laboratory apparatus common for plant extraction,because is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. (López-Bascón & De Castro,2020).Citrus limon commonly name is limon was classified under the family Rutaceae, this plant is evergreen aromatic shrub mostly with thorny branches distributed throughout the temperate and tropical region of the world. The important of this plant is have different properties which is benefit in {health, industry, research, etc.}like the secondary metabolites groups for example {flavonoids, carotenoids, minerals, etc.},as well as the Citrus seeds are an important source of bioactive compounds, it is commonly considered as waste. This part of limon is rich in phenolic compounds that have been demonstrated to possess several valuable properties, antimicrobial activities, particular anti-inflammatory and antioxidant, all the this characteristics made the Citrus limon plant as nutraceuticals source of bioactive compounds (Sridharan et al,2016).

.)Materials and Methods Seed Collection The Citrus limon seeds were collected from the lemon fruit that wall take from our farm in Baghdad. The seeds wall collected and wish with distal water, after that the seeds dried and powdered in mixture. The powder was used for extraction with Wight {200g}Hot water seeds extraction In this method we used soxhlet extractions was performed using 100g of Citrus limon seeds powder loaded into a thimble and placed in the extraction chamber. The soxhlet parameters in this study was distilled water with amount five hundred milliliters, the water solvent was poured into around_ bottom flask. The solvent was heated to 90°, this process took time two hours (Ozel& Kaymaz,2004). After the extraction process a rotary vacuum evaporator in conditions below atmospheric pressure and temperature in the range (35°_ 40°) was used to recover the solvent from the extract the final extract Wight 20mL.Cold water seeds extraction In this case we used soxhlet extractions was performed using 100g of Citrus limon seeds powder loaded into a thimble and placed in the extraction chamber. The soxhlet parameters in this study was distilled water with amount five hundred milliliters, the water solvent was poured into around_ bottom flask. The solvent was heated to 25°, this process took time two hours (Ahmed et al,2019). After the extraction process a rotary vacuum evaporator in conditions below atmospheric pressure and temperature in the range (15°_ 20°) was used to recover the solvent from the extract the final extract Wight 20mL.The flavonoids test {1ml} from each extraction was taken and butted in test tube, and added few drops of dilute (NaOH) solution. After five minutes the yellow color was appeared in the test tube which means positive result (Harborne, 1973). The color was diapered when we addition a few drop of dilute acid, the result was presence of flavonoids. The alkaloids test {1ml} from each extraction was taken and butted in conical flask and added ammonia solution {3ml}for identification the alkaloid groups. It was allowed to stand for {10 minute} to evaluated free alkaloids. The {10ml} of chloroform was added to the conical flask, after that we shake by hand and filtered the solution. The chloroform was evaporated from the crude extract by water bath, and added Mayer's reagent {3ml} (Mattocks & Jukes, 1987).The change in color for cream precipitation that showed alkaloids was presence. The saponins test {1ml} from each extraction was taken and butted in a test tube, and diluted with {20ml} of distilled water. The test tube was shaken by hand for {20 minute} (Kensil et al, 1991). The foam layer was appeared in the top of test tube. This foam layer showed saponins was presence. The tannins test {2.5 ml} from each extraction was taken and butted in a test tube, the two extracts were dissolved in {5 ml} of distilled water in a water bath and used filter paper to filtered the solution. The ferric chloride (Fe Cl₃) was added and filtered by filter paper. The result was does not present blue_ green or blue _black green choler (Elmaki et al,1999). The result of this process was nonexistence of tannins in two extract.

Table 1 : Secondary metabolites groups tests of water Citrus limon seeds extracts

Sequence	Secondary metabolites groups	Type of extracts	
		Hot water seeds extraction	Cold water seeds extraction
1	flavonoids test	+	+
•	Test guide	Yellow color	Yellow color
2	Alkaloids test	+	+
•	Test guide	Cream color	Cream color
3	Saponins test	+	+

•	Test guide	Foam layer	Foam layer
4	Tannins test	-	-
•	Test guide	No color	No color
- Guide for present secondary organic compounds + Guide for absent secondary organic compounds			

Quantitative Of Flavonoids The flavonoids quantitative determination we used {10 ml} from each extraction were taken and butted in beaker and mixed with {100ml} of {80%} aqueous methanol at room temperature. The result solution was filtered by Whatman filter paper No42 {125mm}. The filtrate was transferred into a crucible and evaporated into dryness over in water bath (Crozier et al ,1997),after that weighed to a constant weight. The flavonoid percentage of two samples was calculated

Quantitative Of Alkaloids The alkaloid quantitative determination we used {5ml} from each extraction were taken and butted in beaker with {200ml} of {10%} acetic acid and added {200ml} ethanol ,the solution was mixed carefully and covered ,after that the solution was left for {4h}. The solution was filtered and construction in water bath. Concentrated ammonium hydroxide was added by drop wise to the concentrated solution(Tsuchiya et al ,1999) ,after that the precipitation process was completed .The precipitated solution was collected and washed with dilute ammonium hydroxide ,and then filtered . The result was alkaloid , which was dried and turned to powder in mixture ,after that the powder was weighted. The alkaloid percentage of two samples was calculated .

Quantitative Of Saponins The saponins quantitative determination we used {20 ml} from each extraction were taken and scattered in {200 ml} of {20%} ethanol. The suspension was butted in water bath for four hours with continuous stirring at about {50°}. The result mixture was filtrated by filter paper and the remainder re _extracted with another{200ml} of {20%} ethanol. The result material was reduced to {40ml} by used water bath in {90°}. The concentrate was transferred into a {250ml} separating funnel and {20ml} of diethyl ether was added and shaken strongly. The watery layer was retrieved while the ether layer was removed. The cleaning process was reiterated. {60ml} of n _butanol with two extracts were washed twice with {10ml} of {5%} watery sodium chloride . The remaining solution was heated in water bath . After vaporization the two samples were dried in the oven to have constant weight (Cheok et al ,2014) . The saponin percentage of two samples was calculated .

Table 2 : Quantitative of secondary metabolite groups of water Citrus limon seeds extracts

Sequence	Secondary metabolite groups	Type of extract	
		Hot water seeds extraction	Cold water seeds extraction
1	Flavonoids	5.3 mg / g	4.8 mg / g
2	Alkaloids	4.2 mg / g	3.5 mg / g
3	Saponins	3.9 mg / g	2.8 mg / g

.1) Results and DiscussionIn this study was focused on Citrus limon seeds extracts specially the secondary metabolites groups in this part of fruit. The secondary metabolites groups were present in both extracts of Citrus limon , by using water solvent in two ways ,the first way was used distilled water with 90 temperature two have hot water seeds extract, but the second way was used distilled water with 25 temperature two have cold water seeds extract. The secondary metabolites groups present in Citrus limon extracts have been tabulated in table number {1} ,and this compounds were{ flavonoids, alkaloids and Saponins} in both extracts , when the secondary metabolites groups quantitative in Citrus limon extracts have been tabulated in table number {2} , and the average of secondary metabolites groups was {5.3 mg /g _ 2.8 mg /g} . This groups have multiple benefits like the working as {antioxidants ,antifungals ,a cure for cancer diseases ,etc. } . This benefit was used in different fields like {health field , agriculture field ,scientific research development ,etc.}(Pinar& Rodríguez-Couto,2024).For example , the flavonoids have antioxidants benefit ,because the flavonoids have ability to reduced free radical formation and to scavenge free radicals .This benefit was very important specially the health field (Wicaksana et al ,2025). The saponins have antifungals benefit , because the saponins have ability to destroy the structure of cell membrane by leakage the cell contents, which made inhibited the mycelium growth , reduced aggregation and cell adhesion , and effectively inhibited the formation of biofilm. This benefit was very important specially agriculture field (Zong et al ,2025). The alkaloids have a cure for cancer diseases benefit , because the alkaloids have anti-tumor effect by targeting the microenvironment of tumor. The key of treatment cancer by used alkaloids in immunosuppressive environment and supporting anti-tumor immunity . This benefit was very important specially in scientific research development field (Habib et al,2023). The researchers have ability to separate the secondary metabolites groups from Citrus limon seeds and development human life by possession the benefit of secondary metabolites groups ,which that present in both extracts of Citrus limon seeds.

.)¹ Conclusion The present study was concentrate on Citrus limon fruit specially the seeds ,because this part of plant have many secondary metabolites groups ,and this groups was extracted by used soxhlet with distilled water solvent in two temperatures. The result of extraction process was two types of Citrus limon seeds extracts ,and the two types were {hot water seeds extract , cold water seeds extracts } this process depended on two temperatures {90° , 25°}. The secondary metabolites groups in two type of extracts {flavonoids , alkaloids , saponins} , but the quantitative of this groups was different ,because the percentage of hot water seeds extracts groups were more than percentage of cold water seeds extracts groups. The result of this paper leads the researchers to focusing on secondary metabolite groups of hot water seeds extract in Citrus limon ,and used it in different life fields of people to improve and protect the human health and the environment. Acknowledgment I would like to thanks to Dr. Fadhl Ahmed (Assistant professor) in Al_ Farabi University for the publish this paper and I would like thanks for Shaima Mohammed (Assistant teacher) in Baghdad University to help me in chemical analyses of Citrus limon seeds extracts .

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