

Comparison Between Two Extraction Methods on Total Extract with Primary Investigation of Phytochemical Compounds of Some Medicinal Plants used in Treatment of Urinary Tract Disease.

Abbas Moussa Kashmar¹ and Estabraq H. Naser²

1: Agriculture college /Karbala university, Karbala. Iraq.

2: Pharmacy college /Karbala university, Karbala. Iraq.

Key words: Urinary tract infection, fennel, parsley, corn silk, phytochemical compounds.

Received (November), **Accepted** ()

ABSTRACT

Phytochemical investigation and urinary tract infection (UTI) treatment of parsley seeds, fennel seeds, and corn silk were studied. The aim of this study was conducted to investigation the best method of extraction on total extract, with preliminary screening of phytochemical compounds of parsley seeds (*Petroselinum sativum*), fennel seeds (*Foeniculum vulgare* Mill.) and corn silk (*Zea mays* L.) to treat urinary tract diseases. The parts of each plant were extracted by two different methods, hot method by using distilled water and cold method was done by maceration with 90% ethanol at room temperature. In both methods the extract was dried under reduced pressure by rotary evaporator. Preliminary investigation of phytochemical compounds was done by using alkaline reagent test for flavonoids, foam test for saponins, terpenoids test for terpenoids, fehling's reagent for reducing sugar and Dragendroff's reagent for alkaloids. The qualitative identification was done by TLC. The results showed that the percentage yields of crud extracts by boiling with distilled water were higher than that obtained from cold maceration with 90% ethanol. The percentage of phytochemical components, flavonoids, saponins, reducing sugar, terpenoids and alkaloids of water extracts were higher than that in ethanolic extracts.

The effect of extracts in the treatment of UTI displayed that the combination of three plants water extracts were stronger than ethanolic extract. Based on our knowledge this is the first study on the effect of extracts from *P. sativum*, *F. vulgare*, and *Z. mays* in the treatment of UTI.

المقارنة بين طريقتي استخلاص على المستخلص الكلي مع التشخيص الاولي للمركبات الكيمونباتية لبعض النباتات الطبية المستخدمة في علاج التهاب المسالك البولية

عباس موسى كاشمر¹, استبرق حسين ناصر²

1. كلية الزراعة /جامعة كربلاء, كربلاء. العراق.

2. كلية الصيدلة /جامعة كربلاء, كربلاء. العراق.

الكلمات الافتتاحية: التهاب المسالك البولية, حبة الحلوة, المعدنوس, كفضة الذرة, المركبات الكيمونباتية.

الخلاصة:

تمت دراسة تشخيص المكونات الكيمونباتية وقابلية المستخلصات لنباتات المعدنوس, حبة الحلوة, حرير الذرة في علاج التهابات المسالك البولية لنبات المعدنوس, حبة الحلوة, وكفشة الذرة. تهدف هذه الدراسة إلى البحث عن أفضل طريقة لاستخلاص للحصول على اعلى نسبة من المستخلص الكلي , مع الفحص الأولي للمركبات الكيمو نباتية من بذور المعدنوس *Petroselinum sativum* وحرير الذرة *Zea mays L.* وبذور الحبة الحلوة *Foeniculum vulgare Mill.* لعلاج امراض المسالك البولية. أجزاء كل نبات تم استخلاصها بطريقتين مختلفتين، الطريقة الحارة باستخدام الماء المقطر والطريقة الباردة تمت بواسطة التنقيع مع الايثانول 90% بدرجة حرارة الغرفة. في كلا الطريقتين المستخلص تم تجفيفه تحت ضغط منخفض بواسطة المبخر الدوار. تم إجراء كشف أولي للمركبات الكيميائية النباتية باستخدام اختبار الكاشف القلوي لمركبات الفلافونويد، واختبار الرغوة للصابونيات، واختبار تيربينويد للترينويدات، وكاشف فهلينك للسكر المختزل وكاشف دراجندروف للقلويدات. التشخيص الأولي تم بواسطة صفائح الطبقة الرقيقة الكروماتوغرافيا. اظهرت النتائج ان نسبة المستخلصات الخام بواسطة الغليان بالماء المقطر كانت اعلى من تلك التي تم الحصول عليها من التنقيع البارد مع الايثانول بنسبة 90%. التشخيص الأولي اظهر ان نسبة المكونات الكيميائية النباتية، الفلافونويدات، الصابونيات، والسكر المختزل، والتيربينويد والقلويدات من مستخلصات المياه اعلى من تلك الموجودة في المستخلصات الإيثانولية. تأثير المستخلصات في علاج التهاب المسالك البولية أظهر أن الجمع بين مستخلصات المياه كانت أقوى من مستخلصات الإيثانول. بناء على معرفتنا هذه هي الدراسة الأولى عن تأثير مستخلصات المعدنوس, حبة الحلوة, وحرير الذرة في علاج التهاب المسالك البولية.

Introduction

Plants have been used for medicinal purposes long before recorded history. ⁽¹⁾ Recently the world health organization estimated that 80% of people world were rely on herbal medicines for some part of their primary health care. ^(2,3) About 150 million patients in the world were diagnosed with UTI each year. An alternate therapy of medicinal plants in the management of UTI was very necessitate due to increase the resistance of antibiotics between bacterial pathogens ⁽⁴⁾.

There are different medicinal plants were used in traditional medicine in Iraq for treatment of many diseases of urinary tract system such as parsley seeds, corn silk, and fennel seeds. ⁽⁵⁾

Parsley (*Petroselinum sativum L.*) which belong to Umbelliferae family and considered as medicinal plant used in traditional medicine for urinary tract, stones and infection. ^(6,7) It's used as diuretic ⁽⁸⁾ and for the prevention and treatment of kidney gravel. ⁽⁹⁾ Phytochemical analysis of parsley plant showed the presence of flavonoids, carotenoids, ascorbic acid, myristicin, apiole, terpenoids and coumarins, plathalides and tocopherol. ⁽¹⁰⁾

Fennel (*Foeniculum vulgre mill.*) is a biennial medicinal plant belonging to the family Apiaceae (umbelliferae) ⁽¹¹⁾. The fennel fruits its used in traditional medicine as a diuretic, analgesic, and antioxidant activity ^(12,13). The chemical constituents of fennel fruits include essential oil, fatty acid, phenylpropanoids, tannins, flavonoids, cardiac glycosides, saponins and other types of compounds ⁽¹⁴⁾

Corn silk (cs) is a collection of the stigmas (fine, soft, yellowish, threads) from the female flowers of the maize plant ⁽¹⁵⁾ (*Zea mays L.*) which belongs to Gramineae family. Corn silk used for treatment of infections and cystitis, aids in the passage of stones and others related from kidney to the renal disease ⁽¹⁶⁾. Corn silk extract composition is very important and this importance due to the content of flavonoids. ⁽¹⁷⁾ Also contains alkaloids, saponins, volatile oil, mucilage, vitamin C, K and E, Minerals especially starch, K, glucose, gluten, cellulose, fat,

maizenic acid, dextrin, silica and phosphate of lime. ⁽¹⁸⁾ Therefore the aim of this study was conducted to investigate the best extraction method and phytochemical screening of main active compounds like flavonoids, terpenoids, coumarin and the effect of these extracts in the treatment of urinary tract infection.

Materials and methods

Plant samples preparation:

Corn silk, fennel, and Parsley seed were obtained from Al-Waady al-aKhter for medicinal plant office in Baghdad. All samples were identified and authenticated by department of pharmacognosy and medicinal plants of college of pharmacy /Al-Mustansiriyah University. Plant samples were kept at the department of pharmacognosy / college of pharmacy / university of karbala.

Equipment and chemicals

The instruments used were rotatory evaporator (Buchi Rotatory evaporator) R-205 swiss), sonicator (Branson sonifier, USA), thin layer chromatography (TLC) aluminum plates pre-coated with Silica gel GF 60 & silica gel GF 254; layer thickness 0.25 mm; 20 x20 cm aluminum cards; made by Merck-Germany, sensitive electrical balance sartorius /Germany, Oven memmert 854 / Germany, Hot plate: Horst achtung /Germany water bath: memmert / Germany and centrifuge: china. The chemicals used in this study were Ammonia 25% , n-butanol, chloroform , copper sulfate, Ethanol 90%, ethyl acetate, ferric chloride, Glacial acetic acid, n-hexane, hydrochloric acid, Iodine solution, picric acid, Potassium– bismuth iodide, potassium hydroxide, sodium potassium tartarate and sulfuric acid (96 – 98 %)

Extraction

The dried plant was powdered in a mechanical grinder, each powdered plant was extracted by two different method:

Extraction method No.1

Two hundred grams of powdered of each corn silk, fennel fruits, and parsley seeds were placed in a beaker separatory and extracted by boiling with 1000 ml of distilled water for 15 minutes on heater, then the aqueous extracts cooled at room temperature and filtered, the filtrates evaporate to dryness under reduced pressure by rotatory evaporator at 40°C to give crude extracts. ^(12,19)

Extraction method No.2

Two hundred grams of fennel seeds was soaked in a beaker and defatted with 500 ml of n-hexane for three days then the defatted plant materials was dried at room temperature and re-extracted with 250 ml of ethanol 90% for five days by cold maceration process while the corn silk and parsley seeds powder were macerated with ethanol 90 % for five days, then the ethanolic extracts were filtered, the filtrates evaporated to dryness under reduced pressure by using rotatory evaporator at temperature didn't exceed 40°C to give a crude extract. ⁽²⁰⁾

Preliminary phytochemical investigation was done by alkaline reagent test for flavonoids, foam test for saponins, terpenoids test for terpenoids, fehling's reagent for reducing sugar and Dragendroff's reagent for alkaloids. (21,22,23,24,25)

Qualitative identification

By thin layer chromatography silica gel plate that is ready made GF254 (20x20) cm, thickness 0.25 (MERCK) were used, this plate was activated in an oven at 110°C for 30 minutes before used. Developing solvent systems saturated in glass tank (22.5 cm X 22cm X 7 cm), the developing system added to this tank and the former lined with filter paper to accelerate saturation process, then cover the tank with glass lid and allowed to stand for 45 minutes before use for saturation.

The developing system that used for identification is: S1 (chloroform: methanol (4:1). Each compound appear as single spot in the developing solvent. They have the same color and the R_f value of the standard one after visualized by UV (254 and 366) nm. (26)Vanillin reagent was used for identification of flavonoids. It was prepared by dissolve 0.5 gm of vanillin in 85 ml methanol, 10 ml acetic acid, 5ml concentrated sulfuric acid. The developing plate sprayed with this reagent and heated in an oven at 100°C for 5-10 min.

Results and discussion

The results showed that the percentage of yield of crude extract from extraction by method No.1 was higher than that obtained from extraction by method No.2 (table 1)

Table (1): percentage yield of each crude extract, obtained from extraction methods No.1, and No.2:

Extraction methods	% yield of crude extract of fennel	% yield of crude extract of parsely	% yield of crude extract of corn silk
Method No.1	18.73	38.74	5.16
Method No.2	1.66	2.02	0.83

The preliminary investigation revealed the presence of flavonoids, saponins, reducing sugar, terpenoids and alkaloids in all plant samples were used in this study but different in their concentrations table (2)

Table (2) chemical identifications of the flavonoids, saponins, reducing sugar, terpenoids, and Alklaoids.

Test name	Fennel	Parsley	Corn silk
Flavonoid test	++	+++	+
Saponin test	+	++	+++
Reducing sugar test	++	+++	+
Terpenoid test	+	+	+
Alkaloidal test	+	+	+

The results of identification by TLC were presented in Figure (1)

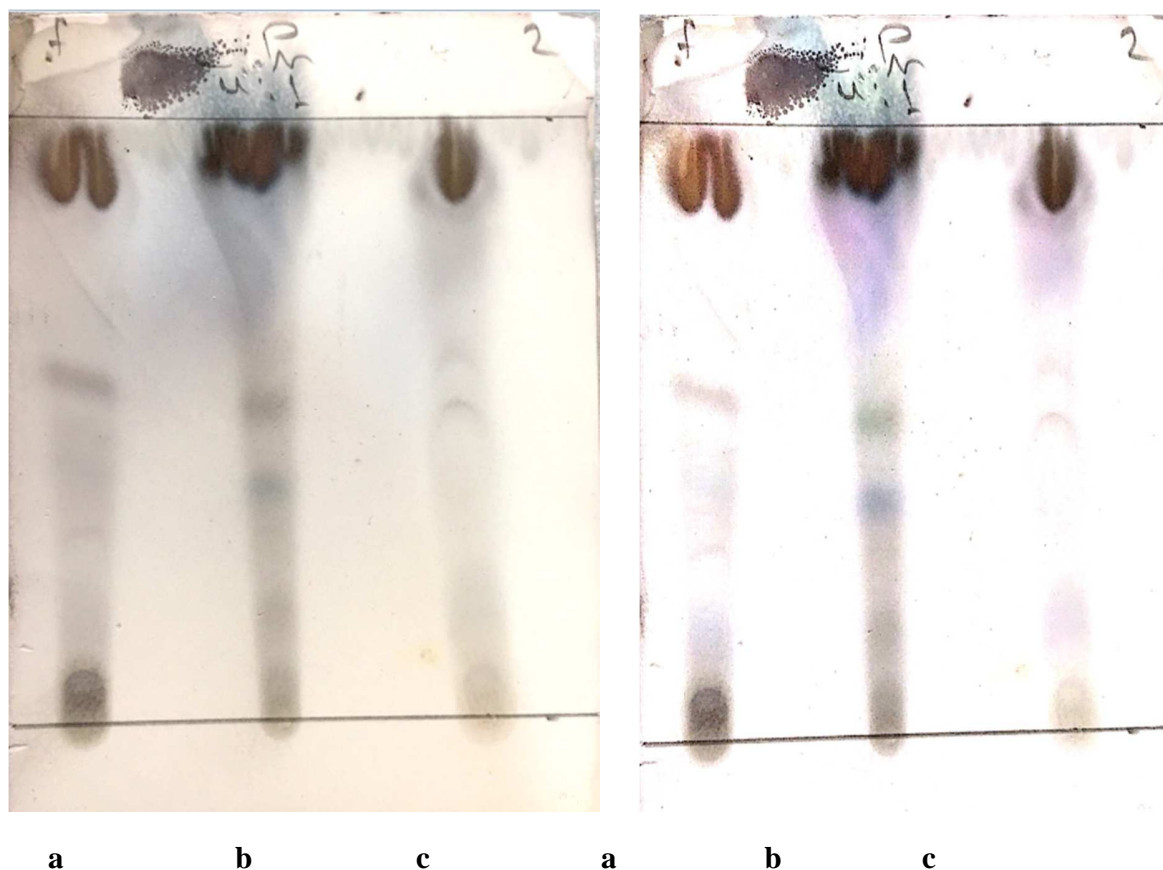


Figure (1) TLC identification of crude extract of *Foeniculum vulgare* (a) and *Petroselinum crispum* (b) and *Zea mayes* (c) respectively.

The Rf values of fennel fruits, corn silk, parsley seeds extracts were identified by TLC were presented in table 3,4and 5 respectively.

Table (3) R_f values of each spot of *Foeniculum vulgare* in the crude extracts by using the developing solvent systems in TLC plate.

Spot no.	R_f value
1	0.55
2	0.41
3	0.16

Table (4) R_f values of each spot of *Petroselinum crispum* in the crude extracts by using the developing solvent systems in TLC plate.

Spot no.	R_f value
1	0.54
2	0.38
3	0.18

Table (5) R_f values of each spot of *Zea mays* in the crude extracts by using the developing solvent systems in TLC plate.

Spot no.	R_f value
1	0.55
2	0.48
3	0.18

The more crude extract weight obtained when use the hot method was belong to the more polarity of water than ethanol. Also the heating increased the solubility of active compounds inside the plant cells then they pass to the solvent. Although the differences of active compounds concentration among plants were used in this study was belong to different factors such as type of soil, light, water or irrigation, time of gathering, genetic and biosynthesis of active compounds.

The combination of these three plants was effective in the treatment of UTI and stones due to each plant has role in this treatment as parsley has antioxidant activity due to flavonoid compounds which mean they eradicates the free radicals in body which cause oxidative stress in cells. Fennel cause soothing of the muscle and tissue of the urinary tract, also increase urine flow and finely the elimination of waste products from the urinary tract was increase. Corn silk help in the passage of stone from kidney.

Conclusion

The plants were used in this study contained the most important of active compounds especially flavonoids, glycosides that considered as antioxidant agent. This results were showed that the uses of these plants in treatment of urinary tract disease by boiling with distilled water was the best method.

Acknowledgements

The authors thanks Pharmacognosy department, college of pharmacy, kerbala university especially Dr. Salam Ahmed for their financial supports to complete this study.

References:

1. Al-jubori, Ibrahim s., (2007) Response of *Foeniculum vulgare* Mill. For field practices and their effect on parameters of growth, yield and active compounds, **Ph. Thesis, agricultural College, Baghdad university, Iraq.**
2. Shaza. M. Ahmed, Aisha Z., and Almagboul, (2016), Phytochemical screening of *Petroselinum crispum* (Mill.) Fuss and in vitro evaluation of its antimicrobial activity against some uropathogens, **Arabian journal of medicinal and aromatic plants**, 23; 2(2): 87-88.
3. JH Doughari, (2006), Antimicrobial Activity of *Tamarindus indica* Linn, **Tropical Journal of Pharmaceutical Research**, 5 (2): 597-603.
4. Ahmed Salih Sahib, Imad Hashim Mohammed, and Saba Jasim Hamdan, (2012), Use of aqueous extract of corn silk in the treatment of urinary tract infection, **Journal of Intercultural Ethnopharmacology**, 1(2): 93-96.
5. Huda S. A. A. Mohamed, Warda S. Abdelgadir, and Aisha Z. I. Almagboul, (2015) In vitro antimicrobial activity of Anise seed (*Pimpinella anisum* L.), **International Journal of Advanced Research**, 3(1):359-367.
6. Lopez MG, Sanchez-Mendoza IR, and Alejo O. (1999) Comparative study of volatile components and fatty acids of plants and in vitro cultures of parsley (*Petroselinum crispum* (Mill)), **J. Agric. Food Chem**, 47: 3292–3296.
7. Ouis Nawel, Hariri Ahmed, and El Abed Douniazad, (2014) Phytochemical analysis and antimicrobial bioactivity of the Algerian parsley essential oil (*Petroselinum crispum*), **African Journal of Microbiology Research**, 12; 8(11): 1157-1169.
8. Kreydiyyeh SI and Usta J, (2002) Diuretic effect and mechanism of action of parsley, **J Ethnopharmacol**, 79(3): 353-357.
9. Kreydiyyeh SI, Usta J, Kaouk I et.al. (2001) The mechanism underlying the laxative properties of parsley extract, **Phytomedicine**, 8(5): 382-388.
10. Sidra Mahmood, Shahzad Hussain, and Farnaz Malik, (2014) Critique of medicinal conspicuousness of Parsley(*Petroselinum crispum*): A culinary herb of Mediterranean region, **Pak. J. Pharm. Sci.**, 27(1): 193-202.
11. Ejigu Bayu (2012) Phytochemical and antimicrobial investigations of leaves of Fennel herb (*Foeniculum vulgare*) extracts. **Haramaya University.**

12. Somayyeh Sadrefozalayi, Farah Farokhi (2014) Effect of the aqueous extract of *Foeniculum vulgare* (fennel) on the kidney in experimental PCOS female rats, **Avicenna J Phytomed**, 2014; 4(2): 110-117.
13. Hassan Mahmoudi, Mohammad Reza Arabestani, Mohammad Molavi, et. al., (2016) The Study Effects Antimicrobial of *Foeniculum vulgare mill* and *Achilles mille folium* Plant on Bacterial Pathogens Causing Urinary Tract Infections and Nosocomial Infection, **International Journal of Pharmacognosy and Phytochemical Research**, 8(9): 1549-1554.
14. Weiping He, and Baokang Huang, (2011) A review of chemistry and bioactivities of a medicinal spice: *Foeniculum vulgare*, **Journal of Medicinal Plants Research**, 5(16): 3595-3600.
15. Thoudam Bhaigyabati, Kirithika T, Ramya J, Usha K., (2011) Phytochemical Constituents and Antioxidant Activity of Various Extracts of Corn Silk (*Zea mays. L*), **Research Journal of Pharmaceutical, Biological and Chemical Sciences**, 2(4): 986-993.
16. M.A. SOLIHAN, A.R. NURHANAN, W.A. WAN AMIR NIZAM et. al., (2015) Aqueous Extract of Cornsilk Confers Mild Diuretic Activity in Normal Rats, **Sains Malaysiana**, 44(8): 1167-1174.
17. Khairunnisa Hasanudin, Puziah Hashim, and Shuhaimi Mustafa, (2012) Corn Silk (*Stigma Maydis*) in Healthcare: A Phytochemical and Pharmacological Review, **Molecules**, 17: 9697-9715.
18. Parle Milind, and Dhamijia Isha, (2013) ZEA MAIZE: A MODREN CRAZE, **International Research Journal of Pharmacy**, 4(6): 39-43.
19. Nabila M. Rashwan, (2012) Biological Study on the Effect of Arginine and Parsley on Renal Toxicity in Rats, **World Journal of Medical Sciences**, 7 (4): 264-269.
20. Sreemoyee Chatterjee, Nandini Goswami, and Pradeep Bhatnagar, (2012) Estimation of Phenolic Components and *in vitro* Antioxidant Activity of Fennel (*Foeniculum vulgare*) and Ajwain (*Trachyspermum ammi*) seeds, **Advances in Bioresearch**, 3(2): 109-118.
21. Onwukaeme, D.N.; Ikuegbreweha, T.B; Asonye, C.C., (2007) Evaluation of phytochemical constituents, Antibacterial activities and Effect exudate of pycanthus Angolensis weld wrab (Myristicaceae) on corneal ulcers in rabbits, **Tropical Journal of Pharmaceutical Research**, 6(2):725-730.
22. Victor, B.K.; Al. Shaikh Hamed, W.M.A., (2014) Preliminary Phytochemical Screening and Evaluation of *in vitro* antioxidant activity of Iraqi species of *Hypericum perforatum* arial part, **International research Journal of Pharmacy**, 5(5):369-373.
23. Prashant , T.; Bimlesh , K.; Mandeep, K.; Gurpreet, K.; Harleen K., (2011) Phytochemical screening and extraction: A review, **International pharmaceutical science**, 1(1): 98-106.
24. Meena Sahu, Devshree Vermaand, and K.K. Harris, (2014) Phytochemical analysis of the leaf, stem, and seed extract of *Cajanus cajan L .* (Dicotyledoneae: Fabaceae), **World Journal of Pharmacy and Pharmaceutical Sciences**, 3(8): 694-733.
25. Neetu S Jamwal, Sunil Kumar, and A.C Rana, (2013) Phytochemical and pharmacological review on *Foeniculum vulgare*, **PHARMA SCIENCE MONITOR**, 4(3): 327-341.
26. Egon Stahl, (1962) Thin layer chromatography a laboratory handbook, **Springer**: New York.