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.

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

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 investigate 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 Dragendorff’s reagent for alkaloids. The qualitative identification was done by TLC. The results showed that the percentage yields of crude 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.
الخلاصة:

تمت دراسة تشكيل المكونات الكيميائية وقابلية المستخلصات لنباتات المعदنوس، حبة الحلوة، وحرص الزرعة في علاج المفاصل البولية لنباتات المعدنس. حبة الحلوة، وحرص الزراعة منذ البداية إلى البحث عن أفضل طريقة للحصول على أعلى نسبة من المكونات الكلية، مع الفحص الأولي للمركبات الكيميائية من بذور المعدنس، فواكه حبوب اللوز، وحرص الزراعة Mill. وفصيلة البولية Zea mays L. وفصيلة البولية Foeniculum vulgare Mill. أجريت دراسة لمعرفة أفضل طريقة لفحص مركبات النبات. وتم استخدام النباتات المختارة باستخدام 90% نبض حارة الغرفة في كل الطرق المختلفة. تم تجفيف النباتات بعد فحص بواسطة المبيض الدوار. تم إجراء كشف أولي لمكونات النباتات الكيميائية باستخدام اختبار الكشف اللفيزي لمركبات اللف، واختبار الرغوة للصابونيات، واختبار تيربينويد للتربينويدات، وكشف فهل يلك للسكر المخزنة وكشف أدراج في اللف. stataً للفحص الأولي، تم بواستاء صناع طاقة الرقيقة الكرومكاتافية. أظهرت النتائج أن نسبة المستخلصات الخام بواستاء الغليان بإشرب الماء المتجرأ كانت أعلى من تلك التي تم الحصول عليها من التلفيق خماد مع الابنول بنسبة 90%. النباتات الكيميائية النباتية، اللف، اللف، السبل، المستخلصات، والتربينويد والتربينويدات من مواد النباتات أظهر أن النباتات الكيميائية النباتية، اللف، اللف، السبل، مستخلصات الأعلام المخزنة من تلك الموجودة في مستخلصات الابنول. يتأثر النباتات في علاج المفاصل البولية، أظهر أن النباتات الكيميائية النباتية، اللف، اللف، السبل، مستخلصات الماء أقوى من مستخلصات الابنول. بدأ على معرفتنا هذه في الدراسة الأولى عن تأثير مستخلصات الماء. حبة الحلوة، وحرص الزراعة في علاج المفاصل البولية.

 소개

نباتات الطبيعة تم استخدامها في الطب البديل منذ الأزل. (1) وفقًا للمنظمة العالمية للصحة، كان 80% من السكان تعتمدون عليه من النباتات الطبية. (2,3) وفقًا للإحصائيات، يوجد حوالي 150 مليون مرض شهق في العالم كل عام. (4) لذا، استُخدمت النباتات الطبية كجزء من العلاج الكيميائي. (5)

تم استخدام نباتات كبراء، وفاصلي، وكسوة الكرومات كعلاج لل合法权益. (6,7) بذور كبراء، وفاصلي، وكسوة الكرومات تم استخدامها كدواء مولع، ودواء مولع، ودواء مولع. (8) دراسة النباتات الكيميائية النباتية، اللف، اللف، السبل، مستخلصات الماء أقوى من مستخلصات الابنول. بدأ على معرفتنا هذه في الدراسة الأولى عن تأثير مستخلصات الماء. حبة الحلوة، وحرص الزراعة في علاج المفاصل 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 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 vulgare mill.) is a biennial medicinal plant belonging to the family Apiaceae (ummbelliferae) (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 Graminaceae 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,
maizeneic 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 sonofier, 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 sartorious /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 40ºc 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 an 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 40ºc to give a crud 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 Dragendorff’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 110ºc 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 so lvent. They have the same color and the Rf 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 100ºc 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:**

<table>
<thead>
<tr>
<th>Extraction methods</th>
<th>% yield of crude extract of fennel</th>
<th>% yield of crude extract of parsley</th>
<th>% yield of crude extract of corn silk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method No.1</td>
<td>18.73</td>
<td>38.74</td>
<td>5.16</td>
</tr>
<tr>
<td>Method No.2</td>
<td>1.66</td>
<td>2.02</td>
<td>0.83</td>
</tr>
</tbody>
</table>

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 Alkaloids.

<table>
<thead>
<tr>
<th>Test name</th>
<th>Fennel</th>
<th>Parsley</th>
<th>Corn silk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid test</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Saponin test</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Reducing sugar test</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloidal test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

![TLC identification of crude extract of Foeniculum vulgare (a) and Petroselinum crispum (b) and Zea mayes (c) respectively.](image)

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

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>$R_f$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>2</td>
<td>0.41</td>
</tr>
<tr>
<td>3</td>
<td>0.16</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>$R_f$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.54</td>
</tr>
<tr>
<td>2</td>
<td>0.38</td>
</tr>
<tr>
<td>3</td>
<td>0.18</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>$R_f$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>2</td>
<td>0.48</td>
</tr>
<tr>
<td>3</td>
<td>0.18</td>
</tr>
</tbody>
</table>

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

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References:


