

Research Article

Standardization the Crude Extracts of all *Urtica* plant Species Growing in Palestine for Quality Control of Cosmeceutical and Pharmaceutical Formulations

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ABSTRACT

Background: The safety and the therapeutic efficacy of plants medications are mainly dependent on the quality controls and standards by which they are made for, to give powerful actions when prescribing them. Medicinal plants and their extracts have been used as sources of medicine, cosmetics and food in virtually all nations and cultures and the usage of plants as a source of cosmetics and medications among different population seems to never stop, even with the recent chemical synthetic medicines revolution. **Objective:** This study aimed to evaluate the standard of crude extracts yields of the active constituents for all species of *Urtica* plants leaves (*Urtica kioviensis* Rogow., *Urtica membranacea* Poir. ex Savigny, *Urtica pilulifera* L. and *Urtica urens* L.) growing wildy in Palestine and used for treatment of various diseases as well as used in cosmetics and food. **Method:** Serial exhaustive extraction method using polar and nonpolar solvents for four species of *Urtica* leaves without boiling to avoid the hydrolysis of the active phytochemical compound. **Results:** The best percentage of the aqueous extract yield was for *Urtica kioviensis* 609mg (24.36% of the total starting powder weight), while the best organic extract yield was also for *Urtica kioviensis* 72mg (2.88% of the total starting powder weight). **Conclusion:** *Urtica kioviensis*, *Urtica pilulifera*, *Urtica urens* aqueous serial exhaustive extraction yield and *Urtica kioviensis* organic exhaustive extraction yield can pass the quality control for manufacturing of cosmetics formulations and pharmaceutical preparations.

Keywords: Standardization; Serial extraction; *Urtica kioviensis*; *Urtica pilulifera*; *Urtica urens*; *Urtica membranacea*

INTRODUCTION

Most of the all world's populations especially in the developing countries still depend on plant-based medicines for their primary health care according to the World Health Organization estimations. Actually this is a clear indication for the value of plants products in the treatment of diseases and maintenance of health, as well as being therapeutic alternatives throughout the world, still a very important factor in the 21st century^{1,2}.

Recent years have witnessed a renewed interest in plants as cosmeceuticals and as pharmaceuticals in the western world. This trended interest open new channels into the discovery of new physiologically and biologically active compounds by the pharmaceutical and cosmeceuticals industry. The process of drug and cosmetics development from plants involves the isolation, identification and extraction of active components of crude extracts or whole plants and, in some cases, semisynthesis of equivalent active compounds³.

Now a day many studies show that about 25% of the medications prescribed around the world derived from plants. Of the 252 medications considered as essential and basic by the WHO, 11% are actually isolated from herbal origin and a significant number of synthetic chemical medications semi-synthesized from natural

molecules precursors. Examples of important drugs obtained from plant origin are atropine from *Atropa belladonna* L., vincristine from *Catharanthus* spp., papaverine, morphine and codeine from *Papaver somniferum* L. and a lot of other natural medications⁴.

The utilization of medicinal and non medicinal plants in developing countries has been a gradual revival of interest in recent years because herbal medicines have been reported more safely or with a few adverse side effects especially in comparison with synthetic chemicals drugs. Thus a search for new medications and cosmeceuticals with cheaper and better substitutes from herbal origin is a natural excellent choice. The therapeutic values of these plants depended on some chemical molecules that produce physiological and biological activities⁵.

Nettle (Stinging nettle), *Urtica kioviensis* Rogow., *Urtica membranacea* Poir. ex Savigny, *Urtica pilulifera* L. and *Urtica urens* L. (Fig.1) are annual herbaceous plants belonged to family Urticaceae which naturally growing in the mountains, pathways, fields and wildwood in Palestine. They are grown in mild climate areas, bottom of barriers, between cultivated plants, street, and water runnels in addition to all that they have a wide distribution in the world⁶.

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Figure 1: A. *Urtica kioviensis*, B. *Urtica membranacea*, C. *Urtica pilulifera*, D. *Urtica urens*

Table 1: *Urtica* plant species in Palestine and their voucher specimen codes

<i>Urtica</i> plants species growing wildly in Palestine	Voucher specimen codes
<i>Urtica kioviensis</i>	Pharm-PCT-2559
<i>Urtica membranacea</i>	Pharm-PCT-2560
<i>Urtica pilulifera</i>	Pharm-PCT-2561
<i>Urtica urens</i>	Pharm-PCT-2562

Nettle leaves are stinging, have serrated edge and dark green color. The leaves are 2–4 cm long, oval, and core in shape and can cause burning and blushing of skin if touched. The flowers of nettle are small and green usually cultivated from May to September. The fruits of nettle are arid and single germ⁷.

Nettle leaves are rich with a micronutrient like all green vegetables and considered a nutritious food but these leaves should be cooked or steamed before consumption to get rid of the stinging hairs, which contain acetic acid, formic acid, histamine, butyric acid, leukotrienes, acetylcholine, 5-hydroxytryptamine and other irritant compounds^{6,8-10}.

The skin contact with the leaves hairs leads to a mildly painful itching, sting and numbness for a period lasting

from minutes to days also may develop of an erythematous macule but the nettle leaves do not cause this reaction in medicinal extracts if the hairs are destroyed in preparing of them¹¹⁻¹³.

Stinging nettle contain lectins and polysaccharides (important particularly in prostate disease)¹⁴, also contain lignans phytochemicals such as (-)-3,4-divanillyltetrahydrofuran (useful in benign prostatic hyperplasia) as well as contain Bis (2-ethyl hexyl) maleate, Dibutyl phtaleate, Neophytadiene, 1,2-benzenocli carboxylic acid, Phtaleic acid and other classes of natural phytochemicals like alkaloids, flavonoids, tannins, glycosides, proteins and phenolic compounds¹⁵⁻¹⁷.

In the folk medicine Nettle leaves are used for the treatments of eczema, digestion and sexual disorders, muscle pains, mild menorrhagia, gout, anemia, hair loss, hypertension, arthritis, hay fever and in the treatment of symptomatic benign prostate hyperplasia as well as used as cleaning tonic, blood purifier, hypoglycemic, antiasthmatic, depurative, astringent and antidandruff^{11,18-24}.

In cosmetics the plant used for manufacturing of shampoo, astringent and deodorizing body spray, cleansers solutions and hair conditioning cream^{25,26}.

Table 2: The weights of the resulted extracts

The serial extracts	Urtica kioviensis extract weight in mg	Urtica membranacea extract weight in mg	Urtica pilulifera extract weight in mg	Urtica urens extract weight in mg
The organic extract	72	68	45	62
The first aqueous	433	343	421	411
The second aqueous	176	123	161	122
Total of the aqueous extracts	609	466	582	533

The recent evidence based study shows that *Urtica* can be used for treatment of benign prostatic hyperplasia and other urinary tract conditions^{27,28}, while the whole plant clinically approved their effect for treat of neuralgia, arthritis and other related conditions²⁹⁻³².

Other recent scientific studies indicate that Nettle extracts have antibacterial effects on the gram positive and gram negative bacteria several times more than chemical materials^{33,34}, antiviral (viruses such as those causing hepatitis and aids)³⁵ and antifungal activities³⁶.

Also useful in treatment of cardiovascular diseases (vascular types), improve lipid profiles by inhibition of platelet aggregation^{37,38} and allergic rhinitis (it may be useful for allergic diseases of all types)³⁹.

Table 3: The percentages of the resulted yields extracts

Urtica plant species in Palestine	The percentage of the organic exhaustive extraction yields	The percentage of the aqueous serial exhaustive extraction yields
<i>Urtica kioviensis</i>	2.88	24.36
<i>Urtica membranacea</i>	2.72	18.64
<i>Urtica pilulifera</i>	1.8	23.28
<i>Urtica urens</i>	2.48	21.32

MATERIAL AND METHODS

Collection, identification and treatment of the four Urtica species samples:

The leaves of *Urtica kioviensis*, *Urtica membranacea*, *Urtica pilulifera* and *Urtica urens* were collected from different regions of Palestine between March and April 2014. The plant was botanically identified by Dr. Nidal Jaradat from the Pharmacy Department at An-Najah National University. Voucher specimen was deposited in the Herbarium of the Pharmaceutical Chemistry and Technology Division (Laboratory of Pharmacognosy) and the plants codes are presented in (Table 1)

Later the leaves of four species were washed and cleaned with soft clothes to remove all traces of mold, dust and insects, then dried in shade at 25-30°C, with continuous overturn to prevent mould. Weighed, ground mechanical grinder, placed in airtight bottles and stored in the desiccators to be used later for extraction⁴⁰.

Chemicals & Instruments

Methanol, n- hexane, ethanol, triple distilled water, shaker device (Memmert Shaking Incubator, Germany), rotary evaporator (Heidolph OB2000 Heidolph VV2000, Germany), freeze dryer (Mill rock technology, model BT85, Danfoss, china), grinder (Moulinex model, Uno, China), balance (Rad wag, AS 220 / c/2, Poland), filter paper (MACHRERY-NAGEL, MN 617 and Whatman no.1)

The first extraction

The leaves of the four plants samples were dried in the shade for about 2 weeks, at room temperature, until they became completely dry. Then 25 gram of the leaves of the four plants species were obtained and cut into small pieces, then powdered in a mechanical grinder. The 25 gram of the powdered plant, were suspended in 50 ml n-hexane which is relatively safe, cheap, largely unreactive and easily evaporated non-polar (hydrophobic) solvent, and 250 ml of 50% ethanol in triple distilled water (to ensure sterility) in a bottle, with continuous shaking (200 round per minute) at 25°C for 72 hours in the shaking incubator. After that, the mixture was filtered by Whitman's No.1 filter paper using the Buchner funnel. The plant materials that had been accumulated on the filter paper were re-extracted again (2nd extraction).

The liquid filtrate was separated by separatory funnel into 2 phases: lower phase which has higher density (aqueous phase) and upper phase which has lower density (organic phase). The aqueous phase was collected first and kept in a volumetric flask at room temperature till the next step (obtaining the powder of aqueous extract). The organic phase was collected second and placed in a pre-weighed glass beaker, which was placed in the hood at room temperature in order to evaporate the solvent (n-hexane), and to obtain the organic extract. The beaker with the organic extract was weighed again after evaporation; the weight of the organic extract was determined by calculating the difference of the weights and was kept in a sterile brown bottle at 4°C in the refrigerator till later use.

The second extraction

This extraction was only for the aqueous extract. The plant materials that accumulated on the filter paper after the first filtration were re-extracted again, by adding 250 ml of 50% ethanol in triple distilled water, with continuous shaking for 72 hours in the shaking incubator at 25°C as before. A second filtration for the mixture was done by using Whitman's No.1 filter paper on the Buchner funnel. The second aqueous phase was collected after filtration and kept in a volumetric flask at room temperature.

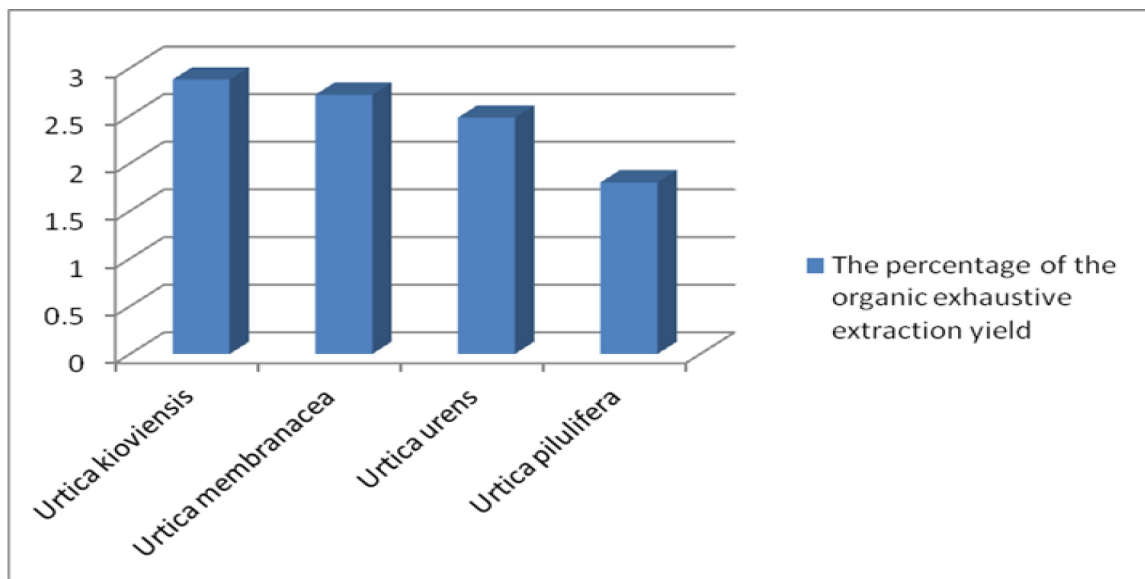


Figure 2: The percentage of the organic exhaustive extraction yields for four *Urtica* plant species

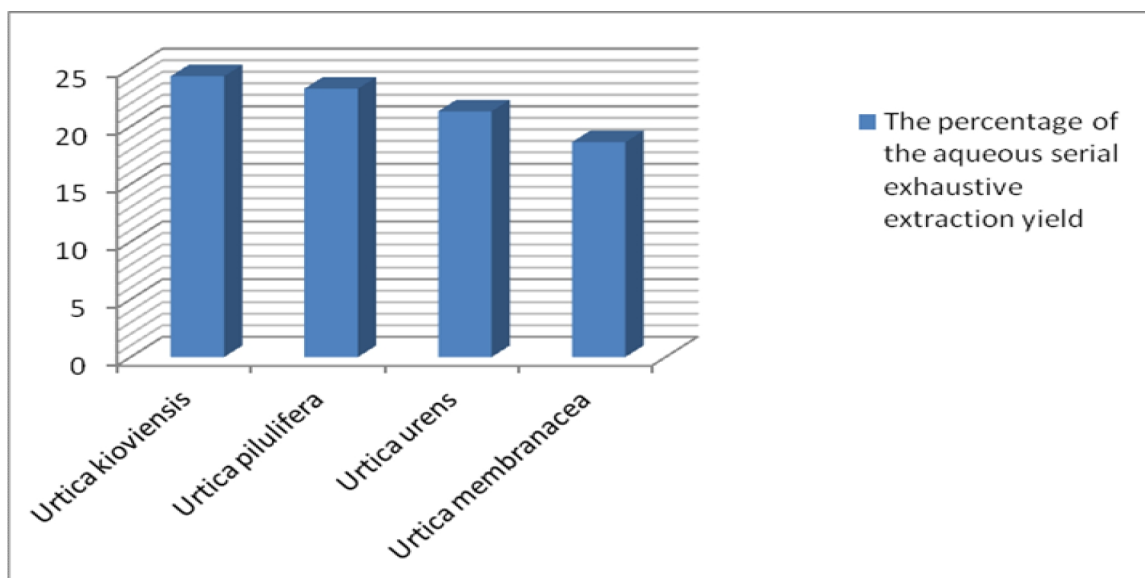


Figure 3: The percentage of the aqueous exhaustive extraction yields for four *Urtica* plant species

The rotary evaporator was used for 1 hour at 40°C to evaporate any leftover organic solvents from both aqueous phases obtained from the first and second extraction. Then both aqueous extracts were put separately in preweighed freeze dryer bottles and placed on the freeze dryer for 24 hours till they dried completely. Then the freeze dryer bottles were reweighed again, and the dry weight of both extracts was calculated⁴¹. All these procedures repeated four times for each *Urtica* plant species.

RESULTS AND DISCUSSION

The aqueous and organic extracts

Twenty five grams of four species of *Urtica* plant powders were subjected to serial exhaustive extraction.

The weights and percentages of the dried aqueous and organic extracts that were produced from the first and the second extractions are shown in Table 2 and Table 3 and clarified in (Fig. 2) and (Fig.3). The results showed that the best total aqueous extract yield was for *Urtica kioviensis* 609 mg (24.36% of the total starting powder weight), while the best organic extract yield was also for *Urtica kioviensis* 72 mg (2.88% of the total starting powder weight).

The safety and efficacy of herbal medications are dependent upon the standards by which they are made for and our knowledge base when prescribing them, according to the American Herbal Pharmacopoeia and Therapeutic Compendium the aqueous Nettle leaves extract yields must be not less than of 20% while the

organic extracts yields must be not less than 2.5% that means *Urtica kioviensis*, *Urtica pilulifera*, *Urtica urens* aqueous serial exhaustive extraction yield and *Urtica kioviensis* organic exhaustive extraction yield can pass the quality control and standardization process for manufacturing of cosmetics formulations and pharmaceutical preparations.

CONCLUSION

The leaves of *Urtica kioviensis*, *Urtica membranacea*, *Urtica pilulifera* and *Urtica urens* which were collected from different regions of the West Bank/ Palestine, exhaustively extracted by using polar and nonpolar solvents. This research scientifically certified that *Urtica kioviensis* was the best source for further manufacturing of standardized cosmeceuticals and pharmacological active and evidence based pharmaceutical forms. We also recommend researchers to use *Urtica kioviensis* plant for their future scientific researches as well as it's a good source for natural foods supplements, pharmaceuticals and cosmeceuticals industry.

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