Research Article

Formulation and Evaluation of Herbal Tablets and Hard Capsules Containing *Urtica dioica* Soft Extract

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Accepted on: 10-04-2015; **Finalized on:** 31-05-2015.

ABSTRACT

Urtica dioica (Stinging Nettle) is one of the most popular plants, whose dietary and therapeutic benefits have been known since ancient times. The therapeutic activity of nettle leaves extracts in arthritis and osteoarthritis is related basically to the presence of phenolic compounds, and their antioxidant activity. Literatures indicate that plants maturation leads to changes of their chemical composition. So firstly, we compared number of extracting methods to determine the one that gives the highest total content of phenolic compounds. Folin-Ciocalteu method for that was applied. Secondly, the prepared soft extract was inserted in tablets and capsules formulations. Results showed that the optimal extracting method was in using fresh young leaves, Ethanol 95% as extracting liquid, at 70°C with reflux condenser, for five hours. We successfully developed *the Urtica Dioica* herbal tablets using the wet granulation method. The best pharmaceutical formulation, contained calcium carbonate as filler, Magnesium stearate and Sodium benzoate as lubricants, Sodium Carboxy Methyl Cellulose (CMCNa) as Disintegrating agent and Alcoholic Polyvinyl pyrolidone solution as Binder. These granules formulation components were also successfully prepared to be filled later in hard capsules.

Keywords: *Urtica Dioica*, Folin-Ciocalteu, Phenolic compounds, herbal tablets formulation.

INTRODUCTION

There has been a great interest in the last few decades in using plants to cure diseases in general, and to consider it as a main source in the Alternative Medicine to cure the chronic diseases in particular.[1]

These prescriptions that contain compounds refer to chemical groups produced by plants are called Botanical Products.[2,3] Ones of these products are those in their components contain *Urtica Dioica* (Stinging Nettles) Plant, which its leaves for their high content of phenolic compounds, are recommended by World Health Organization (W.H.O) to be used in treating Arthritis and osteoarthritis.[4-6] On the other hand its roots due to the present of Lignans compounds, are recommended by (W.H.O) to be used in Benign Prostatic Hyperplasia (BPH) and other androgen- and estrogen-sensitive conditions.[4-6] National Institution of Health (NIH) indicates to be over than 402 products around the world contain *Urtica Dioica* plant as whole or different parts of it, presented in Pharmaceutical forms as tablets and capsules.[7]

The therapeutic activity of phenolic compounds, as main active ingredients in leaves, has been interestingly studied by scientists.[8] Studies showed that phenolic compounds work as antioxidant and anti-inflammatory agents.[8] Their mechanism of action is to reduce cytokines levels, TNF-α, and CRP, which temporarily allows to reduce the feeling of pain in Arthritis and Osteoarthritis.[9-13] *Urica Dioica* leaves work also as inhibitors for Cox_1 and Cox_2 enzymes to inhibit, as a result, prostaglandins synthesis especially PGD_2.[13,14] An open study showed that oral dosing of *Urtica Dioica* leaf is associated with symptom reduction comparable to that achieved with non- steroidal anti – inflammatory drugs (NSAIDs) that are considered as OTC.[15,16] Another open study showed that nettles leaf (50g of stewed leaves daily with food) potentiated the efficacy of sub-therapeutic doses of NSAIDs as Diclofenac.[11]

The antioxidants mechanism of phenolic compounds is mainly scavenging the free radicals that act by attacking the unsaturated fatty acid in the biological membranes which extend to membranes lipid peroxidation and finally to the cell inactivation or death.[8] Probably this makes *Urtica Dioica* leaves therapeutically active in diabetes mellitus, cardiovascular, inflammation, cancer, osteoporosis and degenerative diseases.[17-21]

It is known that plants maturation leads to changes of their chemical composition.[8,22] Hence, this research aims to prepare extracts from *Urtica Dioica* leaves, determine their total content of phenolic compounds to insert them later in hard pharmaceutical preparations such as tablets and capsules.

MATERIALS AND METHODS

Leaves of *Urtica dioica* were collected from the country of Jableh Region, Lattakia, Syria, in March 2013 (Mature plants), December 2014 (fresh plants). A voucher specimen of each batch was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Lattakia, Syria.

Sodium carbonate anhydrous was purchased from (BDH Laboratory. England), Tannic acid by (Bhiwadi, Rajasthan,

India), Ethanol 95% was provided from (Medical Ethanol95%, Zouheir and Ramez Bounder Co. Lebanon), Folin-Ciocalteu reagent provided from (Sigma-Aldrich, Switzerland).

Extraction Procedure

Ethanol 95% was used as extracting liquid for phenolic compounds.[23] The conventional extraction with and without Heat was used. Two grams of homogenized sample of leaves powder were extracted with 20 ml of Ethanol 95% in a conical flask with magnetic stirrer at 600 rpm at [room temperature(31°C), 40°C and 70 °C], for three different periods of extraction (2,5,9)h in each experiment. The leaves extracts were then filtered (paper No. 89). The extraction process was done in triplicate.

Determination of Total Phenolic Content

Determination of Total Phenolic Compounds, expressed as tannic acid equivalent, was performed according to *Folin-Ciocalteu* method. The chromophore development reaction is based on oxidation of polyphenols *via Folin-Ciocalteu* reagent, which is a mixture of phosphomolybdenic and phosphotungstic acids, in a basic medium. The blue complex thus formed, is assessed by absorbance at 750 nm, and is directly proportional to the total amount of phenols in the medium.[24]

0.2 mL of each extract were taken, followed by the addition of 4 mL of 2% sodium carbonate solution, mixed using the vortex for 5 minutes, then 0.2 mL of Folin-Ciocalteu reagent (diluted 1:1) was added. The flasks were mixed well and left in the dark at room temperature for 30 minutes. The absorbance, then, was read at 750 nm using a UV-Vis spectrophotometer Jasco V-530. Tannic acid was used to prepare a calibration curve in the range (0-100) mg/100mL **(Figure 1)**. The concentration of total phenols was expressed in percentage (mg of Gallic acid equivalents per 100 g of dry plant material) (mg GAE \100g_{dw})[24].

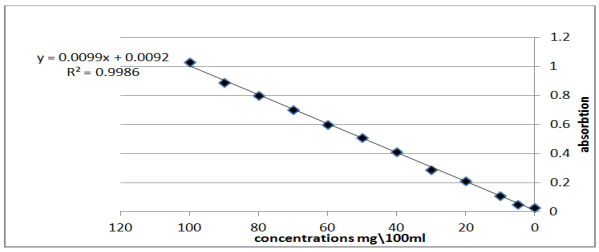

Figure 1: Standard Calibration Curves for Tannic acid

Formulation and Evaluation of Tablets

The soft plant extracts (≤7.5% moisture) were mixed with different excipients using wet granulation method to prepare later solid pharmaceutical forms; these prepared granules of each form were compressed into tablets using Compressing machine, Wick PR1\Austria. One granulation formulation (C1) was prepared to be filled later in capsules.[25] The details of composition of these formulations were given in **(Table 1)**. Calcium Carbonate was provided from Qualikems, India. Sodium Carboxy methyl cellulose, Mg stearates and Talc were provided from atocomi, India. Polyvinylpyrolidone, Sodium Benzoate and Aerosil were provided from Bihwadi Rajasthan, India.

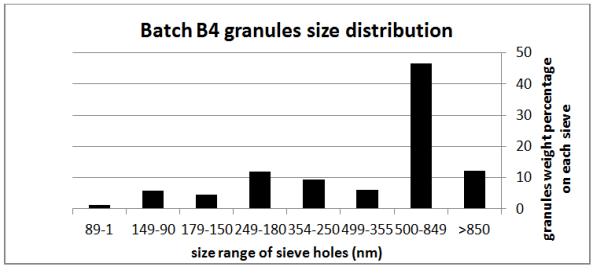

Figure 2: Batch B4 granules size distribution

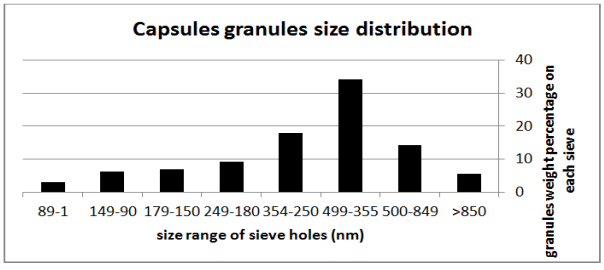

Figure 3: Capsules granules size distribution

Table 1: Formulations of group (A) of granules containing Lactose as Filler and group (B) of granules containing Calcium carbonate as Filler.

Form No. Component (mg)	A1	A2	A3	B1	B2	B3	B4	C1
Herbal Extract	100	100	100	100	100	100	100	50
lactose	300	300	400	-	-	-	-	-
Calcium Carbonate	-	-	-	300	500	400	372.5	120
CMCNa	10%	10%	10%	10%	10%	10%	10%	10%
Alcoholic PVP 15%	QE	-	-	-	-	-	-	-
Alcoholic PVP 30%	-	QE	QE	QE	QE	QE	QE	QE
Mg Stearate	5%	5%	5%	0.5%	0.5%	2%	4%	4%
Na benzoate	-	-	-	-	-	-	5%	5%
Talc	2%	2%	2%	2%	2%	-	-	-
Aerosil	-	-	-	-	-	2%	-	-

Table 2: The Total content of Phenolic compounds in young leaves**.** (mg GAE\100g_{dw})

Heat (°C) \ Time (hours)	2	5	9
Room temperature 31 °C	229.090 ± 14.443	651.090 ± 10.069	455.350 ± 6.500
40 °C	354.550 ± 11.832	720.710 ± 10.914	574.380 ± 10.011
70 °C	518.910 ± 9.077	831.330 ± 23.015	283.230 ± 11.815

Table 3: The Total content of phenolic compounds in mature leaves.(mg GAE\100g_{dw})

Heat (°C) \ Time (hours)	2	5	9
Room Temperature 31 °C	106.54 ± 14.25	127.61 ± 14.04	198.53 ± 19.81
40 °C	128.07 ± 10.47	208.50 ± 16.35	220.36 ± 14.42
70 °C	160.19 ±23.13	240.76 ± 18.73	201.88 ± 21.60

Table 4: Granules Compressibility & Flow ability of all Batches.

Formulation\Parameter		BD	TD	(HR)	(CI%)	Flow ability as (HR)	Flowability as (CI%)
Batches containing Lactose as Filler	A1	0.37	0.53	1.42	30.18%	bad	weak
	A2	0.36	0.54	1.50	33.3%	bad	weak
	A3	0.46	0.67	1.45	31.3%	bad	weak
Batches containing Calcium Carbonate as Filler	B1	0.48	0.63	1.30	23.0%	bad	weak
	B2	0.43	0.57	1.32	24.5%	bad	weak
	B3	0.47	0.59	1.23	20.3%	good	weak
	B4	0.48	0.54	1.066	6.6%	good	excellent
	C1	0.55	0.59	1.058	-	good	-

The granules of each form, prepared by wet granulation method, were tested for their flow ability by calculating Hausner's Ratio and Carr's Index after measuring Bulk and Tapped densities using SOTAX–Tap Density Tester(USP)–TD_2V 230 315 mAT.[26] Granules sizes distribution test was done after adding the external phase of excipients using Fritsch industriestr 8 D-55743Idar-Oberstein\Germany.

The prepared tablets and capsules were evaluated for their appearance, weight variation, disintegration time and content variation range of phenolic compounds test. Tablets also underwent the Friability and hardness tests.[27-29]

In weight variation study, twenty tablets were selected at a random and average weight was calculated. Then individual tablets were weighed and weight was compared with an average weight. Same do with the content of Capsules.

Content variation range of phenolic compounds test was done for both ten tablets and ten capsules, by dissolving each milled tablet and the content of each capsule in 20ml Ethanol, filtered, then total phenolic compounds content was determined using Folin-Ciocalteu method.

Hardness Tester

Erweka D-63150: GmnH\ TBH.200, (Germany) was used for determining of the hardness of tablets. Ten tablets were each placed in contact between the plungers, and the handle was pressed, the force of the fracture was recorded.

The friability of tablets was determined using Friability Tester: LOGAN instrument corp. FAB-2. Ten tablets were accurately weighed together and the tablets had any dust removed before the test. After 4 minutes of rotation at 25 rpm, any loose dust from the tablets was removed then weight the tablets again and calculate it's friability.

In the disintegration time study, Six tablets were tested. Each tablet was put into 900 ml Hcl solution (0.1N) at 37±2 °C. Time required for complete dispersion of a tablet was measured with the help of Disentegration test device: Erweka ZT 52\Germany. Same procedure goes for six capsules.

RESULTS AND DISCUSSION

Young leaves contained higher content of phenolic compounds than the mature leaves. The total content of phenolic compounds in young leaves range was [229.09-821.33] (mg GAE\100g_{dw}), while the total content of phenolic compounds in mature leaves was [106.54-240.76] (mg GAE\100g_{dw}). (**Tables 2 & 3**)

The highest content of total phenolic compounds was found in young leaves of *Urtica dioica* extracted using Ethanol 95%, at 70 °C, for 5 hours, with the present of magnetic stirrer provides 600rpm, that reached (821.33 ± 23.01) mg GAE\100g_{dw}.

While the lowest content was in mature leaves extracted using same procedures, but at room temperature (31 °C), and for 2 hours, that reached (106.54±14.25) mg GAE \100g_{dw}.

Our results indicated same content range of phenolic compounds in comparison to related results in literature [44-1410]mg GAE\100g_{dw}, or[24.1-36.78]mg\1$g_{d.extract}$[30,31]. This can be explained by a sudden drop of

phenolcarboxylic acids in leaves harvested at plant flowering stage; mature plants.[22]

Our results agreed with (MOLDOVAN*L) about the content of phenolic compounds in *Urtica Dioica*, but they expressed their result as Caffeic acid Equivalent, their result was (9009 ± 2.82 mg Caffeic acid Equivalent \100 g_{dw}).[33]

On the other hand, our result were less than (Kukric Z. Z.) whose their study conducted by using the conventional method extracting with ethanol 80% for half an hour, at room temperature (25 °C), following different analytical procedure of Folin-Ciocalteu method. Their result showed that the total content of phenolic compounds amounted to 20837 mg GAE\100g_{dw}.[34]

Same time, our results were less than a study conducted by (IOANA N.), their result of total content of phenolic compounds was [7012.5 – 1444.6] mg GAE\100g_{dw} using ethanol 50% as extracting liquid with reflect condenser for 30 minutes. These last two studies followed different procedure of Folin-Ciocalteu method that also differs from each other.[8,34]

Evaluation of Prepared Granules and Tablets

The results obtained on various parameters of pre-formulation studies of granules were found satisfactory only for formulation B4 and for the Capsules granules formulation C1. Capping and sticking was observed for the trial (A1-A3 & B1-B3). Hence this trial was rejected, and the other tests were applied only on B4 & C1 batches. From the compressibility index (CL%) and Hausner's ratio (HR) values obtained for granules of all the batches, B4 granules were found to have good flow properties. (**Table 4**)

This result can be explained by the high concentration of combined lubricants used in B4&C1 batches that reached to 4% for Mg Stearates (Hydrophobic lubricant) and 5% for Sodium Benzoate (Hydrophilic lubricant).[35,36]

In Granules sizes distribution test, the granules sizes of Batch B4 arranged between [500-849]nm in 46.56 % (**Figure 2**) and the granules sizes, to be later filled in empty capsules, ranged between [355-499]nm in 34.09%. (**Figure 3**)

Figure 4: Tablets Appearance

The prepared capsules were homogenized colored and the snaps are well sealed. The prepared tablets of batch B4 were spherical, with smooth surface but non-homogenized colored (green with some dots of white). (**Figure 4**)

This can be related to the different in colors between the granules; that got their color from the green chlorophyll in the extract as it is leaves extract, and the external phase excipients; their original color is white.

As a result these tablets need to be coated. The maximum weight variation of the B4 tablets was ±4.6%, which falls within the acceptable weight variation range of ±5%, hence the tablets of batch B4 passed the weight variation test. The maximum weight variation of the Capsules was ±7.54%, which falls within the acceptable weight variation range of ±10%, hence the Capsules passed the weight variation test.

Hardness for tablets of batch B4 was in the range of [5.76-13.5] kg/cm², which falls above the limit of not less than 3.5 kg/cm². Friability value for tablets of the batch B4 was 0.338% which is lower than 1%.

Disintegration time is an important parameter of tablets and capsules as it's the first stage for active ingredients to be released. In this study, the tablets of batch B4 disintegrated within 5 minutes**,** and the capsules disintegrated within 6:12 min. Both are less than the ideal disintegration time that is within 15 minutes for tablets, and 30 minutes for capsules.

The maximum content variation range of the B4 tablets was [86.45-110.78]% which falls within the acceptable content variable range of [85-115]%, hence the tablets of batch B4 passed the content variation test. The maximum content variation range of the capsules was [89.2-103.7]% which falls within the acceptable content variable range of [85-115]%, hence the capsules passed the content variation test.

CONCLUSION

As we can conclude from this work that the best method for phenolic compounds extraction was in using young leaves, Ethanol 95% as extracting liquid, at 70°C for 5 hours.

This agreed with Rolson and colab 2003 and the best granules formulation with to be later compressed into tablets or to be filled in hard capsules was in using calcium carbonate as filler, CMCNa as disintegration agent, Alcoholic PVP as binder, Mg stearates and Na benzoate as lubricants for *Urtica Dioica* leaves soft extract.

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Source of Support: Nil, **Conflict of Interest:** None.