

Standardization of extraction process for *Rumex vesicarius* L.

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Abstract

The use of bioactive compounds in different commercial sectors needs the most appropriate and standard method to extract the active components from plant material. Thus standardization of the extraction process is an important step for the establishment of a consistent biological activity, a consistent chemical profile or for quality assurance in production and manufacturing of herbal drugs or any herbal formulations. In the present study, hot soxhlet extraction and cold maceration extraction methods using petroleum ether, chloroform, methanol, aqueous as solvents have been developed for extraction of bioactive compounds from *Rumex vesicarius* L. Among the extraction methods applied, hot soxhlet extraction method was found to be effective in terms of the percentage of yield compared to cold maceration extraction method. And also the percentage of extract varies with the solvent and duration of extraction process. Thus extraction time and the solvent systems were also standardized for *Rumex vesicarius* L. to extract the compounds.

Index terms: *Rumex vesicarius* L., Extraction, Cold maceration, Soxhlet extraction

Introduction

Phytochemicals are the plant derived chemicals and have the capability of disease prevention, thus beneficial to human health [1]. These bioactive compounds produced by medicinal plants act either through interfering in the metabolites of infecting microbes or on different systems of animals including man. The bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction, either microbe are pathogenic or symbiotic. Plants natural constituents can be derived from any part of plant like bark, leaves, flowers, roots fruits, seeds etc. i.e. any part of plant may contain active components [2]. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for the synthesis of chemical substance. Thus it is important to identify these bioactive compounds in plants, isolate, purify and characterize active ingredients in crude extracts by various analytical methods. The extraction and purification of secondary metabolites is difficult as they are synthesized in specialized cell types and at distinct developmental stages.

organic substances accumulated by plants, their chemical structure, biosynthesis, metabolism, natural distribution and biological function. These methods should help in separation, purification and identification of different constituents present in plants. In medicinal plant analysis, extraction of components from the source is the first step. Extraction is the separation of medicinally active compound from the other inactive component of plant tissue using selective solvents through standard procedures. During extraction solvents diffuse into solid plant material and solubilize compounds with similar polarity [3].

Rumex vesicarius Linn. is a branched succulent herb which belongs to the family polygonaceae and is distributed in India. It is one of green vegetable, medicinally valuable plant and is commonly called as "Bladder dock". The Whole plant is medicinally important and cures several diseases *Rumex vesicarius* Linn. traditionally used as aperient, diuretic and cooling agent. The plant juice is useful in curing stomach heat, toothache, checks nausea and promotes appetite. Fruits are aperients and diuretic, eaten fresh against Jaundice, hepatic conditions, constipation and indigestion, roasted seeds are prescribed in dysentery [4]. Hence, an effort has been made to standardize extraction method for *Rumex vesicarius* L.

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Materials and Methods

Plant material collection and authentication

The plant *Rumex vesicarius* Linn. was collected in the month of July- August of every season from village kusnoor in Gulbarga district Karnataka. The specimen plant material

The herbal medicine is a complicated system of mixture of components. Several methods are needed to understand the

of species has been identified with the help of flora of the presidency of Madras [5], the flora of Karnataka [6] and the flora of Gulbarga District [7]. A voucher specimen was submitted in the herbarium at Department of Botany Gulbarga University Gulbarga for authentication with voucher number HGUG- 5012.

Drying and Pulverization

The whole plant of *Rumex vesicarius* Linn., were washed thoroughly and subjected to shade dried. The shade dried plant is pulverized and stored separately in an air tight container for future use.

Extraction of powdered plant material

The *Rumex vesicarius* L. Powder was subjected to extraction by two methods

1. Hot Soxhlet extraction method
2. Cold Maceration (at room temperature) method.

Hot Soxhlet Extraction Method

In this method, the whole or coarsely powdered plant material of *Rumex vesicarius* L is successively extracted by solvent like petroleum ether, chloroform, methanol and water in increasing polarity order for different period of time (6h, 8h, 10h, 12h). The powder is placed "thimble" in chamber of the Soxhlet apparatus. The extracting solvent in flasks is heated, and its vapors condense in condenser. The condensed extractant drips into the thimble containing the powder, and extracts it by contact. When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber siphon drop into flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The extract thus obtained were filtered and concentrated to dryness, weighed and stored for further use [8]. The yield of the extract is calculated by using the following formula

$$\text{Yield (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Cold Maceration Extraction Method

In this process, the whole or coarsely powdered plant material of *Rumex vesicarius* L. is successively extracted by placing the powder in a stoppered container with the solvent (petroleum ether, chloroform, methanol, water) and allowed to stand at room temperature for a different period of time (6h, 12h, 24h, 48h) with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing. The solvents like petroleum ether, chloroform, methanol and water were used in the ratio of 1:6 according to the increasing polarity. All the extracts

were evaporated to dryness, weighed and stored for future use [8]. The yield of the extract is calculated by using the following formula

$$\text{Yield (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Statistical Analysis

Statistical analysis was performed using Graph pad (version 6.04). The data were expressed as means \pm SD.

Results and Discussion

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans [8]. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [9]

Successful extraction begins with careful selection and preparation of plant samples, and thorough review of the appropriate literature for indications of which protocols are suitable for a particular class of compounds or plant species. During the extraction of plant material, it is important to minimize interference from compounds that may co extract with the target compounds, and to avoid contamination of the extract, as well as to prevent decomposition of important metabolites or artifact formation as a result of extraction conditions or solvent impurities [9]. In the present study, standardization of extraction process for *Rumex vesicarius* L. by two conventional extraction techniques is demonstrated.

Hot soxhlet extraction

The percentage of extracted compounds from *Rumex vesicarius* L. using petroleum ether, chloroform, methanol and water were compared for 6, 8, 10 and 12 h of period in order to optimize the extraction condition (Table. 1).

Table. 1 Percentage of extract yield produced by Soxhlet Extraction technique.

Solvent	Petroleum ether	Chloroform	Methanol	Water
Time (h)				
6	1.92±0.20	1.4±1.62	7.9±0.53	26.0±2.24
8	2.21±0.30	1.60±2.70	8.7±1.28	25.2±3.21
10	2.35±0.71	1.31±1.15	10.1±2.50	22.6±1.19
12	2.01±0.50	1.20±0.64	11.9±1.26	22.5±2.68

Values are the mean ±SD of 3 values

The non polar solvent petroleum ether extracted the highest yield (2.35%) at 10h. The chloroform yield was found to be highest (1.60%) at 8 h. Methanol extract was found to be highest (11.9%) at 12h. For water which is the most polar solvent the highest yield (26.0%) is obtained at 6 h. The highly polar solvents (e.g. water) and non polar (e.g. petroleum ether) are not appropriate for extracting high polar content. Moreover, the use of water as the only solvent yields to an extract with a high content of impurities along with polar compounds which could interfere in the identification and quantification.

Chloroform could not be a suitable solvent in extracting polar compounds like phenols due to its nonpolar entity, thus it is understood that the methanol extracts contain higher polar compounds than the water. Many studies have confirmed that the other plant species of polar solvents produce a higher yield of phenolic concentration compared with the non-polar ones [10].

The results recorded in Table 1. also suggest that there is a certain correlation between time and yield extraction. In case of methanol, as time increases (from 6 h till 12 h) the extraction product increases, but with petroleum ether it increases from 6 h till 10 h then it remains constant. For chloroform it increases from 6 h till 8 h and the decreases with increase in time. With respect for the extraction with water, as time increases, extraction product decreases from 6 h till 12 h.

Thus we can conclude that the optimal extraction time depended on solvent type. This observation was well explained by Fick's second law of diffusion, the final equilibrium will be achieved between the solute concentrations in the plant matrix and in the bulk solution (solvent) after a certain time meaning that an excessive extraction time is not useful to extract more compounds and prolonged extraction process might lead to oxidation due to light or oxygen exposure (11).

Cold maceration extraction

In cold maceration, the solvent type and the extraction time have an effect on the extraction (Table 2). The percentage of yield for petroleum ether, chloroform, methanol and water was found to be highest (0.62%, 1.5%, 2.6%, 11.01%) at 48 h. The results suggest that there is a correlation between the increasing time and percentage of yield. In case of cold maceration extraction of *Rumex vesicarius* L. there is an increase in the yield with an increase in time (6h till 24h) with different solvents (petroleum ether, chloroform, methanol and water). These results were explained and supported by Fick's second law of diffusion [11].

Table. 2 Percentage of extract yield produced by Cold Maceration Extraction technique.

Solvent	Petroleum ether	Chloroform	Methanol	Water
Time (h)				
6	0.21±0.46	0.72±0.52	0.9±0.31	4.2±0.6
12	0.32±0.57	0.85±0.76	1.5±0.60	7.5±1.05
24	0.51±0.50	1.2±0.11	2.2±0.53	9.8±0.32
48	0.62±0.35	1.5±0.30	2.6±0.36	11.01±1.01

Values are the mean ±SD of 3 values

Thus the yield of the extract depends on the type of extraction method and the solvent system selected. The selection of solvent plays an important role and is taken into consideration which depends on the type of component to be extracted. The specific solvent will extracts the specific phytochemical compound. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents which are soluble in a particular solvent. [12]

And also the comparison of the two extraction methods indicated that the yield of extract by cold maceration extraction is less compared to that of the hot extraction soxhlet. Non standardized procedures of extraction may lead to the degradation of phytochemicals present in the plants and may lead to the variations thus leading to the lack of reproducibility [9].

The possible reason may be, in case of hot soxhlet extraction the most volatile parts of the plant may be damaged or lost with exposure to heat . And also successful prediction of botanical compounds from plant material is largely dependent on the type of the solvent used and the method of extraction followed [13].

Conclusion

The standardization of the extraction process is very important for the isolation of bioactive compound. In the present work the percentage yield of *Rumex vesicarius* L. varies with the type of the solvent and the duration of extraction time. Furthermore the soxhlet method has the highest extraction yield when compared to the cold maceration extraction method. The result also reveals that the methanol can be used as a suitable solvent for extraction of polar compounds.

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