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Research Article

OPTIMIZATION OF EXTRACTION METHOD FOR *IPOMOEA AQUATICA* FORSSK. [INDIAN RIVER SPINACH] FROM ITS WHOLE PLANT

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ABSTRACT

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Key Words:

Extraction, Maceration, hydroalcoholic, *Ipomoea aquatica*, Convolvulaceae and Yield.

The study was targeted to optimize the terrific extraction method for straining out maximum yield of phytochemicals occurs in the preferred plant, to decide the solvents and its ratio used for opted process being "Cold maceration" as well and to weigh the percentage yield of excerpt by means of the selected solvents and its measure. The official method was found to be best for extracting thermo labile substances also. The solvents were selected depend on its utility, polarity and suitability. The proportionality of the solvents was fixed by calculating the extractive value practicing a short term technique that is "Ultra sonication" at 40.0-50.0°C for 1.0 hour. The concluded portion of menstruum was depleted with large quantity of weighed powder for 7 days softening at room temperature. The % yield of aqueous and alcoholic extracts was found to be 25.80%w/w and 7.40%w/w respectively by Ultra sonication. The quotient was fixed as 50:50 v/v of water and ethanol. Then the cold susurration was experimented out with hydroalcoholic mixture for 7 days taking 350.0g of drug and 2000.0ml of menstruum. The yield of extract was determined as 28.91%w/w. The juice prepared could be utilized for making natural formulations since it is non toxic and stable from fungal infection. The product might be applicable for detection of active secondary metabolites which are being the part of plants by both qualitatively and quantitatively fit in herbal research run with all health sciences quality control departments to meet global standards in herbal formulation development.

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INTRODUCTION

Ipomoea aquatica belonging to the family Convolvulaceae is a semi-amphibious, equatorial plant ripened as a vegetable for its tender boughs and leaves. It is beginned all over the tropical and subtropical domains of the planet, although it is not acknowledged where it was emerged. This is recognized in English as water spinach, river spinach, water morning



Figure 1 Ipomoea aquatica Forssk.

glory, water convolvulus, or by the more cryptic names; Chinese spinach, Chinese Watercress, Chinese convolvulus, swamp cabbage or *kangkong* in Southeast Asia; In South India, the leaves are finely minced and mixed with grated coconut to prepare *thoran*, a dish in Kerala. The same dish in Tamil Nadu is prepared as *thuvaiyal* or as *kootu* (R. B. H. Will *et al*, 1996; B.H. Chen, 1992)

Extraction Methods

The appropriate concentration and the functioning of the active ingredients accommodated in to the plants were brought about by extracting the constituents with the siutable solvents, choosed in line with the solubility and stability of the valuable materials. Few significant extraction procedures are percolation, digestion, infusion, decoction and maceration (Das K, 2010).

Percolation

The plant material is damped exceeding their arrangement in the percolator with a desired volume of menstruum. It is placed in a sealed decanter and leave to exist for roughly four hours. After then it ought to be efficiently placed in the percolator so as to permit the even aisle and the complete contact of fluid. The percolator needs to be filled with liquid and capped. The bottom outlet is opened until get a typical dropping. More menstruum is added to enclose and soak all the material in the percolator kept for 24 hours. The wet mass is pressured to extract the maximum residual fluid retained and augmented with sufficient solvent to get the proper fraction; it is filtered or clarified by decantation.

Digestion

It is a form of softening with slight warming during the process, provided that the temperature does not alter the active ingredients of plant material and there is greater efficiency in the use of menstruum. The mostly used temperatures are between 35° C and 40° C., although can rise to no higher than 50° C. This method is tried with the tougher plant parts or those contain poorly soluble substances.

Infusion

It is a dilute solution of freely soluble constituents of the raw drug and is advisable for the aromatic drugs, to avert volatile oils evaporation.

Decoction

It is applied for active contents that don't alter with heat. The drug is boiled with water for 15 to 60 minutes in such process. The decoctions are prepared for using in the moment and shouldn't be stored for more than 24 hours (Amita Pandey *et al*, 2014; Azwanida NN, 2015).

The other methods are Plant tissue homogenization, Serial exhaustive extraction, Soxhlet extraction, Sonication, Aqueous Alcoholic Extraction by Fermentation, Counter-current Extraction, Ultrasound Extraction (Sonication) or Ultrasound-assisted extraction (UAE) or sonication extraction, Phytonics Process, Microwave assisted extraction (MAE), Accelerated solvent extraction (ASE), Supercritical fluid extraction (SFE) and Steam Distillation.

Maceration

The word 'maceration' means 'softening' and employed in the preparation of tinctures, extracts and concentrated infusions. This is a smooth method of crude drug extraction and is official in Indian Pharmacopoeia.

In this process, the material to be extracted is placed in a closed vessel and suitable menstruum is added and left for 7 days with occasional shaking. The liquid is then strained off and the solid residue (Marc) is pressed to remove the solution as much as possible. The liquids are mixed and cleared up by filtration.

Types of maceration

Modified maceration

This quick operation is necessarily used for extracting unorganized drugs. Ex: gums, resins etc. Here, the soluble constituents are directly exposed to men strum due to lack of cellular structure.

Multiple maceration

This is applied to achieve maximum extraction by practicing portions of total volume of menstruum for successive maceration. The drug: menstrum ratio is low. The volume of men strum for each maceration can be estimated as follows, for double maceration, Volume of menstruum (total volume – volume retained drug)/2 + for 1st maceration = volume retained by drug. For triple maceration, Volume of menstruum for 1st maceration = (total volume - volume retained by drug)/3 + volume retained by drug (S.S. Khadabadi *et al*, 2013; Vibha Porwal *et al*, 2012).

Vacuum extraction

This process employs a specially designed maceration vessel with arrangement for connecting it to vacuum line. This increases the permeability of the cell walls considerably and facilitates extraction in a much shorter time (Stephen Olaribigbe Majekodunmi, 2015).

Uses

The application of this process for an important medicinal plant *Ipomoea aquatica* helps in revealing its phytochemicals which are formed in the primary and secondary metabolism of the herb. The metabolic products are extracted into suitable solvents in what those are soluble. Mostly therapeutically active secondary metabolites of the plant will come into polar solvents like alcohol, water, etc. Thereby extraction is found as the most substantial basic approach to recognize the phytochemical potential of a natural source (Veena Sharma *et al*, 2014; C. K. Kokate *et al*, 2015; Khandelwal K. R., 2002).

Literatures

Phytochemical Studies on the Flowers of *Ipomoea Aquatica* was determined (K.Parimala *et al*, 2013). Antibacterial activity of Dibutyl Phthalate: A secondary metabolite isolated from *Ipomoea carnea* stem was studied (Elija Khatiwora *et al*, 2012). Pharmacognostical Investigations on *Ipomoea Aquatica* Forsk was reported (Mital N. Manvar, 2011). In-vitro free radical scavenging activity studies of extracts and isolated compounds of *Eugenia jambolana* lam. Seeds were evaluated (Sasikala M. *et al*, 2016). Chemotaxonomical and pharmacological review on medicinal plants in temperate region was depicted (Sasikala M. *et al*, 2017)

MATERIALS AND METHODS

Materials

The targeted plant contents are given in the table 1. The instruments, chemicals/reagents and glass wares/apparatus effective for the study are delineated in the table 2, table 3 and table 04 accordingly.

Table 1	Plant	details
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S. No.	Parameters	Subject
1.	Plant Name	Water Spinach, River Spinach
2.	Botanical Name	Ipomoea aquatica Forssk.
3.	Family	Convolvulaceae
4.	Location	Parambikulam – Aliyar Riverine, Pollachi
5.	Part of the plant	Whole plant
6.	Authentication No.	BSI/SRC/5/23/2017/Tech./3269
7.	Place of Authentication	BSI, Coimbatore-641003, Tamil Nadu, India

	S. No. Name of the Instrument			Model Na	ame		
	1.	Precisio	n Balance	Wensa	Wensar		
	2.	Hot	plate	Cinter	Cintex		
	3.	Ultra S	Sonicator	Labma	n		
	4.	Electrical	Water bath	Technie	20		
Table 3 Chemicals/Reagents used							
S.No.	Name of t	he Reagent	t Comp	any	Location		
	Petroleur	n Benzine					
1.	boiling rai	nge 60.0°C-	Merck Spe	cialities	alities Mumbai –		
	80.0°C GR	(Petroleum	Private Limited		400 018		
	etl	her)					
			Jiangsu	Huaxi			
2.	Ethanol .	AR 99.9%	9% International Trade China				
	Co., Ltd.						
3.	Distille	ed water					
Table 4 Glass wares/Apparatus used							
S. No.	Io. Name of the Glassware Capacity Brand N				nd Name		
1.	Round botto	med flask	1000.0ml	R	iviera		
2.	Funn	el	Medium Size	Sh Boros	silicate Glass		
3.	Beak	er	1000.0ml	Borosil	licate Glass		
4.	Measuring	cylinder	10.0ml	R	iviera		
5.	Measuring	cylinder	50.0ml	Sh Boros	silicate Glass		
6.	China	dish	Big & Small siz	e Chines	Chinese Porcelain		
7.	Stirr	er	Small size	Sh Boros	Sh Borosilicate Glass		
8.	Conical	flask	250.0ml	Borosil	Borosilicate Glass		

Table 2 Instruments used

Miscellaneous

Aluminium foil, Muslin cloth, Filter paper and butter paper

Methods

Plant Collection, drying and powdering

The plant was aggregated from Parambikulam-Aliyar Riverine in Pollachi, Tamil Nadu. The collected portions of the plant were washed with distilled water three times and allowed to dry under shade keeping over the news paper. Then the half dried parts were cut into small pieces using stainless steel knife and put up beneath shadow only for drying completely. It took 22 days for complete drying. The dried material was pulverized into coarse powder by means of manual blender. The powdered plant material was stored in air tight containers at 4.0° C for further use. The weight of powder obtained was 450.0g.

Selection of solvents for extraction

The solvents were chosen based on polarity and nature of the active phyto constituents. Among them, water has greater polarity in which more polar compounds dissolve. But merely aqueous extract leads fungal growth. Hence, to overcome this disadvantage, the choice of next better solvent for extraction proceeded. Many literatures state that huge number of effective components will come into the solvent alcohol. When comparing with methanol, ethanol is non toxic. Moreover, it acts as a preservative in the extract also and the same was opted as another solvent for maceration.

Determination of Extractive value

Before starting maceration process, extractive values with the preferred menstruum were estimated to set the ratio in view of ensuring better extraction. 5.0g of drug powder was weighed and was taken in a 250.0ml conical flask. Petroleum ether was added to remove the fatty matters associated with the powder. The solvent retained was evaporated at room temperature after

rinsing for few minutes. Then the dried defatted powder was immersed in 100.0ml of distilled water. The same procedure was done for the second solvent ethanol. The two solutions were closed with aluminium foil and kept in an ultra sonicator between 40.0-50.0°C for 1.0 hour. Afterwards, the culls were concentrated at 40.0°C. The concentrated excerpts were transferred into respective accurately previously weighed porcelain dishes and evaporated at 40.0°C. It took two days for thorough evaporation. The weight of dried extracts was noticed. The colour of the aqueous and alcoholic of those was brown and green subsequently.

Calculation

Aqueous extract

Weight of empty petridish (W_1) Weight of petridish + extract $(W_2$ Weight of extract alone $(W_2 - W_1)$ Percentage yield of extract	: 59.30g): 60.59g): 1.29g Weight of extract obtained Weight of powder taken : $\frac{1.29}{5.0} \times 100$: 25.80%w/w		
Alcoholic extract			
Weight of empty petridish (W_1)	: 59.55g		
Weight of petridish + extract (W ₂): 59.92g		
Weight of extract alone $(W_2 - W_1)$): 0.37g		
Percentage yield of extract :	$: \frac{\text{Weight of extract obtained}}{\text{Weight of powder taken}} \times 100$		
	5.0		
	: /.40%W/W		

Maceration

350.0g of coarse powder of drug was balanced and was taken in a 5000.0ml round bottomed flask. Petroleum ether was drenched to abolish the fatty matters associated with the particle. The solvent possessed was evaporated at room temperature after rinsing for few minutes. Then the dried defatted powder was immersed in 2000.0ml of solvents which comprises 1000.0ml of distilled water and 1000.0ml of ethanol. The content was closed with aluminium foil and kept for cold maceration at ambient temperature with occasional shaking to bring about rapid equilibrium between intra and extra cellular fluids thereby bringing fresh menstruum to the particle surface for further extraction. The contents would take 3 days for imbibitions and another 4 days for extraction. Wherefore, totally the content was kept for 7 days of maceration. The volume of menstruum retained after extraction was 1700.0ml.

After the mentioned period, the content of extraction was constrained through a muslin cloth. The marc was separated from the menstruum. The extract was divided into two portions in two separate 1000.0ml beakers and kept at 40.0°C for concentration. After 4 days, the concentrated extract was poured into a previously weighed china dish of big size for evaporation at the same temperature. After 3 days, the completely dried extract was cooled to room temperature and weighed. The percentage yield was calculated.

Calculation

Hydroalcoholic extract

Weight of empty petridish (W_1) : 59.46g Weight of petridish + extract (W_2) : 160.66g

Weight of extract alone $(W_2 - W_1)$: 101.2g <u>Weight of extract obtained</u> × 100 Weight of powder taken $\frac{101.2}{350.0} \times 100$ 28.91%w/w.

RESULTS AND DISCUSSION

The extraction schemes are pictured in figure 02 and figure 03.

Steps Involved in the Maceration System



Figure 2 Drying and powdering of the plant



Figure 3 Extraction Scheme

The percentage yield of aqueous and ethanolic extracts was found to be 25.80%w/w and 7.40%w/w respectively. From the results of extractive values, the yield was higher in water than in alcohol. Even then the yield was immense in water excerpt, it could not be predicted that the big number of active phyto principles come into the fluid when compared to alcohol. Hence, giving equal importance to both the solvents, the proportionality was established as 50:50 v/v which represents 50 volumes of each liquids.

Table 5 Results	of Extraction	Mechanism
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S. No.	Name of the Plant	Name of the Extract	Colour of the Extract	Method of Extraction	Weight of the powder (g)	Weight of Extract (g)	% Yield of Extract (%w/w)
1.	x	Aqueous	Brown	Ultra sonication	5.0	01.29	25.80
2.	aquatica	Alcoholic	Green	Ultra sonication	5.0	0.37	7.40
3.	FOISSK.	Hydroalcoholic	Dark brown	Cold Maceration	350.0	101.2	28.91

Then the cold maceration was experimented out with hydroalcoholic menstruum for 7 days taking 350.0g of drug and 2000.0ml of menstruum. The percentage vield of extract was calculated as 28.91%w/w.

CONCLUSION

Among all the sophisticated, modernized extraction methods, the cold maceration was outstanding and the same was optimized for Ipomoea aquatica Forssk. from its whole plant by using 50% v/v ethanol in distilled water. This technique helps to extract out thermolabile polar elements along with other active fundamentals from the powdered drug which is not possible with other techniques like percolation, sonication, digestion, decoction, etc. The extract processed could be used for executing herbal drugs as it is non toxic and stable from fungal contamination. The product might be pertinent for detection of functional secondary metabolites which are present by qualitatively and quantitatively in the herbal research in all health sciences QC/QA branches to meet global standards in herbal formulation development.

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