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Phytochemical investigation and standardization of extracts of flowers of *Woodfordia fruticosa*; a preliminary study

Y. H. Syed[1]*, M. Khan[2], J. Bhuvaneshwari[3], J. A. Ansari[1]

[1]*Department of Pharmacognosy, MESCO College of Pharmacy, Hyderabad-500006 A.P. India;*
[2]*Department of Pharmacognosy, Oriental College of Pharmacy, Mumbai, Maharashtra, India;*
[3]*Department of Pharmacognosy, Al-Ameen College of Pharmacy, Bangalore, Karnataka, India.*

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Abstract

The present study deals with the preliminary physico-chemical, qualitative and quantitative phytochemical investigation of three samples of *Woodfordia fruticosa* flowers from different geographical areas. The dried flowers were extracted successively with various solvents with increasing polarity and all the extracts were subjected to phytochemical screening for the identification of phytoconstituents. The results of qualitative examination revealed the presence of carbohydrates, proteins and amino acids, glycosides, alkaloids, polyphenols, saponins, phytosterols, fixed oils, gums and mucilage, flavonoids and resins. The quantitative analyses further substantiated the findings that alkaloids, saponins and polyphenols are present in the drug. Of significance was the total polyphenol content. The results obtained can be used for the genuine identification of the plant from its adulterants and subsequent screening for potent bioactivity.

Keywords: Woodfordia fruticosa, Proximate Analysis, Phytochemical, Polyphenols.

INTRODUCTION

The guidance of ethnopharmacological knowledge in studying natural products has significantly contributed to the discovery of novel chemical structures and mechanisms of action[1].

The flowers and leaves of the plant *Woodfordia fruticosa* Kurz (Lythraceae) have been a source of great value in traditional systems of medicines. The species are long-lived shrubs, typically 2-3 m in height with a sprawling, "untidy" appearance, the result of an irregular sympodial branching pattern. The several primary stems, which arise at or near the base, produce branches of various lengths that often are long and drooping [2]. It is found in the Gangetic plains and also throughout India, ascending to 1500 m in the Himalayas and cultivated in gardens too[3].

Woodfordia fruticosa flowers are an important ingredient in Ayurvedic preparations of various "Asavas" and "Aristhas"[5]. Aristhas are believed to be general health tonics in nature, having overall health stimulating properties via ameliorating and/or delaying one or other systemic disorders. Of the 18 aristhas mentioned in the Indian Ministry of Health and Family Welfare's monograph (CCRIMH, 1978), 17 have been found to contain *Woodfordia fruticosa*. According to the Indian systems of medicine, flowers of this plant have pungent, acrid, cooling, toxic, alexiteric properties and are used as a sedative and as an anthelmintic. These flowers are also useful in fevers, thirst, blood diseases, dysentery, toothache,.leprosy, , leucorrhea, , and menorrhagia. *Charaka* and *Sushruta* used sweetened decoction of flowers for fever, haemothermia, persistent dysentery; included *Dhaataki* in conception-promoting group of herbs. Powder of *Dhaataki* flowers, mixed with honey, was prescribed for leucorrhoea [6]. Dried flowers are powdered and dusted over ulcers and wounds to eliminate discharge and promote granulation. The dried flowers are an astringent tonic in disorders of the haemorrhoids, mucous membranes and in derangements of the liver and also considered a safe stimulant in pregnancy[7].

According to *Yogaratnakara*, one of the most renowned treatises on Indian medicine and local traditional knowledge the flowers of *Woodfordia fruticosa* have been used as a substitute for *Glycyrrhiza glabra*. *Yogaratnakara* states that "*Abhave madhuyashyastu dhatki prayogyatu",* meaning, the flowers of *Dhataki* can be used as a substitute for *Yashtimadhu* [8].

*Corresponding author *E - mail:* yousufhussain2000@yahoo.com

An Ayurvedic medicine called '*Balarishta*', containing *Woodfordia fruticosa* flowers as one of the chief constituents which belongs to the '*Asava*' and '*Aristha*' group, is indicated in burning sensation in the stomach[9].

This plant has attracted many researchers in the modern times too. Of the 87 research papers reviewed, 79 research papers describe the ethno botanical significance of the plant [10].

A series of publications have appeared on the characterization of the phytoconstituents showing potent antiulcer activity. Of particular relevance are the phenolic compounds [11-13], saponins [14,15] and alkaloids [16].

Each drug is unique in its own physical and chemical characteristics which separates it from various other closely related drugs. Standardization and validation of the physico-chemical properties of a drug is essential to maintain its integrity and purity.

In light of the above facts, we have made an attempt to screen the phytochemical aspects of *Woodfordia fruticosa* flowers and standardize the extracts with reference to the reported chemical constituents.

MATERIALS AND METHODS

Plant Material

The three drug samples of the dried flowers of *Woodfordia fruticosa* were collected from the local market in Hyderabad, Bangalore and Nahan, Himachal Pradesh respectively.

The drugs were authenticated by qualified plant taxonomists at the Plant Anatomy Research Centre, Chennai, Tamil Nadu Voucher no. PARC/2012/1433. The reference samples were deposited at Raw Drug Collection center of MESCO College of Pharmacy, Mustaidpura, Hyderabad

Proximate analysis

The determination of Physicochemical parameters like moisture content, total ash value, acid insoluble ash value, alcohol soluble extractive values and water soluble extractive values was done

Determination of moisture content[17]

Principle

The purchase of crude drugs containing excess water not only becomes uneconomical but also in combination with a suitable temperature, this moisture will lead to the activation of enzymes followed by the proliferation of microorganisms. The moisture balance works on the principle of the loss in weight of the sample being tested, principally due to the water content and sometimes due to small amounts of other volatile materials contributing to the weight loss. The moisture balance is suitable when large numbers of samples are handled as it combines both the drying process and weight recording [18].

Procedure

Moisture content was determined using Infrared moisture balance Model-M-3A Deluxe Voltag-230VAC (Advance Research Instrument Co).

Five grams of the sample was uniformly distributed on the sample pan with the help of a spatula and the temperature was set at 105^0C the reading in percentage was directly read from the scale. Respective moisture content (%) for all the samples were calculated and results are shown in Table 2.

Determination of ash values: Total ash value[19]

Principle

The method for determination of total ash is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological ash", which is residue of extraneous matter (e.g. sand and soil) adhering to the plant surface. When vegetable drugs are incinerated, they leave an inorganic ash in some plants called the total ash. This is of importance and indicates the extent of care taken in the preparation of the drug. Carbon must be removed at a low temperature (450 ºC) as possible because alkali chlorides, which may be volatile at high temperatures, would otherwise be lost. The total ash usually contains carbonates, phosphates, silicates and silica.

Procedure

Four grams of the ground air-dried sample was weighed into previously ignited, dried and tarred silica crucible. The material was spread evenly as a thin layer. Kept on a gas burner under a low flame and ignited slowly to obtain a carbonized residue. It was then placed in the muffle furnace and the temperature of the muffle was adjusted to 450-500 ^{0}C and heated for 3 hours, cooled in

a desiccator and weighed. The ash value was calculated and expressed as % Ash and is recorded in Table 2.

Table-2 Determination of Proximate Analysis of *Woodfordia fruticosa*

Physico chemical Parameters	***1. W. fruticosa***	***2. W. fruticosa***	***3. W. fruticosa***	**Limits[4]**
Moisture content	8.0%	7.2%	7.2%	Not more than 8%
Total Ash	5.12%	5.58%	6.33%	Not more than 10%
Acid insoluble ash	0.56%	0.71%	0.85%	Not more than 1%
Alcohol soluble extractive	23.00%	20.41%	19%	Not less than 7%
Water soluble extractive	38.49%	36.14%	32.36%	Not less than 28%

1. *W. fruticosa* sample – Local market in Hyderabad, Andhra Pradesh
2. *W. fruticosa* sample - Local market in Bangalore, Karnataka
3. *W. fruticosa* sample – Local market in Nahaan, Himachal Pradesh

Acid insoluble ash[19]

Principle

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Total ash treated with dilute hydrochloric acid reacts with minerals to form soluble salts and the residue which consists mainly of silica is the acid insoluble ash.

Procedure

To the Silica crucible containing the total ash obtained 25 ml of hydrochloric acid (~70g/l) TS was added, covered with a watch glass and boiled gently for 5 minutes on a hot plate. The watch glass was rinsed with 5 ml of hot water and these washings added to the crucible and filtered. The insoluble matter was collected on an ashless filter paper by filtration. The filter paper was rinsed repeatedly with hot water until the filtrate was neutral /free from acid. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight in the muffle furnace at 450-500 ºC. The silica crucible was removed from the muffle furnace and allowed to cool in a dessicator for 30 minutes, and then weighed without delay. The content of acid insoluble ash was calculated and results are shown in Table-2.

Determination of Extractive Values[20]

Principle

The determination of water and alcohol soluble extractive value is used as a means of evaluating the quality and purity of drugs whose constituents cannot be readily estimated by other means. This solvent extraction of the drug can be done by a discontinuous method i.e., maceration with cold sol90vent or by a continuous extraction method such as Soxhlet extraction. These methods are based upon the principle of solid-liquid extraction. In the solid-liquid extraction, the solvent initially diffuses into plant cells and the metabolites are then dissolved in the solvent and transferred out of the cells [1].

Procedure

Alcohol soluble and Water soluble extractive value.

The air dried drug weighing 5 g, coarsely powdered, was macerated with 100 ml of 90 % ethanol or water in a glass stoppered flask for 24 hours; the contents were shaken frequently during the first 6 hours and allowed to stand for 18 hours. There after it was filtered rapidly, taking precautions against loss of ethanol. 25 ml of the filtrate was evaporated to dryness on a water bath in a

tarred flat bottomed petri plate. 2ml of alcohol was added to dry residue and dried again on water bath, dried at 105 ^{0}C for 1 hour in a hot air oven, and weighed. The percentage of ethanol soluble and water soluble extractive was calculated with reference to the air-dried drug and results are shown in Table 2.

Calculation: -The Percentage of Alcohol soluble extractive value = B - A X 4 X 100 / W, where, A=Empty weight of the dish (g), B=Weight of dish + residue (g), W=Weight

Preparation of the *W. fruticosa* extracts

The powdered drug (100 gm.) was extracted successively using a Soxhlet extractor with 200ml each of n-hexane, benzene, chloroform, ethyl acetate, acetone, ethanol and water. Extracts were filtered, concentrated and after complete solvent evaporation, each of these solvent extract was weighed and preserved at 5°C in an airtight bottle until further use (Table-3). These extracts were subjected to phytochemical screening.[21]

Table-3 Percentage yield of the extracts obtained by successive solvent extraction

Sr. no.	Extract	Weight	%w/w
1	n-hexane	304 mg	0.40
2	benzene	520 mg	0.70
3	chloroform	370 mg	0.50
4	ethyl acetate	624 mg	0.83
5	acetone	10.17 gm	13.56
6	ethanol	9.92 gm	13.22
7	aqueous	13.37 gm	17.82

Phytochemical screening[21]

The n-hexane, benzene, chloroform, ethyl acetate, acetone, ethanol and water extracts were subjected to preliminary phytochemical analysis for the identification of carbohydrates, proteins and amino acids, glycosides, alkaloids, polyphenols, saponins, phytosterols, fixed oils, gums and mucilage, flavonoids and resins (Table 4).

Quantitation

To further substantiate the findings of the preliminary identification tests, quantification of important phytoconstituents was carried out. The drug was subjected to analysis for total alkaloids[22], determination for total saponins[23] and total polyphenols[25].

Table 4. Phytochemical composition of the extracts of *Woodfordia fruticosa flowers*

Chemical Constituent / Solvent	n-hexane	Benzene	$CHCl_3$	Ethyl acetate	Acetone	Ethanol	Water
Carbohydrates	-	-	+	+	+	+	+
Proteins and amino acids	-	-	-	-	-	+	+
Gums and mucilages	-	-	-	-	-	+	+
Saponins	-	-	-	-	-	-	+
Alkaloids	-	-	+	-	-	+	+
Glycosides	-	-	-	-	+	+	+
Polyphenols	-	-	-	-	+	+	+
Fixed oils and Fats	-	-	+	-	+	+	+
Flavonoids	-	-	+	-	+	+	+
Phytosterol	+	-	-	-	+	+	-
Resins	-	-	-	-	-	-	-

Determination of total alkaloidal content[22]

Principle

Alkaloids are weak bases. Alkaloids in the plant material are extracted based on their solubility. Alkaloidal bases are soluble in non-polar solvents like chloroform and ether while their salts are soluble in water. The total alkaloidal content was determined by the procedure described in USP.

Procedure

Coarse powdered drug (10 g) was moistened with 5 % ammonia, and mixed by means of a stirring rod, and allowed to stand for 5 minutes. Then the solvent, a mixture chloroform and ethanol (30:70) was added and the drug was allowed to macerate for 6 to 12 hours. The drug was subjected to reflux with sufficient quantity of solvent and the drug was extracted until extraction was complete. The filtrate was concentrated and again extracted with 2 % sulphuric acid twice. The combined aqueous extract was further extracted with chloroform using a separating funnel. The acid solution was rendered alkaline with 10 % ammonia. The alkaline solution was extracted with chloroform (2-3 times). The chloroform layer was evaporated and dried at 105 ^{0}C to constant weight. The trace of chloroform solvent was removed by the addition of few ml of neutralised alcohol, followed by evaporation at low temperature.

Determination of total Saponins[23]

Principle

Saponins are characterized by their haemolytic activity and foamimg properties. The presence of both polar (sugar) and nonpolar (steroid or triterpene) groups provides saponins with strong surface-active properties [24].

Procedure

For the determination of total saponins 5 g of plant material was accurately weighed and transferred to a round bottom flask. It was refluxed with 50 ml of petroleum ether (40-60° or 60-80°) for 1 hour on a water bath to remove fatty matter. It was filtered and the dried marc was further extracted with successive quantities of 50ml each of methanol until the solution became colourless (complete extraction). The combined methanol extracts were concentrated to 10ml volume by direct evaporation over a water bath. Acetone (5-100ml) was then added slowly until complete precipitation of saponins occurred. It was filtered through a previously weighed Whatman filter paper No.1. After air drying for some time, the residue was dried in a hot air oven at 80-90 °C until a constant weight had been obtained.

Calculation: % of saponins = B - C / A x 100, where, A=Sample weight in g, B=Weight of tarred dish + saponins, C=Weight of tarred dish.

Determination of total polyphenol content[25]

Principle

In the presence of sodium carbonate phenolic compounds react with the Folin-Denis reagent and forms blue colour which is measured at 700 nm. The total Polyphenol content was determined by the procedure described by Schanderi et al, 1970.

Procedure

The powdered material (0.5 g) was weighed and was transferred to round bottom flask. 75 ml of water was added to it. The solution was boiled gently for 30 minutes. The solution was centrifuged at 200 rpm for 20 min and supernatant was collected in 100 ml volumetric flask and volume was made up to 100 ml with distilled water. 1 ml of sample extract was transferred to 100 ml volumetric flask containing 75 ml of distilled water. 5 ml of Folin-Denis and 10 ml of sodium carbonate solution was added, volume was made up to 100 ml with distilled water. The solution was shaken well and after 30 min the absorbance was measured at 700 nm. The blank was prepared without sample. The standard graph was prepared using 10-100 µg tannic acid. Total Polyphenol content of the sample was calculated as tannic acid equivalents from the standard graph and expressed as %. The polyphenol content of the drug is shown in Table 5.

Table 5. Quantification of selected phytoconstituents

Contents	**Extract of *W. fruticosa* flowers**
Total alkaloid (% w/w)	0.19
Total Saponins (% w/w)	4.99
Total Polyphenol content (mg TAE/g)	180

RESULTS AND DISCUSSION

The morphology of the flowers was in accordance with the reported literature[3],[4]. The morphological identification of drugs was done and the results are shown in Table 1. The photographs of *Woodfordia fruticosa* are shown in Figure 1.

Table 1. Data showing morphological features of *Woodfordia fruticosa* flowers.

Characters	***Woodfordia fruticosa flowers***
Colour	Reddish brown, Deep orange-red
Odour	Characteristic
Taste	Pungent
Size	1.2 cm long, occurs as single or in bunches of 2-15
Shape	Slightly curved and striate, ending in a 6 – toothed oblique mouth

Figure-1 Flowers of *Woodfordia fruticosa*

Proximate analysis

The Moisture content, total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive values for *W. fruticosa* were determined (Table 2). All the parameters were found to be within the prescribed limits[3],[4].

Extracts

In our study the yields (% w/w of the powdered drug) of seven extracts were found to be 304 mg (0.40% w/w), 520 mg (0.70% w/w), 370 mg (0.50% w/w), 624 mg (0.83% w/w), 10.17 gm (13.56% w/w), 9.92 gm (13.22% w/w) and 13.37 gm (17.82% w/w) for n-hexane, benzene, chloroform, ethyl acetate, acetone, ethanol and aqueous extracts respectively (Table 3). The maximum yield was observed for the aqueous extract.

Phytochemical screening

All the seven extracts were screened for phytoconstituents as shown in Table 3. It was observed that *carbohydrates* were found to be present in all the extracts except n-hexane and benzene extracts. *Proteins and amino acids and gums and mucilages* were found to be present only in the alcoholic and aqueous extracts. The chloroform, acetone, ethanol and water extracts showed the presence of *fixed oils and fats* and *flavonoids*. The tests for a*lkaloids* showed positive reaction in chloroform, acetone, ethanol and water extracts. It was notable to observe the presence of *glycosides* and *polyphenols* in acetone, ethanol and water extracts. *Phytosterols* were identified in the n-hexane, acetone and alcoholic extracts. *Resins* were found to be absent in all the extracts.

Quantification

The quantitative analyses supported our findings that alkaloids are present in the drug, but in a lesser proportion when compared to saponins and polyphenols. The total alkaloid content was calculated to be 0.19% w/w in the chloroform extract. The total saponins in the methanolic extract were found to be considerably more than the alkaloidal content i.e., 4.99% w/w. Of significance was the total polyphenol content of 180 mg TAE/g (Table-5). However, further study is necessary to isolate and quantify active constituents present in the flowers of *W. fruticosa* by sophisticated techniques.

CONCLUSIONS

This preliminary approach for the phytochemical screening and standardization of extracts of flowers of *Woodfordia fruticosa* reveals an interesting insight into the importance of such studies. With support from traditional knowledge and subsequent scientific documentation, this plant appears to be a promising drug for new drug discoveries. Nevertheless, there is more room for study of this plant in isolating, characterizing and quantifying the active constituents present in the flowers of *Woodfordia fruticosa* by sophisticated techniques.

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