ORIGINAL ARTICLE

Preliminary phytochemical and physicochemical investigation of *Woodfordia fruticosa* (linn) kurz root

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Abstract

Introduction: The objective is to investigate the preliminary phytochemical and physicochemical properties of *Woodfordia fruticosa* Kurz roots. *W. fruticosa*, commonly called as Dhavi, is a large beautiful tree, about 10 ft long, belongs to the family Lythraceae. These studies were performed because, before any experimental and clinical trial, phytoconstituents present in the plant should be known. **Material and Methods:** The root of *W. Fruticosa* contains protein, fixed oil, glycoside, carbohydrate, terpenoid, steroid, tannin, and saponin. The physiochemical analysis of *W. fruticosa*root was also performed which includes ash value, loss on drying and extractive value determination. **Result and Discussion:** Total ash value, acid-insoluble ash, and water-soluble ash value were 6.5 % w/w, 2 % w/w, and 0.5 % w/w. Loss on drying was 4.4 % w/w, and extractive value was 3.4 % w/v in water and 6.4 % w/v in ethanol. **Conclusion:** This study revealed that these parameters will be useful in the identification and quality control of the genuine plant material or crude drug.

Key words: Glycosides, Physicochemical, Phytochemical, *Woodfordia fruticosa*

INTRODUCTION

Many researchers and scientist are on the verge of discovering of natural products exploiting natural products are the most consistently successful source of drug leads. More than half of drug molecules are natural derived compounds. It provides more structural and therapeutically diversity than synthetic products. There are so many natural products which have to be discovered, but the main challenge is to right access on this chemical diversity which is useful in severe ailments such as cancer and diabetes heart diseases. For the treatment of various types of disease, medicinal plants have been used from the primitive times. Plants contain different types of an active constituent that can be used for the therapeutic purpose or as precursors for pharmaceutical synthesis. During recent times, herbal therapies have become more popular due to low cost more effectiveness, easy availability, and fewer side effects.[1] As a result, global market demand for herbs and their products has increased tremendously in recent years. Therefore, quality control of herbal products for the purpose of efficacy and safety is essential.[2] Authentication and development of standardization parameter are essential for any crude drug and their formulation. The World Health Organization (WHO) has prescribed a number of standardization parameter for the quality control of medicinal plant materials. Quality control is determined on the basis of identity, purity, content, chemical, physical, and/or biological properties, as well as by manufacturing process.[3] Thus, in this research work attempt has been made for standardization of *Woodfordia fruticosa* root by investigating its physicochemical and qualitative phytochemical properties.

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Figure 1: Woodfordia fruticosa

Figure 2: Woodfordia fruticosa root

W. fruticosa [Figures 1 and 2] commonly called as Dhavi, Dhavari, Dhatki, etc. It is a plant with medicinal properties and belongs to the family Lythraceae. The brown bark has fibers peeling off from the hairy stem. The stalkless, ovate, and lanceolate leaves are placed opposite to each other and sometimes in whorls of three arising at the nodes. It mainly grows in the hill region of India and also grows in the middle of India. The small, red, bell-shaped flowers arise from along the stems. The seeds are brown in color and flowers are brilliant red in color. Flowers of this plant are the most effective fermentation agents in Ayurvedic medicines.[4,5]

The flowers of *W. fruticosa* contain phenolic compounds, tannin, especially hydrolyzable tannins and flavonoids. The other phenolic constituents present in flower are steroids hecogenin and meso-inositol. Leaves of this plant contain triterpenoids lupeol which is triterpenoid in nature. Other constituents present in leaves are betulin, betulinic acid, oleanolic acid, and ursolic acid. Gallic acid is found to present in leaves and stems, ellagic acid in leaves and flowers and the new constituent norbergenin in stems, etc.[4] The dried flowers of this plant are reported to be used for the treatment of hemorrhoids, dysentery, diarrhea, liver diseases, piles, mucous membranes related disorder, leukorrhoea, menorrhagia, ulcers, wounds, burning sensations, skin diseases, fever, herpes, etc.[5,6]

According to the literature survey, it was found that there is a lack of research on *W. fruticosa (Linn.) Kurz* root. Hence, the present study attempted to investigate preliminary phytochemical and physicochemical properties *W. fruticosa (Linn.) Kurz* root.

Plant Material

Selection of Plant

Woodfordia fruticosa was used traditionally in the rural areas from the long back for the different purposes like that prevention of uterine secretion after child birth. And there is lack of literature research on the root of Woodfordia fruticosa. Also the Literature survey revealed that the phytochemical and physicochemical investigation of root of W. fruticosa has yet not been studied. On the basis of literature survey this plant selected to investigate its preliminary phytochemical and physicochemical properties.[7,8]

MATERIALS AND METHODS

Collection and Identification

The roots of *W. fruticosa* were collected from Barra village of district Janjgir-Champa (C.G.) in the month of November 2017 and the voucher sample of the plant authenticated by CSIR-NISCAIR, New Delhi, with voucher sample number NISCAIR/RHMD/Consult/2018/3207-08.

Washing, Drying, and Grinding

The root of *W. fruticosa* was collected and washed thoroughly with water to remove dust and sand and then dried under shade. The dried roots were ground into a coarse powder with the help of simple mechanical grinder and sieved with 10/44 mesh to get a coarse powder.

Preparation of Plant Extract

Successive soxhlet extraction

About 25 g of the air-dried powdered root was successively extracted with the petroleum ether, benzene, chloroform, acetone, ethanol, and water in a Soxhlet extractor. Each time before extracting with the next solvent, the powdered root has been dried at below 50°C. The different extracts obtained were concentrated by distilling off the solvent and then evaporated to dryness on the water bath. The extracts obtained with each solvent were weighed, and percentage was calculated with reference to the weight of the air-dried root. The color and consistency of the extracts were noted.[5] The nature

and percentage yield of various extracts of *W. fruticosa* by successive solvent extraction is shown in Table 1.

Direct Extraction Method

For direct extraction method, 25 g of powdered root sample is extracted using Soxhlet extraction using ethanol as a solvent. The extract obtained was evaporated to remove excess solvent and dried on desiccators after drying measure extractive percentage yield and for further analysis.

Phytochemical analysis of *W. fruticosa* root extract

The various extracts which are obtained by successive solvent extraction of *W. fruticosa* roots such as petroleum ether extract, benzene extract, chloroform extract, acetone

Table 1: Nature and percentage yield of various extracts of *Woodfordia fruticosa* root

Extract	Colour	Consistency	Percentage yield % w/w
Successive solvent extract			
Petroleum ether (60–80°)	Black	Sticky	0.8
Benzene	Dark brown red	Nonsticky	0.68
Chloroform	Dark Brown	Sticky	0.95
Acetone	Reddish Brown	Crystalline	3.66
Ethanol (95%)	Reddish Brown	Crystalline	6.61
Water	Reddish	Crystalline	1.62
Direct ethanolic extract			
Ethanol	Reddish Brown	Nonsticky	11.6

Table 2: Qualitative chemical examination of various extracts

Test/Reagent used	Successive solvent extracts						Direct extraction
	Petroleum ether extract	Benzene extract	Chloroform extract	Acetone extract	Ethanol extract	Water extract	Ethanol extract
Alkaloids							
Dragendorff's	-	-	-	-	-	-	-
Mayer's reagents	-	-	-	-	-	-	-
Amino acid and Proteins							
Millon's reagent	-	-	-	-	-	+	-
Carbohydrates							
Molisch's test	-	-	-	-	-	+	-
Fixed oil and fats							
Saponification test	+	+	-	-	-	-	-
Flavonoids							
Shinoda test	-	-	-	-	-	-	-
Glycosides							
Borntrager's test	-	-	-	-	+	+	+
Legal's test	-	-	-	-	+	+	+
Gum and Mucilage							
Molisch's test	-	-	-	-	-	+	-
Tannins (Phenolics)							
Gelatin test	-	-	-	+	+	+	+
Steroids and triterpenoids							
Salkowski test	-	-	-	+	-	-	-
Saponins							
Foam test	-	-	-	-	+	-	+

+ indicates positive reaction, - indicates negative reaction

extract, ethanol, and aqueous extract were then subjected to qualitative tests for the identification of different types of phytoconstituent such as alkaloids, amino acids flavonoids, fixed oils, steroids, terpenoids, carbohydrates, gums and mucilage, tannins, polyphenols, and glycosides.[9] Presence and absence of different phytoconstituents are shown in Table 2.

Detection of Alkaloid

A small portion of each extracts obtained after successive solvent extraction was subjected to few drops of dilute hydrochloric acid and filtered. Then, the extract was filtered and tested for alkaloid carefully with various alkaloidal reagents.

Detection of Proteins

Millon's test: 2 ml of Millon's reagent was added to the test solution of different successive extract; white precipitate indicated the presence of amino acids.

Detection of Carbohydrates

Molisch's test: Alcoholic α-naphthol was added to each successive extracts, and few drops of concentrated sulfuric acid added through sides of the test tube. Purple to violet color ring appeared at the junction.

Detection of Fat and Fixed Oils

Saponification test:Various extracts were treated with a small amount of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein separately and heated on a water bath for 1-2 h. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Detection of for Steroids and Terpenoids

Liebermann–Burchard test: To each successive extracts add 10 ml of methanol and heated for about 30 min. The mixture was filtered while hot, and a further 5 ml of the solvent was added, heated and soluble materials were rinsed away. The solvent was evaporated off by rotary evaporator and triturate with ether in a test tube, and a few drops of Liebermann–Burchard reagent were added carefully. Blue-green ring between layers indicated the presence of steroids while pink, purple ring indicated the presence of terpenes.

Detection of Flavonoids

Shinoda test: In the extracts, few magnesium turnings and concentrated hydrochloric acid were added drop-wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

Detection of Glycosides

Borntrager's test: To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to its pink color indicated the presence of glycosides.

Legal's test: About 50 mg of the different extracts were dissolved in pyridine; sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Presence of glycoside was indicated by pink color.

Detection of Gum and Mucilage

Molisch's test: Few drops of alcoholic α-naphthol solution were added to the extract, shaken the mixture and 1 ml of concentrated sulfuric acid was added from the sides of the test tube well. A violet ring appears at the junction which indicates the presence of carbohydrates.

Detection of Tannins (Phenolic Compounds)

Gelatin test: Small amount of different successive extract was dissolved in 5 ml of distilled water, and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to the small amount of extract. White precipitates appear which indicates the presence of phenolic compounds.

Detection of Steroids and Triterpenoids

The extract was treated in chloroform with few drops of concentrated sulfuric acid; the mixture was shaken well and allowed to stand for some time, the red color appeared at the lower layer indicated the presence of steroids and formation of yellow color at lower layer indicated the presence of triterpenoids.

Frothing Test for Saponins

The extracts were transferred into a test tube, shaken vigorously, left to stand for 10 min and the result noted. A thick persistent froth indicated the presence of saponins.

PHYSICOCHEMICAL DETERMINATION OF ROOT

The air-dried coarsely powdered root of *W. fruticosa* (Linn.) *Kurz* was evaluated for its physicochemical parameters such as the ash values (total ash, water soluble ash, and acid insoluble ash), extractive values (water-soluble extractive value and

Table 3: Determination of Moisture contents of *Woodfordia fruticosa* root

Initial weight of Powder+China dish	Final weight of Powder+China dish	Weight difference b/w Initial and final weight	Percentage of moisture content (%)
104.83 g	104.61 g	0.22 g	4.4

alcohol-soluble extractive values), and moisture content (loss on drying) according to the WHO guidelines.[10-12]

DETERMINATION OF MOISTURE CONTENT

An empty China dish was kept in a hot air oven for 30 min and then in desiccators for 30 min to ensure the moisture is completely removed from the evaporating dish, and it becomes completely moisture free. Weighed the empty China dish and tarred on weighing balance and then 2 g of *W. fruticosa* (Linn.) *Kurz* root powder was weighed in a tarred evaporating dish. Again weighed the evaporating dish containing powder and recorded the value as the weight of the powder drug with China dish before drying. The sample was dried in an oven at 105°C in a hot air oven for 30 min. After 30 min, it was placed in desiccators for cooling and weighed. The above procedure was carried out till weight reach a constant value. Moisture content is determined by subtracting the weight of empty China dish with constant value of China dish containing crude drug obtained after the drying. The experiment was repeated 3 times, and the result was calculated as a loss of weight in percent. The result is shown in Table 3.

Determination of Alcohol-soluble Extractive Value

About 5 g of *W. fruticosa* (Linn.) Kurz root powder was taken and macerated with 100 ml of alcohol (95%) in a closed flask for 24 h. It was shaken frequently for the first 6 h and allowed to stand for 18 h. The macerate was filtered rapidly taking precautions against loss of solvent. A reflux condenser was attached to the flask and boiled gently for 1 h. The mixture was then filtered, 25 ml of the filtrate transferred to a tarred Petri dish and evaporated to dryness over a water-bath. The Petri dish was then dried in an oven at 105°C for 6 h, dried in desiccators (silica gel) for 30 min and weighed. The experiment was done in triplicate, and the content of extractable matter was then calculated in mg per gm with reference to air-dried material and expressed as a percentage. The result is shown in Table 4.

Determination of Water-soluble Extractive Value

About 5 g of the air-dried powder was taken and macerated with 100 ml of distilled water in a closed flask for 24 h. It was shaken frequently for the first 6 h and allowed to stand for the next 18 h. The macerate was filtered rapidly; 25 ml of this filtrate was evaporated to dryness in a tarred flat bottom shallow dish, and dried at 105°C for 6 h, to constant weight and weighed. The percentage of water-soluble extractive value had been calculated with reference to the air-dried powder. The result is shown in Table 4.

Table 4: Determination of Extractive Values (%) of *Woodfordia fruticosa* (Linn) Kurz

Solvent used	Quantity of dried extract obtained (g)	Extractive value in Percentage (%)
Ethanol	0.32	6.4
Water	0.17	3.4

Table 5: Ash Values (%) of the Root Powder of *Woodfordia fruticosa* root

Ash value parameter	Quantity of ash (g)	Percentage of ash (%)
Total ash	0.13	6.5
Acid-insoluble ash	0.04	2
Water-soluble ash	0.01	0.5

Ash Values Determination

The quality of a drug can be determined by the ash left after ignition. There are different methods which measure the ash: Total ash, acid-insoluble ash, and water-soluble ash.

Determination of Total Ash

About 2 g of grounded root powder was accurately weighed and taken into a previously ignited and tarred crucible. The root powder was spread in an even layer in the crucible and ignited in a muffle furnace for about 6 h by gradually increasing the heat to 500–600°C until it was white indicating that it was free from carbon. The time taken for this process is about 6 h. After complete ignition crucible was removed from the muffle furnace and cooled in desiccators and weighed. The content of total ash was calculated as mg per gm of air-dried material and expressed as a percentage. The experiment was carried out in triplicate. The average value of total ash is shown in Table 5.

Determination of Acid-insoluble Ash

About 25 ml of dilute hydrochloric acid was added to the crucible for dissolving the total ash obtained from the above procedure and covered with a watch glass. It was boiled gently

for 5 min. The watch glass was rinsed with 5 ml of hot water, and the liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the crucible, dried on a hot plate, and ignited to a constant weight. The crucible was cooled in desiccators and weighed. The content of acid-insoluble ash was calculated in mg per gm of air-dried material and expressed as a percentage. The experiment was carried out in triplicate. The content of acid-insoluble ash was calculated as a percentage of ash with reference to the air-dried root powder. The result showed in Table 5.

Determination of Water-soluble Ash

The total ash was boiled with 25 ml of water for 5 min. The insoluble matter was collected on an ashless filter paper, and then the residue was washed with hot distilled water and ignited for 15 min at a temperature not exceeding 450˚C. The total weight of insoluble matter was subtracted from the weight of the total ash. Their difference in weights represented the water-soluble ash. The percentage of water-soluble ash should be calculated with reference to the air-dried root powder. The experiment was carried in triplicate. The result showed in Table 5.

RESULTS AND DISCUSSION

Adulteration is one of the major problems due to the absence of standards of the proper identification and quality control of the drug. Skill hand and cost factors for pharmaceuticals purposes, the quality of medicine must be as high as that of other medicinal preparations. Quality refers to the intrinsic value of the drug, the number of medicinal principles or active constituents present. This study of phytochemical and physicochemical investigation of *W. fruticosa* root revealed a set of parameters which may enable those who handle this plant to maintain its quality control.

Physicochemical Investigation

The study of physicochemical parameters is useful to determine the physiological and nonphysiological adulteration. Determination of physical constant of any drug is important for the detection of adulteration and improper storage or handling. The moisture content of the drug will help to determine the possibility of microbial growth.[13] In root very less amount of moisture was present; it was only 4.4 % (w/w). The total ash value indicates the level of mineral in the drug. The insoluble ash value gives the idea about adulteration of the drug with the siliceous element.[14] The result of total ash value found in the root was 6.5% (w/w). The results acid insoluble ash value and water soluble ash value were 2% and 0.5%. Alcohol soluble and water-soluble extractive value was 6.4% (w/v) and 3.4% (w/v). The values obtained after the physical evaluation of the crude drug or powdered crude drug such as loss on drying, ash values, and extractive values serve as standard data shown in Table 1 for the identification and quality control of this plant for future study.[13,14]

Phytochemical Screening

The preliminary qualitative test is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development. The phytochemicals found in the different extracts were tannin, glycoside, phytosterol, fixed oil and fat, carbohydrate, saponin, and gums. Detail results of qualitative phytochemical screening shown in Table 2.

CONCLUSION

W. fruticosa plant was collected, and herbarium of the plant was prepared and authenticated. The collected root was dried and ground and performed the successive solvent extraction. The phytochemical analysis of different successive extract was performed. The successive solvent extract of the root of *W. fruticosa* represents that ethanol is a suitable solvent for the extraction of the majority of constituents. In the ethanol highest extract has been obtained 6.61% compared to the other solvents they are, respectively, acetone 3.66%, water 1.62%, chloroform 0.95%, petroleum ether 0.8%, and benzene 0.68%. Various physicochemical parameters were also determined such as that moisture content, extraction value (alcohol and water-soluble extractive value), and ash value (total ash value, acid-insoluble ash value, and water-soluble ash value). The different physicochemical parameters are also showing good results. These physical parameters will help to identify the authenticity of the drug. In the conclusion, it will serve as standard data for the quality control of the preparation containing this plant in future and also helpful in distinction from other allied species and adulterants. A preliminary screening test may be useful in the detection of bioactive principle having therapeutic properties. Their further studies are recommended to isolate and characterize the chemical constituents which may be responsible for the pharmacological activities of the *W. fruticosa* root. These work findings may help all researchers, pharmacological investigators and dosage form formulators and pharmaceutical scientists to develop a new way in the field of health science.

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