INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 January, 2014; received in revised form, 20 March, 2014; accepted, 26 April, 2014; published 01 July, 2014

PHYSIOCHEMICAL EVALUATION, PHYTOCHEMICAL SCREENING AND CHROMATO-GRAPHIC FINGERPRINT PROFILE OF *WOODFORDIA FRUTICOSA* (L.) KURZ EXTRACTS

Neha Grover, Rishikesh Meena* and Vidya Patni

Plant Tissue Culture and Biotechnology laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Keywords:
Woodfordia fruticosa, hemorrhoids, standardization, phytochemicals, physicochemical, tannins.

Correspondence to Author:
Dr. Rishikesh Meena
Assistant Profeesor
Department of Botany,
University of Rajasthan,
Jaipur-302055, Rajasthan, India.

E-mail : rishi_1180@yahoo.com

ABSTRACT: *Woodfordia fruticosa* (L.) Kurz. is a plant with vast medicinal properties. The plant and its parts are reported to be used for the treatment of hemorrhoids, dysentery, diarrhoea, liver diseases, piles, leucorrhoea, menorrhagia, ulcers, wounds, skin diseases, fever, headache, herpes, etc. The present work attempts to evaluate the physicochemical and preliminary phytochemical screening using various extracts of *W. fruticosa* (Family-Lythraceae). The herbal standardization was carried out on the basis of physico-chemical properties. Physicochemical parameters including ash values, extractive values and fluorescence analysis were evaluated. The qualitative phytochemical screening using various solvent extracts of plant revealed the presence of most of the biologically active phytochemicals in the methanolic extract of *Woodfordia fruticosa* leaves. TLC profiling of *in vivo* and *in vitro* plant part extracts gives an idea about the presence and confirmation of various phytochemicals. TLC screening showed the presence of ellagic acid (Rf 0.54), gallic acid (Rf 0.61), quercetin (Rf 0.8), myricetin (Rf 0.96) and β-sitosterol (Rf 0.58). Results confirmed the presence of therapeutically potent compounds in the leaf extract predominantly tannins and terpenoids.

INTRODUCTION: Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug [1]. The future development of the pharmacognostic analysis of herbal drugs is largely dependent upon reliable methodologies for correct identification, standardization and quality assurance of herbal drugs [2].

Evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(7).2772-82

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(7).2772-82

Over the years the nature and degree of evaluation of crude drugs has undergone a systematic changes. Initially, the crude drugs were identified by comparison only with the standard description available. Due to advancement in the chemical knowledge of crude drugs, at present, evaluation also includes method of estimating active constituents present in the crude drug. The detection of active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude plant extracts and is very important with regard to their potential pharmacological effects [3]. With the advent of separation techniques and instrumental analysis, it is possible to perform physical evaluation of a crude drug, which could be both of qualitative and quantitative in nature [4]. The plant may be considered a biosynthetic laboratory not only for the chemical compound such as carbohydrate,

proteins and lipids that are utilized as food by man but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc. that exerts a physiologic effect. The compounds that are responsible for therapeutic effect are usually secondary metabolite. The plant material may be subjected to preliminary phyto-chemical screening for the detection of various plant constituents [5].

***Woodfordia fruticosa* (Linn.) Kurz**. is a rare, much branched, beautiful shrub, with fluted stems and long, spreading branches, 1-3 m high. Fire flamed Bush (*Woodfordia fruticosa* (L.) Kurz.) commonly called as Dhavari, Dhatki, etc. is a plant with medicinal properties and belongs to the family Lythraceae. Flowers of this plant are the most effective fermentation agents in ayurvedic medicines [6]. It is used both internally as well as externally. The dried flowers of this plant are reported to be used for the treatment of hemorrhoids, dysentery, diarrhoea, liver diseases, piles, disorders of mucous membranes, leucorrhoea, menorrhagia, ulcers, wounds, burning sensations, skin diseases, fever, headache, herpes, etc. [7]. They are often added to the Ayurvedic Arishtas to cause alcoholic fermentation [8].

Externally, the powder of dried flowers is sprinkled on the wounds to alleviate the burning sensation, arrest bleeding and to promote healing. The juice of its fresh flowers applied on the forehead, reduces headache, especially due to pitta. To facilitate dental eruption in children, the powder of its dried flowers is massaged on the gums. The leaves of *Woodfordia fruticosa* possess antibiotic activity *in vitro* against *Micrococcus pyogenes* var. *aureus* as well as sedative properties.

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth [9]. So, the objective of the present investigation was to screen the physicochemical characteristic and also to determine the possible phytochemical components from *Woodfordia fruticosa* that would be attributed to the biological activity by phytochemical and TLC screening of various plant extracts.

MATERIALS AND METHOD:

Collection of Plant Material: The plant samples were collected from Haridwar, Rishikesh and Jhadol (Udaipur) and voucher specimens authenticated and deposited in the Rajasthan University's Herbarium (RUBL20635). They were established in the nursery of Department of Botany, University of Rajasthan and used for the study.

Preparation of plant extracts: The plant material of *Woodfordia fruticosa* was collected and washed with water to remove dust and sand, shade dried at room temperature. Extracts were prepared by the method of [10]. The dried plant materials were grounded into fine powder in an electric blender and subsequently sieved for obtaining fine powder. These powdered materials were used for further physiochemical and fluorescent analysis.

Physico-chemical standardization of leaves:

Determination of Ash value: Ash content in the investigated plant species *Woodfordia fruticosa* was calculated by the methods given below:

Total ash: When the powdered drug is ignited at 7000ºC a white colored ash is obtained. It contains inorganic salts in oxide form.

Water soluble ash: Water soluble ash contains inorganic salts which are soluble in water.

Acid insoluble ash: It contains inorganic salts which are not soluble in water as well as in 10% hydrochloric acid example, salts of silicate etc.

Determination of total ash: Five grams of the ground plant material was taken in a silica crucible previously ignited and weighed. The ground plant material was then spread in a fine even layer on the bottom of the crucible. It was then incinerated in a muffle furnace by gradually increasing the heat not exceeding dull red heat until free from carbon and then cooled and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water. The residue was collected on an ashless filter paper which was then incinerated. The ash percentage was calculated with reference to the air dried material [11].

Where, w_1 = weight of crucible (g); w_2 = weight of crucible with the ash (g); w = weight of dried sample (g)

Determination of Water Soluble Ash: 100 mg of ash was boiled for five minutes with 10 ml of distilled water. The insoluble matter was collected in a silica crucible or on an ash less filter paper. It was washed with hot water and then ignited to constant weight at low temperatures. The weight of the insoluble matter was subtracted from the weight of the ash. The percentage of water soluble ash was calculated with reference to the amount of ash taken[11].

Determination of Acid Insoluble Ash: The total ash was boiled for five minutes with 25 ml of 10% HCl. The insoluble ash was collected in a silica crucible or on an ash less filter paper. It was washed with hot water and then ignited and weighed. The weight of the insoluble matter was subtracted from the weight of ash. The difference in weight represents the acid insoluble ash. The percentage of acid insoluble ash was calculated with reference to the amount of ash taken [11].

Determination of extractive value with different solvents: Estimation of extractive value was done according to the method of [12]. A known quantity of powdered drug was taken. Extraction was made in soxhlet apparatus with different solvents i.e. petroleum ether, ethyl acetate, benzene, methyl alcohol, chloroform and ethyl alcohol. The extract was filtered and the solvent was evaporated, accurate weight of the extract was taken. The percentage (%) was calculated with reference to air dried drug.

Fluorescence analysis: Fluorescence analysis of the drug was conducted by the method of [13]. Methanol, ethanol, benzene, hexane, chloroform, 1N NaOH, ethanolic NaOH, HCl, 50% H_2SO_4, acetone and water were taken and treated individually with desired quantity (1g) of the plant material, After 24 hours, fluorescence of each extraction was observed and recorded both in visible light and UV light.

Phytochemical analysis: For phytochemical screening, 3 gm of fine powdered sample was weighed and soaked separately in 15 ml of different solvents (Ethyl acetate, Methanol, Benzene, Ethanol and Chloroform) in the ratio of 1:5 weight by volume (w/v). These were allowed to stand for 24 hrs at ambient room temperature. The soaked plant powder was filtered and used as crude extract. Different crude extracts of this plant were stored in a refrigerator and used as such for qualitative phytochemical analysis.

Crude extracts of the plants were prepared and stored in a refrigerator and used for the phytochemical tests. These extracts were tested for the presence of various bioactive compounds which are given below:-

Test for Flavonoids: To one ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow color was produced in the plant extract, which became colorless on addition of a few drops of dilute acid, indicating the presence of flavonoids.

Test for Glycosides: To the test solution in alcohol, 1 ml concentrated H_2SO_4 was added and after hydrolysis of the test solution the presence of sugar was determined with the help of Fehling's solution. A black-red precipitate indicated the presence of glycosides.

Test for Reducing Sugars: To the test solution, 2ml of Fehling's reagent was added followed by 3ml of water, formation of Red-Orange color showed the presence of reducing sugars.

Test for Sterols/Terpenes: Salkowski Test: Extract was treated in chloroform with few drops of conc. sulfuric acid, shaken well and allowed to stand for some time, red color appeared at the lower layer indicating the presence of steroids and formation of yellow colored lower layer indicated the presence of triterpenoids.

Test for Tannins: To the test solution, water and 2ml of 5% $FeCl_3$ was added, formation of blue-black precipitate indicated the presence of tannins.

Test for Anthraquinones: Five ml of the extract solution was hydrolyzed with conc. H_2SO_4 extracted with benzene.

1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

Test for Alkaloids: Test solution was taken with 2 N HCl. Aqueous layer formed was decanted, to which one or few drops of Mayer's reagent (Potassium mercuric iodide solution) was added. White precipitate or turbidity formed showing the presence of alkaloids.

Test for Saponins: The extract was diluted with distilled water and it was agitated for 15 minutes. The formation of layer of stable persistent foam/froth showed the presence of saponins.

Test for Vit C/Ascorbic acid: To the test solution, 2 ml of water, 0.1 gram of sodium bicarbonate and about 20 mg ferrous sulphate was added, shaken and allowed to stand. A deep violet color was produced. To this 5 ml of 1 M sulphuric acid was added, the color disappeared showing the presence of Vitamin C/ascorbic acid.

Chromatographic fingerprint profiling of present phytochemicals in the plant:

TLC Screening of TANNINS- Gallic acid and Ellagic acid:

***In vivo* plant parts:** Various mature plant parts (leaves and flowers) of *Woodfordia fruticosa* were collected and were washed with tap water to remove dust and dried in shade.

***In vitro* tissue culture:** Nodal stem explants of *Woodfordia fruticosa* were sterilized with 0.1% mercuric chloride solution and inoculated after several rinses with sterile distilled water onto MS[14] medium supplemented with IBA (1.0 mg/l) and BAP (0.5 mg/l). Six week old callus tissue was dried in an oven at 100°C for 15 min. to inactivate enzymes, followed by 60°C till a constant weight was achieved. Tissue samples were powdered and used for extraction.

Extraction procedure: Each of the dried and powdered samples was soxhlet extracted in methanol (100 ml/gm dry weight) on a water bath for 24 hrs. Then, this methanolic extract was filtered and concentrated and transferred to 25-mL volumetric flasks individually and their volumes were made up with methanol.

Chromatographic analysis:

Thin layer chromatography (TLC): The glass plates (20 × 20 cm) coated with silica gel 'G' (0.2-0.3 mm thick and 30gm/60 ml distilled water) were dried at room temperature. The dried plates were activated at 100°C for 30 minutes in an oven and cooled at room temperature. Each of the extracts was applied 1 cm above the edge of tile chromatographic plates along with the markers (standard Tannins – Gallic acid and Ellagic acid) and developed in an air tight chromatographic chamber which was already saturated with a solvent system of Toluene-Ethyl acetate-Formic acid-Methanol (3:3:0.8:0.2 v/v).

Such chromatograms were air-dried, visualized under UV light at 254 nm and the fluorescence or the colours were noted. Bands were detected after spraying with 5% methanolic ferric chloride solution[15]. Plates were also placed in a chamber saturated with I_2 vapours to observe the colour of spots and to locate the spots in unsprayed developed chromatograms, exposure to I_2 vapours also proved useful.

TLC Screening of Flavonoids - Quercitin and Myricetin:

Extraction procedure: The dried and powdered samples were separately Soxhlet extracted in 80% methanol (100 ml/gm dry weight) on a water bath for 24 hrs [16]. The extract filtered and filtrate evaporated or concentrated by heating at 55º C on water bath to get a paste. Then it was taken up in distilled water till it dissolved. Each of the extracts was successively extracted with equal amount of petroleum ether (40°-60°C) (fraction-I), ethyl ether (fraction-II) and ethyl acetate (fraction-III).

Each of the steps was repeated three times to ensure complete extraction in each case. Fraction I was discarded due to the presence of high fatty substances, whereas fraction II was analysed for the free flavonoids in each of the samples.

Fraction III of each of the test samples was dried and hydrolysed by refluxing with 7% H_2SO_4 (10 ml/gm residue) for 5 hours on water bath. The mixture was filtered and the filtrate extracted with ethyl acetate in a separating funnel. The ethyl acetate layer was washed with distilled water till neutrality and dried in vacuo. The residues were taken up in small volumes of ethanol separately and then subjected to various tests for quercetin.

Chromatographic analysis:

Thin layer chromatography (TLC): Ethyl ether and ethyl acetate fractions from each sample were separately applied 1 cm above the edge of the plates along with standard reference compounds (quercetin and myricetin). These glass plates were developed in an airtight chromatography chamber containing about 200 ml of solvent mixture of n-butanol, acetic acid and water (4 : 1 : 5, upper layer). Later the developed chromatograms of each test fraction were first examined under UV light, and then by spraying with 5% ethanolic Ferric Chloride solution

TLC Screening of Sterols - β-sitosterol, Stigmasterol, Lanosterol and Cholestrol:

Extraction procedure: Dried and powdered plant material of *W.fruticosa* and was defatted in petroleum ether (60-80^o C) for 24 h on a water bath. Defatted material was air-dried and hydrolyzed in 30% HCl (v/v) for 4 h. Each hydrolyzed sample was washed with water till pH 7 and dried. The dried preparation was again extracted with benzene for 24 h. The extract was filtered and dried in vacuo. The crude extract was dissolved in chloroform before chromatographic examination.

Chromatographic analysis:

Thin layer chromatography (TLC): The crude extracts were applied separately on silica gel 'G' coated and activated thin glass plates along with standard reference sample of sterols (β-sitosterol, stigmasterol, lanosterol and cholesterol). The plates were developed in different organic solvent mixtures of Toluene-Chloroform-Methanol (4:4:1), Benzene: Ethyl acetate (3:1) and Hexane: Acetone (8: 2). The developed plates were air dried, sprayed with 50% sulphuric acid and subsequently heated at 100°C for 10 minutes. Toluene-Chloroform-Methanol (4:4:1) gave best results in the present investigation. Later, these plates were air-dried and visualized under UV light and fluorescent spots corresponding to that of standard markers were marked. These developed plates were sprayed with 50% H_2SO_4 [17] and anisaldehyde reagent, separately and heated at 110^{o}C for 10 min.

Identification: Each of the isolated compounds was subjected for its melting point, UV and IR spectra studies along with the authentic samples. Later, on the basis of the colour reactions, TLC behavior and the spectral data, the isolated compounds were identified by comparing with that of the reference compounds.

RESULTS AND DISCUSSION:

Physicochemical standardization of leaves:

Determination of Ash value: The determination of ash is often useful for the detection of low-grade products, exhausted drugs and excess of sandy or earthy matter; it is more especially applicable to powdered drugs [18]. The objective of ashing plant material is to remove all traces of organic matter which might otherwise interfere in analytical determination. Ashing at too high temperature may also result in the formation of complex silicates which are not soluble in hydrochloric acid and an apparent loss of some constituents may result from it. The acid insoluble ash, i.e. the ash insoluble in dilute HCl is often of much value than the total ash. The water soluble ash is used to detect the presence of material soluble in water. The water soluble ash shows greater reduction than the total ash and is therefore used as an important indicator for the presence of exhausted material substituted for the genuine articles [19]. Analysis of ash provides the list of various elements present in the plant. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The drug was found to contain an average of total ash 11.34 %, water soluble ash 6.46% and acid insoluble ash 3.29 % (**Table 1** and **Fig. 1)**.

TABLE 1: ASH VALUES (%) OF THE POWDERED MATERIAL OF PLANT

	Total Ash	Water Soluble Ash	Acid Insoluble Ash
	11.36	6.46	3.34
	11.28	6.50	3.2
	11.14	6.44	3.44
	11.34	6.50	3.24
	11.44	6.48	3.3
	11.5	6.42	3.22
Mean	**11.34**	**6.46**	**3.29**
S.D.	0.126	0.032	0.090
S.E.	0.051	0.013	0.036

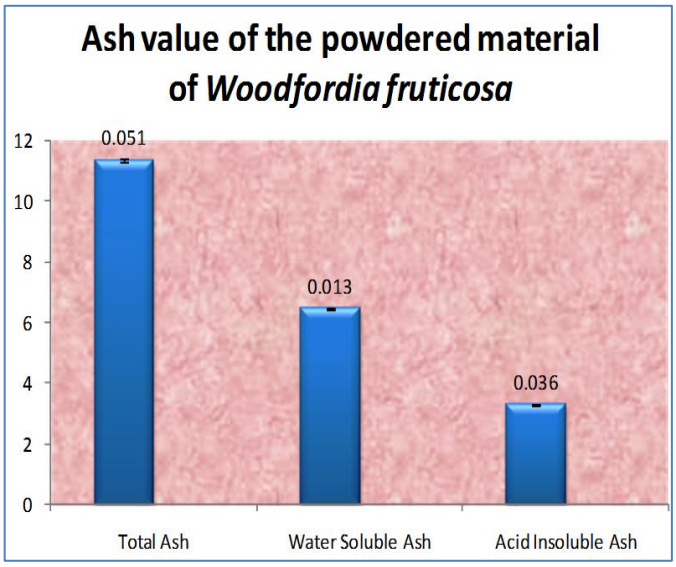


FIG. 1: ASH VALUE OF *W. FRUTICOSA*

Similarly ash value of different plant parts were studied from pharmacognostical point of view in different plants such as *Cardiospermum halicacabum* Linn.[20], *Nigella sativa* Linn.[21], *Berginia ligulata* and *Ammania buccifera* Linn.[22], *Persea macrantha* (Nees) Kosterm[23], *Leucas cephalotes* Spreng [24]. High total ash value indicates that presence of inorganic constituents and very low value of acid insoluble ash denotes the presence of negligible amount of siliceous matter.

The ash value varies within fairly wide limits and is therefore an important parameter for the purpose for evaluation of crude drugs. In certain drug, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted adulterants of drug, some time posses a character that raises the ash value. Ashing involves an oxidation of the components of the product. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. The total ash value, acid insoluble ash value, water-soluble ash values were determined and the results are presented in **Table 1** and **Fig. 2.**

Determination of extractive value with different solvents: Determination of extractive value of *Woodfordia fruticosa* with different solvents is significant because by knowing the extractive value of *Woodfordia fruticosa* with different solvents; isolation and quantification of chemical substances found in plant species can be easily carried out with the solvents of higher extractive values and solvents with lower extractive value may be avoided. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. The variation in extractable matter in various solvents is suggestive of the fact that the formation of the bioactive principle of the medicinal plants is influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive values reveal the presence of polar substance like phenols, tannins and glycosides, as reported by [25].

In the present study, it was found that extractive value was highest when extraction was done with methyl alcohol and lowest with petroleum ether i.e. 15.25 % and 5.82 % respectively. Results are shown in **Table 2 and Fig. 2** Similarly, maximum extraction with methyl alcohol has been observed in *Butea frondosa* Linn. by [26]. Extraction of different plants with different solvents has been done by many workers [27, 28, 29, 30].

TABLE 2: DETERMINATION OF EXTRACTIVE VALUES (%) OF *WOODFORDIA FRUTICOSA* (LINN.) KURZ. IN DIFFERENT SOLVENTS

S. No.	Ethyl alcohol	Methyl Alcohol	Petroleum ether	Benzene	Ethyl acetate	*Chloroform*
1	10.8	16.6	6.4	9.16	7.2	8.6
2	12	16.72	6.0	8.8	6.6	8.4
3	11.4	14.6	5.6	9.6	8.4	8.2
4	11.6	15.36	5.2	9.2	7.8	8
5	11	13	5.9	9.8	7.6	8.32
Mean	**11.36**	**15.25**	**5.82**	**9.312**	**7.52**	**8.304**
S.D.	0.477	1.53	0.44	0.393	0.67	0.223
S.E.	0.213	0.68	0.20	0.175	0.30	0.100

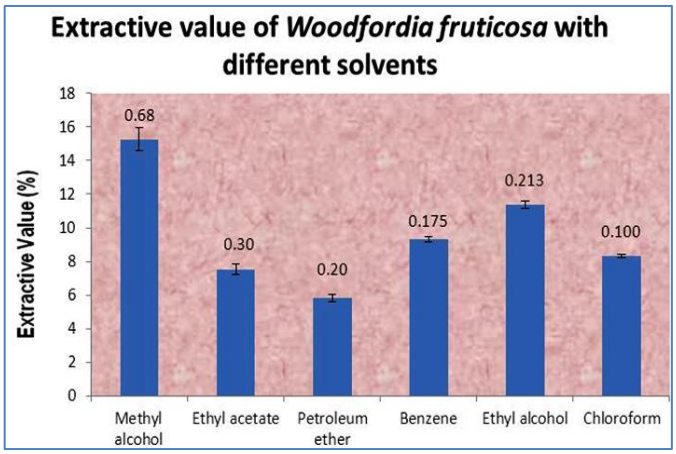


FIG 2: EXTRACTIVE VALUE OF *W. FRUTICOSA*

Fluorescence analysis: Fluorescence studies help in the identification of drugs that may be more or less difficult to distinguish. In a mixture of drugs of two or more species we can judge the identification by the use of estimates of intensity of fluorescence, the comparison of the unknown should be made with a sample of known identity. In the present investigations, fluorescence analysis of dried powder of *Woodfordia fruticosa* in different organic solvents produced a marked difference in color between visible light and UV light (**Table 3**).

Color variation was observed in visible light and UV light and it can be used as a standard parameter for quality control of the drug. The quality control is necessary if plant products are to fill the needs for cheap and reliable medicines or if natural products are to be used as template for new drug molecules. Similarly fluorescence analysis was done in *Lagascea mollis* [31]; *Eugenia singampattiana* Bedd. [32]; *Naringi crenulata* (Roxb) Nicols. [33]. **Table 3** shows a detailed fluorescence behavior of crude drug powder.

TABLE 3: FLUORESCENCE ANALYSIS OF *WOODFORDIA FRUTICOSA*

Name of Extract	Visible light	UV light
Drug powder	Green	Dark green
Drug powder in methanol	Greenish brown	Greenish dark brown
Drug powder in ethanol	Dark greenish black	Greenish brown
Drug powder in benzene	Yellowish Brown	Greenish black
Drug powder in hexane	Yellowish brown	Fluorescent Greenish black
Drug powder in chloroform	Blackish green	Bluish black
Drug powder in 1N NaOH	Blackish red	Brownish black
Drug powder in ethanolic NaOH	Yellowish brown	Light greenish brown
Drug powder in HCl	Golden yellow	Greenish brown
Drug powder in 50% H_2SO_4	Black	Black
Drug powder in acetone	Greenish brown	Greenish brown
Water	Pale yellow	Light green

Phytochemical analysis: Preliminary qualitative test is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development [34]**.** Herbal drug standardization is an essential step in order to assess the quality of drugs, based on the concentration of their active principle, physical and chemical standards. Moreover, a plant extract may contain several thousand different secondary metabolites but phytochemical analysis will reveal only a narrow spectrum of its constituents. Successive isolation of bioactive compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

The preliminary phytochemical analysis indicates the nature of phytoconstituents present in different solvent extract.

Results are presented in **Table 3**. In the qualitative phytochemical screening using various solvent extracts of plant, it was found that most of the biologically active phytochemicals were present in the methanolic extract of *Woodfordia fruticosa* leaves. In other words, the results confirmed the presence of therapeutically potent compounds in leaf extract of *Woodfordia fruticosa*. It revealed that tannins and terpenoids were predominantly found in all the five extracts, followed by flavonoids which were found in four extracts and anthraquinone, saponins, glycosides and vitamin C /ascorbic acid were found in three extracts. Alkaloids were not found in all the five extracts used for phytochemical screening.

The ethyl acetate extract of flower of *Woodfordia fruticosa* showed the presence of terpenoids, tannins, flavonoids and vitamin C/ ascorbic acid. Methanol extract of flower showed the presence of tannins, terpenoids, flavonoids, reducing sugar, glycosides, anthraquinone, saponin and vitamin C/ascorbic acid. The benzene extract of flower exhibited the presence of anthraquinone, tannins, terpenoids, reducing sugar and flavonoids. Flavonoids, tannins, terpenoids, glycosides and vitamin C/ascorbic acid were found in ethanol extract. Flavonoids and terpenoids were screened in chloroform extract of flower. Glycosides were found in methanol and ethanol extract only and reducing sugar only in methanol and benzene extract. Saponin found only in methanol extract and vitamin C/ascorbic acid only in ethyl acetate, methanol and ethanol extract. Alkaloids were not found in all the five extracts used for phytochemical screening.

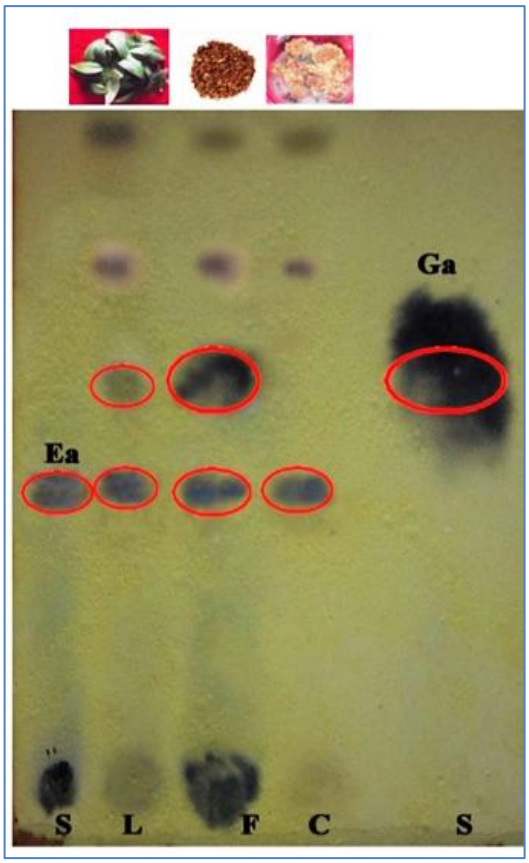
Chromatographic fingerprint profiling of present phytochemicals in the plant

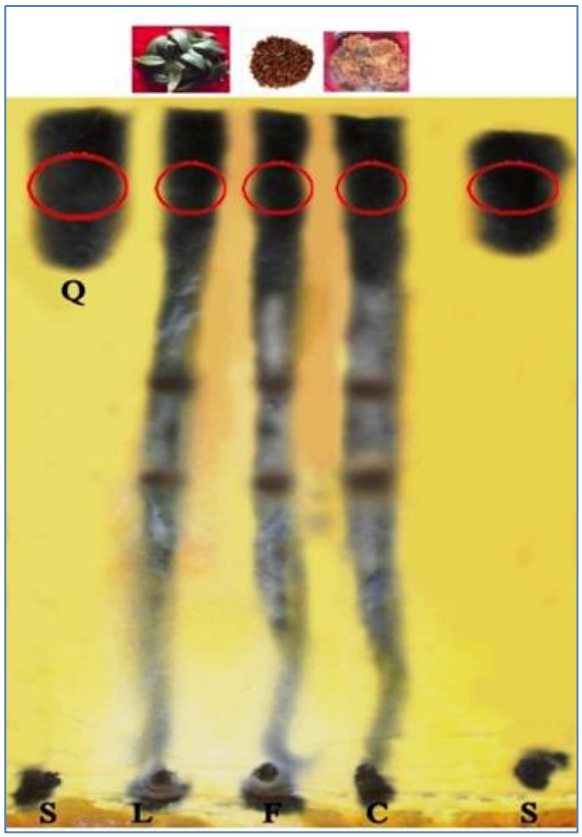
The chromatographic and spectroscopic techniques have proved very useful in isolation and proper identification of the active constituents in the plant extracts[35]. TLC profiling of all plant extracts have resulted in directing towards the presence of a number of phytochemicals. Various phytochemicals have different Rf values in different solvent system.

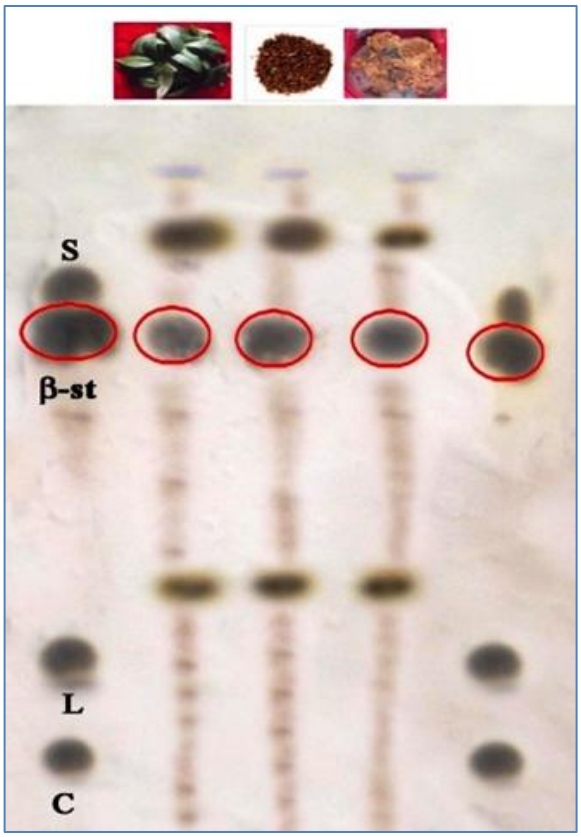
Different Rf (Retention factor) value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals[36].

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. As per phytochemical screening in the present investigation, TLC screening of tannins showed the presence of four spots having Rf value as 0.54, 0.61, 0.8 and 0.96, and three spots in the callus extracts of the plant with Rf 0.54, 0.8 and 0.96, out of which two spots were identified as ellagic acid (Rf 0.54) and gallic acid (Rf 0.61) as they coincided with the markers.

TLC screening of flavonoids revealed the presence of two coinciding spots, identified as quercetin (Rf 0.8) and myricetin (Rf 0.96). TLC screening of sterols revealed the presence of four spots with Rf value 0.96, 0.8, 0.58, 0.54, out of which only one spot coincided with the authentic marker compound of β-sitosterol (Rf 0.58) showing the presence of only one type of sterol compound in the plant samples. Results are shown in **Fig 3.**







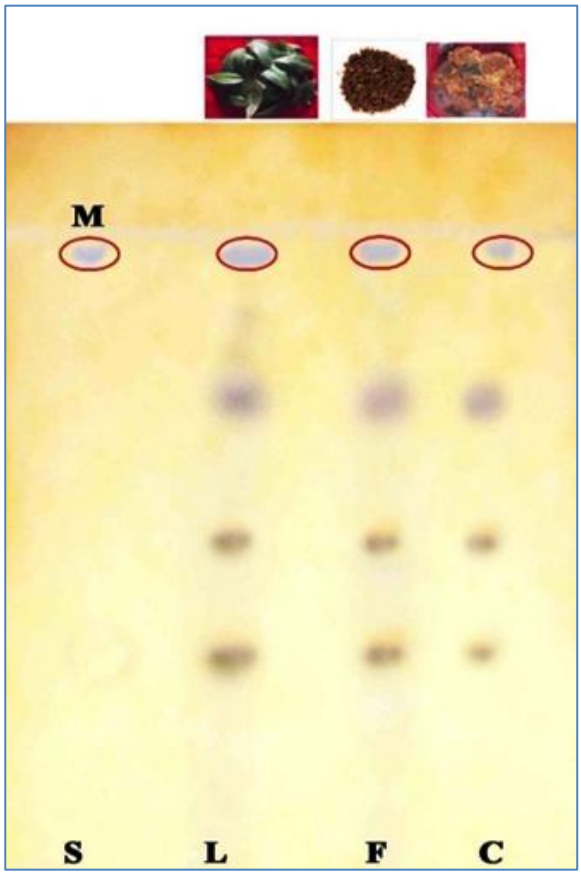


FIG. 3: CHROMATOGRAPHIC PROFILING OF PHYTOCHEMICALS PRESENT IN *W. FRUTICOSA*

CONCLUSION: Plants serve as vast source for varied phytoconstituents exhibiting varied pharmacological property. Identifying such potential plants is of significance in medicine. So it becomes necessary to study the pharmacognostic characteristic of the plant before its use in the field of research and also in pharmaceutical formulation. Moreover it also helps in distinction from other allied species and adulterants.

From the present study, it can be concluded that most of the biologically active phytochemicals were present in the methanolic extract of *Woodfordia fruticosa* leaves. In other words, the results confirmed the presence of therapeutically potent compound in leaf extract of *Woodfordia fruticosa*. The chromatogram of thin layer chromatography showed the presence of various tannins (gallic acid and ellagic acid), flavonoids (quercetin and myricetin) and sterols (beta-sitosterol). The approach given for standardization of any new herbal or medicinal plant includes chemical evaluation and comparison.

For developing an analytical method, pure active chemical constituent should be isolated in further study and identification should be done on the basis of reference standard.

ACKNOWLEDGEMENT: The authors are grateful to Prof. Uma Kant, Professor Emeritus, Department of Botany, University of Rajasthan for guiding and positive feedback during the course of the work. Authors are also thankful Honorable Vice-Chancellor and Head of the Department for providing necessary facilities at Department of Botany and Central lab, University of Rajasthan, Jaipur.

REFERENCES:

1. Sasidharan S, Chen Y, Saravanan D, Sundram K M and Latha LY.: Extraction, isolation and characterization of bioactive compounds from plants extracts. African Journal of Traditional Complementary and Alternative Medicines. 2011; 8(1) : 1-10.
2. Shazia Sultana, Mir Ajab Khan, Mushtaq Ahmad, Asghari Bano, Muhammad Zafar and Zabta Khan Shinwari. Pak. J. Bot. 2011. 43: 141-150.
3. Pascual M E, Carretero M E, Slowing KV and Villar A.: Simplified screening by TLC of plant drugs. Pharmaceutical Biology. 2002; 40(2): 139-143.
4. Kokate CK, Purohit AP, Gokhale SB.: Pharmacognosy. Nirali Prakashan, 38th edition 2007.
5. Kokate CK.: Practical Pharmacognosy. Vallabh Prakashan, 4th edition 1994, Reprint 2004.
6. Kroes BH, Van-der-Berg AJJ, Heystra EA, De-Silva KTD and Labadie RP.: Fermentation in traditional medicine: the impact of *Woodfordia fruticosa* flowers on the immunomodulatory activity and the alcohol and sugar contents of *Nimba arishta* (a portion of fermented *Azadirachta indica* bark). Planta Medica. 1990; 56(6):667.
7. Chadha YR.: The Wealth of India: A dictionary of Indian Raw Materials and Industrial Products- Raw Materials, vol. X. Publication and Information Directorate, CSIR, New Delhi. 1976; 586-687.
8. Atal CK, Bhatia AK, and Singh RP.: Role of *Woodfordia fruticosa* Kurz. (Dhatki) in the preparation of Asavas and Arishtas. J. of Res. in Ayurveda and Siddha III. 1982; 193-199.
9. Mungole Arvind and Chaturvedi Alka: *Hibiscus sabdariffa* L. A rich source of secondary metabolites. International Journal of Pharmaceutical Sciences Review & Research. 2011; 6(1): 83-87.
10. Audu JA, Kela SL, Vnom VV.: Antimicrobial activity of some medicinal plants. J. Econ. Taxon. Bot. 2004; 24: 641-649.
11. Anonymous: British Pharmacopoeia. General medical council, Pharmaceutical Press-17, Bloomsbury square London. 1968; pp- 1209.
12. Regina, SN: Morphological and preliminary phytochemical studies of drug yielding herbs. Ph.D. Thesis, C.S.J.M. University, Kanpur, 2003.
13. Prasad S, Nayar KC and Bhattacharya IC: 'Pharmaceutical studies on Chirata and its substitutes and adulterants. Part 2nd: *Swertia anguistifolia* Buch-Ham. J. Sci. Ind. Res. 1960; 19: 139-145.
14. Murashige T and Skoog F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 1962; 15: 473-497.
15. Wagner H and Bladt S.: Plant Drug Analysis: A thin layer chromatography Atlas. Second edition. Springer-Verlag Berlin Heidelberg New York Tokyo. 1996; 384.
16. Subramanian S S and Nagarajan S.: Flavonoids of the seeds of *Crotlaria retusa* and *C. striata*. Curr. Sci. 1969; 38: 65-68.
17. Bennett PD and Heftmann E.: Thin layer chromatography of steroidal sapogenins. J. Chromatogr. 1962; 9: 353-358.
18. Prajapati N Chetankumar and Patel M. Natvarlal. Physio-chemical and Phytochemical Evaluation of *Leptadenia reticulata* Roots. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2012; 3(4): 1791- 1797.
19. Verma ML: Pharmacognostic studies on certain species of the family Asteraceae. Ph.D. Thesis. Department of Botany, U.O.R., Jaipur (India), 1988.
20. Viji M and Murugesan S.: Phytochemical analysis and antibacterial activity of medicinal plant *Cardiospermum halicacabum* Linn. Journal of Phytology. 2010; 2(1): 68–77.
21. Sharma NK, Ahirwar D, Gupta S and Jhade D.: Pharmacognostic standardization, physico and phytochemical evaluation of *Nigella sativa* Linn. seed. International Journal of Pharmaceutical Sciences and Research. 2011; 2(3): 713-718.
22. Jani Switu, Shukla J Vinay, Harisha CR. Comparative pharmacognostical and phytochemical study on *Bergenia ligulata* Wall. and *Ammania buccifera* Linn. Pharmaceutical Standardization. 2013; 34(4): 406-410.
23. Kulkarni Y A, Gokhale S B, Yele S U, Surana S J and Tatiya A U.: Pharmacognostical studies and preliminary phytochemical investigations on the bark of *Persea macrantha* (Nees) Kosterm. Indian Journal of Natural Products and Resources. 2011; 2(2): 211-217.
24. Mohammad Yusuf Ansari, Abdul Wadud, Ehteshamuddin and Hamida Bano.: Pharmacognostical evaluation of root of Gumma (*Leucas cephalotes* Spreng.). Indian Journal of Natural Products and Resources. 2013; 4(1) : 88-95.
25. Baravalia Y, Nagani K and Chanda S.: Evaluation of pharmacognostic and physicochemical parameters of *Woodfordia fruticosa* Kurz. flowers. Pharmacognosy Journal. 2011; 2(18):13-18.
26. Das C, Dash S and Sahoo DC.: Pharmacognostical and phytochemical investigation of *Butea frondosa* Linn. bark. Der Pharmacia Lettre. 2012; 4 (2):475-482.
27. Devika R and Sajita U.: Phytochemical and pharmacological investigation of *Phyllanthus niruri* – A preliminary screening. J. Phytol. Res. 2007; 20(1) : 133-135.
28. Jahan N, Afaque S H, Khan N A, Ahmed G and Ansari AA.: Physiochemical studies of the gum *Acacia*. Natural Product Radiance. 2008; 7(4): 335-337.
29. Bhattacharya S and Zaman M K.: Pharmacognostical evaluation of *Zanthoxylum nitidum* root. Pharmacognosy Journal. 2009; 1: 15-21.
30. Mohanty A, Das C, Dash S and Sahoo D C.: Physico-chemical and antimicrobial study of polyherbal formulation. International Journal of Comprehensive Pharmacy. 2010; 1(4): 1-3.

31. Suseela L and Prema S.: Pharmacognostic studies on *Lagascea mollis* (Asteraceae). J. Phytol. Res. 2007; 20(1): 95-101.
32. Kala S M J, Balasubramanian T, Mohan V R and Tresina Soris P.: Pharmaco-chemical characterisation of *Eugenia singampattiana* Bedd. Advances in bioresearch 2010; 1(1): 106-109.
33. Sampathkumar S and Ramakrishnan N.: GC-MS analysis of methanolic extract of *Naringi crenulata* (Roxb.) Nicols. Leaves. International Journal of Pharmaceutical Research and Development. 2011; 3(5): 113 – 116.
34. Mallikharjuna P B, Rajanna L N, Seetharam Y N and Sharanabasappa GK.: Phytochemical studies of *Strychonos potatorum* L.F. A medicinal plant. E.J. Chem. 2007; 4: 510-518.
35. Kataria Sahil, Bhardwaj Sudeep, Middha Akanksha.: Standardization of Medicinal Plant materials. International Journal of Research in Ayurveda & Pharmacy. 2011; 2(4): 1100-1109.
36. Talukdar A Das, Choudhary M Dutta, Chakraborty M and Dutta BK.: Phytochemical screening and TLC profiling of plant extracts of *Cyathea gigantean* (Wall. Ex. Hook.) Haltt. And *Cyathea brunoniana*. Wall. Ex. Hook. (Cl. & Bak.). Assam University Journal of Science& Technology: Biological and Environmental Sciences. 2010; 5(1): 70-74.

How to cite this article:
Grover N, Meena R and Patni V: Physiochemical evaluation, phytochemical screening and chromatographic fingerprint profile of *Woodfordia fruticosa* (l.) Kurz extracts. *Int J Pharm Sci Res* 2014; 5(7): 2772-82.doi: 10.13040/IJPSR.0975-8232.5 (7).2772-82.