Evaluation of Physicochemical, Fluorescence Characterisation, Phytochemical investigation and In-vitro Anti-inflammatory activity of Woodfordia fruticosa (L.) Kurz. Methanol flower Extract

Rohit Kumar Bargah[1*], Vikash Kumar Painkra [2] , Bhumika Painkra [3], Karuna Gupta [4] Lebhanti [5]

[1*]Assistant Professor & Head, Department of Chemistry, Govt. S.P.M. College Sitapur Surguja (C.G.) India, 497111.

[2] Research scholar, Department of Chemistry, Govt. S.P.M. College –Sitapur, Surguja (C.G.) INDIA 497111.

[3]Research scholar, Department of Chemistry, Govt. S.P.M. College –Sitapur, Surguja (C.G.) INDIA 497111.

[4]Research scholar, Department of Chemistry, Govt. S.P.M. College –Sitapur, Surguja (C.G.) INDIA 497111.

[5]Research scholar, Department of Chemistry, Govt. S.P.M. College –Sitapur, Surguja (C.G.) INDIA 497111.

[1*]**Address Correspondence**: Dr. Rohit Kumar Bargah, Assistant Professor& Head, Department of Chemistry, Govt. S.P.M. College Sitapur Surguja (C.G.) India,497111,

ABSTRACT

Background and Objectives: Phytochemicals are naturally occurring chemical compounds found in plants, many of which possess medicinal properties. These compounds, though not essential nutrients, can contribute to disease prevention and treatment. Medicinal plants, in turn, are plants that contain these phytochemicals and are used for therapeutic purposes. 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 study aimed to evaluate the physicochemical, Fluorescence characterisation, Phytochemical Screening and Anti-inflammatory properties of methanol extracts of flower of Woodfordia fruticosa (L.) Kurz. (family: Lythraceae).

Methods: The herbal standardization was carried out on the basis of physicochemical properties. Physicochemical parameters including ash values, extractive values and fluorescence analysis were evaluated. The qualitative phytochemical screening using methanol extracts of plant revealed the presence of most of the biologically active phytochemicals in the methanol extract of Woodfordia fruticosa Flower. Anti - inflammatory activity of the flower parts the plant were analysed by an *In-vitro* protein denaturation inhibition assays.

Result: The highest percentage of extractive yield, total ash, water soluble ash and acid insoluble ash were found Woodfordia fruticosa. Fluorescence analysis of flower extract and flower powder of Woodfordia fruticosa showed characteristic coloration with various chemicals. The fluorescent characteristics of the leaf powder with various chemical reagents were examined under visible and UV light. The result revealed the presence of flavonoids, alkaloids, Triterpenoids, Phenolic compound, Steroidal glycosides, saponins, tannins, Reducing Sugar, in methanol extract. Methanol Woodfordia fruticosa flower extract exhibited significant anti-inflammatory activity by inhibiting albumin denaturation. Maximum inhibition of 65.78 % was observed at 500 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 63.15 % at the concentration of 100 µg/ml compared with control.

Conclusion: The current study proves the Physicochemical analysis, fluorescence analysis, phytochemical and anti-inflammatory uses of Woodfordia fruticosa; The results affirmed that methanol extract showed

better anti-inflammatory activity flower extract. However, further studies are required for phytochemical screening to isolate the responsible bioactive compounds and discover the lead molecules from the plant species.

Keywords: Woodfordia fruticosa, Physicochemical analysis, fluorescence characterisation, phytochemical Screening, anti-inflammatory activity, Methanol extract.

1. INTRODUCTION

Herbal medicines are obtained from different parts (leaves, stem, root, flower, etc.) of plants, which have been used extensively everywhere since ancient times for the treatment and cure of different diseases [1]. Since ancient times, medicinal plants have been used for treating human diseases, and their use continues to be prevalent in modern times. Plant-based remedies are extensively engaged in managing conditions such as inflammatory disorders, central nervous system diseases, cancer, viral infections, fungal ailments, and various other health issues [2] Through experimental and clinical investigations, the use of natural products and herbal extracts for treating human diseases and disorders has finally begun to get scientific acceptance [3]. According to the World Health Organization (WHO), nowadays, over 80% of people living in rural areas turn to medicinal plants as an alternative approach to their basic healthcare needs [4].

Woodfordia fruticosa (L.) kurz belongs to the family 'Lythraceae' has been utilized in traditional medicine for treating common ailments since ancient periods. It is conventionally employed against numerous diseases, including cold, toothache, blood infection, leprosy, dysentery, wounds, rheumatic pain, fever, urinary disorders, inflammation, antifertility and menstrual problem [5-6].

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 [7]. 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.[8]. Evidence revealed the significant host-mediated antitumor activities, antioxidant, antibacterial, and analgesic activities of the flower, essential oil, and leaf extracts of W. fruticosa. Despite having scientific verification of the traditional uses, mainly of this plant's flower and leaf parts, more investigation into the pharmacological activities of the bark of W. fruticosa is needed [9-10].

Fig 1: Flowering plant of Woodfordia fruticosa

2. MATERIALS AND METHODS

2.1 Plant Material

Woodfordia fruticosa flowers were collected from the Mainpat Hiis Surguja Chhattisgarh . The plants were authenticated by Prof. Rijwan Ulla, Department

of Botany, Rajeev Gandhi Govt. Autonomous Post Graduate College Ambikapur, Surguja, Chhattisgarh, India. The plant materials were dried under shade by placing in a single layer and coarsely powdered by hand mixer and pass through sieve no 60.

2.2 Preparation of Plant Extract

The plant material of Woodfordia fruticose Flowers was collected and washed with water to remove dust and sand, shade dried at room temperature. The dry samples were chopped into pieces and ground into powder by using a mechanical grinder. The powdered materials were stored in clean plastic bottles until the use and extracted with methanol for 50 hr. Under reduced pressure, all traces of alcohol is eliminated, yielding semisolid extracts.

2.3 Physicochemical analysis

Physicochemical parameters include loss on drying, water content, total ash content, acid-insoluble ash content, water-soluble ash content, water-soluble extract content, and ethanol-soluble content [11-13].

(i) Determination of loss on drying (LOD)

LOD was determined by gravimetric determination. 2–5 g of sample was placed in crucible porcelain, dried at 105 °C for 60 min, then moved into a desiccator. This process was repeated until the constant weight was achieved. LOD was expressed as gram per gram of air-dried (World Health Organization 1998).

(ii) Determination of water content

Water content was determined by gravimetric method. 1 g of sample was heated in the oven at 105ºC for 5 h, and then weighed. The process was continued with 1 h intervals until the difference between 2 consecutive weighings is not more than 0.25% .

(iii) Determination of total ash content

1 g of sample was placed in a silicate crucible and weighed. Sample was spread in an even layer in the crucible, and the material ignited by gradually increasing the heat to 500–600 °C until free from carbon, cooled in a desiccator, and weighed. Repeatedly until a fixed weight is obtained (World Health Organization 1998).

(iv) Determination of acid-insoluble ash content

Ash obtained from ash content testing was boiled with 25 mL HCl (~70 g/l) TS for 5 min. The ash is filtered with non-ash filter paper and washed with 5 mL hot water. The insoluble matter was transferred to the crucible, dried on a hot-plate and ignited to constant weight, and placed in a desiccator for 30 min, then weighed without delay. Content of acid-insoluble ash is calculated in mg per g of air-dried material (World Health Organization 1998).

(v) Determination of water-soluble ash content

Containers containing total ash were added with 25 mL of water and boiled for 5 min. Material that does not soluble is collected into a glass cup or ashless filter paper. Then, it was washed with hot water and ignited in a cup for 15 min at a temperature of 450 °C until the weight remained. The reduction of the residue weight in mg is total ash weight. The water-soluble ash is calculated in mg per g of air-dried material (World Health Organization 1998).

(vi) Determination of water- and ethanol-soluble extract

5 g of extract was macerated with 100 mL of water for 6 h for water-soluble extract determination, and then saturated with CHCl3. For ethanol-soluble determination, it was macerated with ethanol. They were shaken frequently, and allowed to stand for 18 h. The extract produced was filtered and poured into a volumetric flask. 20 mL of extract was transferred to a porcelain cup,

evaporated until dry. The residue was heated using an oven at 105 °C to receive constant weight. The soluble extract was calculated in g per g of air-dried material.

2.4 Sample preparation for fluorescence analysis

Powdered drug of different parts of plant gave different fluorescence under ultraviolet (UV) radiation. Each fluorescence characteristic of the treated sample was observed under visible light and then under UV light of both long and short wavelengths. Therefore, fluorescence evaluation is used for the identification of plant and powdered drug. Some crude drugs are often assessed qualitatively in this way, and it is an important parameter of pharmacognostic evaluation [14].

The flower of Woodfordia fruticosa was dried in the shade to prevent decomposition of active principle and make fine powder for the fluorescence study. A small quantity (1 gm) of dried and finely flower of Woodfordia fruticosa was treated with freshly prepared acids, alkaline solutions, and different solvents. The drug powders were treated with acids (10% HCl, Conc. HCl, Conc. H_2SO_4 , and Conc. HNO_3), alkaline solutions (1 N aqueous NaOH, 1 N alcoholic NaOH, and 5 KOH), other chemicals (5% iodine, 5% $FeCl_3$, and acetone), and distilled water. They were subjected to study the fluorescence analysis in visible light and in short UV light (254 nm) and long UV light (365 nm).

2.5 Phytochemical analysis:

Each extract was subjected to qualitative tests for identification of various constituents like alkaloids, carbohydrates, glycosides, steroids, saponins, flavonoids, tannins and phenolic compounds and proteins. The preliminary phytochemical screenings of extracts were performed according to standard

procedure [15-16]. These extracts were tested for the presence of various bioactive compounds which are given below:-

(i) 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.

(ii) Test for Steroids / Triterpenoids

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.

(iii) Test for Tannins

To the test solution, water and 2ml of 5% FeCl3 was added, formation of blueblack precipitate indicated the presence of tannins.

(iv) **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.

(v) 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.

(vi) Test for Glycosides:

Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(vii) 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.

(viii) 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.

(ix) Test for phenolic compound

A small amount of the plant extract is dissolved in distilled water. A few drops of 5% or 10% neutral ferric chloride solution are added to the solution. A color change to blue, green, or purple indicates the presence of phenolic compounds.

(x) Test for Proteins and Amino acids

(a) Biuret test**:** Added 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color was produced, and then added to the 1ml of the extract. Formation of pinkish or purple violet color indicated the presence of proteins.

(b) Ninhydrin test: Added two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

2.6 Evaluation of in vitro anti-inflammation activity

Inhibition of Albimin denaturation assay

Effect of methanol extract of Woodfordia fruticosa flower on heat-induced bovine serum albumin (BSA) denaturation assay was carried out using a method described by Chandra et al. [17] with minor modifications. The reaction mixtures consist of varying concentrations (100, 200, 300,400 and 500 µg/mL) of reference drug Aspirin (an NSAID), 1% w/v BSA and phosphate buffered saline (PBS, pH 6.4) separately while PBS was used as control. The reaction mixtures were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 70 °C for 5 min. After cooling, turbidity was measured at 660 nm using UV-visible spectrophotometer (Schimadzu Double Beam UV-2600, Japan). The control represents 100% protein denaturation. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition =

(Abs Control –Abs Sample) X 100/ Abs control

3. RESULT AND DISCUSSION

3.1 Physiochemical parameters

Physiochemical parameters of the flower of Woodfordia fruticosa are tabulated in Table 1.1. Different extracts of the powdered flower were prepared for the study of extractive values. Percentage of extractive values was calculated with reference to the air-dried drug. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant material can be easily deteriorated due to fungus. The loss on drying at 105 °C in flower was found to be 10.1%. Total ash value of plant material indicated the amount of minerals and earthy

materials attached to the plant material. Physiochemical parameters of the flower of Woodfordia fruticosa are tabulated in Table 1.1. Studies of physicochemical characterization can serve as a valuable source of information and are usually applied in judging the purity and quality of the drug. The extractive values give an idea about the chemical constitution of the drug.

Table 1.1**:** Physicochemical parameters of flower Woodfordia fruticosa

S.N	Parameters	Results (%w/w)
1	Total Ash	7.57
2	Acid insoluble Ash	1.89
3	Water -soluble Ash	3.56
4	Water soluble extractive value	18.55
5	Methanol soluble extractive value	21.84
6	Loss on Drying	5.32

Woodfordia fruticosa flower ethanol extract has the highest solubility of phytochemicals when extracted with methanol than aqueous extracts tested. Analytical results showed the results are in agreement with Woodfordia fruticosa flower extracts total ash 5.57, acid insoluble 1.89, water-soluble ash 3.56 , water- soluble extractive value 18.55, methanol soluble extractive value 21.84, and loss of drying (moisture of contents) 5.32.

The moisture content of a material plays a significant part in the efficacy and stability of drugs. By heating the sample to a constant weight or calculating the loss in weight on drying, the moisture content is assessed. The moisture content should be controlled or decreased to avoid bacterial attack and chemical

degradation. The moisture content of the plant was found to be in the suggested range. Thus, it can be stored and used for a longer period effectively [18].

Ash values are useful in defining the purity and quality of crude drugs. The conversion of crude material to ash eliminates biological material and avoids its intrusion in analysis. Crude drug material usually results in a residue after full ignition that is called ash. Ash comprises silicates, phosphates, and carbonates of calcium, sodium, magnesium, and potassium.

Acid insoluble ash shows calcium oxalate or silica which becomes inactive during garnering the crop. Sulphuric acid is used to determine sulfated ash by some analysts to get less fusible ash than the usual one. Ash value tests are important to determine and identify the presence of any foreign matter like carbonates, silica and oxalate. Different ash values and extractive values of seed powder are determined to evaluate the purity and quality of the powdered drug. According to British Pharmacopoeia, the accepted range of ash value is 20 %. The study outcomes have shown that ash value is present in the recommended range [19]. The extractive values are the indication of presence of polar compounds like phenols, tannins and glycosides [20].

3.2 Fluorescence Analysis:

Fluorescence spectroscopy is an analytical method based on the fluorescence properties of the sample. It is used for quantity measurement of medicine metabolized and other chemical products**.** Fluorescence characteristics of different solvent extracts under visible, short, and long light were determined and are shown in Table 1.2. Fluorescence behavior of the flower powder with different chemical reagents was analyzed to detect the occurrence of phytoconstituents along with color changes [21].

Table 1.2: Fluorescence behavior of powdered flower treated with different reagents

S.N.	Sample	Short UV 254 nm	Long UV 365 nm	Visible light
1.	Powder as such	Yellow	Sand colour	Sand colour
2.	Powder + Saturated picric acid	Neo Red	Neo Green	Neo Yellow
3.	Powder+ Nitric acid	Neo Red	Dark Blue	Neo Yellow
4.	Powder +HCL	Violet	Neo Green	Brown
5.	Powder+50% sulphuric acid	Neo Green	Neo Green	Neo Green
6.	Powder glacial acetic acid	Neo Green	Dark Blue	Neo Green
7.	Powder + 5% ferric chloride sodium	Dark Green	Dark Blue	Reddish black
8.	Powder +2 N Sodium hydroxide solution	Reddish Yellow	Reddish Green	Blood Red
9.	Powder +Aqueous iodine solution	Neo Green	Neo Green	Neo Red

3.3 Preliminary phytochemical Analysis

The preliminary phytochemical constituents detected in the flower are known to be beneficial in the treatment of infected diseases recently, a number of studies has been carries out on the various phytochemical of plant across the worlds evolutions of phytochemical such as alkaloids, flavonoids, glycosides and amino acid revealed the presence of most of the Woodfordia fruticosa flower constituents in polar extracts. In the present investigation flower of methanol extract of showed the presence of flavonoids derivative, alkaloids, terpenoids, reducing sugars, tannins, saponins, steroids, glycosides, phenolic compound , protein and amino acid. [table1.3]. phenols, mainly the type of flavonoids from some medicinal plants are safe and bioactive and have antioxidants, antibacterial and anti-inflammatory properties [22]. Bioactive compound

formed in various plant parts possess multiple biological effects on human and non-human biota [23].

Table 1.3 :Phytochemical present in Woodfordia fruticosa flowers of methanol extract

S.N	Phytochemical Test	Methanol Extract
1	Carbohydrate	+
2	Protein	-
3	Alkaloid	+
4	Flavonoid	+
5	Steroid	+
6	Amino Acid	+
7	Tannin and Phenolic	+
8	Glycosides	+
9	Saponines	-

Present (+), Absent (-)

3.3 The anti-inflammation activity

The ethical challenges and the nonexistence of rationale to use animals for pharmacological research of new chemical compounds, when other suitable methods are available or could be investigated, pushed us to select the protein denaturation bioassay and membrane stabilization potential for in vitro evaluation of anti- inflammatory property activity of studied plant materials [24].

The inhibition of protein denaturation

Inhibition of albumin denaturation Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of

external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity [25], ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 65.78 % was observed at 500 µg/ml. Aspirin, a standard ant inflammation drug showed the maximum inhibition 63.15 % at the concentration of 100 µg/ml compared with control (Table 1.4).

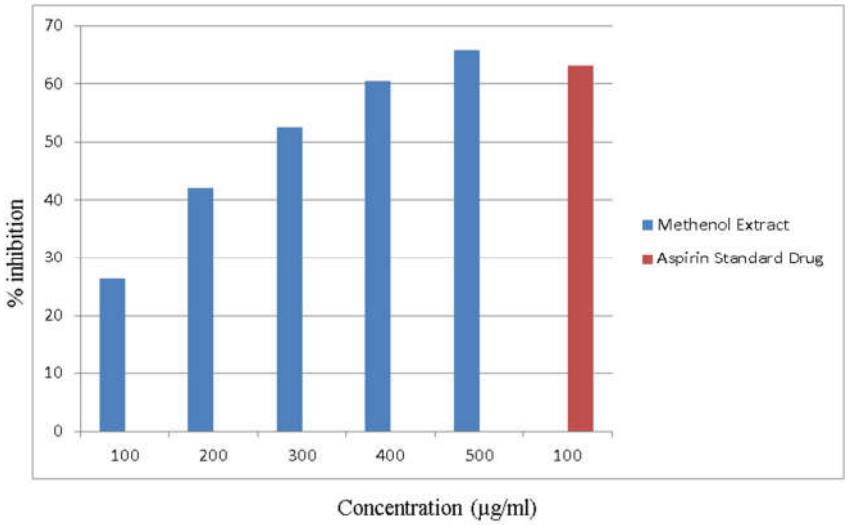
Further research highlights the potential of natural products in the treatment of chronic inflammatory diseases, which is particularly relevant given the increasing prevalence of these conditions. The side effects associated with NSAIDs, including gastrointestinal disturbances and cardiovascular risks, underscore the need to seek safer and more effective alternatives. Methanol extract, with its favorable safety profile and demonstrated efficacy, may represent a promising avenue for the development of anti-inflammatory treatments [26].

Table 1.4: Effect of Woodfordia fruticosa methanolic extract on heat induced protein denaturation

S.N.	Concentration (µg/ml)	Absorbance at 660 nm	% inhibition of protein denaturation
1	100	0.30 ± 0.07	25.00
2	200	0.24 ± 0.03	42.10
3	300	0.19 ± 0.03	52.50
4	400	0.16 ± 0.01	60.00
5	500	0.13± 0.07	67.50

6	100 (Aspirin) Standard anti-inflammation drug	0.10 ± 0.01	75.15
7	Control	0.40 ± 0.04	-

Each value represent the mean ± SD. N=3 experimental group were compared with control p <0.001 considered extremely significant.



Fig, 2: Anti-inflammatory activity of Woodfordia fruticosa methanol extract using albumin Protein denaturation

4. CONCLUSION

Our investigation clearly demonstrates that studied plant materials possess significant bioactive constituents against various antioxidant systems and potent anti-inflammatory. The presence of various Polyphenols especially flavonoid may be responsible of these activities. The present study provides a scientific base for the ethno medicinal claims of plants. Since the development of herbal medicine opens unparalleled opportunities in the treatment of various ailments, the focus on the exercise of the medicinal system by traditional practitioners is escalating day by day. Therefore, the outcome of the present study justified the traditional uses of Woodfordia fruticosa and might provide useful information

with respect to its identification and isolation of potential candidates for further chemical and biological investigation.

5. CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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