



COMPARATIVE *IN VITRO* ANTHELMINTIC AND PHYTOCHEMICAL EVALUATION OF METHANOLIC AND PETROLEUM ETHER EXTRACTS OF *WOODFORDIA FRUTICOSA* (L). FLOWER

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ABSTRACT

The present study aims at preliminary phytochemical investigation and anthelmintic activity of dried flowers of *Woodfordia fruticosa* (L). The dried flowers were extracted individually with methanol and petroleum ether. The extracts were evaluated for phytochemical studies. Preliminary screening of the flowers of *Woodfordia fruticosa* revealed the presence of carbohydrates, tannins and glycosides in major quantities, phenols in moderate quantities and anthraquinones and flavonoids in minor quantities. Methanol extract and Petroleum ether extract of *Woodfordia fruticosa* flower were tested for anthelmintic potential against the Indian earth worm *Pheritima posthuma*. Different concentrations of the extract ranging from 50-100 µg / ml were tested and results expressed as time required for paralysis and death of the worms. Piperazine citrate (10 mg / ml) and Albendazole (20 mg / ml) were used as a reference standards and DMSO (1 %) as the negative control. The methanolic extract of *Woodfordia fruticosa* (L) flower exhibited significant anthelmintic activity as compared to petroleum ether extract.

Keywords: Anthelmintic activity, *Woodfordia fruticosa*, *Pheretima posthuma*, Piperazine, Preliminary phytochemical.

INTRODUCTION

Woodfordia fruticosa is an important traditional medicinal plant belonging to the family Lythraceae. It is extensively used in the preparation of “Ahavas and Arishtas” containing self generated alcohol. The flowers of *Woodfordia fruticosa* Kurz are commonly used for the treatment of several ailments which includes rheumatism, leucorrhoea, menorrhagia, asthma, liver disorder and inflammatory conditions¹. The flowers are astringent, acrid, refrigerant, stimulant, depurative, typtic, uterine sedative, antihelmintic, antibacterial and vulnerary, corrective of a pigments, alexeteric and febrifuge. It has antibacterial², antifertility³ activities. Moreover it can be used as a mordant in the preparation of dyes⁴. A mixture of *Woodfordia fruticosa* powder, honey and rice water is extremely affected in diarrhoea, dysentery and piles. It can be safely used even in pregnancy. The decoction of flowers effectively quenches the excessive thirst, especially in diabetic patients⁵. The flowers of this plant have various medicinal uses, possess high content of tannins and have been used as an astringent tonic in disorders of mucous membrane^{6,7}. The powder of dried flowers is sprinkled over ulcers and wounds to diminish discharge and promote granulation⁸. The flowers also showed hepatoprotective activity⁹. The previously isolated constituents from *Woodfordia fruticosa* flowers are ellagitannin dimmers with astringent and haemostatic properties that affect histamine release¹⁰. Literature review indicates that anthelmintic activity of the flowers of *Woodfordia fruticosa* has not been clinically evaluated so far. The present paper reports the preliminary phytochemical screening and anthelmintic activity of methanolic and petroleum ether extracts of the flowers of *Woodfordia fruticosa* against Earthworms.

MATERIAL AND METHODS

Plant Material

The fresh flowers of *Woodfordia fruticosa* were collected from Assam in the month of January 2013. The plant were identified and authenticated by a taxonomist and Voucher

herbarium specimen was deposited in the Department of Pharmacognosy of ROFEL, Shri G.M.B college of Pharmacy for future reference bearing a No-RPC/RS/009. The plant material was dried in sunlight, pulverized and stored in air tight container and used for further extraction.

Preparation of Extract

Fifty g of shade dried, powder of flowers of *Woodfordia fruticosa* Kurz were extracted successively with 200 ml each of Petroleum ether and Methanol, using a Soxhlet extractor for 48 h separately. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 5°C in an airtight bottle until further use. The percent yield of methanolic extract was 20.8 % w/w and petroleum ether (60 Grade) extract yield 5.1 % w/w.

Preliminary Phytochemical Screening

Chemical tests were carried out on the methanolic and petroleum ether extract using standard procedures to identify the constituents^{11,12}. The plant extract was assayed for the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, steroids, glycosides, carbohydrates and Proteins or amino acids.

Testing for Alkaloids

Each extract (0.5 g) was stirred with 5 mL of 1 % HCL on a steam bath. The solution obtained was filtered and one mL of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extract.

Testing for Tannins and Phenolics

Each extract (0.5 g) was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5 % FeCl₃ reagent were added to the filtrate. Blue-black or blue green colouration or precipitation was taken as an indication of the presence of phenolics and tannins.

Test for Saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and observed for the formation of emulsion.

Test for Steroids

Two ml of acetic anhydride was added to 0.5 ethanolic extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face formed to show positive results for the presence of terpenoids.

Test for Steroid (Liebermann Burchard Reaction)

To extract Chloroform, 2 ml acetic anhydride and 2 drops conc. H₂SO₄ was added from the side of the test tube. No red or blue colour was appeared indicating the absence of steroids.

Legal's test for Glycosides

To the hydrolysate 1 ml of pyridine and few drops of sodium nitropruside solution was added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's Test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonical layer turned pink colour shows the presence of anthraquinone glycosides.

Test for Carbohydrates (Molisch's test)

A small quantity of the extract were dissolved separately in 4 ml of distilled water and filtered. The filtrate was treated with 2-3 drops of 1 % alcoholic alpha naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of the two liquids showed the presence of Carbohydrates.

Test for Amino Acids (Million's test)

The above prepared mixture was treated with Million's reagent. No red colour formed due to absence of proteins or amino acids.

Screening of Anthelmintic Activity

Worm Collection and Authentication

Indian adult earthworms (*Pheretima posthuma*) were collected from moist soil and water logged areas at Vapi, namdha Road in the rainy season and was identified at the Department of Pharmacology, ROFEL, Shri G.M.B College of Pharmacy. Then all collected worms were washed with normal saline to remove all the faecal matter and used for the anthelmintic study. The earthworms of 4-5 cm in length and 0.1-0.3 cm in width were used for all the experimental protocol.

Sample Preparation

Test sample for *in vitro* study were prepared by dissolving and suspending 2.5 g of each extract in minimum amount of DMF and the volume was adjusted to 25 ml with normal saline to obtain a stock solution of concentration of 100 mg / ml. from this stock solution different dilutions were made to get concentration range of 50, 75 and 100 mg / ml.

Drugs and Chemicals

Piperazine citrate (Glaxo Smithkline), Albendazole (BANDY, Mankind Pharma Ltd., New Delhi, India), Chemicals: Methanol A.R (PCL, Pune, India), DMF (PCL, Pune, India), Saline water (Claris Lifesciences Ltd., Ahmedabad, India).

Anthelmintic Activity

The anthelmintic activity was performed according to the method of Ajaiyeoba *et.al*, 2001¹³. On adult Indian earthworm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings^{14,15}. *Pheretima posthuma* worms are easily available and used as suitable model for screening anthelmintic drugs¹⁶. Earthworms were divided into nine groups (6 each). The first group (I) served as normal control which received saline water only. The second (II) group received the standard drug Piperazine citrate at a dose level of 10 mg / ml. Groups (III) group received the standard drug Albendazole at a dose level of 20 mg / ml. Groups (IV) to (VI) received doses of Pet. Ether extracts of 50 mg / ml, 75 mg / ml and 100 mg / ml, respectively. Groups (VII) to (IX) received doses of methanolic extracts of 50 mg / ml, 75 mg / ml and 100 mg / ml, respectively. Observations were made for the time taken to paralyse or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body colour. Piperazine citrate (10 mg / ml) and Albendazole (20 mg / ml) were used as reference standards and normal saline water as control. All the results were expressed as Mean ± S.D of six worms in each group.

RESULTS AND DISCUSSIONS

Preliminary screening of the flowers of *Woodfordia fruticosa* revealed the presence of carbohydrates, tannins and glycosides in major quantities, phenols in moderate quantities and anthraquinones and flavonoids in minor quantities. Tannins were shown to produce anthelmintic activities. Chemically tannins are polyphenolic compounds. It is possible that tannins contained in the flower extracts of *Woodferdia fruticosa* produced similar effects. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. The exact mechanism of the anthelmintic activity of *Woodferdia fruticosa* cannot be explained on the basis of the present result. From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. While there need further study to isolate and revealed the active constituents responsible for the anthelmintic present in the crude extract of flowers of *Woodferdia fruticosa* as well as to establish the mechanism of action.

Table 1: Phytochemical Analysis of Methanolic and Petroleum Ether Extracts of *Woodfordia fruticosa* Flower

S. No.	Constituents	Tests	Petroleum Ether Extract	Methanolic Extract
1	Alkaloids	Mayer's test	-----	---
2	Saponins	Foam test	-----	-----
3	Tanin	Extract + 5 % Fecl3		+++
4	Terpinoid	Salkowski test	-----	-----
5	Steroid	Liebermann burchard reaction	---	-----
6	Glycosides	Legal's test	++	+++
7	Phenolic compound	Extract + 5 % Fecl3	++	++
8	Flavonoids	Residue + lead acetate solution	---	+
9	Carbohydrats	----	++	+++
10	Amino acids	-----	-----	---
11	Anthraquinones	-----		+

+++ Major, ++ Moderate, + Traces, - Absence

Table 2: Anthelmintic Activity of Methanolic and Petroleum Ether Extracts of *Woodfordia fruticosa* Flower

Test substance	Groups	Concentration (mg/ml)	Time taken for paralysis(min.) (Mean± S.D)	Time taken for Death(min.) (Mean± S.D)
Vehicle	I	-----	No paralysis (upto72 minutes)	No paralysis (upto72 minutes)
Piperazine citrate (Std.)	II	10	21.44 ± 0.27	60.01 ± 0.32
Albendazole (Std.)	III	20	32.86 ± 0.21	43.50 ± 0.24
Methanolic extract	IV	50	22.76 ± 1.67	44.62 ± 3.24
	V	75	16.03 ± 2.42	33.02 ± 4.79
	VI	100	12.07 ± 1.66	26.08 ± 3.87
Petroleum ether extract	VII	50	36.19 ± 1.89	56.33 ± 1.98
	VIII	75	26.89 ± 3.43	48.97 ± 2.26
	IX	100	18.36 ± 4.06	36.53 ± 2.54

Each value represents the mean ± SD (n = 6). This activity was Concentration dependent. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms

CONCLUSION

From the above results, it is concluded that *Woodferdia fruticosa* flower used traditionally to treat intestinal worm infections, showed significant anthelmintic activity. Methanolic extract showed better anthelmintic activity in comparison to the Pet. ether extract of *Woodferdia fruticosa* flowers. The phytochemical profile showed the presence of carbohydrates, tannins and glycosides in major quantities, phenols in moderate quantities and anthraquinones and flavonoids in minor quantities. Further studies are required to identify the exact chemical constituents present in the flower extracts of this plant responsible for anthelmintic activity.

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