

FORMULATION AND EVALUATION OF HERBAL DRUG TABLETS OF WOODFORDIA FRUTICOSA AND IN-VITRO EVALUATION OF ITS ANTI-ULCER, ANTI-MICROBIAL AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Aim: The present work aims to develop a tablet formulation of the powdered drug Woodfordia Fruticosa of varied doses and to evaluate the strength of the developed formulation. Woodfordia fruticosa is a herbal plant belonging to Lythraceae family of Sapindales. The powdered form of drug is used widely in Ayurvedic formulations as an excipient to enhance the effect of the active ingredient. **Materials and Methods:** The tablets are formulated using various excipients and evaluated for the strength and quality control tests such as hardness, friability, weight variation and disintegration time. The evaluated tablets were further examined for their medicinal properties. The medicinal usefulness of the drug was hypothesized depending upon the plant's chemical composition. **Results:** Different doses of the tablet were prepared and the physical appearance of the drug was maintained during formulation. The hypothesized usefulness of the drug was checked through in vitro examination for various medicinal properties

like Anti ulcer, Anti Microbial, Anti Inflammatory activities respectively. Suitable methods were selected for the so called In vitro examination of the drugs medicinal properties and the

results obtained were further evaluated to proceed to conclusion. All the methods used for the study were approved by I.P and USP. **Conclusion:** The formulated tablets of *Woodfordia* showed positive results to all the tests and evaluation performed via in-vitro.

KEYWORDS: *Woodfordia fruticosa*, Anti ulcer activity, Anti inflammatory activity, Anti microbial activity.

1. INTRODUCTION

Woodfordia fruticosa commonly known as fire flame bush or dhataki belongs to Lythraceae. It is mainly found in tropical and sub tropical regions. It is rich in chemical compounds like tannins, anthraquinone glycosides, polyphenols and flavanoids. The leaf and flower extracts of *woodfordia fruticosa* possesses significant pharmacological activities. Hence it is mostly used by traditional medicine practitioners of south east asian countries in ayurvedic and unani medicine (Chopra *et al.*, 1956, Att, 1972, Dymock *et al.*, 1995). Among all the parts of plant, flowers possess huge demand in both in domestic and international markets Dhataki is useful in managing leucorrhea, augmenting skin health, improves digestion, promotes oral health, fights respiratory related problems, good at managing diabetes as well as menorrhagia.



Fig. 1: *Woodfordia fruticosa* plant.

2. MATERIALS AND METHODS

Different concentrations of *Woodfordia Fruticosa* (50,100,250,500mg) tablets were prepared using sodium saccharin, magnesium stearate, talc and lactose as excipients.

Preparation of woodfordia fruticosa tablets: Initially, all the ingredients required for the formulation were weighed accurately on the sensitive balance. In the second step, *Woodfordia fruticosa* powder, lactose and sodium saccharin were grinded using mortar and pestle and the

powder mixture was sieved through sieve no. 20. Later, talc and magnesium stearate were added to it and mixed well. From the above powder mixture, ten portions were weighed and tablets were prepared by direct compression method.



Fig. 2: Woodfordia Fruticosa tablets.

Characterization of formulated tablets of woofordia fruticosa

The tablets formulated were subjected to various evaluation parameters to assess their quality. The main parameters observed were

Organoleptic properties of tablets

These properties include colour, odour, taste and appearance of the tablet. They play a major role in patient acceptance of medication.

Weight variation test

Based on USP guidelines, weight variation test is performed by taking weights of 20 tablets individually and comparing them with their total average weight. For the average weight of 130 mg or less than 130mg, the acceptable percentage difference is 10. For more and 130mg and less than 324mg, it is 7.5 and for more than 324mg average weight, it is 5.0.

Friability test: Tablet friability is measured by the use of the Roche friabilator. For this test 10 tablets are weighed and kept in it. They are exposed to continuous rolling and repeated shocks when they fall from 6 inches height inside the apparatus. The instrument is run at 25rpm for 4 min. then the tablets are removed and weighed. The difference in the two weights is used to calculate friability and the value of friability is taken in percentage. It is calculated using the following formula.



Fig. 3: Roche friabilator.

Tablet hardness

Hardness of the tablet can be tested both manually and by using Monsanto hardness tester. Tablet hardness can be measured as the force needed for breaking the tablet when force is applied diametrically. This test is done to know if a tablet can resist the breakage while transporting, storing or during their use.



Fig. 4: Monsanto hardness tester.

Tablet disintegration test

$$\text{Friability (\%)} = \frac{\text{Initial Weight (W1)} - \text{Final Weight (W2)}}{\text{Initial Weight (W1)}} \times 100$$

In disintegration test, the time taken for a batch of tablets to disintegrate under specified conditions is measured. The formulated tablet passes the test if it reaches the end point in time less than the given limit.



Fig. 5: Disintegration apparatus.

In vitro evaluation of anti-ulcer activity

The formulated tablets of *Woodfordia fruticosa* are evaluated for their Anti ulcer activity by In-vitro method. The method of choice for the determination of anti ulcer activity is the Acid neutralization technique.

Procedure: The anti-ulcer activity of *Woodfordia Fruticosa* tablets is explained by Acid-neutralization reaction. The acid neutralizing capacity values for *Woodfordia* tablets are compared with the standard extract of Aluminum Hydroxide-Magnesium hydroxide (AHMH). A single tablet of 50 mg was taken in a beaker and mixed with 3ml of 1N HCl and the volume to made up to 10ml with distilled water and stirred to dissolve the tablet. The contents were transferred into a conical flask and titrated against 0.25 N NaOH. Phenolphthalein solution is used as an indicator and the endpoint is the appearance of pink color. The procedure is repeated by taking the varied concentrations of the tablet and the results are compared with the standard solution of Aluminum Hydroxide-Magnesium hydroxide. The results showed Concentration dependent reduction in anti – ulcer activity.

Calculations

The moles of acid neutralized is calculated by the formula

Moles of acid neutralized = (Vol. of HCl × normality of HCl) - (Vol. of NaOH × normality of NaOH). Acid neutralizing capacity (ANC) Per gram of antacid = Moles of HCl neutralized ÷ Grams of Antacid/extract.

In vitro evaluation of anti microbial activity

Procedure

Bacterial cultures required for the experiment were procured from microbiology lab of St. Paul's college of pharmacy. Bacterial culture included in these studies was *Escherichia coli*. The culture was grown in Mueller Hinton agar (media).

Preparation of agar medium

Requirements: Agar-1.5gm, Beefextract-1gm, NaCl-0.5gm, Peptone-1 gm, Water to make 100ml.

Anti microbial assay

The media prepared was transferred into the dishes. When the media reaches to 40-42°C, the bacterial strain was inoculated carefully to ensure correct homogenization. Different concentrations of **ethanolic** extract of dry *Woodfordia fruticosa* flowers were prepared and tested against *Escherichia coli* using agar well method to identify their antimicrobial activity. Mueller hinton agar plates were prepared and kept aside to solidify. Every plate was seeded with test microorganism and **three** wells were made on every plate using sterile 2.0mm cork borer. Two wells were filled with the test solution i.e., *woodfordia fruticosa* and other well with the standard solution of Omeprazole. Later, the agar plates were incubated at 37°C for 24 hours. After incubation, the plates were removed from incubator and the area of zones of inhibition were measured using scale and mean value for each microbe was recorded.

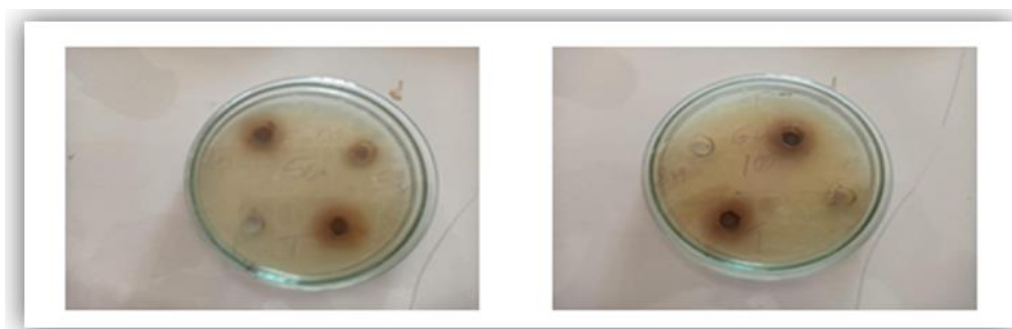
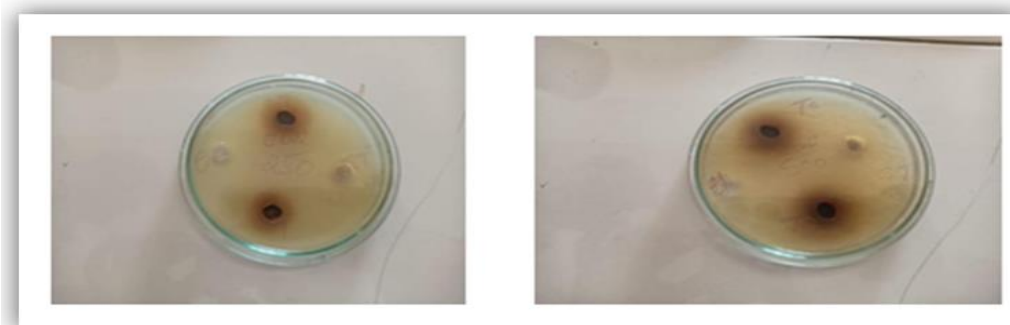


Fig. 6: 150mg Agarwell.

Fig. 7: 200mg Agarwell.

**Fig. 8: 250mg Agarwell.****Fig. 9: 500mg Agarwell.**

In-vitro anti-inflammatory activity

The objective is to evaluate the anti-inflammatory activity of woodfordia fruticosa using In vitro protein denaturation method. Materials required: Diclofenac sodium, Woodfordia fruticosa, Egg Albumin, Phosphate buffer, Distilledwater.

Instruments: Incubator, UV Spectrophotometer

For Invitro anti inflammatory activity, 0.2 ml of fresh hen's egg albumin is taken later 2.8 ml of phosphate buffered saline of pH 6.4 is added to it. Then, 2ml of different concentrations of woodfordia fruticosa were added so that final concentrations became 10, 20, 30, 40, 50, 60, 70, and 80 mg/ml and same quantity of double-distilled water was used as control. Later all the prepared mixtures were incubated at 37 +2°C temperature in a BOD incubator for 15min and later heated at 70°C for five minutes and then allowed to cool. After cooling, the absorbance of all the mixtures were taken at 660nm on SHIMADZU, UV 1800 by using the distilled water as blank and Diclofenac sodium in varying concentrations of 10, 20, 30, 40, 50, 60, 70, and 80mg/mL as reference.

Formula for The percentage inhibition of protein denaturation :

$$\% \text{inhibition} = 100 \times [Vt/VC - 1]$$

Where

Vt= absorbance of the test sample and Vc = absorbance of control.

3. RESULTS AND DISCUSSION

Ten doses of woodfordia fruticosa loaded herbal tablets of varied concentration were formulated and evaluated for their quality using quality determination tests.

Organoleptic properties: The woodfordia fruticosa tablets had grey colour, saunf like smell with sweet taste and good appearance.

Weight variation test

Not more than 2 tablets should be out of upper and lower limit values or should not be twice the limits. If the tablets were within the limits, it passes the test. The weight variation tolerance information is given in Table 1.

Table 1: Weight variation tolerance.

Dose	Weight limit	Average weight
50mg	18.02	360 ±18.02
100mg	17.35	374 ± 17.35
250mg	19.92	398 ±19.92
500mg	25.35	507 ± 25.35

Friability test: The percentage friability of all formulated dosage forms were given in table 2.

Table 2: Percentage friability of each dosage form.

Dose	W1	W2	%friability
50mg	3.570	3.560	0.280%
100mg	3.450	3.400	3.18%
250mg	3.400	3.880	0.3%
500mg	5.02	4.92	1.99%

Hardness test: The results of hardness test were given in table 3.

Table 3: Hardness test results using monsanto hardness tester.

50mgtablet:	$7+8+9/3=24/3=8$
100mgtablet:	$6+7+7/3=20/3=6.6$
250mgtablet:	$7+7+7/3=21/3=7$
500mgtablet:	$4+6+5/3=15/3=5$

Disintegration time: The results of disintegration time of woodfordia tablets is given in table 4.

Table 4: Disintegration time of tablets.

Dose	Disintegration time
50mg	4mins47secs
100mg	6mins12secs
250mg	6mins45secs
500mg	7mins15secs

In-vitro evaluation of anti-ulcer activity

The formulated tablets of woodfordia fruticosa were evaluated for Anti-Ulcer activity by In-vitro method. The method used was the acid neutralization titration. Aluminum hydroxide-Magnesium hydroxide(AH-MH-500) tablet was used as a standard. The Acid neutralizing

capacity of woodfordia fruticosa tablets is given in table5.

Table 5: Acid neutralizing capacity results of Wood for diafruticosa tablets by in–Vitro method.

Concentration of Test sample	Volume of NaOH consumed	M.Eq of acid consumed	ANC per gram of antacid
Woodfordia-50mg	32.4	11.25	112.7
Woodfordia-100mg	25.3	15.6	42.17
Woodfordia-250mg	40.5	9.8	10.22
Woodfordia-500mg	35.4	11.5	6.10
AHMH-500mg	47.2	7.75	11.90

Evaluation of anti-microbial activity of Woodfordia fruticosa

The antimicrobial activity of the different concentrations was checked against E.coli. Zones of inhibition for different concentrations of Woodfordia Fruticosa tablets against gram negative bacteria were measured. The formulated tablets are deemed to possess Anti-microbial properties. The values of zones of inhibition were given in table 6.

Table 6: Zone of inhibition.

Dose	Content	Zone of inhibition
50mg	Control	0cm
	Test	0.2cm
	Standard	0.4cm
100mg	Control	0cm
	Test	0.3cm
	Standard	0.5cm
250mg	Control	0cm
	Test	0.5cm
	Standard	0.6cm
500mg	Control	0cm
	Test	1.0cm
	Standard	0.8cm

Evaluation of Anti inflammatory activity of woodfordiafruticosa tablets: The Absorbance values of tablet formulation and standard drug diclofenac sodium were given in table 9. The calibration curve showing comparison of absorbance of Woodfordia fruticosa against Diclofenac sodium is shown in figure no 7.

Table 7: Absorbance rate.

Concentration (mg/ml)	Absorbance	
	Woodfordia	Diclofenac sodium
10	0.071	0.052

20	0.14	0.096
30	0.191	0.182
40	0.33	0.216
50	0.395	0.292
60	0.567	0.383
70	0.761	0.55
80	0.921	0.734

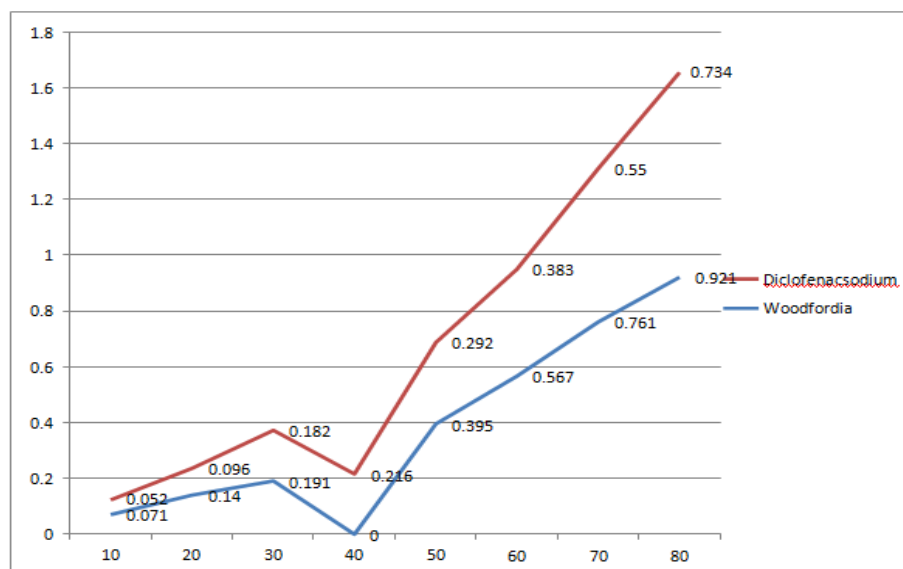


Fig. 7: Calibration curve showing comparison of absorbance of Woodfordia fruticosa against Diclofenac sodium Absorbance of Woodfordia Fruticosa (Test).

Slope Equation and R^2 Value found to be $y = 0.012x - 0.127$ $R^2 = 0.961$ The % of Drug found to be: 109 %

Absorbance of diclofenac sodium (Standard)

Slope Equation and R^2 Value found to be $y = 0.009x - 0.100$ $R^2 = 0.933$

The % of Drug found to be: 115%

%inhibition = $100 \times [Vt/VC - 1] = 100 \times [109/115 - 1] = 95.33 \%$

The whole project involves identification of the usefulness of Woodfordia fruticosa in the field of medicines by carrying out In-vitro experiments. These experiments can serve as a basis for further investigation of the products usefulness in these above aspects.

CONCLUSION

Woodfordia fruticosa tablets were prepared with the aim to provide a concise unit dosage form of the powder which is already being used in Ayurvedic formulation as a churna. The tablets were prepared by direct compression by using necessary excipients. The tablets were

evaluated for hardness by carrying out various tests. The formulated tablets were evaluated for their Anti-ulcer, Anti-inflammatory and Anti-microbial activities using In vitro methods. Based on IP and USP, the evaluation tests of tablets were done on each dose of the tablet. The In-vitro evaluation was done on the tablet to determine its beneficial activities. The tablets had enough hardness to withstand various handling conditions. The results of all the in-vitro tests done on the formulated tablets were active and positive. Finally, it is concluded that these In-vitro test results do not fulfill the norms to make the tablets useful for the said effect and cannot be enough evidence to prove their usefulness in the said condition but instead pave the way for further investigation of the drug in the above aspects. The above results can be helpful for carrying out In-vivo evaluation of the drug for the mentioned activities.

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