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ORIGINAL ARTICLE

Chromatographic Profiling of Ellagic Acid in *Woodfordia fruticosa* Flowers and their Gastroprotective Potential in Ethanol-induced Ulcers in Rats

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ABSTRACT

Background: *Woodfordia fruticosa*, a plant of Indian origin, is extensively used in folk medicine for the treatment of various ailments. **Objective:** The aim of the present study was to standardize the flowers of *W. fruticosa*, Kurz (*Lythraceae*), an important plant of Indian origin and explore the chemical constituents contributing to its anti-ulcer activity. **Materials and Methods:** High-performance thin layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) profiling of the three samples of *W. fruticosa* flowers purchased from three different markets was done using ellagic acid as the biomarker. Two doses of the aqueous extract of the *W. fruticosa* (AEWF) flowers were evaluated for anti-ulcer activity by ethanol-induced ulcer model in Wistar albino rats. Omeprazole was used as the positive control. The parameters used for the assessment of the anti-ulcer potential were total titratable acidity (TTA), ulcer index, and percentage protection. **Results:** The HPTLC and HPLC studies confirmed the presence of ellagic acid in all the three drug samples. The AEWF showed significant reduction in terms of TTA at both doses of 100 mg/kg and 200 mg/kg. The gastroprotection indicated by a lower ulcer index and higher percentage protection was significant for 200 mg/kg dose of AEWF, better than the protection afforded by omeprazole (10 mg/kg). **Conclusion:** The chromatographic profiling and the anti-ulcer studies served as an efficient tool in the characterization of ellagic acid as an important biomarker for the flowers of *W. fruticosa* and a probable contributor to the gastroprotective capacity of the drug. The bioactivity studies further supported the traditional use of *W. fruticosa* in the treatment of ulcers.

Key words: Anti-ulcer activity, ellagic acid, ethanol-induced ulcer, high-performance thin layer chromatography, *Woodfordia fruticosa*

SUMMARY

- HPTLC & HPLC fingerprinting of *W. fruticosa* using ellagic acid as a biomarker.
- Evaluation of *W. fruticosa* for gastroprotection potential in ethanol induced gastric ulcer in rats model.
- Aqueous extract of the drug showed better gastroprotection than the standard drug omeprazole at a dose of 200 mg/kg.

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INTRODUCTION

There is a balance in the stomach between the aggressive digestive capabilities of the acid plus pepsin and the mucosal barrier. The disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance leads to ulceration. Several factors such as increased acid pepsin secretion or impaired bicarbonate neutralization or mucus secretion and precipitated lesions on the mucosal layer are implicated in the pathogenesis of gastric ulcer.[1]

Woodfordia fruticosa is one of two species of the genus *Woodfordia*, Salisbury, in the family *Lythraceae*. It is well-known in many parts of Asia as a plant possessing medicinal values.[2] The generic name honors E. James Alexander Woodford (e), a botanist, physician, and contemporary of Salisbury, Edinburgh.[3] This plant is commonly known by various vernacular names such as fire-flame bush, dhai ke phul, dhataki, and bahupushpi.[4] The species are long-lived shrubs, typically 2–3 m in height, with a sprawling, untidy appearance which is the result of an irregular sympodial branching pattern. The Asian species *W. fruticosa* is distributed principally along the lower Southern slopes of the Himalayas from Kashmir into Yun-nan Province of China and extends southward along the Western Ghats in India.[3] The flowers and leaves of the plant *W. fruticosa* Kurz (*Lythraceae*) have been a source of great value in traditional systems of medicines.[5] The leaves and flowers of *W. fruticosa* are popularly employed in folk medicine for fever, hemothermia, persistent dysentery, menorrhagia, and seminal weakness.[6] The dried flowers are powdered and dusted over ulcers and wounds to eliminate discharge and promote granulation.[7] While chemical investigations on various extracts of the flowers of *W. fruticosa* have shown the presence of several bioactive compounds such as flavonoids, saponins,[8] polyphenols, tannins,[9] and alkaloids.[5] In separate studies, bioactivity studies have demonstrated the gastroprotective, gastric healing, and anti-diarrheal activities of these phytoconstituents, viz., flavonoids,[10] saponins,[11] polyphenols,[12] alkaloids,[13] in other plants.

An herbal product contains multiple constituents that might be responsible for its therapeutic effects. It thus becomes necessary to define as many of the constituents as possible to understand and explain the bioactivity. The concept of "phytoequivalence" has been introduced in Germany to ensure consistency of phytotherapeuticals. According to this concept, a chemical profile for an herbal product is constructed and compared with the profile of a clinically proven reference product.[14]

Thus, the present study is aimed at evaluating the gastroprotective potential of *W. fruticosa* flower extracts. The alcoholic and hydro-alcoholic extracts of *W. fruticosa* flowers along with the ellagic acid standard have

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been subjected to chromatographic analyses followed by bioactivity studies in the conquest to explore the chemical compounds probably responsible for the gastroprotective activity.

MATERIALS AND METHODS

Drugs, chemicals, and materials

The three drug samples of the dried flowers of *W. fruticosa* were collected from the local market in Hyderabad, Telangana; Bangalore, Karnataka; and Nahan, Himachal Pradesh, respectively. Ellagic acid was purchased from Sigma-Aldrich. Potassium dihydrogen orthophosphate and acetonitrile were obtained from Loba Chemie, India, and Merck, India, respectively. Solvents and mobile phases used for high-performance liquid chromatography (HPLC) were of HPLC grade obtained from Rankem, India. All solvents used were of analytical grade.

Thin layer chromatography

Thin layer chromatography (TLC) profiling of the three samples of *W. fruticosa* purchased from Hyderabad, Telangana; Bangalore, Karnataka; and Nahan, Himachal Pradesh, along with the ellagic acid (standard biomarker) were subjected to TLC profiling to optimize the solvent systems and conditions.

Method of extraction

Powdered sample (1 g) was refluxed with 50 ml 70% ethanol for 30 min. The solution was filtered and again the marc was extracted with the same menstruum until colorless. The extracts were pooled and evaporated on a water bath and the residue was redissolved in the same solvent and made up to 50 ml. Freshly prepared extracts were used for TLC studies.[15]

Preparation of standards

Ellagic acid solution was prepared by dissolving 3 mg of the standard in 5 ml of HPLC water (3 mg/5 ml).

Solvent systems

The solvent system toluene: chloroform: ethyl acetate: formic acid (2:6:6:2) designated as solvent system A and toluene: ethyl acetate: methanol: formic acid (6:6:1.6:0.4) designated as solvent system B were optimized for the drug samples with respect to the standard used.[16,17]

High-performance thin layer chromatography fingerprinting

TLC procedure was standardized with the solvent systems A and B which were used for the high-performance TLC (HPTLC) fingerprinting of the three samples of the *W. fruticosa* flowers along with the standard ellagic acid. The sequence of the tracks is given in Table 1. HPTLC fingerprinting analysis was done using the CAMAG linomat 5 sample applicator using micro syringe (100 µl, Hamilton), CAMAG reprostar 3, Twin trough chamber, Dip tank, Win cat software - Version 1.3.3., with a development distance of 70 mm. Precoated silica gel plates 60F254 from Merck (20 cm × 10 cm) were used. Samples and standards were applied as bands on the plates in volumes 10 µl and detection was done under ultraviolet (UV) 254, UV 366, and after derivatization with 5% methanolic ferric chloride reagent.

High-performance liquid chromatography

Standard solution

The stock solution of ellagic acid (standard biomarker) was prepared in HPLC grade water. The stock solution was diluted in the mobile phase to make the final concentration of 3 µg/5 ml.

Method of extraction

The sample of *W. fruticosa* flowers weighing 0.125 g pulverized (16 mesh size) was extracted using HPLC grade water (25 ml) as solvent and sonicated for 30 min. The extract was filtered and made up to 25 ml with the same solvent and filtered through 0.45 µ membrane filter and used for HPLC analysis.[18]

High-performance liquid chromatography conditions

The liquid chromatographic system used was a gradient type HPLC Shimadzu system comprising of a pump (LC-10ATVP, Shimadzu, Japan), a UV-visible detector (SPD-10A Shimadzu) equipped with CLASS-VP6 software, and a rheodyne sample injector fitted with a 20 µl sample loop. The chromatographic separation was carried out on Merck C18 (250 mm × 4.6 mm) column with 5 µ particle size. The solvent system was acetonitrile: 5 mM potassium dihydrogen orthophosphate (KH_2PO_4) (95:5, v/v) buffer adjusted to pH 2.5 with dilute orthophosphoric acid and filtered with Whatman filter paper. The total run time for sample analysis was 18 min with a flow rate of 1.5 ml/min at room temperature. The UV detector was operated at 254 nm. The time program is given in Table 2.

Acute toxicity studies

Acute oral toxicity of the aqueous extract of the *W. fruticosa* (AEWF) in mice was carried out as per OECD guidelines (425). Healthy adult albino mice (25–30 g) were used for the study. They were divided into two groups each containing six animals. Group I animals were treated with distilled water (2 ml/kg), and group II administered with single dose of 2000 mg/kg extract by gastric intubation using a soft catheter. The animals were observed continuously after 2 h and then intermittently at gaps of 1 h and observed for mortality during 48 h study period toxicity (short-term). The short-term profile of the drug was used to determine the dose of the next animals as per OECD guideline 425 with minor modifications. Calculation of the LD_{50} of the test extract was done using AOT 425 software provided by environmental protection agency, USA.[19]

Experimental animals

Thirty male Wistar rats weighing 180–200 g were used for the study. The animals were housed in five groups of six animals in standard laboratory cages and fed on balanced rat chow and water *ad libitum*

Table 1: Application pattern of samples and standard ellagic acid on high-performance thin layer chromatography plate

Band number	Samples	Injection volume (µL)	Amount (µg)
1	*W. fruticosa* sample (HYD)	10	200
2	*W. fruticosa* sample (BNG)	10	200
3	*W. fruticosa* sample (NHN)	10	200
4	Ellagic acid standard	5	3

W. fruticosa: *Woodfordia fruticosa*; HYD: Hyderabad, Telangana, sample of *Woodfordia fruticosa* flowers; BNG: Bangalore, Karnataka, sample of *Woodfordia fruticosa* flowers; NHN: Nahan, Himachal Pradesh, sample of *Woodfordia fruticosa* flowers

Table 2: Time program for gradient elution of *Woodfordia fruticosa* samples and ellagic acid standard in high-performance liquid chromatography

Time (min)	Percentage (KH_2PO_4)	Percentage of acetonitrile
0.01	5.0	95.0
18.0	45.0	55.0
25.0	80.0	20.0
28.0	80.0	20.0
35.0	45.0	55.0
40.0	5.0	95.0
45.0	5.0	95.0

with temperature (22°C ± 3°C) and lighting (12 h light/dark cycle; lights on 0700 h) controlled.[20] All experimental animals were carried out according to the guidelines of the Institutional Animals Ethics Committee, MESCO College of Pharmacy, Hyderabad. The rats in group I served as normal control group which received distilled water (1 ml) orally and were not subjected to ulcer induction. Rats in group II were left untreated after ulcer induction by absolute ethanol (1 ml/200 g of rat) and designated as negative control. Rats in group III were administered with omeprazole (10 mg/kg) as positive control. Rats in group IV and V received the AEWF at doses of 100 mg/kg and 200 mg/kg, respectively.[21]

Selection of dose

The dose was selected as prescribed by The Ayurvedic Pharmacopoeia of India, 2007, i.e. for *W. fruticosa* flowers - 3 – 6 g.[4]

Approximately 4 g of the human dose of raw drug was calculated for rats by using dose conversion factor reported by Gosh.[22]

It is expressed as follows:

Animal dose (for 200 g animal) = Human dose × 0.018, where 0.018 is conversion factor

= 4000 mg × 0.018

= 72 mg/200 g of rat i.e. 360 mg/kg

Ethanol-induced ulcer model

The gastric ulcers were induced in rats by administering absolute ethanol (1 ml/200 g of rat) orally. All the animals fasted for 18 h before administration of ethanol. The rats were treated with the extract/omeprazole (10 mg/kg) orally using gastric lavage, 30 min before administration of 1 ml absolute ethanol. Untreated animals were included as negative controls. The animals were anesthetized 1 h later with anesthetic ether and sacrificed by cervical dislocation. The ulcers were examined by an incision on the stomach along the greater curvature end. Mean ulcer score for each animal was expressed as ulcer index. Percentage protection was also calculated with the ulcer index. Gastric juice was collected and gastric secretion studies were performed in terms of total titratable acidity (TTA).[23-25]

Ulcer score

The ulcers were scored as follows:

0 = Normal colored stomach,

0.5 = Red coloration,

1 = Spot ulcers

1.5 = Hemorrhagic streaks,

2 = Ulcers ≥3 but ≤5,

3 = Ulcers >5

The mean ulcer score was expressed as ulcer index for each animal.

Percentage protection

$$\text{Percentage protection} = \frac{\text{Negative control mean ulcer index} - \text{Test drug mean ulcer index}}{\text{Negative control mean ulcer index}} \times 100$$

Total titratable acidity

The TTA is expressed as follows:

$$\text{TTA} = \frac{\text{volume of NaOH} \times \text{normality}}{0.1} \times 100\,\text{mEq / l / h}$$

Statistical analysis

The data were expressed as the mean ± standard error of mean (SEM), and statistical analysis was performed using the Dunnet's *t*-test and ANOVA.

RESULTS

Preliminary studies

In our previous study, the *W. fruticosa* flowers purchased from three different geographical locations had been subjected to proximate analysis, successive solvent extraction, phytochemical screening, and quantification of total alkaloids, total saponins, and total polyphenols for the assessment of stability, repeatability, accuracy, and chief active phytoconstituents. The results have been discussed in detail in our previous publication.[5]

High-performance thin layer chromatography fingerprinting

Ellagic acid was used as the biomarker for the standardization of the three *W. fruticosa* samples using HPTLC fingerprint profiles. The HPTLC conditions described in the material and methods section allowed good separation for all the three *W. fruticosa* samples. Various solvent systems were tried for the separation. Good separation and resolution was achieved with two solvent systems; toluene: chloroform: ethyl acetate: formic acid (2:6:6:2) and toluene: ethyl acetate: methanol: formic acid (6:6:1.6:0.4) which were designated as solvent systems A and B. Development was done under UV 254, UV 366 nm, and after spraying with 5% methanolic ferric chloride.

The chromatograms developed using solvent system A and solvent system B for the three samples along with the standard ellagic acid are shown in Figures 1 and 2 under UV 254 nm, UV 366 nm, and after spraying with 5% methanolic $FeCl_3$, respectively. The results summarized in Tables 3 and 4 for solvent systems A and B, respectively, reveal that the standard ellagic acid showed a single band at R_f 0.21and 0.30, respectively. Apart from other bands, all the three *W. fruticosa* samples also showed a common corresponding band with the same R_f value as that of ellagic acid. After spraying with 5% methanolic $FeCl_3$, brown bands were observed for ellagic acid and the three *W. fruticosa* samples with solvent system A at the same R_f value. The plate developed with solvent system B showed violet color bands for ellagic acid and the corresponding bands in the three *W. fruticosa* samples after spraying with 5% methanolic $FeCl_3$. The results confirmed the presence of ellagic acid [Tables 3 and 4].

High-performance liquid chromatography fingerprinting

An attempt has been made to standardize the flowers of *W. fruticosa* along with ellagic acid by HPLC. The solvent system composition was optimized to acetonitrile and 5 mM potassium dihydrogen orthophosphate buffer pH 2.5 (95:5, v/v) by considering resolution, peak symmetry, and shape. Typical chromatograms of samples and ellagic acid [Figures 3-6] showed that all compounds were completely separated in 27 min. The chromatographic peaks of the ellagic acid in the three drug samples were identified by comparing their retention time with that of the ellagic acid standard. It can be observed in Table 5 that the peak at R_t = 12.3 min is common among all the drug samples and ellagic acid [Figure 7].

The HPLC method of standardization of *W. fruticosa* described herein is simple, sensitive, and precise and may be of value in standardizing the raw material for preparation of formulations containing this plant.

Acute toxicity studies

The AEWF flowers were found to be safe up to a dose of 2000 mg/kg body weight in adult albino mice.

Anti-ulcer activity

To evaluate the biological effect of *W. fruticosa* flowers for gastroprotective action and the probable contribution of ellagic acid to this effect, the

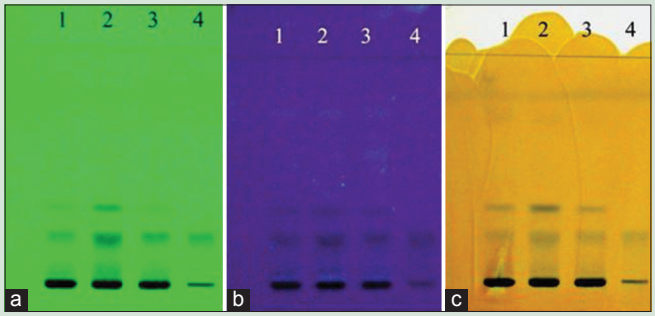

Figure 1: High-performance thin layer chromatography fingerprint of *Woodfordia fruticosa* samples and ellagic acid standard with solvent system A. (a) At ultraviolet 254 nm; (b) at ultraviolet 366 nm; (c) after spraying with 5% methanolic $FeCl_3$ reagent. 1: Hyderabad, Telangana, sample of *Woodfordia fruticosa* flowers; 2: Bangalore, Karnataka, sample of *Woodfordia fruticosa* flowers; 3: Nahan, Himachal Pradesh, sample of *Woodfordia fruticosa flowers*; 4: Standard ellagic acid

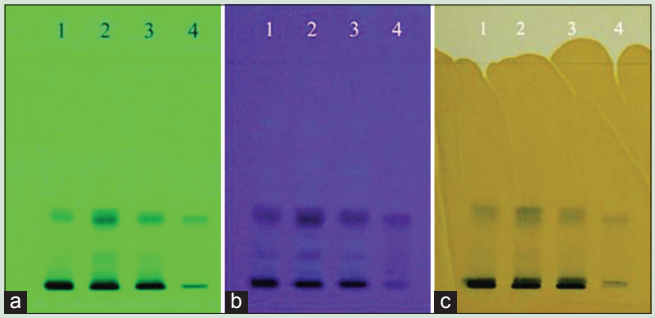

Figure 2: High-performance thin layer chromatography fingerprint of *Woodfordia fruticosa* samples and standard ellagic acid with solvent system B. (a) At ultraviolet 254 nm; (b) at ultraviolet 366 nm; (c) after spraying with 5% methanolic $FeCl_3$ reagent. 1: Hyderabad, Telangana, sample of *Woodfordia fruticosa* flowers; 2: Bangalore, Karnataka, sample of *Woodfordia fruticosa* flowers; 3: Nahan, Himachal Pradesh, sample of *Woodfordia fruticosa flowers*; 4: Standard ellagic acid

aqueous extract of the flowers was subjected to ethanol-induced ulcer model in albino rats. The results of this study of antiulcer properties of *W. fruticosa* in ethanol-induced ulcer model show a significant reduction in TTA in the aqueous extract treated animals [Table 6]. The reduction in TTA is highly significant at both doses of 100 mg/kg as well as 200 mg/kg body weight. With respect to ulcer index, the extract showed gastroprotective effects at both doses of 100 mg/kg and 200 mg/kg body weight [Table 6]. The gastroprotection indicated by a low ulcer index was significant for 200 mg/kg dose of AEWF and has offered better protection than omeprazole 10 mg/kg dose. The AEWF showed significant protection index of 78% and 64% with the doses of 100 and 200 mg/kg, respectively. Omeprazole as reference standard drug showed a protection index of 71% [Table 6].

DISCUSSION

This study deals with the chromatographic fingerprinting of the hydro-alcoholic and aqueous extracts of the flowers of *W. fruticosa* using ellagic acid as a biomarker and investigation of the gastroprotective activity of the water extract of the flowers of *W. fruticosa* on gastric lesions induced by alcohol as well as acid secretion parameters. This is the first report standardizing the different extracts of the flowers of *W. fruticosa* using ellagic acid as a biomarker in HPTLC and HPLC studies.

Standardization of herbal drugs aims at developing authentic analytical methods which can efficiently and reliably profile the phytochemical composition of medicinal plants, along with the quantitative analyses of marker/bioactive compounds and other major constituents. Standardization also serves as a tool for the establishment of a consistent biological activity and chemical profile.[26]

Results of the present chromatographic investigation confirm the presence of an important polyphenol ellagic acid in *W. fruticosa* flowers pointed out in the previous literature.[27] In HPTLC analysis very similar to that of the present study (solvent system A), Heeshma *et al.*[17] reported the presence of a spot at R_f value of 0.20 at UV 254 nm and a bluish-black spot at R_f value of 0.21. In the present study, we compared the spots at UV 254 nm, UV 366 nm, and after derivatization with brown spot of ellagic acid at R_f value of 0.21 in all the three hydro-alcoholic extract samples. To further substantiate the presence of ellagic acid in the extracts of *W. fruticosa* flowers solvent, system B reported by Bagul *et al.*[16] was tried which gave very clear bands at R_f value of 0.30 at UV 254 and 366 nm and a violet spot with 5% methanolic $FeCl_3$ reagent. This solvent system B reported for the quantification of ellagic acid content in a different study yielded precise, specific, and accurate results in the chromatographic profiling of *W. fruticosa* flowers.

Table 3: High-performance thin layer chromatography fingerprinting of *Woodfordia fruticosa* samples and standard ellagic acid with solvent system A

Band number	Samples	R_f values at UV 254 nm	R_f values at UV 366 nm	R_f values of bands with 5% methanolic $FeCl_3$ reagent
1	*W. fruticosa* sample (HYD)	**0.21** and 0.33	0.04 and **0.21** 0.58 and 0.77	**0.21 (brown)**, 0.33 (black)
2	*W. fruticosa* sample (BNG)	**0.21** and 0.33	0.04 and **0.21** 0.58 and 0.77	**0.21 (brown)**, 0.33 (black)
3	*W. fruticosa* sample (NHN)	**0.21** and 0.33	0.04 and **0.21** 0.58 and 0.77	**0.21 (brown)**, 0.33 (black)
4	Ellagic acid standard	**0.21**	**0.21**	**0.21 (brown)**

R_f value marked in bold is common across all samples and standard. *W. fruticosa: Woodfordia fruticosa*; HYD: Hyderabad, Telangana, sample of *Woodfordia fruticosa* flowers; BNG: Bangalore, Karnataka, sample of *Woodfordia fruticosa* flowers; NHN: Nahan, Himachal Pradesh, sample of *Woodfordia fruticosa* flowers

Table 4: High-performance thin layer chromatography fingerprinting of *Woodfordia fruticosa* samples and standard ellagic acid with solvent system B

Band number	Samples	R_f values at UV 254 nm	R_f values at UV 366 nm	R_f values of bands with 5% methanolic $FeCl_3$ reagent
1	*W. fruticosa* sample (HYD)	**0.30** and 0.33	0.06, 0.14, and **0.30** 0.52, 0.71	0.03 (violet), **0.30 (violet)**
2	*W. fruticosa* sample (BNG)	**0.30** and 0.33	0.06, 0.14, and **0.30** 0.52, 0.71	0.03 (violet), **0.30 (violet)**
3	*W. fruticosa* sample (NHN)	**0.30** and 0.33	0.06, 0.14, and **0.30** 0.52, 0.71	0.03 (violet), **0.30 (violet)**
4	Ellagic acid standard	**0.30**	**0.30**	**0.30 (violet)**

R_f values marked in bold are common across all samples and standard. *W. fruticosa*: *Woodfordia fruticosa*; HYD: Hyderabad, Telangana, sample of *Woodfordia fruticosa* flowers; BNG: Bangalore, Karnataka, sample of *Woodfordia fruticosa* flowers; NHN: Nahan, Himachal Pradesh, sample of *Woodfordia fruticosa* flowers

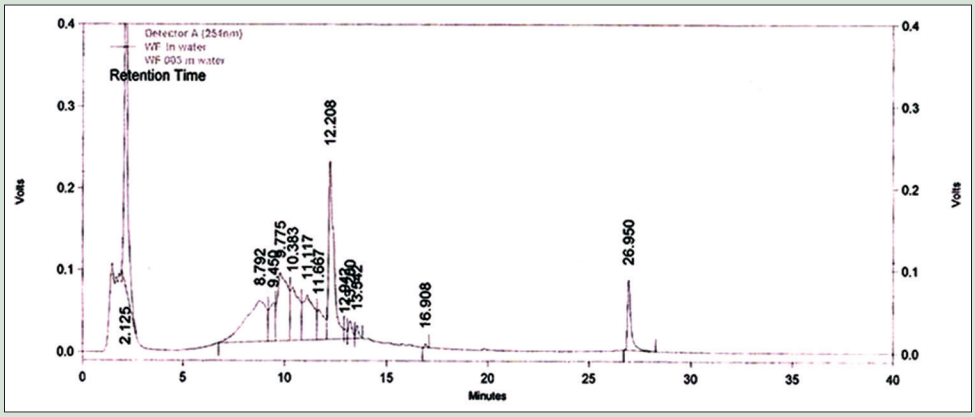

Figure 3: High-performance liquid chromatography chromatogram of aqueous extract of *Woodfordia fruticosa* flower sample (Hyderabad). HYD: Hyderabad, Telangana, sample of *Woodfordia fruticosa* flowers

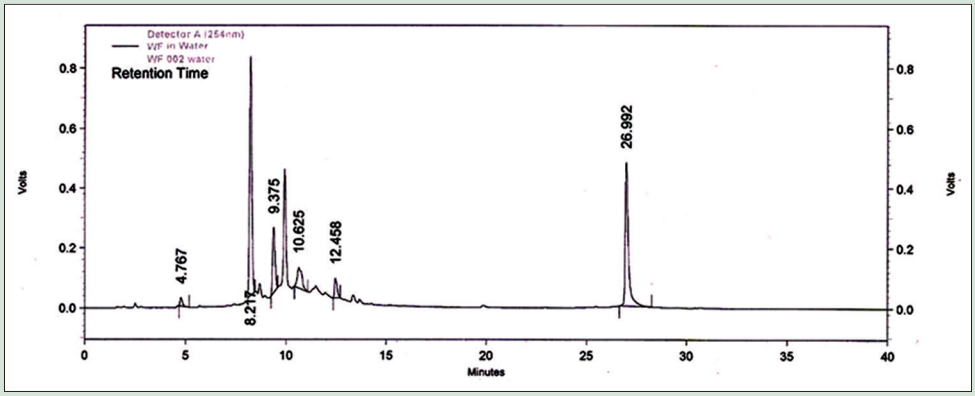

Figure 4: High-performance liquid chromatography chromatogram of aqueous extract of *Woodfordia fruticosa* flower sample (Bangalore). BNG: Bangalore, Karnataka, sample of *Woodfordia fruticosa* flowers

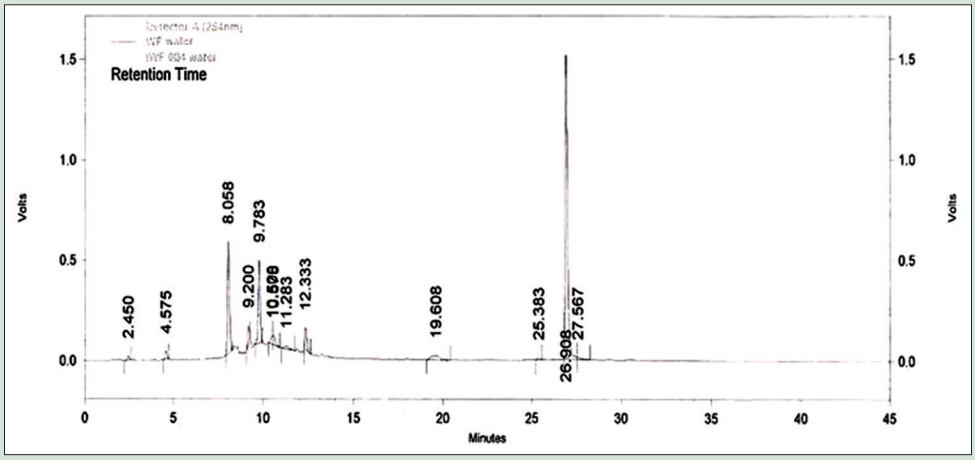

Figure 5: High performance liquid chromatography chromatogram of aqueous extract of *Woodfordia fruticosa* flower sample (Nahan, Himachal Pradesh). NHN: Nahan, Himachal Pradesh, sample of *Woodfordia fruticosa flowers*

The HPLC method developed by Bala *et al*. was tried with respect to resolution of peaks which showed good results.[28] For improving separations in HPLC, gradient elution systems instead of isocratic elution are universally recommended.[29] The optimizing of 5 mM potassium dihydrogen orthophosphate (pH 2.5) – acetonitrile (5:95, v/v) overcame the problem of peak tailing (asymmetry at 10% height of the peak reduced to 1.1) in C18 column with a retention time of 12.3 min which was not accomplished in previous studies. To the best of our knowledge, no paper has been published on HPLC studies of *W. fruticosa* flowers using ellagic acid as a standard.

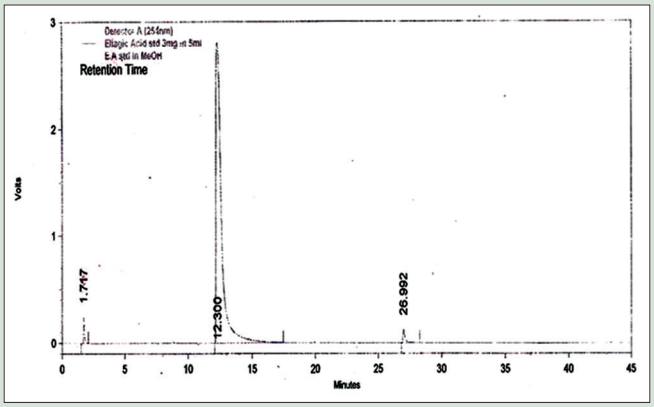

Figure 6: High-performance liquid chromatography chromatogram of standard ellagic acid

Table 5: Comparison of high-performance liquid chromatography peak profiles of the three *Woodfordia fruticosa* flower samples

Number of peaks	Retention time in minutes (R_t)	Area peak (%)			
		W. fruticosa (HYD)	*W. fruticosa* (BNG)	*W. fruticosa* (NHN)	Ellagic acid
1	2.1	36.28	-	-	
2	2.4	-	0.43	-	
3	4.5	-	1.15	-	
4	4.7	-	-	1.51	
5	8.2	-	17.50	40.66	
6	8.7	12.80	-	-	
7	9.3	3.37	2.09	11.98	
8	9.7	10.41	12.80	-	
9	10.3	6.84	1.16	-	
10	10.6	-	1.74	7.48	
11	11.1	7.47	1.06	-	
12	11.6	3.07	-	-	
13	12.3	**13.85**	**3.36**	**3.74**	**97.06**
14	12.9	0.40	-	-	
15	13.2	1.02	-	-	
16	13.5	0.54	-	-	
17	16.9	0.11	-	-	
18	19.6	-	2.71	-	
19	25.3	-	0.37	-	
20	26.9	**3.70**	**54.99**	**34.66**	**1.16**
21	27.5	-	-	-	

Area peak (%) values marked in bold indicate common R_t in all samples and standard; Area peak (%) values highlighted in yellow indicate common R_t in the three samples only. *W. fruticosa*: *Woodfordia fruticosa*; HYD: Hyderabad, Telangana, sample of *Woodfordia fruticosa* flowers; BNG: Bangalore, Karnataka, sample of *Woodfordia fruticosa* flowers; NHN: Nahan, Himachal Pradesh, sample of *Woodfordia fruticosa* flowers

Table 6: Effects of omeprazole and *Woodfordia fruticosa* flowers on total titratable acidity, ulcer index, and percentage protection in ethanol-induced ulcer in rats

Groups	Dose (mg/kg)	Total acidity (mEq/L/h)	Ulcer index	Percentage protection
Normal group	Distilled water	29.4±2.53	No ulcer	-
Negative control	1 mL/animal[a]	66.96±3.01	8.50±0.51	-
Omeprazole	10	26.13±2.06**	2.50±0.31**	71
AEWF	100	35.93±3.26**	3.08±0.35**	64
AEWF	200	21.23±3.01**	1.83±0.27**	78

Statistical analysis; Dunnetts *t*-test and ANOVA; Values as expressed as mean±SEM; **P<0.01; [a]1 mL of absolute alcohol for induction of gastric ulcers. *W. fruticosa*: *Woodfordia fruticosa*; AEWF: Aqueous extract of the *Woodfordia fruticosa*; ANOVA: Analysis of variance; SEM: Standard error of mean

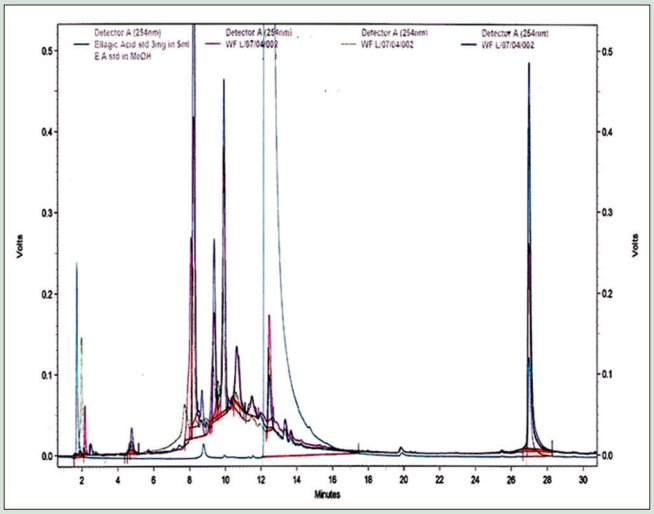

Figure 7: Overlay high-performance liquid chromatography chromatogram of aqueous extracts of *Woodfordia fruticosa* flower samples with standard ellagic acid

The evaluation of the biological activity of the whole plant is one of the most significant parameters in standardization of herbal raw drugs along with the passport data of raw plant parts, botanical authentication, microscopic examination, and chemical profiling by various chromatographic techniques.[30] In the present study, the ethanol-induced ulcer model in male Wistar albino rats was employed for the evaluation of gastroprotective capacity of the flowers of *W. fruticosa*. The genesis of ethanol-induced gastric lesions is multifactorial with the depletion of gastric wall mucous content as one of the involved factors. Another factor to this effect is the significant production of free radicals which results in increased oxidative stress and damage to the cell and cell membrane.[31] Although several factors have been suggested as being responsible for ulceration, recent data[32] indicate the involvement of histamine release in this process, which explains the high efficacy of H2-antagonists in this model. Our results suggest parallelism in the mechanism of action for both standard omeprazole[33] and the test drug (i.e., interaction with H2-receptors of parietal cells). In the experiments presented here, both standard and the test drugs reduced ulcer incidence, in a dose-dependent manner, in the stomach, with a potency of AEWF 200 mg/kg higher than that of omeprazole. Furthermore, alcohol stimulates acid secretion and reduces blood flow leading to microvascular injuries, by the disruption of the vascular endothelium and facilitation of vascular permeability; it also increases activity of xanthine oxidase.[34] The acid secretion studies also exhibited significant reduction in the TTA. Ethanol also triggers imbalances in cellular antioxidant processes. Thus, the ethanol-induced ulcer model is useful for studying the efficacy of potential drugs or testing agents that have cytoprotective and antioxidant activities.[35] The correlation between ellagic acid in particular and the phenolic compounds in general from different sources and their anti-ulcer capacity has been evidenced by several researchers.[36-38] Whether or not ellagic acid present in AEWF 150 and AEWF 300 contributes to the anti-ulcer activity is not possible to determine from this experiment. As AEWF contains flavonoids, saponins, polyphenols, tannins, alkaloids for which bioactivity studies have demonstrated the gastroprotective, gastric healing, and anti-diarrheal activities of these phytoconstituents in separate studies,[5,8,9] we conclude that in addition to ellagic acid these phytoconstituents may be contributing to the anti-ulcer activity in this animal model.

CONCLUSION

The developed HPTLC and HPLC methods proved to be quite simple, precise, and robust. The proposed methods are suitable for the chromatographic profiling of the flowers of *W. fruticosa* with respect to ellagic acid. These methods can be used for the quality control of marketed preparations containing *W. fruticosa* as one of the ingredients. The bioactivity studies further supported the role of ellagic acid as a contributor in the gastroprotective capacity of *W. fruticosa* flowers. Nevertheless, further study is required to isolate, characterize, quantify, and evaluate the other active constituents present in the flowers of *W. fruticosa* for their contribution to the overall gastroprotective potential. Clinical studies should be performed to evaluate the effect as a remedy against gastric ulcer in human.

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Conflicts of interest

There are no conflicts of interest.

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