

Full Length Research Paper

Dermatological potential of crude extracts and different fractions of *Achyranthes aspera* Linn.

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***Achyranthes aspera* (Prickly chaff) seeds contain spines which often cause irritation on the hands of those who collect them. The main objective of this investigation was to evaluate its irritant potential on animal's skin. Skin irritating chemical constituents were separated in the form of various fractions. For this purpose, solvents of different polarities were successively used to extract least-polar compounds (petroleum ether extract), constituents of intermediate polarities (chloroform extract) and polar constituents (methanol extract) from pulverized seeds of *A. aspera*. Eleven fractions were collected from methanol extract of powdered seeds by liquid column chromatography. The irritant potential of these fractions was evaluated on rabbit's skin. Fractions eluted by CHCl₃/MeOH (60:40), CHCl₃/MeOH (40:60) and CHCl₃/MeOH (20:80) showed more irritant potential. These biologically active purified fractions were characterized by ultraviolet (UV) and Fourier transform infrared (FTIR) spectroscopy. The presence of –OH, –COOH, or ketonic group and a double bond in these fractions were liable to be reacted with the cell membrane and cellular contents of both the superficial and deeper layers of epidermis causing irritancy. It was concluded that *A. aspera* contained skin irritant compounds.**

Key words: *Achyranthes aspera*, irritant compounds, liquid column chromatography, ultraviolet (UV) and Fourier transform infrared (FTIR) spectroscopy, solvent extraction.

INTRODUCTION

Dermatitis caused by plants is commonly encountered in practice of dermatology (Wood, 1962). Irritant and sensitizing properties of plants have long been known to Indian, Chinese and Arab physicians. The old Indian barbers made use of the irritant properties of certain plants for cauterization of skin diseases. Plants produce different reactions on coming in contact with the skin, depending on the nature of the plant, the type of skin and other varying factors. Further, it must be realized that certain plants cause irritation at one time and sensitization and photosensitization at another time, depending on the amount and concentration of the irritant

and other environmental factors (Behl, 2004). *Achyranthes aspera* also known as prickly chaff flower and devil's horsewhip, belongs to family Amaranthaceae. It is used as laxative and antiemetic. It is also used for the diseases of heart, flatulence, itching, pain in abdomen and enlargement of abdomen. The whole plant juice is given for 2 to 3 days daily to treat fever. The whole plant decoction along with root is given to treat menorrhagia, diarrhoea, and stomach pain (Aggarwal and Tamarkar, 2002). The flowering spikes or seeds ground into paste with water are used as external application for bites of poisonous insects and reptiles. Spikes with seeds are often used as expectorant. Seeds paste is given to treat Rabies (Ravendra and Martin, 2006). This plant is reported to possess antidiabetic and antirheumatic properties. The root is said to be useful for treatment of pneumonia (Battacharjee, 2001). It has anti-implantation,

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antitumor and antiviral activities (Prakash, 1986; Wadhawa et al., 1986; Yu and Zhang, 1995). *A. aspera* show inhibitory effect towards *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Shigella dysenteriae* (Raman et al., 1996). The seed powder is used for periodontal diseases (Kim et al., 1998). It was published that *A. aspera* is used as antispasmodic, diuretic, purgative, antifungal, cardiac stimulant, anticoagulant, hypertensive, abortifacient and antileprotic (Aggarawal et al., 2002).

Seeds of *A. aspera* contain spines which often cause irritation on the hands of those who collect them. As our efforts to explore the flora of Pakistan (Rasool et al., 2011), the main objective of this investigation was to evaluate its irritant potential on animal's skin.

MATERIALS AND METHODS

Plants and chemicals

Fresh seeds of *A. aspera* were purchased from the local market (Papar Mandi, Lahore, Pakistan). Seeds were authenticated by Dr. Zaheer ud Din, Department of Botany, Government College University, Lahore, Pakistan (Voucher specimen No. 621). Seeds were spread on the laboratory tables and dried under the shade at room temperature for 4 days. Dried seeds were stored in amber colored bottles. They were pulverized by using an electric mill.

Petroleum ether (40 to 60°C), chloroform, methanol and acetone were of analytical grade (BDH Company, England). Silica gel 60 (70 to 230 mesh ASTM) was used for Column Chromatography (E. Merck, Germany).

Instruments

Rotary vacuum evaporator (Tokyo Rikakikai Co., Ltd, Japan), ultraviolet (UV) spectrophotometer (Hitachi-270-30), Fourier transform infrared (FTIR) (Pye-Unicam SP-8-400).

Solvent extraction

Dried pulverized seeds (1 kg) were extracted successively in petroleum ether (40 to 60°C) chloroform and methanol, using 2.5 L of each solvent for soaking. Maceration was carried out in each solvent for four days at room temperature ($25 \pm 2.5^\circ\text{C}$). Solvent of each extracted material was removed with rotary evaporator under reduced pressure and the residues were weighed as shown in Figure 1 (Brain and Turner, 1975).

Column chromatography

A boro-silicate glass column of 50 × 2.5 cm size was used for column chromatography. Column was packed uniformly with 250 g of silica gel (activated by heating at 120°C in an oven for 3 h) by slurry method. Chloroform was used for packing the column. Twenty grams of methanol extract of *A. aspera* was adsorbed on 20 g of silica gel, using chloroform. Chloroform was completely evaporated and the dried silica gel that adsorbed material after pulverization was put on the top of the column. The column was first run with a mixture of chloroform and methanol; then the polarity

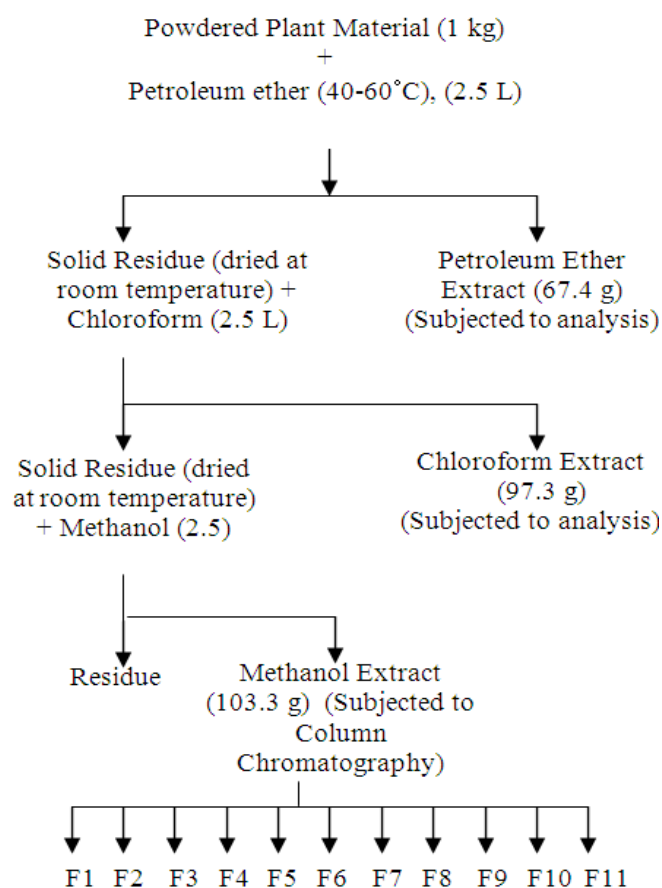


Figure 1. Flow diagram showing solvent extraction and column fractions procedure.

of the system was changed by increasing the quantity of methanol in chloroform (Table 2). Eleven fractions were collected in glass test tubes. These fractions were purified by using prepared thin layer chromatography (TLC) plates (20 × 20 cm by E. Merck, Germany) using solvent systems as shown in Table 2.

Irritancy assay

Preliminary irritancy assay was performed with all three types of solvent extracts on rabbit's ear. This method was originally established by Hecker (1971) for evaluating the irritant principles from croton oil on mice's ears. In this work, the same method was used, but instead of mice, rabbits were used as animal model for assessing the irritancy behaviour, because the irritant reactions produced by different extracts could easily be evaluated and could also be comparable to a similar reaction on other mammalian skins. Many authors used albino rabbits instead of mice for this purpose (Marzulli and Miabch, 1975).

Solutions (20 to 50 µl) from different dilutions were applied to the inner surface of rabbit's ear. Acetone was used as vehicle as well as control. The ears were examined for redness after 15 min of application and then after 30 min intervals, until two examinations indicated that further redness would not occur. Time for maximum erythema was noted. Five dilutions were chosen for the main assay to include one dilution that will give 100% positive response. The

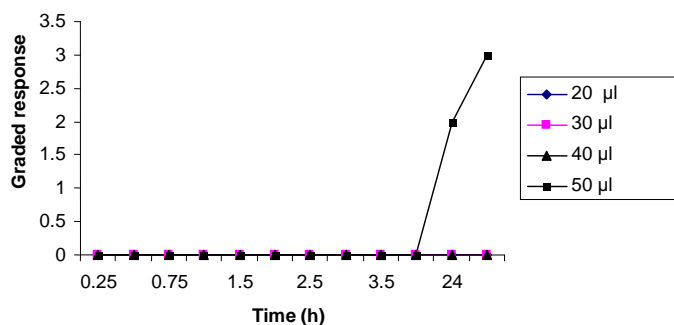


Figure 2. Irritant response of petroleum ether extract. 0 = No reaction; 1 = Doubtful reaction; 2 = Slight reddening of main vessels; 3 = Marked reddening of main vessels; 4 = Intense reddening of the entire ear.

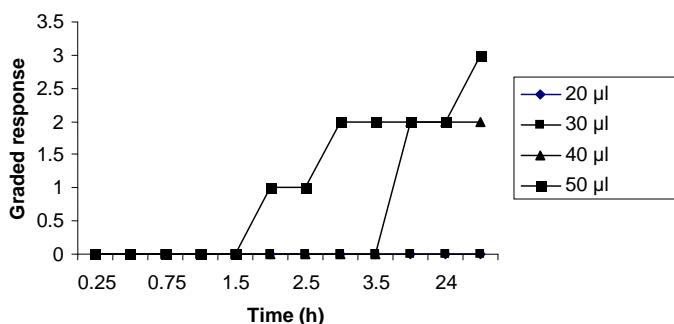


Figure 3. Irritant response of chloroform extract. 0 = No reaction; 1 = Doubtful reaction; 2 = Slight reddening of main vessels; 3 = Marked reddening of main vessels; 4 = Intense reddening of the entire ear.

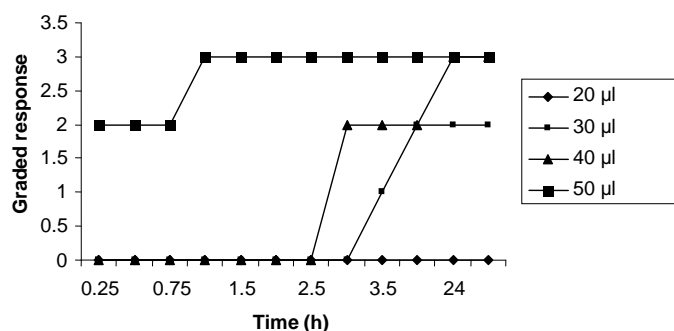


Figure 4. Irritant response of methanolic extract. 0 = No reaction; 1 = Doubtful reaction; 2 = Slight reddening of main vessels; 3 = Marked reddening of main vessels; 4 = Intense reddening of the entire ear.

animals were also examined after 24 and 48 h to ascertain the chronic inflammatory dose.

Evaluation of irritant response of the extracts/fractions was done according to the Table 1. The dose causing an ear redness to the

degree ++ was defined as irritant unit (IU) and expressed in µg/ml per ear (Evans and Schmidt, 1980; Schmidt and Moul, 1983; Hecker, 1971).

Characterization of active purified fractions

Purified fractions 4, 6, 7, 9 and 10 showed more irritant potential. These biologically active purified fractions were characterized by UV and FTIR spectroscopy. Ultraviolet Spectra were recorded on Hitachi-270-30 spectrophotometer using ethanol as a solvent. Infrared Spectra were measured on Pye-Unicam SP-8-400 spectrophotometer using thin film on NaCl disc.

RESULTS

Solvent extraction and percentage yields of extracts

Dried powdered seeds were subjected to successive extraction in three common solvents petroleum ether, chloroform and methanol under the laboratory conditions. Polar components (10.33%) were extracted in methanol and they were higher in yield than others. The components with intermediate polarity (9.73%) were extracted by chloroform. Non-polar constituents (6.74%) extracted in petroleum ether were lowest in yield.

It could be concluded that the seeds of *A. aspera* contained a larger proportion of polar compounds and the compounds with intermediate polarities than the non-polar components.

Separation of fractions

The methanol extract of *A. aspera* which was present in sufficient amount, was subjected to column chromatographic analysis to obtain the fractions, using an increasing quantity of methanol in chloroform. Eleven pooled fractions were collected and purified by Preparative thin-layer chromatography (PTLC) (Table 2).

Irritancy assay

Results of preliminary irritant responses of crude extracts on rabbit's ear have been outlined in Table 3 and Figures 2, 3 and 4. Results of irritant reactions of pooled column fractions of methanol extract on rabbit's ear have been outlined in Table 4 and Figure 5.

Characterization of active purified fractions

The data of UV and FTIR spectra of active purified fractions is shown in Table 5 (Williams and Fleming, 1980; Silverstein et al., 1981).

Table 1. Grading of irritant reactions.

Reaction grade	Explanation
-	No reaction
±	Doubtful reaction, diffused inflammation with no clear visible symptoms.
+	Slight reddening of the main vessels without reddening the area in between.
++	Marked reddening of the main vessels with reddening of the area in between.
+++	Intense reddening of the entire ear often accompanied with macroscopic visible hyperplasia.
++++	Visible exudative lesion with marked epidermal damage.

Table 2. PTLC analysis of pooled column fractions of methanolic extract.

Pooled fraction	Eluting solvent	PTLC solvent systems	No. of Spots	hRf values	Detecting agents		
					UV light	Iodine	Liebr.BurReagent
1	CHCl ₃ (100%)	CHCl ₃ /MeOH (40:60)	1	89	l-pink	l-yel	l-bro
2	CHCl ₃ /MeOH (95:5)	CHCl ₃ /MeOH (30:70)	1	76	d- pink	d-yel	d-bro
3	CHCl ₃ /MeOH (90:10)	CHCl ₃ /MeOH (20:80)	1	85	l-blu	yel	bro
4	CHCl ₃ /MeOH (90:10)	CHCl ₃ /MeOH (10:90)	1	83	l-blu	l-yel	l-bro
5	CHCl ₃ /MeOH (80:20)	CHCl ₃ /MeOH/Ace.A (90:10:1)	1	45	d- pur	d-yel	l-bro
6	CHCl ₃ /MeOH (70:30)	CHCl ₃ /MeOH/Ace.A (80:20:2)	1	41	d-pink	d-yel	l-bro
7	CHCl ₃ /MeOH (60:40)	CHCl ₃ /MeOH/Ace.A (90:10:5)	1	50	l- blu	l-yel	d-bro
8	CHCl ₃ /MeOH (50:50)	CHCl ₃ /MeOH/Ace.A (80:20:5)	1	62	l-pur	yel	l-bro
9	CHCl ₃ /MeOH (40:60)	CHCl ₃ /MeOH/HCl (80:20:2)	1	89	l-pink	l- yel	d-bro
10	CHCl ₃ /MeOH (20:80)	CHCl ₃ /MeOH/HCl (80:20:5)	1	33	d- pink	d-yel	l-bro
11	MeOH (100%)	CHCl ₃ /MeOH/HCl (80:20:7)	1	22	l-pink	l- yel	l-bro

CHCl₃ = chloroform, MeOH = methanol, Ace. A = acetic acid, Liebr. Bur. = liebermann burchad, l-pin = light pink, d-pin = dark pink, l-pur = light purple, l-blu = light blue, yel = yellow color, l-yel = light yellow, d-yel = dark yellow, bro = brown color, l-bro = light brown, d-bro = dark brown

Table 3. Preliminary irritant response of solvent extracts of *A. aspera* on rabbit's ear.

Dose (µl) of 5 mg/ml	Extract	Response after acute time										Chronic time	
		15 min	30 min	45 min	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	3.5 h	4.0 h	24 h	48 h
20	Pet. Ether	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-	-	-
30	Pet. Ether	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	±	+	+	+
40	Pet. Ether	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-	-	+	+	+
	Methanol	-	-	-	-	-	-	-	+	+	+	++	++
50	Pet. Ether	-	-	-	-	-	-	-	-	-	-	+	+
	Chloroform	-	-	-	-	-	±	±	+	+	+	+	++
	Methanol	+	+	+	++	++	++	++	++	++	++	++	+++

- = No reaction; ± = Doubtful reaction; + = Slight reddening of main vessels; ++ = Marked reddening of main vessels; +++ = Intense reddening of the entire ear.

DISCUSSION

The results indicated that two solvent extracts except methanol extract exhibited either no or doubtful irritant

responses when doses of 20 to 40 µl were used, but at 50 µl dose, redness of +, ++ and +++ intensity was observed on rabbit's ears (Table 3). Methanol extract seemed to be more intense irritant than other two

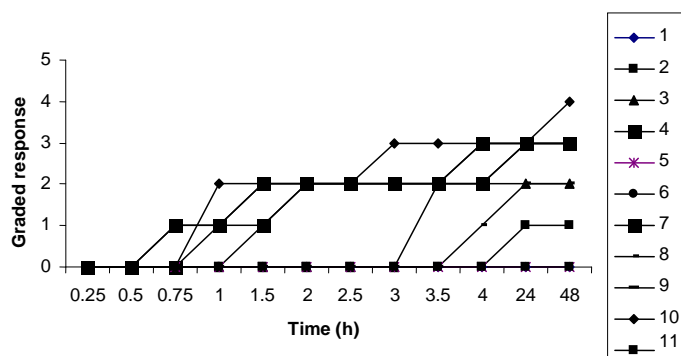
Table 4. Irritant response of pooled column fractions from methanol extract of *A. aspera* on rabbit's ear [dose = 20 μ l of 5 mg/ml].

Fraction	Response after acute time										Chronic time	
	15 min	30 min	45 min	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	3.5 h	4.0 h	24 h	48 h
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	±	±
3	-	-	-	-	-	-	-	-	+	+	+	+
4	-	-	-	±	+	+	+	+	+	++	++	+++
5	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	±	+	+	+	+	++	++	++
7	-	-	±	±	+	+	+	+	+	++	++	++
8	-	-	-	-	-	-	-	-	-	±	+	+
9	-	-	±	±	+	+	+	+	+	++	++	+++
10	-	-	-	+	+	+	+	++	++	++	++	+++
11	-	-	-	-	-	-	-	-	-	-	-	-

- = No reaction; ± = Doubtful reaction; + = Slight reddening of main vessels; ++ = Marked reddening of main vessels; +++ = Intense reddening of the entire ear.

Table 5. UV and FTIR spectra characterization of active fractions.

Active fraction	UV		FTIR (cm^{-1})			
	λ_{max} (nm)	-OH	C=O	-CH ₃	-NH ₂	
4	225	3388	1632	1390,2430	2930	
6	240	-	710,854	1018,1413	2962	
7	275	-	1730	1480,1272	2930	
9	280	3410	1520,1462	1362,1074	2910	
10	250	3660	1733	1410,1174	2914	

**Figure 5.** Irritant response of column fractions from methanolic extract. 0 = No reaction; 1 = Doubtful reaction; 2 = Slight reddening of main vessels; 3 = Marked reddening of main vessels; 4 = Intense reddening of the entire ear.

extracts at this dose level. Chloroform extract exhibited little irritancy. Petroleum ether extract seemed to be nearly inert in its irritant reaction with all the four doses used. It could thus be concluded that polar constituents of *A. aspera* were responsible for such adverse reaction on

rabbit's skin.

Fractions 4, 6, 7, 9 and 10 seemed to contain the most active compounds than the other fractions (Table 4 and Figure 5). These fractions exhibited a strong to moderate irritant response on rabbit's skin. Maximum irritant response was demonstrated by fractions 4, 9 and 10 when the dose of 20 μ l was applied on rabbit's ears. The irritant response of ++ intensity by these fractions were observed after 2 h of their application which continued to increase with time and gained +++ intensity levels in about 4 h. These reactions lasted for about 48 h, and then faded away. On the other hand, two other fractions (6 and 7) demonstrated moderate irritant response (Table 4 and Figure 5). It was further postulated from UV and FTIR spectra results (Table 5) that the fractions 4, 9 and 10 probably penetrated through the skin of rabbit's ear with much ease. The presence of -OH, -COOH, or ketonic group and a double bond in these compounds were liable to be reacted with the cell membrane and cellular content of both the superficial and deeper layers of epidermis. As a result, inflammation of superficial as well as the deeper layers occurred, which probably caused the damage to epidermis. The mechanism of

action of these fractions was probably like the other strong to moderate irritant compounds previously investigated by other workers (Evans and Schmidt, 1980; Schmidt and Moul, 1983). Fractions 6 and 7 displayed a moderate to weak reactions of ++ to + intensity. There might be two possible reasons. Firstly, they penetrated the skin barriers with a little difficulty, thus were not fully available to the skin. Secondly, the nature of their molecules was not strong enough to cause any severe damage to the epidermal tissues of the skin.

Conclusion

Phytochemical investigation indicated that *A. aspera* contains several constituents that can be isolated by column chromatography. Five of the fractions were biologically active and cause skin irritation. The level of activity varies from slight to severe irritation. To the best of our knowledge, irritant activity of crude extracts and fractions of *A. aspera* is first time reported. Furthermore, the mechanism of irritancy can be investigated and different drugs can be formulated for the treatment of this irritancy.

REFERENCES

- Aggarawal SK, Singh R, Hanif M, Singh S, Kumar S (2002). Chemistry and biological activity of *Achyranthes* Species. J. Med. Aromat. Plant Sci. 24:1024-1030.
- Aggarawal SS, Tamrakar BP (2002). Clinically useful herbal drugs, 1st ed. Ahuja Book Company, India. p 135.
- Behl PN, Aggarawal A, Srivastava G (2004). Skin irritant and sensitizing plants of medicinal significance, 3rd ed. CBS publishers and Distributors, India. pp. 6-12.
- Bhattacharjee SK (2001). Hand book of medicinal plants, 3rd edition. Pointer Publishers, India. pp. 11-12.
- Brain KR, Turner TD (1975). The practical evaluation of phytopharmaceuticals. Wright-Scientehnica, Bristol, UK. pp. 1-80.
- Evans FJ, Schmidt RJ (1980). Plants and plant products that induce contact dermatitis. Plant Med. 38:289-316.
- Hecker E (1971). Methods of cancer research. Academic Press, London, pp 1-69.
- Kim MU, Sang N, Suoke Z (1998). Oral composition containing *Achyranthes japonica* extract for periodontal diseases. Kok. Tok. Koh. Jpn. 10:167.
- Marzulli FN, Miabch H (1975). The rabbit as a model for evaluation of skin irritants, a comparison of results obtained on animals and man using repeated skin exposures. Food Cosmet. Toxicol. 13:533-540.
- Prakash AO (1986). Potentialities of some indigenous plants for anti-fertility activity. Pharm. Biol. 24(1):19-24 .
- Raman MH, Farque ABMI, Islam SN (1996). Studies on antibacterial properties of *Achyranthes aspera*. Fitoterapia 67:92-93.
- Rasool N, Rizwan K, Zubair M, Gulzar T, Bukhari IH, Gilani MA, Amjad A (2011). Antioxidant activity of various extracts and organic fractions of *Ziziphus jujuba*. Int. J. Phytomed. 3:346-352.
- Rasool N, Rizwan K, Zubair M, Naveed KR, Imran I, Ahmed VU (2011). Antioxidant potential of different extracts and fractions of *Catharanthus roseus* shoots. Int. J. Phytomed. 3:108-114.
- Ravendra K, Martin P (2006). Ethnomedicinal plants. Agro bios publishers, India. p 211.
- Schmidt RJ, Moul SP (1983). The dermatitic properties of Black bryony (*Tamus communis* L). Contemp. Derm. 9:390-396.
- Silverstein RM, Bassler GC, Morrill TC (1981). Spectrometric identification of organic compounds, 4th Ed. John Wiley & Co., New York. pp. 95-105, 305-310.
- Wadhawa V, Singh M, Gupta DN, Singh C (1986). Contraceptive and hormonal properties of *Achyranthes aspera*. Planta 15:231-233.
- Williams DH, Fleming I (1980). Spectroscopic methods in organic chemistry, 3rd Ed. McGraw-Hill Book Co. Ltd., London. pp. 1-73.
- Wood B (1962). Irritant plants. Transactions of the St. John. Hospital Dermatological Society of London. pp 71-75.
- Yu S, Zhang Y (1995). Effect of *Achyranthes bidentata* polysaccharides on antitumor activity and immune function of S180-bearing mice. Zhonghua Zhong Liu Za Zhi 17(4):275-278.