WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 7, 1091-1100. Research Article ISSN 2277 – 7105

PHYSICO-CHEMICAL AND HPLC ANALYSIS OF THE WHOLE PLANT OF ACHYRANTHES ASPERA (LINN) OBTAINED FROM VINDHYA REGION OF U.P. INDIA

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Article Received on
22 July 2014,

Revised on16 August 2014,
Accepted on 10 Sept 2014

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ABSTRACT

Amaranthaceae is a cosmopolitan family consisting of 64 genera and about 800 species, mostly abundant in tropical regions of America, Africa, and India .The family represented by herbs and few shrubs, contains most of the important allergic species. *Achyranthes aspera* Linn, Family: *Amaranthaceae* or its common name Apamarga is a stiff, erect herb, found commonly as a weed throughout India up to an altitude of 900 m. An abundance of traditional herbal medicine practice has been adopted for the diagnosis, prevention and treatment of various diseases. Many such practices were experimentally proved depicting the scientific insight following their traditional acceptance. At present, outlooks are high about natural products. *Achyranthes aspera* has been reported to passes number of medicinal properties. The current study describes some phytochemical investigations and HPLC analysis undertaken on the whole plant of *Achyranthes aspera* Linn. The samples for research were collected from Vindhya hills region of Utter Pradesh India.

KEY WORDS: *Amaranthaceae*, cosmopolitan, phytochemical, HPLC.

INTRODUCTION

Amaranthaceae is a cosmopolitan family consisting of 64 genera and about 800 species, mostly abundant in tropical regions of America, Africa, and India [14]. The family represented by herbs and few shrubs, contains most of the important allergic species [15, 30]. *Achyranthes aspera* Linn, Family: *Amaranthaceae* or its common name Apamarga is a stiff, erect herb, found commonly as a weed throughout India up to an altitude of 900 m. Stem erect, base woody, angular or ribbed, simple or branched. Leaves are opposite, petiolate, ovate-elliptic-

obovate-rounded, apex usually rounded, finely or softly pubescent on both sides. Flowers are in an auxiliary or terminal spikes, which is more than 50 cm long, greenish white, bracteates and bracteolate. Stamens 5 in number, staminodes are truncate, fimbriate, ovary oblong, sub-compressed and ovule solitary. Fruit easily disarticulate oblong or ovoid and utricle. Seeds are inverse, testa coriaceous, embryo annular and surrounded by floury albumin. The plant has been mentioned in manuscripts of Ayurveda and Chinese medicines. The plant has been reported to passes number of medicinal properties. The herb is widely used to treat various kinds of ailments. The whole plant used as diuretic in renal dropsies and general anasarca[19], in beriberi[9], pneumonia[22], bronchial infection[10], blindness in cattle and rheumatism[23], cough[7], toothache ; roots are used in pneumonia[11], astringent and bowel complaints[21], stomachic and digestive, menstrual disorders[24], antifertility[16], mild astringent, cough, ascites and anasarca[19], bleeding in delivery [8] ; seeds are used as brain tonic, bleeding piles[17]. A decoction of powdered leaves with honey or sugarcandy, is useful in the early stage of diarrhoea and dysentery. Fresh leaves ground in to a paste with jaggery or mixed with black pepper and garlic and made in to pills are used as antiperiodic especially in quartan fevers. Payasam or Kheer made of seeds in milk is a good remedy for diseased brain. The drug is also used in snake-bites [13]. Root is used in atrophy, caries of teeth, emaciation, rheumatism, syphilis spores, and malarial fever [6].

Adulterants / substitutes

A red variety *raktapamargh* and the latter are equated with *Achyranthes bidentata* Bl[5] . The red variety possesses red pigments in the epidermis and outer cortex of the stem while in other respects the anatomical features are alike in both the forms[20].The nature of chemical constituents (steroids and triterpenoids) is also similar in both the varieties. Another plant belonging to the *Amaranthaceae* family is *Cyathula prostrata* (Linn.) Blume. Also gets substituted for *A. aspera* [5].

MATERIALS AND METHODS

Plant materials

Achyranthes aspera Linn were collected in the month of October, from vindhya hills region of the Allahabad district of U.P. India and were authenticated by Dr. Alok Lahri National Botanical Research Institute (N.B.R.I.), Lucknow, U.P., India. Care was taken to select healthy plants and for normal organs.

Physico-chemical studies

The research specimen was subjected to physico-chemical and parameters such as Extractive value [5], Ash value [4], Flurosence analysis [28]. Crude fibre content [29], Foaming index [31] and Total alkaloids [1]

Preliminary phyto-chemical screening

The whole plants were dried in shade at room temperature and screened for the presence of foreign matter. The plant material was ground to a moderately coarse powder in a mechanical grinder. About 200g of the powder was extracted successively with petroleum ether (60 - 80º C bp), ethyl acetate, chloroform and ethanol (95%) using soxhlet apparatus. The extraction with each solvent was carried for 24 h. Finally, the marc left was extracted with water by digesting on a boiling water bath. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The extracts were taken in a tarred porcelain dishes and evaporated to dryness on a water bath and dried at 105º C to a constant weight. The percentage extractives were calculated with reference to air dried drug [12].

Chromatographic studies

TLC analysis

Alcoholic extract, Pet. Ether, Chloroform and ethyl acetate fractions were evaluated by TLC for the presence of alkaloids, phenolic compounds & steroids etc using specific solvent systems & detecting reagents, to substantiate the presence of these constituents, detected in Qualitative chemical tests, & to know how many compounds are present in them[26,27,18] .

Extraction and HPLC Analysis

Extraction and HPLC analysis of constitutes was carried out by following the method [2, 3].

The dried, powdered materials (500 mg) were extracted with ethanol by soxhlet apparatus for 8 hrs at room temperature. Ethanolic extracts were evaporated to dryness in a vacuum oven. For analysis, the remainder was re-dissolved in 1 ml of HPLC grade ethanol and transferred to a polypropylene micro centrifuge tube, vortexed for 30 s and centrifuged for 5 min at 3000 X g. After centrifugation, the clear supernatant was filtered through 0.45 /m nylon membrane filter (Sigma) and was used for the HPLC analysis. The analytical HPLC experiments were performed by National Botanical Research Institute (NBRI) Lucknow, Utter Pradesh, India.

RESULT AND DISCUSSION

Physico-chemical analysis

The aqueous extractive value was higher than the alcoholic extractive value revealing presence of larger amount of water soluble constituents in the whole plant such as plant acids, carbohydrates and phenolic compounds. The total ash value was higher than that of the acid insoluble and water soluble ash value and a decrease in the acid insoluble ash value may be due to presence of smaller quantity of siliceous matters. By conventional procedure, loss on drying was performed showing not less than 8.56% W/W volatile matters.

Fluorescence analysis showed no any specific fluorescence. However, a transition in colour was observed and was reproducible; a pale pink colouration was noted to be predominant.

The percentage extractive of water was higher than the rest of the extractives. Petroleum ether, ethyl acetate and ethanolic extracts showed the presence of phytoconstituents such as triterpenoids and sterols where as ethanolic (95%) and chloroform extracts showed presence of alkaloids; glycosides and phenolic compounds present in chloroform aqueous extracts showed phenolic compounds, and ethanol extract respectively. glycosides, flavonoids, saponins and reducing sugar.

Chromatographic studies

Thin layer chromatographic studies revealed that 9 alkaloids are present in ethanolic extract whereas 7 steroids and terpenoids are present in petroleum ether extrct

Table-1: Physico-chemical analysis of whole plant of *Achyranthes aspera* Linn

Physico-chemical parameter	Mean
Foreign matter	Nil
Percentage extractive*	
A) Ethanol soluble	6.32 ±0.69
B) Water soluble	24.58± 0.81
Ash value % W/W*	
A) Total ash	10.34± 0.44
B) Acid-insoluble ash	0.878± 0.39
C) Water- soluble ash	3.74± 0.46
D) Sulphated ash	15.71± 0.48
Moisture content*	8.56± 0.26
Crude fibre content*	56.07 ± 1.05
Total alkaloids (mg/gm)	6.4 ± 1.95
Foaming index	222.22

*Results are presented as mean ± standard deviation of five counts

Table-2: Percentage yield and physical characteristics of various extract of *A. aspera*

Solvent extract	%w/w	consistency	Fluorescence analysis		
			Visible	Long U.V.	Short U.V
Pet. Ether (60-70^{0}c)	1.796	Sticky	yellowish-green	blue	green
Ethyl acetate	1.829	Sticky	Green	brown	green
Chloroform	2.57	Powder	brown	Dark brown	brown
Ethanol (95%)	5.28	Sticky	grey	bluish	grey
Water	6.008	Dry	orange	Light green	Dark green

Table-3: Qualitative chemical analysis of various extract of whole plant of *Achyranthes aspera*

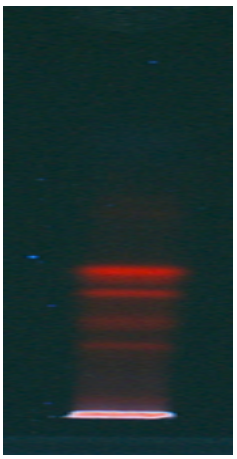
Chemical constituents	Successive solvent extract					Extractive	
	Pet. ether	Ethyl acetate	chloroform	ethanol	water	Ethanol extractive	Aqueous extractive
Carbohydrates	-ve	-ve	+ve	-ve	+ve	-ve	+ve
Alkaloids	-ve	-ve	+ve	+ve	-ve	+ve	-ve
Glycosides	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Tannin	-ve	+ve	-ve	-ve	+ve	-ve	+ve
Phenolic	-ve	-ve	-ve	+ve	-ve	+ve	-ve
Steroids	+ve	+ve	-ve	+ve	-ve	+ve	-ve
Terpenoids	+ve	+ve	+ve	+ve	-ve	+ve	-ve
Proteins	-ve	-ve	-ve	+ve	-ve	+ve	-ve
Saponin	-ve	-ve	-ve	-ve	+ve	-ve	+ve

+ Ve Present, - ve absent

Table-4: TLC evaluation of various extracts

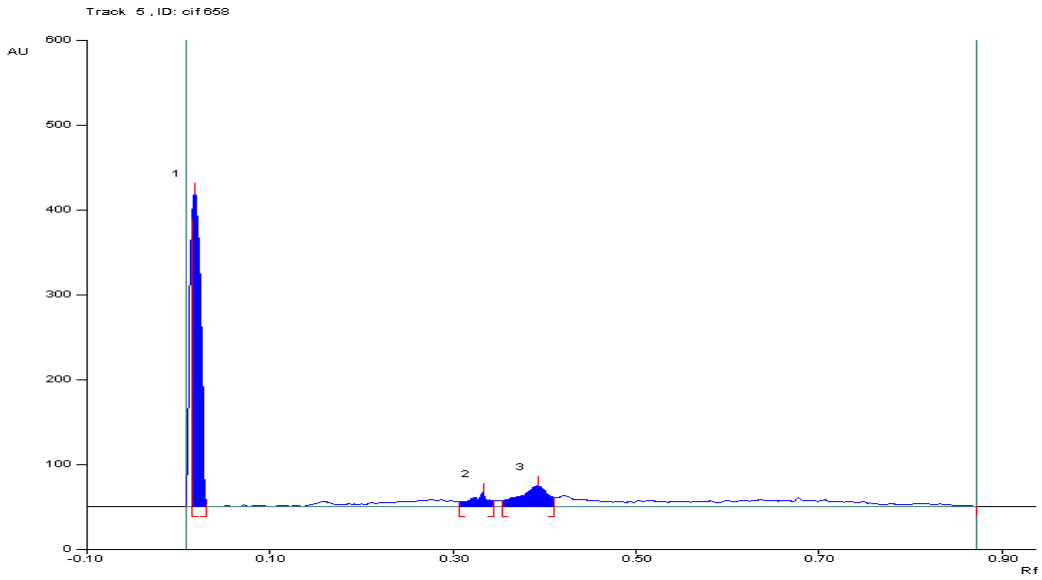
Extract	Evaluation for	Mobile phase	Evaluation of the chromatogram		
			Visualization	No. of spots	Rf values
Ethanol	alkaloids	ethyl acetate: methanol: glacial acetic acid (75:20:5)	iodine saturated chamber	9	0.095,0.14,0.19,0.33, 0.28, 0.51, 0.60, 0.66, 0.90
Pet. ether	steroids & terpenoids	toluene: ethyl acetate: glacial acetic acid (4.5:0.5:0.1)	10% sulfuric acid in ethanol	7	0.96, 0.76, 0.38, 0.33, 0.25, 0.13, 0.07
Ethyl acetate	steroids & terpenoids	toluene: ethyl acetate: glacial acetic acid (4.5:0.5:0.1)	10% sulfuric acid in ethanol	7	0.57, 0.51, 0.45, 0.38, 0.37, 0.34, 0.25
Ethanol	steroids & terpenoids	toluene: ethyl acetate: glacial acetic acid (4.5:0.5:0.1)	10% sulfuric acid in ethanol	3	0.75, 0.49, 0.24
Ethyl acetate	Tannins	ethyl acetate: methanol: water (72.5:13.5:10)	vanillin- HCl acid	4	0.85, 0.40, 0.35, 0.28
Ethanol	flavonoids	Chloroform :acetone: GAA (75:16.5:8.5)	1% aluminium chloride sol^{n} in ethanol followed by ammonia vapour, yellow fluorescence in U.V. light.	2	0.88, 0.81

- **HPTLC Finger Print Profile of *Achyranthes aspera***
- **Application**-Linomat 5 Applicator (Camag)
- **Volume applied**-2 µl
- **Solvent System**-Toluene: Ethyl Acetate :Formic acid (4.5:0.5:0.1)
- **TLC plate Development**-Pre-saturated Camag Twin Trough Chamber
- **Visualization:** observe the developed plate under UV light at 254 nm, 366 nm and visible light after spraying with 10% sulphuric acid solution in ethanol
- **Scan wavelength:** 529 nm



UV 365 nm

Figure-1: HPTLC finger printing of ethanolic extract



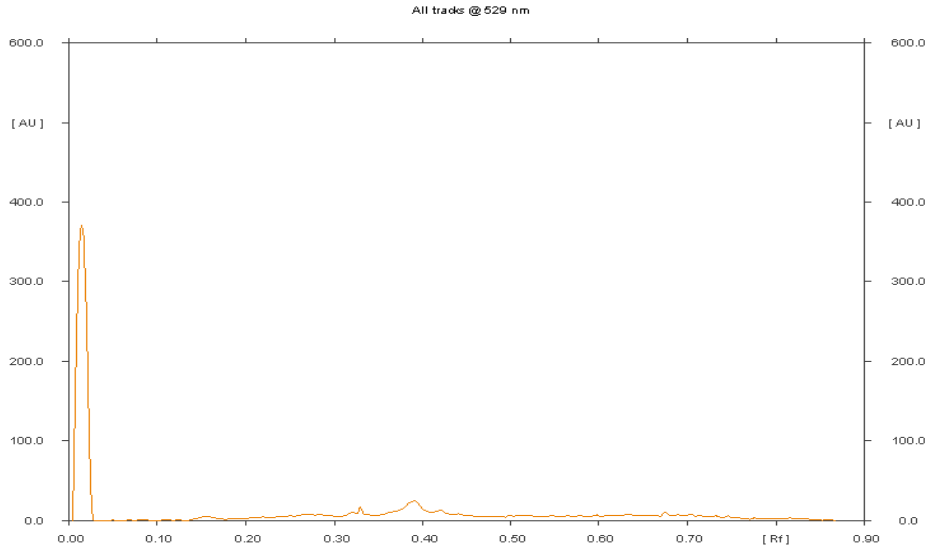


Figure-2: HPTLC finger print profile of Ethanolic extract of *Achyranthes aspera* in Toluene: Ethyl acetate: Formic acid (4.5:0.5:0.1) Solvent system

CONCLUSION

The whole plant of Achyranthes aspera collected from Vindhya hills Utter Pradesh India subjected for physic-chemical and phytochemical analysis. The objective of investigations was to ease the identification of the chemical nature of the species. The presence of valuable phytoconstitutes demand further studies of the species.

ACKNOWLEDGEMENTS

I pay our respect to Dr. Satyawan Singh Ex-director C.D.R.I. Lucknow, Mr. Sanjay yadav, Asst. prof. S.I.T.M. Lucknow for their valuable suggestion and Dr. D.V. Amla N.B.R.I. Lucknow for H.P.L.C. analysis. Also, I express my gratitude to our management for the facilities provided.

REFERENCES

1. Adeniyi SA, Orjiekwe CL, Ehiagbonare JE. Determination of alkaloids and oxalates in some selected food samples in Nigeria. African Journal of Biotechnology, 2009; 8(1): 110-112.
2. Ali M. Chemical investigation of *Achyranthes aspera* Linn. Oriental J Chem., 1993; 9: 84- 85.
3. Ali M. Techniques in terpenoids isolation. Delhi; Birla Publisher: 2001.
4. Anonymous. British Pharmacopoeia, Ministry of Health and Social Services for Northern.vol. II, Ireland: 1988, pp A139 -A140.

5. Anonymous. Pharmacopoeia of India, Ministry of Health and Family Welfare.vol.II, New Delhi; The Controller of Publications: 1996:A47 - A89.
6. Bhatnagar LS, Singh VK, Pandey G. Medico-botanical studies on the flora of Ghaigaon forests, Gwalior, Madhya Pradesh. J Res Indian Med, 1973; 8: 67-100.
7. Chopra R N. Indigenous drugs of India. 2nd ed., Calcutta; U N Dhar & Sons Pvt. Ltd.:1958: 493.
8. Dwivedi S N. Ethnobotanical studies and conservational strategies of wild and natural resources of Rewa district of Madhya Pradesh. J. Econ. Tax. Bot, 2003; 27(1): 233-234.
9. Dwivedi S N. Herbal remedies among tribals of Sidhi district of Madhya Pradesh. J. Econ. Tax, 2004; 28(3): 675-686.
10. Gopalachari R, Dhar ML. Studies in the constitution of the saponin from the seeds of *Achyranthes aspera*: Part I – Identification of the sapogenin. J Sci Ind Res, 1958; 17(B): 276-278.
11. Haerdi F, Eingerborenon D. Acta Tropic Suppl, 1964; 8(1): 278.
12. Harbone J.B. Phytochemical methods.3rd ed., London; Chapman and Hall: 2005:49-52.
13. Jain SK, Defilipps A Robert. Medicinal Plants of India. vol.-I; Reference publication: 1991:101-103.
14. Lawerence GHM. Taxonomy of vascular plants. New Yark; Macmillan Publ. Co.:1989.
15. Lewis WH, Vinay P, Zenger VE. Airborne and Allergic Pollen of North America. London; Johns Hopkins Univ. Press, Baltimore: 1983.
16. Malhi BS, Trivedi VP .Vegetable Antifertility drugs of India. Quert J Crude Drug Res, 1922; 12(3): 1922.
17. Misra MN, Bihari RJ. *Achyranthes aspera* Linn. Sci. Tech, 1964; 7B (3): 195.
18. Mukherjee, PK. Quality control herbal drugs: An approach to evaluation of botanicals. New Delhi; Business Horizons: 2002.
19. Nadkarni KM. Indian Materia medica. Vol–I, Bombay; popular prakashan: 2005: 21-22.
20. Prasad S, Bhattacharya IC. J. Sci. Industr. Res, 1961; 20C:246.
21. Quisumbing E. Traditional medicinal plant *Achyranthes aspera*, Tech Bull, Phillips Dep. Agri Natur Res., 1951; 16.
22. Rangari VD. Pharmacognosy and Phytochemistry Part II. Ist edition,Delhi; Carrer publication:2006: 179-180.
23. Shankar MR. Nagarjun, 1979; 29(1): 9.
24. Singh KK, Palvi SK, Singh HB. Folklore uses of Amaranthaceae family from Nara desert, Pakistan. Ind J. For, 1981; 4(2): 115.

25. Sivarajan VV and Balachandran I. Ayurvedic Drugs and their Plant Sources. New Delhi; Oxford and IBH Publishing Co. Pvt. Ltd.:1994: 47.
26. Stahl Engon. Apparatus and General Techniques in TLC. Thin layer chromatography edited by Egon Stahl. London; George Allen & Unwin Ltd.:1969: 52-86.
27. Wagner H, Bladet S, Zgainski EM. Plant Drug Analysis**,** A TLC Atlas.1st ed., New York; Springer Verlag Berlin Heidelberg: 1994.
28. Wahi, A.K., Khosa, R.L., Mohan, Y. Bot Research, 1981; 3: 205.
29. Wallis TE. Textbook of Pharmacognosy. 5th ed., New Delhi; CBS Publishers & Distributors: 1985.
30. Woodhouse RP. "Antigenic Analysis by Gel Diffusion, III, Pollens of the Amaranth-Chenopod Group." Ann. Allergy, 1957; 15: 527-536.
31. World Health Organization. Quality control Methods for Medicinal Plant Materials. Geneva. A.I.T.B.S.Publishers & Distributors, Delhi: 1998.