Reprinted from

International Journal of Health Research

Peer-reviewed Online Journal

http://www.ijhr.org

Abstracting/Indexing
African Index Medicus, Open-J-Gate, Directory of Open Access Journals (DOAJ), Socolar (China's largest online database), EBSCO, Index Corpenicus

PORACOM
Academic Publishers

e216p55

International Journal of Health Research

The *International Journal of Health Research* is an online international journal allowing free unlimited access to abstract and full-text of published articles. The journal is devoted to the promotion of health sciences and related disciplines (including medicine, pharmacy, nursing, biotechnology, cell and molecular biology, and related engineering fields). It seeks particularly (but not exclusively) to encourage multidisciplinary research and collaboration among scientists, the industry and the healthcare professionals. It will also provide an international forum for the communication and evaluation of data, methods and findings in health sciences and related disciplines. The journal welcomes original research papers, reviews and case reports on current topics of special interest and relevance. All manuscripts will be subject to rapid peer review. Those of high quality (not previously published and not under consideration for publication) will be published without delay. The maximum length of manuscripts should normally be 10,000 words (20 single-spaced typewritten pages) for review, 6,000 words for research articles, 3,000 for technical notes, case reports, commentaries and short communications.

Submission of Manuscript: The *International Journal of Health Research* uses a journal management software to allow authors track the changes to their submission. All manuscripts must be in MS Word and in English and should be submitted online at http://www.ijhr.org. Authors who do not want to submit online or cannot submit online should send their manuscript by e-mail attachment (in single file) to the editorial office below. Submission of a manuscript is an indication that the content has not been published or under consideration for publication elsewhere. Authors may submit the names of expert reviewers or those they do not want to review their papers.

Enquiries:

The Editorial Office
International Journal of Health Research
Dean's Office, College of Medicine
Madonna University, Elele Campus, Rivers State
E-mail: editor_ijhr@yahoo.com or editor@ijhr.org

PORACOM
Academic Publishers

International Journal of Health Research, March 2009; 2(1): 59-64 (e216p57-62)

Available at http://www.ijhr.org

Open Access
Online Journal

Original Research Article

Preliminary Studies on Diuretic Effect of *Hygrophila auriculata* (Schum) Heine in Rats

Received: 25-Aug-08 **Revised:** 02-Jan-09 **Accepted:** 02-Jan-09

Abstract

***Purpose*:** *Hygrophila auriculata* is a traditional folk medicine widely used in the treatment of urinary infection, gout, hepatic obstruction and as a diuretic. This study was conducted to examine the diuretic effect of whole plant extracts and its fractions.
Methods: The diuretic effect was examined by treating different groups of *wistar albino rats* with single (200 mg/kg) oral doses of alcoholic extract/fractions. Frusemide (10 mg/kg) was used as positive control in the study. The urine volume and electrolyte concentration (Na^+, K^+ and Cl^-) were measured.
Results: Out of the different fractions and extract, the n-butanol fraction (200 mg/kg) significantly and markedly increased the urine output ($p < 0.01$). The pattern of diuresis induced by the n-butanol fraction was almost similar to that produced by the frusemide.
Conclusion: *Hygrophila auriculata* plant extract posesses diuretic effects in rats.

Keywords: *Hygrophila auriculata,* Diuretics, Electrolytes, Terpenoid.

Md Sarfaraj Hussain*[1]
KFH Nazeer Ahmed[1]
Md Zaheen Hasan Ansari[2]

*[1]Department of Pharmacognosy and Phytochemistry, Vel's College of Pharmacy, Pallavaram, Chennai- 600 117, India.
[2]Department of Pharmaceutical chemistry, Jamia Hamdard, New Delhi 110062.

For Correspondence:

Tel: +91-9889902496

E-mail: sarfarajpharma@gmail.com or sarfaraj_pharma@yahoomail.com

Introduction

The modern era of diuretic therapy began in 1949 when sulphanilamide was discovered to possess diuretic and natriuretic properties[1]. Diuretic agents have very wide application in the treatment of various chronical diseases associated with edema. They are generally prescribed for the treatment of hypertension, congestive heart failure, glaucoma, diabetes insipidus and liver aliments.

Hygrophila auriculata (Schum) Heine. (synonym: *Asteracantha longifolia Nees, Barleria auriculata schum, Barleria longifolia linn.,* family: Acanthaceae) is described in the ayurvedic literature as Ikshura, Ikshagandha and Kokilasha, having eyes like kokila or Indian cuckoo. The plant is widely distributed throughout India, Sri Lanka, Burma, Malaysia, and Nepal. It is classified in ayurvedic system of medicine as Seethaveryam, mathuravipaka and uses for the treatment of diabetes and dysentery etc.[2,3]

Earlier studies have shown that the plant possesses antitumor [4,5], anti-nociceptive[6], antibacterial[7,8], antioxidant[9], hepatoprorective[10,11,12], hypoglycemic[13], and haematinic[14] effects. Constituents previously isolated from the plant include flavonoids (apigenin 7-0- glucuronide, apigenin 7-0-glucoside)[15], alkaloids (asteracanthine and asteracanthicine)[16], aliphatic esters (25- oxo - hentricontyl acetate, methyl -8-hexyltetracosanoate)[17], minerals (Fe, Cu, Co)[18], sterols (stimagsterol)[19], triterpenes (lupeol, hentricotane, betulin, luteolin, luteolin -7 - 0 –retinosides)[20, 21, 17] and essential oils[16]. It has been shown that the plant, *Hygrophila auriculata,* is unique for flavonoids and terpenoids contents[15].

At present, there was no known scientific study reported in available literature sources that has been carried out so far on diuretic activity of the plant extract and different semi purified active fractions. Therefore, this study is aimed at exploring the plant, *H. auriculata* and its semi purified active fractions for their therapeutic potentials in diuresis.

Materials and Methods

Collection of Plant materials

The whole plant of *H. auriculata* was collected from the field in Paddapai, Kancheepuram district, Tamilnadu, India, in the month of December 2006. The plant material was authenticated by Dr S Jayaraman, Plant Anatomy Research Center, Chennai, Tamilnadu; a voucher specimen no Parc 55/2007 has been deposited at the herbarium unit of the Department of Pharmacognosy, Vel's College of Pharmacy, Pallavaram, Chennai, India.

Extraction and fractionation

The air-dried whole plants of *H. auriculata* were made into coarse powder. The coarse powder (500 g) was then extracted with distilled water and 95% w/v alcohol separately by cold maceration process for 24 hr and 72 hr, respectively. Both extracts were filtered through muslin cloth and evaporated under reduced pressure and vacuum. The yield of the aqueous extract was 43% w/w and alcoholic extract was 29% w/w. Alcoholic extract was suspended in water and successively fractionated with petroleum ether, chloroform, ethyl acetate and n-butanol to produce 5.8%, 3.9%, 2.1% and 1.2% w/w fractional yields from each of the solvents, respectively.

Phytochemical screening and high performance thin layer chromatography (HPTLC) fingerprinting

To fractionate the bioconstituents, the alcoholic extract was fractionated with various solvents from petroleum ether, chloroform, ethylacetate and ethylacetate while the insoluble fraction was fractionated with n-butanol. Phytochemcial test on n-butanol fraction showed the presence of terpenoids. Distinct chemoprofile of this

fraction was developed using HPTLC with pre-coated TLC plate of silica gel 60 F_{254} (E. Merck, India). Ten microliters of the butanolic fraction was spotted in the form of a band using CAMAG Linomat-V Automatic Spotter (CAMAG, Switzerland). The TLC pattern developed using n-hexane : ethyl acetate (7:3) as a solvent system. Then the plates were scanned in CAMAG TLC Scanner-3 and the peaks were recorded at a wavelength of 366 nm.

Toxicity studies

Ethical approval for this study was obtained from the institutional animal ethical committee (IAEC) of CPCSEA (Committee for the purpose of control and supervision of experiments on animals) with approval reg. no. CPCSEA/12-12-00/PH-07-14. Wistar albino rats (200 ± 50 g) of either sex were used in this investigation. The animals were kept in the propylene cages and maintained at a temperature of 25 ± 2 ℃ and had free access to food and water *ad libitum*. For sub-chronic and acute toxicity studies, the rats were divided into two groups of six animals each. First groups served as normal control. The second group of rats were fasted overnight and given water *ad libitum* while food was withheld 3 - 4 hr after oral administration of the n-butanol fraction. The doses of the fraction ranged from 2 - 5 g/kg body weight, orally. All animals exhibited normal behavior with no macroscopic changes in viscera[23].

Evaluation of diuretic activity

The screening was performed according to the method described by Lipschitz *et al*. (1943)[24]. Male healthy Wistar albino rats (150-200 g) were divided into different groups of 6 animals each and kept in standardized environmental conditions. Animals were deprived of food 18 hr before the experiment. The first group of animals, serving as control, received normal saline (25 ml/kg body weight, p.o.); the second group received frusemide (10 mg/kg, p.o.) and other groups received doses of extracts/fractions (200 mg/kg each), in normal saline. Immediately after administration, the animals were placed in metabolic cages, specially designed to separate urine and faeces, kept at 20±1 ℃. The volume of urine collected was measured at the end of 5 hr and the total urine volume, and concentrations of Na^+, K^+ and Cl^- in the urine were determined.

Analytical procedures

Na^+ and K^+ concentrations were determined by flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+[17]. Cl^- concentration was estimated by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator[25, 26].

Statistical Analysis

The results were expressed as mean value ± standard error of mean (S.E.M.), where number of replica (n) is 6. Statistically significant differences between treatment groups were evaluated using analysis of variance (ANOVA) followed by Dunnett's test. At 95% confidence interval, p values less than 0.05 were considered as significant.

Results

Preliminary phytochemical screening of both aqueous and alcoholic extract and different fractions of alcoholic extract revealed the presence of flavonoids, terpenoids and tannins as major phytoconstituents present in alcoholic extracts/fractions. Phytochemcial tests on n-butanol and ethyl-acetate fraction showed the presence of terpenoids (with observed Rf values from TLC in concordance with HPTLC finger prints[22]), flavonoid, and phenolic compounds.

The results of different diuretic parameters for preliminary phytochemical screening of semi purified active fractions are shown in

Table 1. Frusemide treated animals significantly ($p < 0.01$) increased the urinary output (by 387%) and electrolyte excretion of Na^+ (by 154%), K^+ (by 188%) and Cl^- (by 137%) as compared to control. Alcoholic extract also showed good diuretic action ($p < 0.05$). n-butanol fraction significantly ($p < 0.01$) increased the urinary output (by 316%) and electrolytic excretion of Na^+ (by 140%) and K^+ (by 177%), without significant renal excretion of Cl^- as compared to control. The diuretic actions of other fractions like petroleum ether, chloroform and ethylacetate were not significant. The observed Na^+/K^+ ratio for frusemide, alcoholic extract and n-butanol fraction were 1.42, 1.55 and 1.37, respectively, as compared to 1.74 for control.

Discussion

In this study, pharmacological evaluation of diuretic action of alcoholic extracts of *H. auriculata* were evaluated using frusemide (a high-ceiling loop diuretic) under controlled laboratory conditions. As diuretic therapy may lead to number of life-threatening electrolytic disorders and toxicities, so safety profile studies was carried out following a sub chronic administration of extracts. Results showed that there was absence of mortality and overt signs of toxicity. This would amplify the heterogeneous array of diuretic curatives available for safe and effective treatment of edema and cardiovascular diseases[27].

The n-butanol fraction induced diuresis was strong and accompanied with high natriuresis, chloruresis, and kaliuresis ($p < 0.01$). Further there was low Na^+/ K^+ ratio, so the n-butanol fraction seem to be acting like loop diuretics which inhibits Na^+, K^+ and Cl^- co-transport at thick ascending loop of Henle. K^+ excretion was increased perhaps due to high Na^+ load reaching the distal tube. However, alcoholic extract induced both marked natriuresis and kaliuresis ($p < 0.05$), but the Na^+/ K^+ ratio was more than that of frusemide, indicating the weak kaliuresis or K^+ saving property of alcoholic extract.[28] Since the phytochemical screening revealed that alcoholic extract comprise certain other low polar constituents besides the terpenoids, there is a chance of interference among the different constituents. Also, the co-extracted constituents of alcoholic extracts may interfere with absorption, distribution, binding to receptor of the active component(s) or have opposite overt effects on same physiological function, thereby decreasing the cumulative diuretic activity of alcoholic extract as compare with n-butanol fraction.[29] The above results raise the possibility of existence of diuretic activity by inhibiting tubular reabsorption of water and sodium ion. The above result is a good indicator for the efficacy of a *H. auriculata a*s diuretics.

Based on the phytochemical investigations,

Table 1: Diuretic activity of alcoholic extract and its fractions of *Hygrophila auriculata* (Schum) Heine

Group	Dose	Total urine output (ml)	Electrolytes concentration *(mmol/ L)*			
			Na^+	K^+	Cl^-	Na^+/ K^+
Control	25 ml/kg	3.1±0.61	78.33±3.33	45±2.88	105.83±6.50	1.74
Frusemide	10 mg/kg	12.0±0.99 **	120.83±4.72 **	85±4.83 **	145±6.05 **	1.42
Alcoholic extract	200 mg/kg	5.7±0.61 *	100.00±6.45 *	64.16±5.23 *	130±6.58 *	1.55
Pet. ether	200 mg/kg	4.2±0.58	75.00±5.32	53.33±3.80	110±6.58	1.40
Chloroform	200 mg/kg	3.6±0.50	80.83±4.90	40±4.28	115±5.91	2.02
Ethylacetate	200 mg/kg	2.8±0.23	73.33±5.72	50.83±3.51	108.33±4.41	1.44
n-butanol	200 mg/kg	9.8±0.62 **	110.00±4.65 **	80±6.45 **	124.16±4.16	1.37

Values are expressed as mean ± SEM (n = 6); * P<0.05 and * *p < 0.01 compared with control (ANOVA followed by Dunnett's t-test).

and pattern of urinary excretion, it seems that the terpenoid present in n-butanol fraction may be responsible for diuretic action of *H. auriculata.* However, advance experimentation is under way in our laboratory to elucidate the specific mechanism of action.

Conclusion

The extract of *H. auriculata* has diuretic effect supporting the ethnopharmacological use as diuretics.. This effect may be explored in the use of the plant in the management of some cardiovascular diseases.

Ackowledgements

We are grateful to the Dr IK Ganesh, Chairman of the Vel's Group of Institutions, for his assistance and encouragement. We extend our sincere thanks to Dr S Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu, for providing authenticated sample of *Hygrophila auriculata* (Schum) Heine.

References

1. Schwartz WB. Effect of sulphanilamide on salt and water excretion in congestive heart failure, N Engl J Med 1949; 240:173.
2. Nadkarni AK. Indian Material Medica Popular. Vol 1. Prakashan Private Ltd, Bombay, India, 1978, 667- 669p.
3. 3. Chopra RN, Nayer SL & Chopra IC. Glossary of Indian Medicinal plants, National Institute of science and Communication.C.S.I.R Publication, New Delhi, India. 1956, pp 330.
4. Ahmed S, Rahman A, Mathur M, Sultana S. Anti–Tumor Promoting activity of *Asteracantha longifolia* against experimental Hepatocarcinogenesis in rats. J Food Chem Toxicol 2001; 39:19-28.
5. Mazumdar UK, Gupta M, Maiti S, Mukherjee D. Anti-tumour activity of *Hygrophila Spinosa* in Ehrlich ascites carcinoma and Sarcoma -180 induced mice, Indian J Experiment Biol. 1997; 35:473-477.
6. Shanmugasundaram P, Venkatraman S. Anti-nociceptive activity of *Hygrophila auriculata* (schum) Heine. Afr J Trad CAM 2005; 2(1):62-69.
7. Boily Y, Vnpuyvelde L. Screening of medicinal plant of Rwanda for antimicrobial activity. J Ethanopharmacol 1986; 16: 1-13.
8. Valientic AJ, vanhoof L, Totte J, Lasure A, vanden Berghe D, Rwangaboo PC, Mvukiyuwami J. Screening of hundred Rwandese medicinal plants for antimicrobial ana antiviral properties. J Ethanopharmacol 1995; 46:31-47.
9. Sunil Kumar KC. Klausmuller Medicinal plants from Nepal; Evalution of inhibitors of lipid preoxidation in biological membranes, J Ethanopharmacol 1999; 64:135-139.
10. Anubha Singh and Handa SS. Hepatoprotective activity *of Apium graveolens* ana *Hygrophila auriculata* against paracetamol and thioacetamide intoxication in rats. J Ethanopharmacol 1995; 49:119-126.
11. Hewawasam RP, Jayatilaka K, Pathirana C and Mudduwa LKB. Protective Effect of *Asteracantha longifolia* extract in mouse liver injury induced by carbon tetra chloride and para cetamol, J Pharmacy and Pharmacology. 2003; 55:1413-1418.
12. Shanmugasundaram P, Venkatraman S. Hepatoprotective effect of *Hygrophila auriculata* (K.Schum) Heine root extract. J Ethanopharmacol 2006; 104:124-126.
13. Fernando MR, Nalinie Wickramasinghe SMD, Thabrew MI, Ariyananda PL, Karunamayake Effect of *Artrocarpus Heterophyllus* and *Asteracantha logifolia* on glucose tolorence in normal human subject and in maturity onset diabetic patients. J Ethanopharmacol 1991; 31: 277-282.
14. Gomes A, Das M and Dasgupta SC. Haematinic effect *of Hygrophila spinosa. T Andersonon* experimental rodents. Indian J Experiment Biol 2001; 39(4):381-382.
15. Bairaj P and Nagarajan S. Apigenin-7-0-glucuronide from the flowers of *Asteracantha longifolia* Nees. Indian Drugs 1982; 150-152
16. Parashar VV and Harikishan Singh, Investigation of *Astercantha longifolia Nees*. Indian Journal of pharmacology, 1965; 27(4):109-113.
17. Misra TN, Singh, RS, Pandey, HS, Singh, BK, Pande. Constituents *Asteracantha Lonngifolia Nees. FItoterapia* 2001; 72(2):194-196.
18. Choudhary BK and Bandyopdhyay. Important of mineral content and Medicinal properties of *Moringa oleifera* and *Hygrophila auriculata.* Sachitra Ayurved. 1998; 50 (7):543-549.
19. Quasim C, Dutta NL. Reported the Prescence of Stigmasterol in the root of *Asteracantha longifolia Nees.* J Indian Chem Soc. 1967; 44: 82.
20. Govindachari T R, Nagarajan K, Pai BR. J Indian Sci Res 1957;16B72.

21. Nair NC and Henry. AN Flora of Tamilnadu. India. vol-I. Botanical Survey of India. Southern Circle, Coimbatore, India, 1983; 184p.
22. Ali M. Text book of Pharmacognosy. 2nd edition. CBS Publication, New Dehli, 1998; 52 -68p.
23. Turner R A. The Organisation of Screening. In: Screening Methods in Pharmacology, Vol. I. Academic Press, London, 1965; 21p.
24. Haddian W and A Kerpscar. Bioassay of diuretics. J Pharmacol Exp Ther 1943; 79: 97-110
25. Beckette AH, Stenlake JB. Practical Pharmaceutical Chemistry. Part I - 1st ed. CBS Publishers and Distributors, New Delhi, 1997; 197p.
26. Jeffery GH, Bassett J, Mendham J, Denny RC. Vogel's Textbook of Quantitative Chemical Analysis. 5th ed. Addison Westley Longman Ltd., England, 1989; 801p.
27. Maghrani M, Zeggwagh N, Haloui M, Eddouks M. Acute diuretic effect of aqueous extract of *Retama raetam* in normal rats. J Ethnopharmacol 2005; 99: 31–35.
28. Ratan WD, Pieris KP, Samaratunga U, Jayakody J. Diuretic activity of *Spilanthes acmella* in rats. J Ethnopharmacol 2004; 91: 317–320.
29. Nedi T, Nekonen N, Urga K. Diuretic effect of crude extract of *Carissa edulis* in rats. J Ethnopharmacol 2004; 95: 57–61.