Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 7 (6): 611-614, 2008
ISSN 1682-3974
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A Comparative Evaluation of Phytochemical Fingerprints of *Asteracantha longifolia* Nees. Using HPTLC

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Abstract: Chromatographic techniques can be used to document phytochemical fingerprints and quantitate chemical markers to identify morphological and geographical variations in the herbal raw material. In this context, phytochemical profile of *Asteracantha longifolia* Nees. (syn. *Hygrophila spinosa* T. Anders.; *Hygrophila auriculata* [K. Schum.] Heine), an important medicinal herb, was developed using HPTLC technique and was successfully used for evaluating regional and morphological variations. The HPTLC fingerprints were also used for the quantitation of two bioactive markers β-sitosterol and Lupeol in the plant powder. These phytochemical markers were also evaluated from different parts of the plant and from the whole plant collected from different geographical regions. Maximum content of Lupeol was found to be in roots (0.25%), while maximum content of β-sitosterol was found to be in the leaves (0.069%) of *Asteracantha longifolia* Nees.

Key words: *Asteracantha longifolia* Nees., HPTLC, Lupeol, β-sitosterol, quality control

INTRODUCTION

Asteracantha longifolia Nees. (syn. *Hygrophila spinosa* T. Anders.; *Hygrophila auriculata* [K. Schum.] Heine) (known as Kokilaksa in Sanskrit and Talmakhana in Hindi, family-Acanthaceae) is a common weed growing in marshy and water logged areas. The plant is an important medicinal herb, widely distributed in India and is used by local population for different medicinal purposes. It is also used commercially as an ingredient of some Over-The-Counter (OTC) formulations used in liver disorder and those prescribed as general tonic. The herb has been reported to contain chemical constituents such as β-sitosterol, Lupeol (Saleem *et al*., 2005; Mazumdar and Sengupta, 1978; Tiwari *et al*., 1967), linoleic acid and oleic acid as main constituents along with other fatty acids, polysaccharides, histidine and phenyl alanine (Haq and Nabi, 1978). Lupeol and β-sitosterol both have been reported to have antipyretic (Ali, 1967), hepatoprotective (Shailajan *et al*., 2005; Singh and Handa, 1995), antioxidant, anticancer (Ahmed *et al*., 2001) and macrofilaricidal (Chatterjee *et al*., 1992) activities. Roots of *Asteracantha longifolia* Nees. are used as a diuretic (Joshi, 2000; Warrier, 1995), seeds as aphrodisiac tonic in Unani medicine (Rastogi and Mehrotra, 1991) and leaves are popularly used for hepatoprotection, against anemia and in female reproductive dysfunction. Whole plant powder of *Asteracantha longifolia* Nees. is used as a tonic against debility (Joshi, 2000; Warrier, 1995). Diuretic activity of *Asteracantha longifolia* Nees. is attributed to Lupeol (Elisandra and Diones, 2005). Lupeol is also a potential phytochemical in controlling arthritis (Geetha and Varalakshmi, 1999). It also acts as chemo preventive and immunomodulatory (Anton *et al*., 1993). β-sitosterol has therapeutic action in female reproductive disorders (Ryokkynen *et al*., 2005).

Since different parts of *Asteracantha longifolia* Nees. find use for different clinical indications, it is important to establish quality of the plant raw material for its constituent plant part composition. In the present investigation, chromatographic fingerprint of *Asteracantha longifolia* Nees. whole plant powder has been developed by HPTLC method. This method is found to be rapid, sensitive, precise, accurate and has been applied for simultaneous quantitation (Sutar *et al*., 2002) of Lupeol and β-sitosterol from different plant parts of *Asteracantha longifolia* Nees. collected from Thane and from whole plant powder of *Asteracantha longifolia* Nees. collected from different geographical regions. Thane being close to Mumbai, collection of large quantity of plant material for different parts was easier. The HPTLC fingerprints have been successfully used to distinguish the powders of different parts of *Asteracantha longifolia* Nees. The HPTLC method can therefore help distinctively identify the source of the plant powder raw material and also its constituent plant part.

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MATERIALS AND METHODS

Whole plants of *Asteracantha longifolia* Nees. were collected in winter (October-November 2007) during flowering season from Thane Maharashtra and from different regions viz. Patalganga, Mahad, Karjat, Kolhapur, Dehradun and Nazimabad. Herbaria of the whole plant were authenticated from National Institute of Science Communication (NISCOM), New Delhi and National Botanical research Institute (NBRI), Lucknow. The plants were collected, washed and dried in an oven at 45°C for four days, powdered and sieved through BSS mesh No. 85. Standard β-sitosterol (99% purity) and Lupeol (97% purity) were procured from Sigma-Aldrich Chemie Gmbh (Aldrich Division, Steinheim, Federal Republic of Germany). The solvents toluene, ethyl acetate and methanol of analytical grade purchased from Qualigens Fine Chemicals, Mumbai, India were used for the analysis.

A TLC scanner with computer system and Cats 3 Version Software were obtained from Camag (Muttenz, Switzerland). The source of radiation was mercury lamp. Camag Linomat IV was used as applicator. Separation was done on silica gel F_{254} HPTLC precoated plate procured from Merck (Darmstaldt, Germany).

Different parts of the plant were powdered separately, similarly plants collected from different regions were powdered and used for analysis. Thousand milligram of dried powder was accurately weighed and placed in a stoppered tube and 10 mL of methanol was added, the sample was vortexed for 1-2 min and left to stand overnight at room temperature (28±2°C). The contents of the tube were filtered through Whatmann No. 41 paper (E. Merck, Mumbai, India) and the filtrate was used for experimental work.

Chromatography

Procedure: Chromatography was performed on silica gel F_{254} HPTLC pre-coated plate. Samples (10 μL) were applied on the plates as band of 7 mm width with the help of a Camag Linomat IV sample applicator at the distance of 14 mm from the edge of the plates. The mobile phase constituted of toluene-ethyl acetate-methanol, 15 + 3 + 1.5 (v/v). The plates were developed to a distance of 80 mm in a Camag twin-trough chamber previously equilibrated with mobile phase for 30 min. The chromatographic conditions had been previously optimized to achieve the best resolution and peak shape. After development, plates were derivatised in Liebermann-Burchard reagent and heated at 105°C for 15 min and densitometric evaluation of the plates was performed at 366 nm in fluorescence/ reflectance mode using mercury lamp with a Camag Scanner II in conjunction with Cats 3 Version Software. The chromatographic plate of Lupeol and β-sitosterol standard with different morphological parts and geographical regions of *Asteracantha longifolia* Nees. are shown in Fig. 1 and 2 shows phytochemical fingerprint of *Asteracantha longifolia* Nees. with Lupeol and β-sitosterol standards.

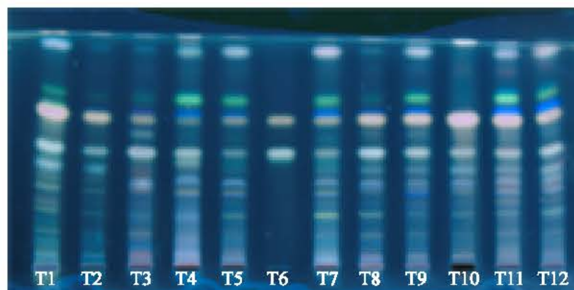

Fig. 1: The chromatographic plate of standard Lupeol and β-sitosterol with *Asteracantha longifolia* Nees. Methanolic extracts of-

- T1: *Asteracantha longifolia* Nees. (Root)
- T2: *Asteracantha longifolia* Nees. (Stem)
- T3: *Asteracantha longifolia* Nees. (Leaf)
- T4: *Asteracantha longifolia* Nees. (Seed)
- T5: *Asteracantha longifolia* Nees. (Thane)
- T6: Standard β-sitosterol + Lupeol
- T7: *Asteracantha longifolia* Nees. (Patalganga)
- T8: *Asteracantha longifolia* Nees. (Mahad)
- T9: *Asteracantha longifolia* Nees. (Karjat)
- T10: *Asteracantha longifolia* Nees. (Kolhapur)
- T11: *Asteracantha longifolia* Nees. (Dehradun)
- T12: *Asteracantha longifolia* Nees. (Nazimabad)

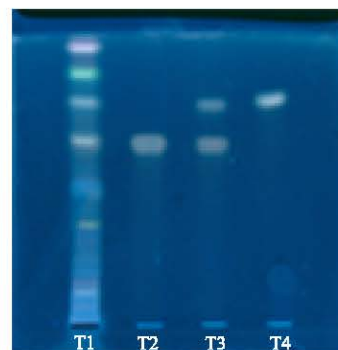

Fig. 2: The chromatographic plate of standard Lupeol and β-sitosterol with *Asteracantha longifolia* Nees.

- T1: Methanolic extract of whole plant powder of *Asteracantha longifolia* Nees.
- T2: β-sitosterol
- T3: Standard β-sitosterol + Lupeol
- T4: Lupeol

Assay procedure: The solution of 0.5 mL of 50 ppm (50 μg mL^{-1}) Lupeol was mixed with 0.5 mL of 50 ppm (50 μg mL^{-1}) β-sitosterol and 10 μL of plant extract were spotted on a HPTLC plate. The amount of Lupeol and β-sitosterol present in the plant extract was calculated by comparison of area measured for the sample to that for the respective standard.

The assay procedure described earlier was repeated three times. The results of assay are given on Table 1a, b and 2a, b.

RESULTS AND DISCUSSION

Different parts of *Asteracantha longifolia* Nees. are prescribed in different indications and the whole plant is used as a tonic for general debility. Since different parts of same plant have different therapeutic applications, it is important to distinctively identify the plant part constituent of the plant powder. In this study HPTLC fingerprint patterns have been therefore, evolved for powders of different plant parts of *Asteracantha longifolia* Nees. Presence of Lupeol and β-sitosterol standards were detected and quantitated accurately using silica gel F_{254} HPTLC pre-coated plates with the mobile phase toluene-ethyl acetate-methanol, 15+ 3+ 1.5 (v/v). Developed chromatograms showed distinct phytochemical variations in morphological parts as well as geographical regions of collection of plant material (Fig. 1). It showed variation in content of Lupeol, which was found to be maximum in roots collected from Thane region (0.25%) and in the whole plant powder it was maximum in the plants collected from Kolhapur (0.237%). β-sitosterol was found to be maximum in leaves collected from Thane region (0.069%) and in whole plant collected from Mahad region (0.067%) (Table 1a, b, 2a, b). Lupeol content is found to be maximum in the roots while β-sitosterol content is maximum in the leaves of *Asteracantha longifolia* Nees. This also justifies the therapeutic use of roots as a diuretic and the use of leaves in the management of female reproductive dysfunction (Joshi, 2000; Warrier, 1995). The HPTLC technique thus developed demonstrates variations in phytochemical

Table 1a: Results of assay of β-sitosterol in different parts of *Asteracantha longifolia* (Nees.)

Sample of *Asteracantha longifolia* Nees.	Weight of sample (mg)	Amount of β-sitosterol present in sample (μg mg^{-1})	Average (%) content of β-sitosterol	RSD (%) n = 3
Root	1000	0.607	0.060	0.594
Stem	1000	0.292	0.029	0.228
Leaf	1000	0.690	0.069	0.071
Seed	1000	0.316	0.031	0.113

Table 1b: Results of assay of Lupeol in different parts of *Asteracantha longifolia* (Nees.)

Sample of *Asteracantha longifolia* Nees.	Weight of sample (mg)	Amount of Lupeol present in sample (μg mg^{-1})	Average (%) content of Lupeol	RSD (%) n = 3
Root	1000	2.500	0.250	0.230
Stem	1000	1.215	0.121	0.767
Leaf	1000	0.513	0.051	0.490
Seed	1000	0.440	0.044	0.226

Table 2a: Results of assay of β-sitosterol in *Asteracantha longifolia* Nees. collected from different regions

Sample of *Asteracantha longifolia* Nees. collected from different regions	Weight of sample (mg)	Amount of β-sitosterol present in sample (μg mg^{-1})	Average (%) content of β-sitosterol	RSD (%) n = 3
Mahad	1000	0.670	0.067	0.296
Karjat	1000	0.464	0.046	0.646
Nazimabad	1000	0.409	0.040	0.282
Dehradun	1000	0.383	0.038	0.519
Kolhapur	1000	0.329	0.032	0.611
Patalganga	1000	0.177	0.018	1.424
Thane	1000	0.129	0.013	0.899

Table 2b: Results of assay of Lupeol in *Asteracantha longifolia* Nees. collected from different regions

Sample of *Asteracantha longifolia* Nees. collected from different regions	Weight of Sample (mg)	Amount of Lupeol present in sample (μg mg^{-1})	Average (%) content of Lupeol	RSD (%) n = 7
Kolhapur	1000	2.370	0.237	0.207
Karjat	1000	1.620	0.162	0.325
Mahad	1000	1.492	0.149	0.316
Patalganga	1000	0.831	0.083	0.315
Dehradun	1000	0.743	0.074	0.269
Thane	1000	0.489	0.0489	0.718
Nazimabad	1000	0.395	0.039	0.506

markers which help to identify the difference in phytochemical constituents due to the geographical region of collection and due to the constituent part of the plant in medicinal plant raw material.

CONCLUSION

In this study it has been demonstrated that HPTLC fingerprint can be employed to identify the source of the plant powder to the specific part of the plant that is constituent in the powder. Further, quantitation of two phytochemical markers namely Lupeol and β-sitosterol using HPTLC from the powder of different plant parts has also been established. The results indicate that the plant parts show specific variations in their content of these phytochemical markers. Distinctive phytochemical variations have also been demonstrated in the whole plant powder sourced from various geographical regions of India. HPTLC fingerprint, as standardized in this study can therefore, be successfully applied to identify the region of collection of the plant. Moreover, the phytochemical markers Lupeol and β-sitosterol, also show region specific variation in their concentrations. Lupeol content is maximum in the whole plant powder sourced from Kolhapur while β-sitosterol content is maximum in whole plant powder sourced from Mahad. Therefore, as a diuretic, the whole plant from Kolhapur may be recommended while as a uterine tonic the whole plant from Mahad may be recommended.

High performance thin layer chromatography (HPTLC) as reported in this study provides a chromatographic fingerprint of phytochemicals and is suitable for confirming the identity and purity of medicinal plant raw materials.

REFERENCES

Ahmed, S., A. Rahman, M. Mathur, M. Athar and S. Sultana, 2001. Anti-tumor promoting activity of *Asteracantha longifolia* against experimental hepatocarcinogenesis in rats. Food Chem. Toxicol., 39: 19-28.

Ali, MA., 1967. Chemical investigation on the seeds of *Hygrophila spinosa*. Pak. J. Sci. Ind. Res., 10: 82-83.

Anton, R., Y. Jiang, B. Weniger, J.P. Beck and L. Rivier, 1993. Pharmacognosy of *Mimosa tenuiflora* (willd.) poiret. J. Ethanoph., 38: 145-152.

Chatterjee, R.K., N. Fatma, P. Kalpnamurthy, P. Sinha, D.K. Kulshrestha and B.N. Dhawan, 1992. Macrofilaricidal activity of the stem bark of *Streblus asper* and its major active constituents. Drug Dev. Res., 26: 67-68.

Elisandra, C.S. and A.D. Diones, 2005. Constituents of *Moquinia kingii*. Braz. J. Phar. Sci., 41: 63-66.

Geetha, T. and P. Varalakshmi, 1999. Effect of lupeol and lupeol linoleate on lysosomal enzymes and collagen in adjuvant-induced arthritis in rats. Acta Phys. Hung., 77: 197-207.

Haq, Q.N. and M.N. Nabi, 1978. Studies on oil from the seeds of *Hygrophila spinosa* . Bangladesh J. Sci. Ind. Res., 13: 29-32.

Joshi S.G., 2000. Medicinal Plants. 1st Edn. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 211..

Mazumdar, U.K. and A. Sengupta, 1978. Triglyceride composition of *Hygrophila spinosa* seed oil. Indian J. Pharm. Sci., 40: 119-120.

Rastogi, R.P. and B.N. Mehrotra, 1991. Compendium of Indian Medicinal Plants. Vol. 2, CDRI Lucknow and PID, New Delhi, pp: 381.

Ryokkynen, A., P. Nieminen, A.M. Mustonein, T. Pyykonen and J. Asikainen *et al*., 2005. Phytoestrogens alter the reproductive organ development in the mink (mustela vision). Toxicol Applied Pharmacol., 202: 132-139.

Saleem, M., K. Mee-Hyang, Y. Jung-Mi, M.A. Vaqar and K. Naghma *et al*., 2005. A novel dietary triterpene lupeol induces fas-mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibits tumor growth in a xenograft model. Cancer Res., 65: 11203-11213.

Shailajan, S., C. Naresh, R.T. Sane and M. Sasikumar, 2005. Effect of *Asteracantha longifolia* Nees. against CCl_4 induced liver dysfunction in rat. Indian J. Exp. Biol., 43: 68-75.

Singh, A. and S.S. Handa, 1995. Hepatoprotective activity of *Apium graveolens* and *Hygrophila auriculata* against paracetamol and thioacetamide intoxification in rats. J. Ethanop., 49: 119-126.

Sutar, A.C., D.P. Sohoni, M.M. Banavaliker and M.K. Blyani, 2002. HPTLC methods for quantitative estimation of genistein and daidzein with its Glycosides in *Glycine max*. Indian Drugs, 39: 434-434.

Tiwari, R.D., K.C. Srivastava and P.D. Sattsangi, 1967. Examination of the fixed oil from the seeds of *Hygrophila spinosa*. Indian J. Applied Chem., 30: 58-59.

Warrier, P.K., 1995. Indian Medicinal Plants. In: A Compendium of 500 Species, Vol. 3, Arya, V.S., (Ed.). Orient Longman Ltd., Kottakkal, France, pp: 442.