International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 807-814, Oct-Dec 2009

PLANAR CHROMATOGRAPHIC STUDIES ON *ABIES WEBBIANA* LEAVES

Ashoke Kumar Ghosh and Sanjib Bhattacharya*

Bengal School of Technology (A College of Pharmacy), Sugandha, Delhi road, Hooghly 712102, West Bengal, India.

Corres.author: sakkwai@yahoo.com

ABSTRACT: *Abies webbiana* Lindl. (Pinaceae), commonly called *Talispatra* in Bengali and Hindi, *Talispatram* in Sanskrit and Indian Silver Fir in English, is a large evergreen tree found in the Himalayan region from Kashmir to Assam in India. In India, this plant has been traditionally used as a drug for several ailments. The leaves of this plants have different indications in Ayurveda, the most ancient traditional system of Indian medicine. In our present investigation, thorough comparative planar chromatographic (one dimensional ascending thin layer chromatography) studies of *A. webbiana* leaves were carried out by using different solvent extracts and different mobile phases along with preliminary phytochemical analysis of each extracts to track and visualize the chemical diversity in *A. webbiana* leaves. Several types of compounds were detected viz. amino acids, flavonoids, saponins, tannins, alkaloids, lipids, triterpenoids and steroids. The percentage yield of phosphate buffer (pH 7.4) extract was found to be the maximum (51.0 % w/w). These preliminary information may be useful in different relevant applications in future days.

Key words: *Abies webbiana*, planar chromatography, phytochemical.

INTRODUCTION

The plants are the biosynthetic laboratories where different secondary metabolites are produced and stored. These secondary metabolites are several classes of organic chemical compounds e.g. alkaloids, amino acids, carbohydrates, fixed oils, flavonoids, glycosides, gums, resins, saponins, sugars, tannins, terpenoids, volatile oils, wax, etc. which are responsible for their medicinal properties. Depending on the nature and quantity of these chemical compounds the plants show their variations in medicinal activities[1]. There are a large number of traditionally used plants on earth having a wide range of medicinal efficacies.

Abies webbiana Lindl. (Pinaceae), commonly called *Talispatra* in Bengali and Hindi, *Talispatram* in Sanskrit and Indian Silver Fir in English, is a large tall evergreen tree occurring in the Himalayan region from Kashmir to Assam in India and in the state of Sikkim (India) in particular. It is also found in Afghanistan (Hindu Kush range), Tibet (China), Nepal, in Karakoram Range and Bhutan at an altitude of 2500-4000 m [2]. In Ayurveda, the most ancient traditional system of Indian medicine, this plant had been described for using against swasa (chronic obstructive pulmonary diseases), kasa (cough), gulma (tumour), agnimandya (hypochlorhydria), amadosha (amoebiasis), hikka (hiccup), chhardi (vomiting), krimi (helminthiasis) and mukharoga (mouth disorders)[3]. The leaves of this plant have been traditionally used for their carminative, stomachic, expectorant, decongestant, antiseptic, astringent, antihyperglycemic, female antifertlity, febrifuge and anti-spasmodic properties. The decoctions of the leaves are useful orally in cases of cough, phthisis, asthma, chronic bronchitis and catarrh of the bladder and other pulmonary infections. Furthermore, leaves of the plant have been used traditionally for its chemotherapeutic efficacies in several ailments like rheumatism, hoarseness, chronic bronchitis and other pulmonary affections [4-7].

Previously it has been reported the extracts from the leaves of the plant have antibacterial, mast cell stabilizing, anxiolytic, anti-tumour, anti-inflammatory, antitussive and CNS depressant actions [8-13]. Some active principles mainly monoterpenes (from essential oil), flavonoids, phytosterols and diterpene glycosides (taxol kike compounds) have also been detected in the leaves. Anti-inflammatory effect was exhibited by (+)-pinitol, isolated from leaves of the plant [4,7,14-16]. From previous chemical investigations it appears clear that the leaves of *A. webbiana* contain constituents of different chemical

classes and it is obvious that not a single active constituent is responsible for its diverse biological activities. However, a detailed comparative planar chromatographic (thin layer chromatography, TLC) study of the different extracts of its leaves has not been reported so far. Hence, it was necessary for us to perform thorough TLC studies along with phytochemical studies of different leaf extracts using different mobile phases in pursuit of the expected chemical diversity and the most enriched extract(s).

EXPERIMENTAL

PLANT MATERIAL

A. webbiana leaves were collected from the mature trees grown at Gangtok, Sikkim, India during the month of October-November and were identified at Botanical Survey of India, Shibpur, Howrah, West Bengal, India. The voucher specimen (No. AW-I) was preserved for future reference. The leaves with branches were dried under shade, then the leaves were separated from branches and dried at 40 ^{o}C for 1 h, and then pulverized by a mechanical grinder. The powder was then passed through 40 mesh sieve and stored in a well closed vessel until use. The various extracts were made from the above said powdered crude plant material.

REAGENTS AND CHEMICALS

All reagents and chemicals used for testing were analytical grade obtained from Ranbaxy Fine Chemicals Ltd., New Delhi and Loba Chemie, Mumbai, India. Silica gel G for TLC was procured from Sisco Research Lab. Pvt. Ltd., Mumbai, India.

EXTRACTION METHODS

EXTRACTION BY DISTILLED WATER (PH 6.0)

The powdered leaves were dried below 40 ºC for half an hour prior to use for extraction. 100 g of this powder was taken in a 1 lit. round bottom flask and extracted with 500 ml of distilled water of pH 6.0 in a Soxhlet extractor for 40 h. The filtrate was taken away from the marc. The marc was again extracted with 500 ml of distilled water of pH 6.0. All the extracted solutions were collected together and was dried on sand bath upto the extent of semisolid mass. This was further dried over water bath to get a solid mass of brownish red colour. This dried solid mass was kept in refrigerator for further use. The yield and results of phytochemical investigation are reported in Table 1.

EXTRACTION BY HCL BUFFER (PH 2.4)

The same procedure as the extraction with distilled water was adopted for this case also. But instead of using distilled water of pH 6.0, 500 ml HCl buffer of pH 2.4 was used for the extraction of 100 g of powdered leaves of *A. webbiana*. Ultimately a solid mass of red colour was obtained. The yield and results of phytochemical investigation are reported in Table 1.

EXTRACTION BY PHOSPHATE BUFFER (PH 7.4)

Instead of distilled water and HCl buffer, here 500 ml phosphate buffer of pH 7.4 was used to extract 100 g of powdered leaves of *A. webbiana*. Finally a solid mass of blackish brown colour with fine aroma was obtained. The yield and results of phytochemical investigation are given in Table 1.

SUCCESSIVE SOLVENT EXTRACTIONS[17,18]

The powdered leaves of *A. webbiana* was dried first at below 40 ^{o}C for half an hour. 100 g of that powder was taken to extract successively with 500 ml of Petroleum ether (40^{o}-60^{o}C), 500 ml of Chloroform, 500 ml of Ethyl acetate and 500 ml of Methanol in a Soxhlet apparatus.

After drying the petroleum ether extract, a dark green solid mass was obtained. The mass was stored in refrigerator for future purpose. The marc was dried under controlled temperature and allowed for extraction by chloroform in Soxhlet.

After drying the chloroform extract, a brownish red coloured solid mass was obtained. The mass was stored in refrigerator for future use. The marc was taken out and dried under controlled temperature and then that was allowed for extraction by ethyl acetate in Soxhlet.

In the same manner as done in the above cases the ethyl acetate extract was dried to get a mass of red colour. That mass was stored in refrigerator for future testing. The marc was taken out and used to extract by methanol in Soxhlet. The methanol extract was dried to get a dark reddish brown coloured mass, which was stored in refrigerator for future use.

The report of yield and results of phytochemical investigations are given in Table 1. A schematic diagram of successive extractions by petroleum ether, chloroform, ethyl acetate and methanol with respective yield values of solid mass from the powdered leaves of *A. webbiana* is depicted in Fig.1.

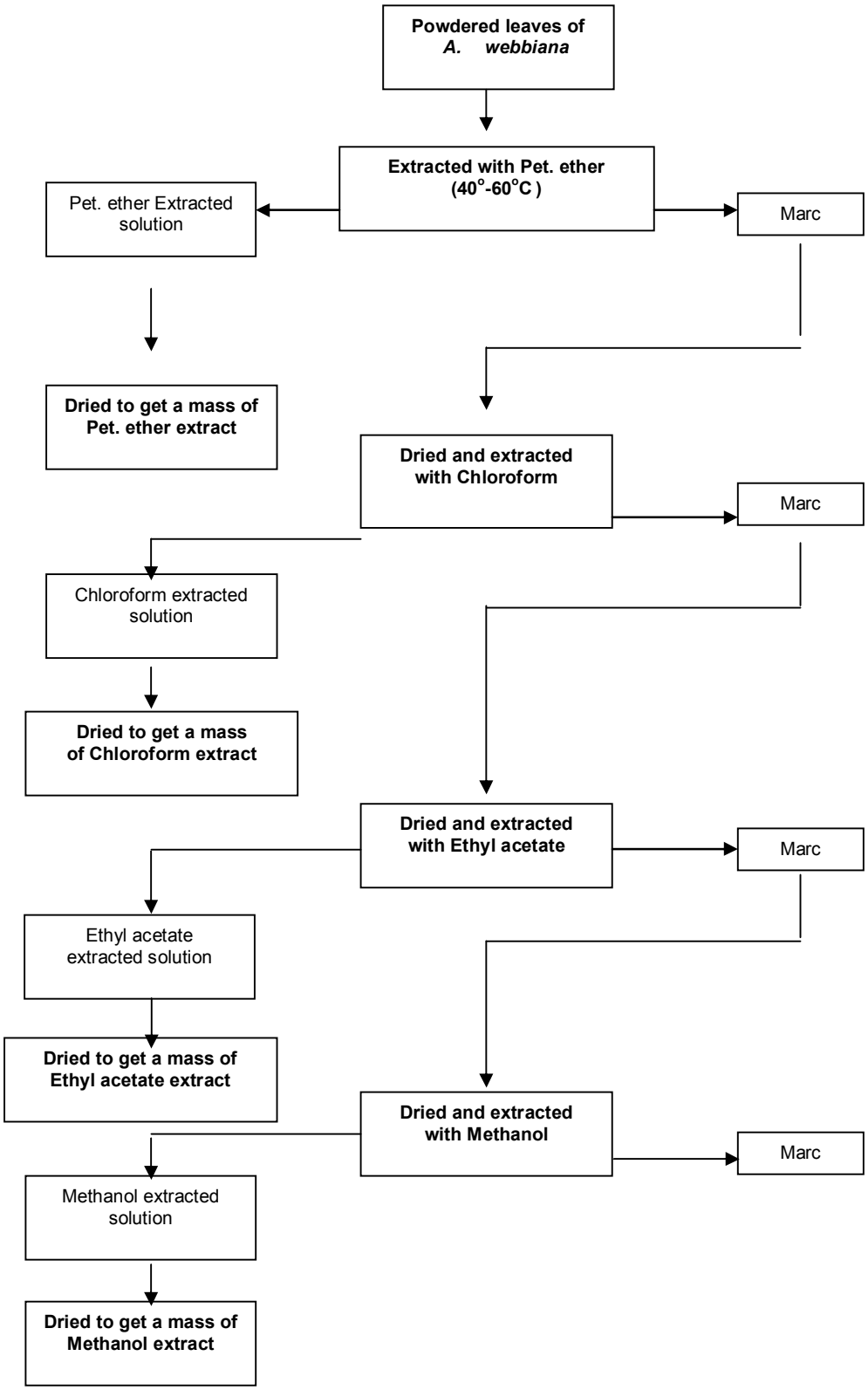

Fig. 1: Schematic diagram of successive extractions of powdered leaves of *A. webbiana*.

PLANAR CHROMATOGRAPHIC (TLC) STUDIES[19,20]

PREPARATION OF TLC PLATES

Silica gel G for TLC was used for the preparation of the TLC plates. Slurry was prepared with distilled water and coated on glass plates of 20 × 5 cm size with the help of conventional spreader, to a layer thickness of 0.25 mm. The plates were first air dried, then activated at the temperature of 110 ºC for 30 minutes. The plates were allowed to cool, then and kept in a desiccator for future use. With a pencil, two small notches were etched to the adsorbent layer from about 2 cm of the bottom of the plate. The notches on the edges of the plate, and each notch were in the same distance up from the bottom of the plate. The base line was little bit above from the solvent level in the chamber.

SPOTTING THE SAMPLES OF DIFFERENT EXTRACTS

Samples of aqueous and non-aqueous extracts were spotted on silica gel G coated activated plates with the help of capillary tubes. The narrow tip of the drawn-out capillary tube was used to touch the liquid extract sample and drawn up about 5 mm of the solution. The samples were spotted carefully without disturbing the surface of the adsorbent and the spots were about 3-3.5 mm in diameter.

DEVELOPMENT OF CHROMATOGRAM

One dimensional ascending development method was followed and the chromatograms were developed in closed development chambers using different solvent systems at an angle of 70° at room temperature (~30^{o}C). The development chamber was left covered with the lid tightly. In each case the solvent system was allowed to run to a distance of 10 cm from the base line of application of the extracts on the plates. The time required for development of chromatogram varied from 30 to 45 minutes. After completion of the run, the plates were removed from the closed chamber; the solvent fronts were traced quickly with a pencil and dried in air. These plates were viewed under UV light before and after spraying of several spray reagents. In all cases, the plates were heated at 105°C for 5-10 minutes after spraying. The colours of the spots developed and their hRf values were inspected in ordinary daylight as well as under long wave UV light.

VISUALIZATION OF THE SPOTS

The developed chromatograms were viewed under the UV viewer lamp before and after exposed to iodine vapour or concentrated H_2SO_4. In all cases, the plates were heated at 105º C for 10 minutes. The colours of spots developed and their hRf values were inspected in day light as well as long wave ultra-violate light (UV, 366 nm). The results of the TLC studies are presented in the Table 2 - 7 .

RESULTS AND DISCUSSION

Chromatography represents a group of methods for separating molecular mixtures that depend on the differential affinities of the solute between two immiscible phases. Among the various methods for separating plant constituents, the chromatographic procedure is the one of the most commonly used techniques of general application[21]. Planar liquid chromatography (PLC) or simply planar chromatography involves the separation of mixtures of organic compounds on thin layers of adsorbents that are usually coated on glass, plastic, or aluminium sheets. The most common form of planar liquid chromatography is thin layer chromatography (TLC) and the most common form of TLC is one dimensional ascending TLC; and this particular technique is the easiest, cheapest and most widely used method for the characterization of natural products and their preparations[22,23]. In addition to qualitative detection, TLC also provides semi-quantitative information on the major active constituents of a crude drug or its preparation, thus enabling an assessment of drug quality. Most importantly TLC provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of crude drugs and for detecting adulterations and substitutions. In this way planar chromatographic techniques can be utilized to analyze plant drugs, their combinations and formulations [24,25].

Plant extracts are generally very complex and comprise mixtures of neutral, acidic, basic, lipophilic, hydrophilic, and amphiphilic (e.g., amino acids) compounds and, as a consequence, there is rarely one TLC method that can practically serve for all eventualities. The chemical constituents determine the extraction conditions and solvent systems necessary for a significant TLC characterization of the crude drug [23, 25].

The results of TLC analysis on petroleum ether, chloroform, ethyl acetate, methanol and different aqueous extracts of leaves of *Abies webbiana*, along with the colour of the spots under long wave UV light and after spraying with specific reagents and the preliminary phytochemical assessment of extracts clearly indicated the presence of different types of constituents (Table 1) in different extracts.

The chemical evaluation as well as chromatographic separation and evaluation (Table 1 - 7) revealed the presence of a multitude of chemical compounds in the leaves of *A. webbiana*. The phosphate buffer (pH 7.4) extract exhibited remarkably maximum yield (51.0 % w/w)), whereas petroleum ether, chloroform and methanol extracts yielded maximum numbers of spots on TLC. Despite showing maximum yield the TLC profile of phosphate buffer extract was not much encouraging, hence it appears necessary to carry out further column chromatographic or solubility based fractionation of this extract. The chemical compounds/constituents were found to be present in distilled water (pH 6.0), in the HCl buffer (pH 2.4) and phosphate buffer (pH 7.4) extracts were found to be similar (i.e. amino acid, flavonoid, saponin and tannin) . However, alkaloids were only detected in the chloroform and methanol extracts. Triterpenoids and steroids were found in petroleum ether, ethyl acetate and methanol

extract. Flavonoids were detected in all extracts. It may be said that phosphate buffer extraction helps to extract more and more the specified constituents which technique may be helpful in industries for its effective extraction and that requires further attention and studies in future. Present preliminary investigation may be helpful for specific TLC fingerprinting, quality assurance and monitoring of the crude drug and its formulations, in developing a suitable commercial extraction method and in further phytochemical and pharmacological studies.

Table 1. Phytochemical screening of several extracts of powdered leaves of *A. webbiana.*

Extraction solvents employed	Colours of the Extracts	Yields of the extracts (% w/w)	Constituents present
Distilled water (pH 6.0)	Brownish red	11.5	Amino acids, flavonoids,Saponins and tannins.
HCl-buffer soln. (pH 2.4)	Red	19.5	Amino acids, flavonoids,saponins and tannins.
Phosphate-buffer soln. (pH 7.4)	Blackish brown	51.0	Amino acids, flavonoids,saponins and tannins.
Petroleum ether (40^{o}-60^{o}C)	Dark green	5.5	Lipids, flavonoids, triterpenoids, and steroids.
Chloroform	Brownish red	6.75	Alkaloids, flavonoids.
Ethyl acetate	Red	7.6	Amino acids, flavonoids, tannins. Triterpenoids and steroids
Methanol	Reddish brown	14.73	Alkaloids, amino acids,flavonoid, saponins, triterpenoids and steroids.

Table 2. TLC of petroleum ether (40º- 60º C) extract of *A. webbiana* leaves.

No. of spots	Colour of the spot under long wave UV light (366nm)				Colour of the spot after spraying I_2 vapour				hRf value			
	Solvent systems				Solvent systems				Solvent systems			
	1	2	3	4	1	2	3	4	1	2	3	4
1	R	Y	DR	R	B	LY	G	G	50	46	87	81
2	YG	G	R	DR	G	P	G	DG	57	77	63	72
3	-	G	-	P	-	R	-	-	-	90	-	63

Solvent system: 1. Ethyl acetate: methanol: water (75:25:5)**,** 2. Chloroform: methanol (95:5), 3. Chloroform: acetone (90:10), 4. Chloroform: benzene (70:30).
Abbreviations: R: red; YG: yellowish green; Y: yellow; G: green; DR: dark red; P: pink; B: brown; DG: deep green; LG: light green; LY: light yellow; PL: purple; RB: reddish brown.

Table 3. TLC of chloroform extract of *A. webbiana* leaves.

No. of spots	Colour of the spot under long wave UV light (366nm)				Colour of the spot after spraying I_2 vapour				hRf value			
	Solvent systems				Solvent systems				Solvent systems			
	1	2	3	4	1	2	3	4	1	2	3	4
1	R	LY	DB	R	B	Y	RB	G	46	53	98	38
2	G	B	R	YG	YG	YG	YB	G	93	92	98	72
3	-	-	Y	-	-	-	*G*	-	-	-	63	-

Solvent system: 1. Ethyl acetate: methanol: water (75:25:5)**,** 2. Chloroform: methanol (95:5), 3. Chloroform: acetone (90:10), 4. Chloroform: benzene (70:30).
Abbreviations: R: red; YG: yellowish green; Y: yellow; G: green; DR: dark red; P: pink; B: brown; DG: deep green; LG: light green; LY: light yellow; PL: purple; RB: reddish brown.

Table 4. TLC of ethyl acetate extract of *A. webbiana* leaves.

No. of spots	Colour of the spot under long wave UV light (366nm)			Colour of the spot after spraying conc. H_2SO_4 vapour			hRf value		
	Solvent systems			Solvent systems			Solvent systems		
	1	2	3	1	2	3	1	2	3
1	R	DB	P	P	G	B	84	72	84
2	-	YG	-	-	G	-	-	82	-

Solvent system: 1. Diethyl ether: benzene (9:1), 2. Benzene: diethyl ether (5:5), 3. Benzene: diethyl ether (9:1).
Abbreviations: R: red; YG: yellowish green; Y: yellow; G: green; DR: dark red; P: pink; B: brown; DG: deep green; LG: light green; LY: light yellow; PL: purple; RB: reddish brown.

Table 5. TLC of methanol extract of *A. webbiana* leaves.

No. of spots	Colour of the spot under long wave UV light (366nm)				Colour of the spot after spraying I_2 vapour				hRf value			
	Solvent systems				Solvent systems				Solvent systems			
	1	2	3	4	1	2	3	4	1	2	3	4
1	R	C	DB	P	B	LY	RB	G	53	46	98	38
2	R	B	Y	YG	YG	G	YB	G	88	88	38	34
3	-	-	B	-	-	-	G	-	-	-	58	-

Solvent system: 1. Ethyl acetate: methanol: water (75:25:5)**,** 2. Chloroform: methanol (95:5), 3. Chloroform: acetone (90:10), 4. Chloroform: benzene (70:30).
Abbreviations: R: red; YG: yellowish green; Y: yellow; G: green; DR: dark red; P: pink; B: brown; DG: deep green; LG: light green; LY: light yellow; PL: purple; RB: reddish brown.

Table 6. TLC of water (pH 6.0) extract of *A. webbiana* leaves.

No. of spots	Colour of the spot under long wave UV light (366nm)	Colour of the spot after spraying conc.H_2SO_4	hRf value
1	R	B	71.6
2	PG	P	52

Solvent system: Butanol: methanol (5:5).
Abbreviations: R: red; YG: yellowish green; Y: yellow; G: green; DR: dark red; P: pink; B: brown; DG: deep green; LG: light green; LY: light yellow; PL: purple; RB: reddish brown.

Table 7. TLC of phosphate buffer (pH 7.4) extract of *A. webbiana* leaves.

No. of spots	Color of the spot in day light	Colour of the spot in 365 nm	Colour of the spot in 254 nm	Colour of the spot after spraying with conc. H_2SO_4			hRf value
				In day light	In 365 nm	In 254 nm	
1	LB	BR	BG	P	YG	GB	83.3
2	-	W	-	-	Y	-	71.6

Solvent system: Butanol: methanol (5:5).
Abbreviations: YG: yellowish green; Y: yellow; P: pink; LB: light brown; BR: blackish red; W: white; BG: blackish green; GB: greenish black.

ACKNOWLEDGEMENT

One of the authors acknowledges Prof. (Dr.) Tarun Jha of Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India, for using the Natural Science Laboratory for performing a part of the present work at the said department.

REFERENCES

1. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Edinburgh: W.B. Saunders; 2002, 150.
2. Anonymous. Ayurvedic Pharmacopoeia of India. Part I, Vol. IV, 1st ed., Govt. of India, New Delhi, 2004, 124-125.
3. Ganguly HC, Kar AK. College Botany. Vol. II. Books and Allied (P) Ltd., Kolkata, 1999, 1102-1103.
4. Anonymous. The Wealth of India, Raw Materials. Vol. IA. Publication and Information Directorate, CSIR, New Delhi, 1976, 16-17.

5. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. III. 2nd ed. Bishen Singh Mahendra Pal Singh, New Delhi, 1933, 2392-2393.
6. Nadkarni KM, Nadkarni AK, Chopra RN. Indian Materia Medica. Vol. I. Popular Prakashan, Bombay, 1976, 3-4.
7. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. Springer, Berlin, Heidelberg, 2007, 2.
8. Ghosh AK, Srikanth K, Jha T. Inhibitory effect of *Abies webbiana* on tumour cells in mice. Indian J Nat Prod 2001; 7: 17–19.
9. Singh, R.K., Bhattacharya, S.K. and Acharya, S.B., 2000. Pharmacological activity of *Abies pindrow*. J Ethnopharmacol 2000; 73 (1-2): 47–51.
10. Kumar V, Singh RK, Jaiswal AK, Bhattacharya SK, Acharya SB. Anxiolytic activity of *Abies pindrow* Royal leaves: an experimental study. Indian J Exp Biol 2000; 38: 343-346.
11. Nayak SS, Ghosh AK, Srikanth K, Debnath B, Jha T. Antitussive activity of *Abies webbiana* Lindl. leaf extract against sulphur dioxide-induced cough reflex in mice. Phytotherapy Res 2003; 17 (8): 930-932.
12. Nayak SS, Ghosh AK, Debnath B, Vishnoi SP, Jha T. Synergistic effect of methanol extract of *Abies webbiana* leaves on sleeping time induced by standard sedatives in mice and anti-inflammatory activity of extracts in rats. J Ethnopharmacol 2004; 93 (2-3): 397-402.
13. Vishnoi SP. Ghosh AK, Debnath B, Samanta S, Gayen S, Jha T. Antibacterial activity of *Abies webbiana*. Fitoterapia 2007; 78 (2): 153-155.
14. Singh RK, Pandey BL, Tripathi M, Pandey VB. Anti-inflammatory effect of (+) pinitol. Fitoterapia 2000; 72: 168-170.
15. Chatterjee A, Pakrashi SC. The Treatise on Indian Medicinal Plants. Vol. I. Publication and Information Directorate, CSIR, New Delhi, 1991, 2.
16. Tiwari KP, Minocha PK. A Chalcone glycoside from *Abies pindrow*. Phytochemistry, 1980: 19: 2501-2503.
17. Kokate CK. Practical Pharmacognosy. 4th ed. Vallabh Prakashan, New Delhi, 1994, 107-111.
18. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 13th ed. Nirali Prakashan, Pune, 2005, 149-153.
19. Lala PK. Practical Pharmacognosy, 1st ed. Lina, Calcutta, 1981, 5-14.
20. Stahl E. Apparatus and General Techniques. In: Stahl E, editor. Thin Layer Chromatography, A Laboratory Handbook. 2nd ed. Springer-Verlag, Berlin, Heidelberg, New York,1969, 52-85.
21. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 34th ed. Nirali Prakashan, Pune, 2006, 114.
22. Gibbons S, Gray AI . Isolation by Planar Chromatography. In: Cannell RJP, editor. Natural Products Isolation. 1st ed. Humana Press Inc, Totowa, New Jersey, 1998, 209.
23. Gibbons S. An Introduction to Planar Chromatography. In: Sarker SD, Latif Z, Gray AI, editor. Natural Products Isolation. 2nd ed. Humana Press Inc, Totowa, New Jersey, 2006, 77-78.
24. Bhattacharya S, Zaman MK. Pharmacognostical evaluation of *Zanthoxylum nitidum* bark. Int J Pharm Tech Res 2009; 1 (2): 292-298.
25. Mukherjee PK. Quality Control of Herbal Drugs: An approach to evaluation of botanicals. 1st ed. Business Horizons, New Delhi, 2002, 427-428.

*****