IJPSR (2016), Vol. 7, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 August, 2015; received in revised form, 25 October, 2015; accepted, 06 December, 2015; published 01 February, 2016

PHARMACOGNOSTICAL AND PHYSICO-CHEMICAL STANDARDISATION OF WHOLE PLANT OF *ADIANTUM CAPILLUS VENERIS* LINN

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Keywords:

Adiantum capillus-veneris Linn, Pharmacognostic standardisation, Phytochemical analysis, TLC Profiling.

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ABSTRACT: Traditional knowledge and ethno-botanical use of plants have been widely acknowledged all over the world. The cicatrizing properties of extracts obtained from this plant have been scientifically studied, attributing the main biological activity to its tannin and flavonoid content. Recent commercialization of the plant drug *Adiantum capillus veneris* Linn. requires pharmacognostical information to develop quality-control methods for raw materials and extracts produced with this plant drug. Macro and micro-morphological parameters were established to authenticate the genuine drug that allowed detection of adulterants usually found in commercial samples of this plant material. All the parameters were studied according to WHO guidelines and Indian Pharmacopoeia. These morphological characteristics can be used for rapid identification of the drug and are particularly useful in the case of powdered materials. Physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, sulphated ash, crude fibre content, loss on drying, percentage of foreign matter and extractive values were determined. Preliminary phytochemical screening in different solvents showed the presence of steroids, flavonoids, terpenoids, fats, tannins and phenolic compounds. TLC profiling of plant extracts gives an idea about the presence of various phytochemicals. The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications.

INTRODUCTION: *Adiantum capillus-veneris* Linn. (Hansraj) belonging to the *Adiantaceae* family is a kind of medicinal and ornamental fern widely distributed throughout the world [1, 2]. It is a delicate graceful fern, small rhizomatous, erect and perrenial herb up to 30 cm tall with long polished black stripes [3, 4, 5].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(2).773-82

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (2).773-82

This fern is cultivated as an ornamental plant in Japan and Europe because of its beautiful evergreen frond [2]. *Adiantum capillus veneris* Linn. is one of the most common species with potential importance for medicinal and nutritive purpose [6]. Ethnomedicinally, the genus has been used as tonic and diuretic; in treatment of cold, fever, cough and bronchial disorders, as stimulant, emollient, purgative, demulcent, general tonic and hair tonic, in addition to skin diseases, tumors of spleen, liver and other viscera in treatment of jaundice and hepatitis and many other uses [7]. It has been used in tea for respiratory diseases and as syrup for severe cough. Also, it promotes hair growth and makes the color of hair black. No side effects of this herb are

reported, but it is contraindicated in pregnancy [8]. Concerning the phytoconstituents, the literature revealed the presence of flavonoids, sulphate esters of hydroxycinnamic acid, sugars and different classes of triterpenoids, sterols, bitter material, mucilage, tannins and ester [7, 8]. Because of the high level content of flavonoids and phenols presented in it, the biological properties attributed to this species including anti-inflammatory, anti-infective and anti-tumours may originate from these components and the probable functional mechanism were antimicrobial and antioxidant effects [9].

In spite of the numerous medicinal uses attributed to this plant, pharmacognosy information about this plant has not been published. Keeping in view, an attempt has been made to standardize the ethnopharmacologically useful whole plant of *Adiantum capillus veneris* Linn. widely used in central India, based on pharmacognostical and physicochemical characteristics.

MATERIALS AND METHODS:

Plant Material Collection and Authentication:
Disease free dried plant of *Adiantum capillus veneris* Linn. were collected from a commercialized source Verdure Herbals, New Delhi. The plant was authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, New-Delhi, under the voucher specimen No.- NISCAIR/RHMD/Consult/2011-12/1792/92 and specimen was submitted to the department of Pharmacognosy and Phytochemistry, Hindu College of Pharmacy, Sonepat, Haryana (India).

Chemicals and Instruments:
Solvents viz. petroleum ether, chloroform, methanol, acetone and reagents, viz. phloroglucinol, glycerin, chloral hydrate, iodine and sodium hydroxide were procured from RFCL, Mumbai, India. Compound microscope, Camera Lucida, Stage and eyepiece micrometer, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Microphotographs were taken using Labomed ATC-200 microscope attached with Sony digital camera.

Preparation of Extracts:
The collected dried sample of whole plant successively extracted with different solvents ranging from non-polar to polar solvents viz. petroleum ether, chloroform, methanol and water so as to get the respective extracts. All the extracts were filtered individually, evaporated to dryness using the rotary evaporator, weighed and percentage yields were calculated. Colour and consistency of the extracts were observed.

Macroscopic and Microscopic Evaluation:
The shape, size, colour, odour, taste, surface texture and fracture characteristics of the leaf and stem were determined. Microscopy was done by taking the thin hand sections of the stem. The thin sections were cleared with chloral hydrate solution and stained with phloroglucinol and hydrochloric acid, then mounted in glycerin for the identification of various regions. Powder of the dried whole plant was separately treated with phloroglucinol, hydrochloric acid and glycerin to study various characteristics [10, 11].

Fluorescence Analysis:
The powdered material and different extracts were exposed to visible and ultraviolet light (U.V. short and U.V. long) to study their fluorescence behaviour [12, 13].

Physicochemical Parameters and Phytochemical Evaluation:
The moisture content, total ash, water soluble ash, acid insoluble ash, sulphated ash, crude fibre content , alcohol and water soluble extractive values were determined as a part of its physicochemical parameters [11, 14]. Petroleum ether, chloroform, methanol and aqueous extracts were subjected to phytochemical analysis for the presence of various secondary phytoconstituents using standard procedures [15, 16, 17].

Thin layer chromatography of extracts:
Thin layer chromatographic (TLC) analysis of different crude extracts was done on analytical plates over silica gel (TLC-grade; Merck India). Different solvent systems were tried for development of chromatographic separation. The plate was immersed slowly into a development

chamber and allowed to develop in the saturated chromatography chamber. The plate was left in the chamber for about 20-30 minutes for the separation of active ingredients. Various spots were viewed under ultraviolet (UV) light at 254 & 365 nm and iodine chamber [18]. Finally, the colour and the distance of the unknown spots were calculated.

RESULTS:

Macroscopic and Microscopic Evaluation: Morphologically, the stems of *Adiantum capillus veneris* Linn. appeared dark purplish to black in colour having aromatic odour, wiry and 10-20 cm long and 2 mm diameter in size, sub erect in shape, smooth in texture, soft in touch; while leaves showed dark green colour, characteristic odour, wedge or fan shaped, 1.2-2.0 cm length and 1.25-2.5 cm breadth, smooth touch, slightly bitter taste, veins spread in a fan like manner in the lamina and cuneate base **Fig.1**and **Table1.**

Transverse section of stem showed the presence of thick walled heavily cutinized epidermis and hypodermis followed by the ground tissue composed of parenchymatous cells with air spaces. In the ground tissue, meristeles are present. Cortex parenchymatous and contains starch grains; stele consists of single layered endodermis followed by pericycle; xylem triarch, surrounded by phloem (**Fig. 2 & 3**). The analysis of powder showed the presence of homosporous tetrahedral spore, multicellular uniseriate covering trichome, cork cells, xylem vessels, lignified fibres, sporangium with incomplete annulus. The annulus is composed of 18-25 cells. The cells of the annulus are heavily thickened (**Fig. 4, 5, 6**).

TABLE 1: MORPHOLOGY OF *ADIANTUM CAPILLUS VENERIS* LINN. DRIED LEAF AND STEM

Parameters	Leaf	Stem
Color	Bright green	Dark purplish to black
Shape	Wedge or fan shaped	Sub erect
Odour	Fragrant smell	Aromatic
Length	1.2-2.0 cm	10-20 cm
Breadth	1.25-2.5 cm	2 mm in diameter
Base	Cuneate	Scaly
Texture	Thin	Smooth
Taste	Slightly bitter	Slightly bitter
Touch	Smooth	Soft

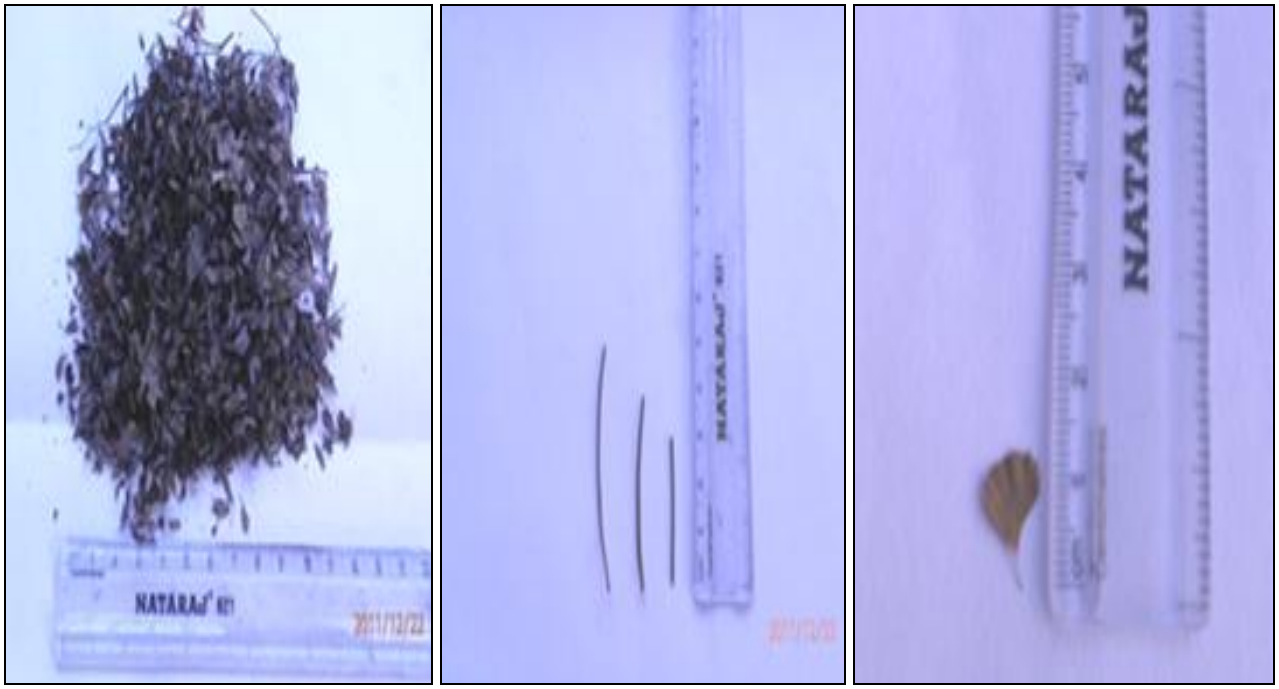

FIG.1: MORPHOLOGY OF WHOLE PLANT, STEM AND LEAF OF *ADIANTUM CAPILLUS VENERIS* LINN.

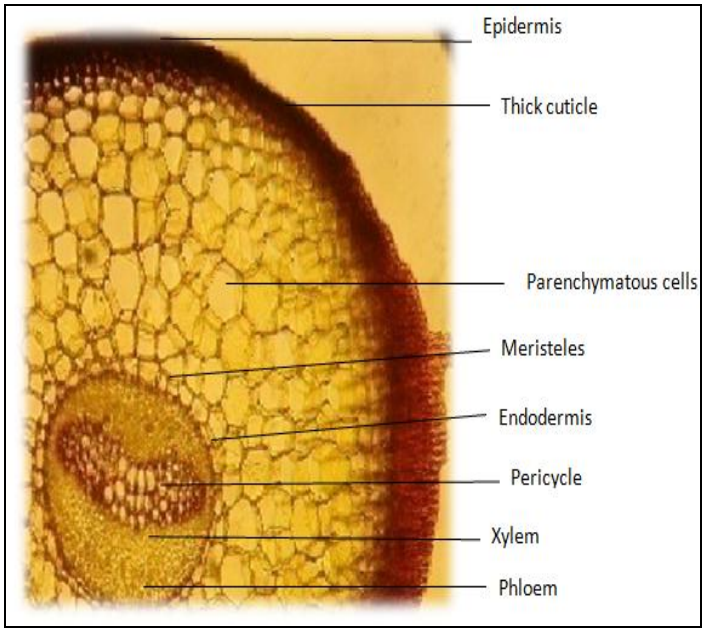

FIG.2: T.S OF *ADIANTUM CAPILLUS VENERIS* LINN. STEM

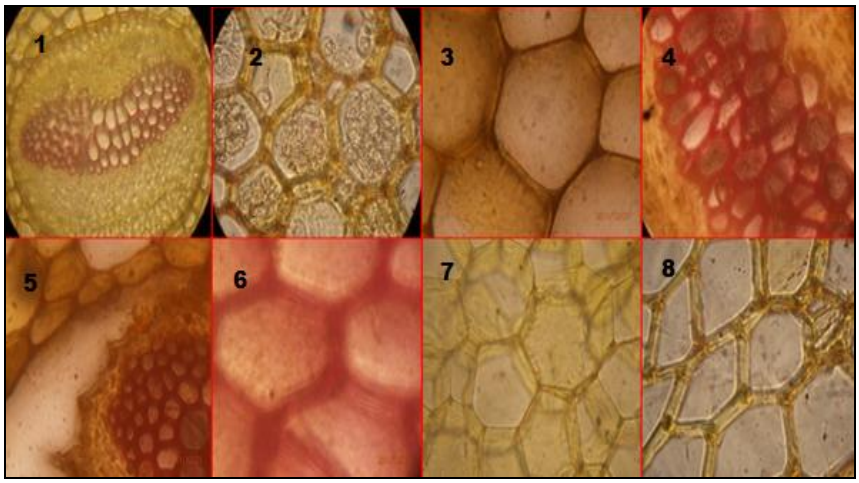

FIG.3: T.S OF *ADIANTUM CAPILLUS VENERIS* LINN. STEM SHOWING; 1, 4, 5, 6: MERISTELES; 2: PARENCHYMATOUS CORTEX CONTAINING STARCH GRAINS; 3, 7, 8: PARENCHYMATOUS CELLS

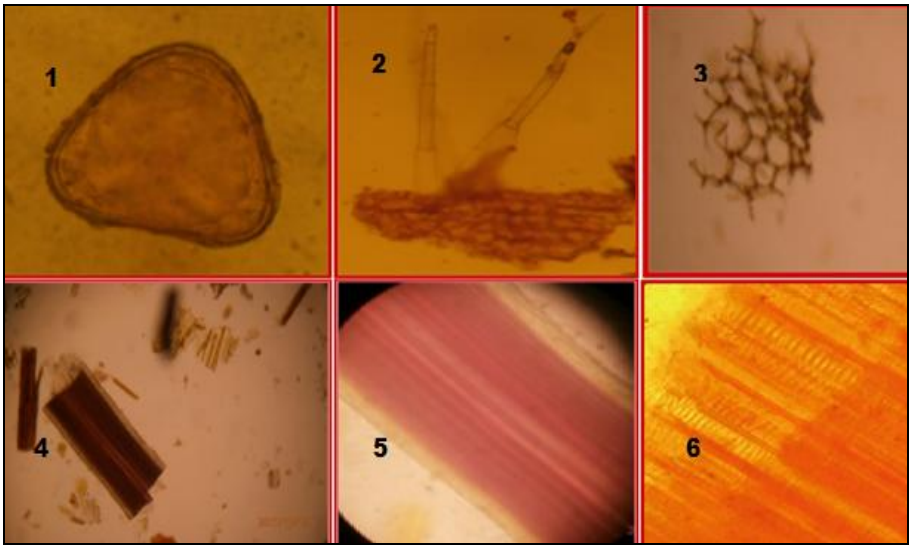

FIG.4: POWDER MICROSCOPY SHOWING; 1: HOMOSPOROUS TETRAHEDRAL SPORE; 2: MULTICELLULAR UNISERIATE COVERING TRICHOME; 3: CORK CELLS; 4, 5 AND 6: XYLEM VESSELS

FIG.5: POWDER MICROSCOPY SHOWING SPORANGIUM

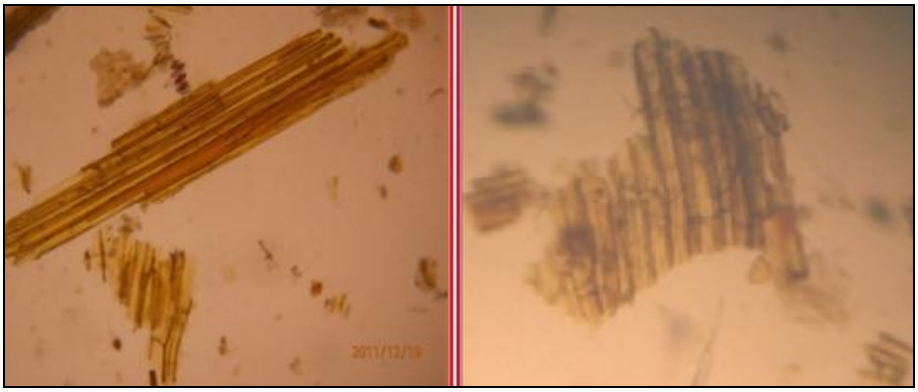

FIG.6: POWDER MICROSCOPY SHOWING LIGNIFIED FIBRES

Fluorescence Study:

Fluorescence analysis of the various solvent extracts and powdered drug after treatment with different reagents like 1N NaOH in methanol, 1N NaOH in water, 1N HCl, 50% H_2SO_4, 50% HNO_3, 50% HCL was observed in the day light and UV light and colours were observed. The results are shown in **Table 1** and **Table 2**.

Physicochemical Evaluation:

Physicochemical parameters are important parameters in detecting adulteration and are adopted to confirm the purity and quality of drug. Ash values are particularly important parameter as it shows the presence and absence of foreign matters like metallic salts or silica etc. The percentage of total ash, acid insoluble ash, water soluble ash and sulphated ash were carried out. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble, alcohol soluble extractive values were calculated. The results are tabulated in **Table 4**.

Preliminary Phytochemical Evaluation:

Phytochemical screening showed the presence of fats, flavonoids, steroids, saponins, tannins and phenolic compounds (**Table 5**).

TABLE 2: PERCENTAGE YIELD, COLOUR, CONSISTENCY AND FLUORESCENCE NATURE OF THE SUCCESSIVE EXTRACTS OF WHOLE PLANT OF *ADIANTUM CAPILLUS VENERIS* LINN.

Solvent	% yield (w/w)	Consistency	Under visible light	Under short wavelength (254nm)	Under long wavelength (360nm)
Pet ether	4.76%	Sticky Solid	Greenish Black	Black	Blackish green
Chloroform	2.05%	Sticky Solid	Greenish Black	Black	Blackish green
Methanol	5.35%	Semisolid	Brownish yellow	Black	Blackish green
Aqueous	10.92%	Sticky Semisolid	Brownish Black	Black	Blackish green

TABLE 3: FLUORESCENCE NATURE OF STEM, LEAF AND POWDERED WHOLE PLANT OF *ADIANTUM CAPILLUS VENERIS* LINN.

Reagent used	Colour under day light			Colour under short wavelength (254nm)			Colour under long wavelength (360nm)		
	Stem	Leaf	Whole plant	Stem	Leaf	Whole plant	Stem	Leaf	Whole plant
Direct powder	Brown	Brown	Green	Black	Black	Black	Dark green	Dark green	Dark green
Acetic acid	Yellowish brown	Brown	Brown	Black	Black	Black	Dark green	Dark green	Dark green
1N HCl	Brown	Brown	Brown	Black	Black	Black	Dark green	Brown	Dark green
50% HCL	Yellowish brown	Brown	Brown	Black	Black	Black	Dark green	Dark green	Dark green
50% H_2S0_4	Brown	Brown	Brown	Black	Black	Black	Dark green	Dark green	Dark green
1N $HN0_3$	Brown	Brown	Brown	Black	Black	Black	Dark green	Dark green	Dark green
50% $HN0_3$	Brown	Brown	Brown	Black	Black	Black	Dark green	Dark green	Dark green
Iodine water	Yellowish brown	Brown	Brown	Black	Black	Black	Dark green	Dark green	Dark green
1N NaOH in water	Brown	Brown	Brownish black	Black	Black	Black	Dark green	Dark green	Dark green
1N NaOH in alcohol	Brownish yellow	Yellowish brown	Dark green	Black	Black	Black	Dark green	Dark green	Blackish green
Picric acid	Yellowish brown	Yellowish brown	Brown	Black	Black	Black	Dark green	Dark green	Dark green
5% $Fecl_3$ in water	Brown	Brown	Brownish green	Black	Black	Black	Dark green	Dark green	Dark green
5% $Fecl_3$ in alcohol	Yellowish brown	Black	Yellowish black	Black	Black	Black	Dark green	Dark green	Yellowish black

TABLE 4: PHYSICOCHEMICAL PARAMETERS OF *ADIANTUM CAPILLUS VENERIS* LINN.

Parameters	Stem	Leaf	Whole plant
Foreign organic matter (w/w)	1.2%	0.8%	2%
Total ash (w/w)	8.5%	6.5%	9.5%
Water soluble ash (w/w)	2%	5.5%	8%
Acid insoluble ash (w/w)	3%	4.5%	4.5%
Sulfated ash (w/w)	4.5%	6%	8%
Alcohol soluble extractive (w/w)	7.2%	6.4%	5.6%
Water soluble extractive (w/w)	16%	12%	7.6%
Moisture content (w/w)	1.5%	0.5%	3.5%
Crude fibre content (w/w)	8%	2%	14%
Swelling index (ml)	1 ml	0.5 ml	2 ml
Foaming index (ml)	Less than 100 ml	Less than 100 ml	Less than 100 ml

TABLE 5: PRELIMINARY PHYTOCHEMICAL SCREENING OF WHOLE PLANT EXTRACTS OF *ADIANTUM CAPILLUS VENERIS* LINN.

Test for Constituents	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	-ve	-ve	-ve	-ve
Carbohydrates	-ve	-ve	-ve	-ve
Flavonoids	-ve	-ve	+ve	-ve
Phenolics & Tannins	-ve	-ve	+ve	+ve
Proteins & Amino acid	-ve	-ve	-ve	-ve
Mucilage	-ve	-ve	-ve	-ve
Steroids	+ve	-ve	-ve	+ve
Terpenoids	-ve	-ve	+ve	+ve
Glycosides	-ve	-ve	-ve	-ve
Saponins	-ve	-ve	+ve	+ve
Fats & Fixed oil	+ve	-ve	-ve	-ve

+ = Presence of constituent, - = Absence of constituent

TABLE 6: TLC RESULTS OF CRUDE PETROLEUM ETHER EXTRACT OF WHOLE PLANT OF *ADIANTUM CAPILLUS VENERIS* LINN.

Solvent system	Visualising agent	Number of component	Distance travelled by solute	Distance travelled by solvent	R_f Value
Toluene: Acetone (95:5)	Day light	6	3.2 cm	15.5 cm	0.20
			8.1 cm	15.5 cm	0.52
			9.2 cm	15.5 cm	0.59
			11.1 cm	15.5 cm	0.71
			12.2 cm	15.5 cm	0.78
			14 cm	15.5 cm	0.90
Benzene: Petroleum ether: Ethyl acetate (85: 13: 2)	Iodine chamber	4	10.2 cm	16.5 cm	0.61
			11.5 cm	16.5 cm	0.69
			12.8 cm	16.5 cm	0.77
			14.2 cm	16.5 cm	0.86
Petroleum ether: Chloroform: Toluene (20: 70: 10)	Iodine chamber	4	2.3 cm	14 cm	0.16
			5.5 cm	14 cm	0.39
			10 cm	14 cm	0.71
			12.5 cm	14 cm	0.89

FIG.7: TLC PLATES OF CRUDE PETROLEUM ETHER EXTRACT

TABLE 7: TLC RESULTS OF CRUDE CHLOROFORM EXTRACT OF WHOLE PLANT OF *ADIANTUM CAPILLUS VENERIS* LINN.

Solvent system	Visualising agent	Number of component	Distance travelled by solute	Distance travelled by solvent	R_f Value
Petroleum ether: Chloroform (25: 75)	Iodine chamber	5	3.4 cm	14 cm	0.24
			7.3 cm	14 cm	0.52
			8.2 cm	14 cm	0.58
			9.5 cm	14 cm	0.67
			12.3 cm	14 cm	0.87
Toluene: Ethyl acetate (6: 1)	Day light	5	3 cm	16.5 cm	0.18
			12 cm	16.5 cm	0.72
			12.8 cm	16.5 cm	0.77
			14 cm	16.5 cm	0.84
			15.1 cm	16.5 cm	0.91
Petroleum ether: Chloroform (50: 50)	Iodine chamber	4	2.5 cm	12.5 cm	0.2
			4.5 cm	12.5 cm	0.36
			8.9 cm	12.5 cm	0.71
			11 cm	12.5 cm	0.88

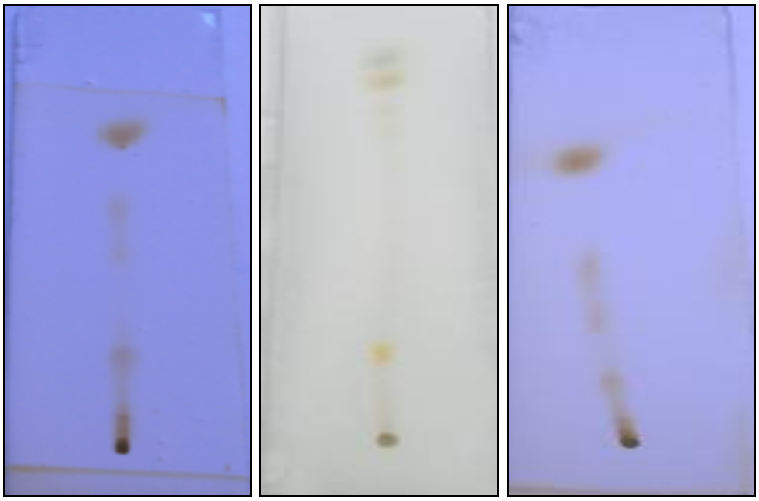

FIG. 8: TLC PLATES OF CRUDE CHLOROFORM EXTRACT

DISCUSSION: Despite the availability of hyphenated analytical techniques, identification and evaluation of plant drugs by pharmacognostical and physico-chemical parameter study is still more reliable, accurate and inexpensive. According to world health organization (WHO), the macroscopic and microscopic determination of the plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [19].

In the present work the macroscopic and microscopic study of whole plant of *Adiantum capillus veneris* Linn. was carried out. The results of macroscopic study might be useful for distinguishing it from its substitutes and adulterants. Microscopic evaluation allows more detailed examination of crude drug and enables to identify the organised structural features such as epidermis, starch grains and parenchymatous cells.
The physico-chemical parameters are helpful in judging the purity and quality of the drug. The percentage of active chemical constituents in crude drugs is usually mentioned on air-dried basis. Hence the moisture content of a drug should be determined and also should be controlled to make the solution of definite strength. The moisture content of a drug should be minimised in order to prevent decomposition of crude drug either due to chemical change or due to microbial contamination.

Ash values were used to detect the presence of any siliceous contamination and water soluble salts. These values are important quantitative standards as it is useful in determining authenticity and purity of drugs. Lower content of total ashes in the results indicate low level of carbonates, Phosphates, silicates and silica. The total ash value for a crude drug is not always reliable, since there is possibility of presence of non-physiological substances. The water soluble extractives indicate the presence of water soluble matters such as alkaloid, amino acids, carbohydrate, mucilage, triterpenoid and flavonoids. These organic ligands possess promising biological activities, which can be utilised to develop potential drugs.

The results of fluorescence analysis of leaf, stem and whole plant powder showed their characteristic fluorescent colour in different organic and inorganic solvents. The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range of daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by treating with different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [20].

The results of preliminary phytochemical screening showed the presence of various phytochemical compounds in the whole plant which are known to have various therapeutic importance in medical

sciences. For instance steroids, terpenoids, flavonoids, saponins, tannins and alkaloids have anti-inflammatory effects. Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities [21, 22]. Rupasinghe et al reported saponins possess hypocholesterolemic and antidiabetic properties [23]. The terpenoids have also been shown to decrease blood sugar level in animal studies [24]. Steroids and triterpenoids showed the analgesic properties. The steroids and saponins are responsible for central nervous system activities [25].

In the present state of affairs, TLC profiling of crude extracts in different solvent system indicated the presence of diverse type of phytochemicals in these plant. Different R_f values of the compound also reflects an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

The leaf, stem and whole plant under study can be utilised as a potential source of useful therapeutics and the outcome data will be beneficial for quantitative and qualitative standardisation of herbal preparations containing *Adiantum capillus veneris* Linn. Further studies are in progress on whole plant in order to isolate, identify, characterise and elucidate the structure of bioactive compounds along with exploration of their pharmacological activity.

CONCLUSION: In the present investigation various standardisation parameters such as macroscopy, microscopy, physicochemical constants, preliminary phytochemical investigation and TLC profiles of sequential extraction of sample in petroleum ether, chloroform and methanol extracts were studied, which are being reported for the first time in this plant, could be helpful in authentication and preparation of a suitable monograph for the proper identification of whole plant of *Adiantum capillus veneris* Linn.

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How to cite this article:
Ranjan V and Vats M: Pharmacognostical and Physico-Chemical Standardisation of Whole Plant of *Adiantum Capillus Veneris* Linn. Int J Pharm Sci Res 2016; 7(2): 773-82.doi: 10.13040/IJPSR.0975-8232.7(2).773-82.