

HPTLC Finger Printing Studies of Medicinal Plant
Adiantum Capillus-Veneris L.

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Abstract

Adiantum capillus-veneris L. is commonly known as herbaceous plant belong to the family *Adiantaceae.*, Hindi- Hansraj, Mubaraka, Purusa, Urdu-Parsiashan, Kumaoan-Mubaraka, Kashmiri-Duntuli, Arabic-Shairuljin, Shiruljin and in English-Southern maidenhair fern, Maidenhair fern, Venus hair fern It is an important medicinal plant native to the United States America, Eurasia, the Levant in Western Asia, Australasia and Asian region, North east India region and west Bengal etc. Quality assurance, quality control parameters studies to evaluate and fix the drug validation, authenticate quality standards development. Active phytochemical constituents present in the plant triterpenoids, aoleananes, phenyl propanoids, carbohydrates, carotenoids, alicyclics and flavonoids like rutin, quercetin, quercetin-3-O-glucoside, querciturone, soquercitrin, nicotiflorin, naringin, astragalin, populnin, procyanidin, prodelphinidin, and kaempferol-3-sulfate. TLC/ HPTLC studies of chloroform and alcohol extracts showed various spots / peaks at 254nm, 366nm and derivatized plates (Vanillin-sulphuric acid reagent), Quality assurance and Quality control parameter. The evaluated HPTLC. research data's will serve as referential supports of pharmacopoeial standard research development of the plant in the near future for any analytical and biological research studies.

Keywords: *Adiantum capillus-veneris L.*, TLC/HPTLC research studies, Quality Assurance, Quality Control parameters.

Introduction

Adiantum capillus-veneris L. commonly known as herbaceous plant belongs to the family Adiantaceae is a kind of medicinal and ornamental fern widely distributed throughout the world. *Adiantum capillus-veneris* grows from 6 to 12 inch (15 to 30 cm) in height; its fronds arising in clusters from creeping rhizomes 15 to 60 cm tall, with very delicate, light green fronds much subdivided into pinnae 4.5 to 8 mm long and broad; the frond rachis is black and wiry,(Shown in Fig.1(a),(b) and (c) respectively.

It is most frequently used for treating problems related to the Diuretic, stimulant, emollient, purgative, demulcent, general tonic and hair tonic. It is used in treatment of cold, fever, cough and bronchial disorders, tumour of spleen, liver and other viscera, treatment of jaundice and hepatitis.(Yumkham *et al.*,2018) It is known as herbal fern which is used in many regions as a herbal medicine for a variety of problems. It is in the treatment of bronchitis in folklore medicine in China. In Kurdistan dried maidenhair fern is rehydrated and boiled in water then the filtrate is used as a drink to get rid of kidney stones, because it is used as a diuretic. it is also used for detoxifying the liver and shortness of breath. In the Philippines fronds are used as treatment for chest disease, and also used for cold, coughs and difficulty of breathing in Iraq, and Iran. (Nakane *et al.*,1999) It has been used for respiratory and urinary disorders. Found to be useful for helping to clear up coughs, and for congestion, and hoarseness. It is also used as syrup in various regions in central and South America, in Amazon and Peruvian as diuretic also in France a syrup from the fronds is used to reduce mucus and cough which is called "Sirop de Capillaire,". And finally England use's true maidenhair for some disease such as asthma, hair loss and shortness of breath (Ansari, and Ekhlesi, 2012).

It is listed as a cultivated plant for economic purposes by many countries (Chong *et al.* 2009). A total of nine (9) species including three (3) sub-species and two (2) races of *Adiantum* have been recorded from the seven states of NE India in the present investigation. They are *A. capillus-veneris*, *A. caudatum*, *A. edgeworthii*, *A. flabellulatum*, *A. hispidulum*, *A. incisum*, *A. peruvianum*, *A. philippense* and *A. raddianum*. Four species (*A. philippense*, *A. flabellulatum*, *A. caudatum* and *A. capillus-veneris*) are very common in the entire NE India and grow along stream banks, brick-canals, humus deposited stones etc.

During our survey, we collected three subspecies of *A. philippense* showing diverse in sterile fronds ranging from entire in *A. philippense* subsp. intermedium, sub-entire in *A. philippense* subsp. philippense to deep-lobed pinnae in *A. philippense* subsp. teestae. In between the two races of *A. capillus-veneris*, the dissectum race is rare and mostly confined to the higher altitude (approx. 1000 m a.s.l.).

Adiantum capillus-veneris L. called in Hindi- Hansraj, Mubarak, Purusa, Urdu-Parsiashan, Kumaoan-Mubarak, Kashmiri-Duntuli, Arabic-Shairuljin, Shiruljin and in English-Southern maidenhair fern, Maidenhair fern, Venus hair fern. It is an important medicinal plant native to the southern half of the United States from California to the Atlantic coast, through Mexico and Central America, to South America. It is also native to Eurasia, the Levant in Western Asia (Bahrain, Iraq, Jordan, Kuwait, Lebanon, Oman, State of Palestine, Qatar, Saudi Arabia, Syrian Arab Republic, United Arab Emirates and Yemen etc.) and Australasia. Found in Asia region such as India (North east India region - Arunachal Pradesh, Sikkim, Manipur, Mizoram, Meghalaya, Nagaland and Tripura and west Bengal etc.) *Adiantum capillus-veneris* L. (Hansraj) have been extensively used in traditional system of medicine for centuries. Native to South America, however it has become widespread throughout the world and begun to naturalized in many places (Asia, Africa and Pacific) as an ornamental fern.

Three (3) maiden ferns (*A. peruvianum*, *A. hispidulum* and *A. raddianum*) are exclusively cultivated as ornamental plants and sold in florist shop/ local ferneries/horticultural firms at the rate of Rs. 200–500. In some areas (Barpeta, Heigang Laitumkhrah, Shillong), these ferns have escaped from cultivation and begin to naturalize in their surroundings. According to Shaffer–Fehre (2006), *A. raddianum* has the potential of becoming an invasive weed in rice fields and tea gardens. In Hawaii, natural population of *A. capillus-veneris* has been replaced by *A. raddianum* in the past few decades (Wilson 1996). The Himalayan species, *A. edgeworthii* was found growing only in three states (Arunachal Pradesh, Manipur and Nagaland), while *A. incisum* were collected from Manipur and Assam. Two more maidens (*A. pedatum* L. and *A. venustum* D. Don.) were recorded from Assam by Borthakur *et al.* (2001), and (Yumkham *et al.*, 2018).

Bioactive Phytochemical constituents and compounds: Their impertinent and remarkable medicinal character is due to the presence of various compounds like leaves extract contain flavanoids like kaempferol-3-sulfate, procyanidin, rutin, naringin, prodelphinidin, rhodoxanthin etc. (Imperato 1982). Akabori and Hasegawa (1969) also reported presence of astragin, quercetin, isoquercetin and nicotiflorin. For the first time, oleanane compounds like olean-12-en-3-one and olean-18-en-3-one were isolated from *A. capillus-veneris* (Nakane *et al.*, 1999). From *A. capillus-veneris*, numerous compounds were isolated. It includes 21-hydroxyadiantone, fern-9(11)-en-12-one, isoadiantone and hydroxyadiantone which are triterpenoids in nature (Ansari and Ekhlesi-Kazaj, 2012) Other important bioactive compounds attributing to their medicinal property include 16-hentriacontanone, hentriacontane, isoquercetin, neohop-13(18)-ene, (Kshirsagar and Mehta 1972; Tsuzuki *et al.* 2001). As the genus *Adiantum* is used as a medicinal herb in many parts of the world from ancient time, many researches and scientists on their pharmacological activity were initiated by different scientific communities. From *A. edgeworthii*, neohop-12-ene, hop-22(29)-ene and 2,6-di-tert-butyl p-cresol were isolated (Shiojima and Ageta 1994; From *A. caudatum*, important steroids like β -sitosterol and daucosterol were reported by Gupta *et al.* (1990). triterpenes, flavonoids, alicyclic acids, phenyl propanoids, lipids, sterols etc. As many as 124 bioactive compounds have been isolated from the genus (Pan *et al.*, 2011). From *A. flabellulatum*, three essential oils (nonanoic acid, n-decanoic acid, and 6, 10, 14-trimethyl-2-pentadecanone) were isolated from the rhizome and young fronds (Kang *et al.* 2009). This includes kaempferol-3-glucoside, isohopane-type triterpenoid, fern-9(11)-en-25-oic acid, filicenol B, 6-oxoferen-9(11)-ene, 3 β -acetoxy-21 α -H-hop-22(29)ene, 22, 29n-Epoxy-30-norhopane-13 β -ol (Mukherjee *et al.* 2001, 2003; Reddy *et al.* 2001). Ji *et al.* 2008; Ageta *et al.* 1968). In highly ornamental ferns like *A. peruvianum* and *A. raddianum*, little research is done to assess their medicinal property. However, Singh *et al.* (2008) Another prominent medicinal maiden fern, *A. incisum* showed presence of multiple bioactive compounds like hentriacontane, adiantone, isoadiantone, adininaonol, adiantuoleanone, β -sitosterol, ferene, 17-pentatriacontene, neophytadiene, hexadecanoic acid and 2,3-hydroxyfernene (Sengottuvel *et al.* 2015; Hayat *et al.* 2002). An essential oil containing n-nonanal as a chief constituent was isolated from the fronds and 2, 6-di-tert-butyl p-cresol from rhizome of *A.*

edgeworthii by Ji *et al.* (2008). The literature of *Adiantum capillus-veneris* L.(Hansraj) on phyto-chemical studies of reveals the presence of triterpenoids, aoleananes, phenylpropanoids, carbo -hydrates, carotenoids, alicyclics and flavonoids like rutin, quercetin, quercetin-3-O-glucoside, querciturone, isoquercitrin, nicotiflorin, naringin, astragalin, populnin, procyanidin, prodelphinidin, and kaempferol-3-sulfate. (Yumkham *et al.*,2018)

The present study was conducted to evaluate the pharmacognostical parameters viz., macroscopy and microscopy, HPTLC finger printing (Sagar *et al.*, 2020; Meena *et al.*,2017)

Material and method:

Herbal drug was procured from Delhi and Ghaziabad market and identified by botanist using pharmacopoeial standards (Johnson, 1940). The physico-chemical studies of the drug were carried out according UPI and for HPTLC profile DESAGA sample applicator was used and photographs were taken with the help of DESAGA photo-documentation system.

Results and Discussion:

Pharmacognostical Studies, Macroscopic Features

The drug is made up of aerial parts of *Adiantum capillus-veneris* L. stem and size ranges from 10 to 16 cm long and 1.5 mm broad and sub erect with aromatic and bitter taste (Slightly), whereas the size of the leaf range from 1.0 to 1.8 cm long and 1.10 to 2.15 cm breadth with wedge and fan shaped having fragrant smell and slightly bitter taste. Shown in Fig.-1(a.) Aerial parts, Fig.-1(b.) Leaf parts, Fig.-1(c.) Herbarium sheet of *Adiantum capillus-veneris* L. respectively

Analytical Studies

Physico-chemical Parameters : The parameters such as the amount of foreign matter, loss on drying at 105°C, total ash content of the sample, amount of water soluble ash, amount of acid insoluble ash, amount of water soluble extractive, alcohol soluble and hexane soluble extractive of the sample are useful in establishing quality profile of *Adiantum capillus-veneris* L.

High Performance Thin Layer Chromatography Fingerprinting Analysis (HPTLC): The drug samples (2g) were soaked in chloroform and alcohol separately for 18 hours and refluxed for 10 minutes on water bath and filtered through Whatman No.1 filter paper. The filtrates were concentrates and made up to 10 ml in volumetric flask with respective solvents (Saxena and Yadav, 1983). HPTLC analysis was carried out as per the standard method. (Wagner and Bladt, 1996).

Safety Parameters: The microbial load and heavy metal parameters were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxins were estimated by Kobra cell techniques using Agilent HPLC instruments as per ASTA method (Anonymous, 1997). The heavy metals were analyzed by Atomic Absorption Spectroscopy (Anonymous, 2005) and pesticide residues were analyzed using GC-MS Agilent instruments equipped with Mass selective detector as per AOAC method (Anonymous, 2005; Sagar *et al.*, 2020; Meena *et al.*, 2017)

High Performance Thin Layer Chromatography (HPTLC) fingerprinting was performed on 10 cm × 10 cm TLC plates pre-coated with 0.25 μm thin layers of silica gel 60 F₂₅₄ (Merck). The chloroform extract of the sample was applied on the plates as bands 10 mm wide. Linear ascending development to a distance of 80 mm with *Toluene: Ethyl acetate* (8 : 2 v/v) as mobile phase was performed in a twin-trough glass chamber (20 cm × 10cm) previously saturated with vapours of mobile phase for 20 minutes. Allow the plate to dry in air and examine under UV (366nm). Observe 13 major fluorescent spots at R_f 0.10, 0.12, 0.20, 0.23, 0.25, 0.29, 0.37, 0.41, 0.44, 0.56, 0.62, 0.69 & 0.77 (red). Under UV (254nm), observe 04 spots at R_f 0.20, 0.61, 0.66 & 0.73 (green). Dip the plate in 1% *Vanillin - Sulphuric acid* reagent followed by heating at 105°C for 5 minutes and examine under visible light. Observe 11 major spots at R_f 0.13 (pinkish purple), 0.19 (green), 0.23, 0.31 (pinkish grey), 0.45 (purple), 0.49 (pinkish purple), 0.58 (yellow), 0.60 (green), 0.62 (pink), 0.67 (light green) & 0.75 (green)., Shown in Table-1, Fig.-2 respectively.

Apply *Ethanol* extract on precoated aluminium TLC plate of silica gel 60 F₂₅₄ using HPTLC automatic sample applicator. Develop the plate in *Toluene - Ethyl acetate* (8: 2) solvent system. Allow the plate to dry in air and examine under UV (366nm). Observe 14 major

fluorescent spots at R_f 0.10, 0.13, 0.15, 0.18, 0.26, 0.33, 0.41, 0.45, 0.47, 0.56, 0.61, 0.65, 0.75 & 0.85 (red). Under UV (254nm), observe 04 spots at R_f 0.25, 0.68, 0.72 & 0.78 (green). Dip the plate in 1% *Vanillin - Sulphuric acid* reagent followed by heating at 105°C for 5 minutes and examine under visible light. Observe 09 major spots at R_f 0.28 (olive green), 0.32 (light brown), 0.40 (bluish grey), 0.53 (pinkish purple), 0.62 (yellow), 0.66 (violet), 0.67 (blue), 0.76 (yellow) & 0.81 (green) ., Shown in Table-2, Fig-3 respectively.

Conclusion

In the present study various parameters such as pharmacognostical, physico-chemical, HPTLC finger printing and WHO parameters of *Adiantum capillus-veneris* L.(Hansraj) plant were carried out and can be laid down as reference standards of the drug and evaluated phytochemical research data will serve as referential supports, pharmacopeial standard research development of the plant in the near future for any advance pharmacological, analytical and biological research studies. It can be concluded that the single drug *Adiantum capillus-veneris* L.(Hansraj) is safe and free from any toxic, hazardous substance.

Table-1: Rf Values of Chloroform Extract

Solvent system	Rf Values		
	254nm	366nm	After Derivatization
Toluene : Ethyl acetate (8.0 : 2.0, v/v)	0.20 (Green)	0.10 (Red)	0.13 (Pinkish purple)
	0.61 (Green)	0.12 (Red)	0.19 (Green)
	0.66 (Green)	0.23 (Red)	0.23 (Pinkish Grey)
	0.73 (Green)	0.25 (Red)	0.31 (Pinkish Grey)
		0.29 (Red)	0.45 (Purple)
		0.27 (Red)	0.49 (Pinkish purple)
		0.37 (Red)	0.58 (Yellow)

	0.41 (Red)	0.60 (Green)
	0.44 (Red)	0.62 (Pink)
	0.56 (Red)	0.67 (Light green)
	0.62 (Red)	0.75 (Green)
	0.69 (Red)	
	0.77 (Red)	

Table-2: Rf Values of Alcohol Extract

Solvent system	Rf Values		
	254nm	366nm	After Derivatization
Toluene : Ethyl acetate (8.0 : 2.0, v/v)	0.25 (Green)	0.10 (Red)	0.28 (Olive green)
	0.68 (Green)	0.13 (Red)	0.32 (Light brown)
	0.72 (Green)	0.15 (Red)	0.40 (Bluish grey)
	0.78 (Green)	0.18 (Red)	0.53 (Pinkish purple)
		0.26 (Red)	0.62 (Yellow)
		0.33 (Red)	0.66 (Violet)
		0.41 (Red)	0.67 (Blue)
		0.45 (Red)	0.76 (Yellow)
		0.47 (Red)	0.81 (Green)
		0.56 (Red)	
		0.61 (Red)	
		0.65 (Red)	
		0.75 (Red)	
	0.85 (Red)		

Fingers :



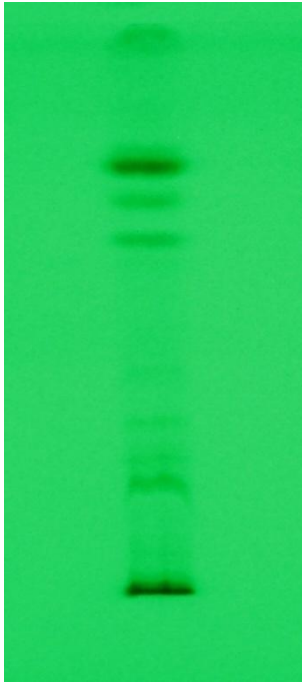
Fig.-1a. Aerial parts of *Adiantum capillus-veneris* L.

Fig.-1b. Leaf parts of *Adiantum capillus-veneris* L.

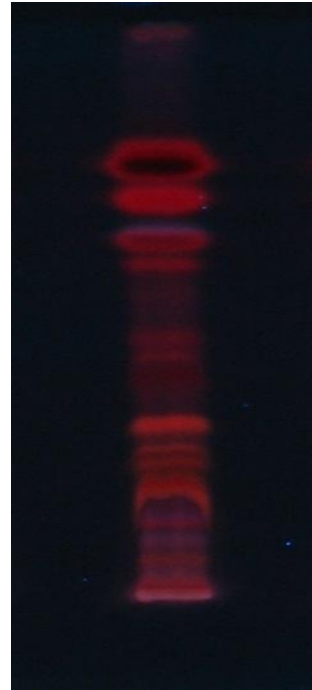


Fig.-1c. Herbarium sheet of *Adiantum capillus-veneris* L.

Fig.-2: HPTLC pic. of *Chloroform* extract of *Adiantum capillus-veneris* L.(Hansraj) plant:



UV 254nm



UV 366nm

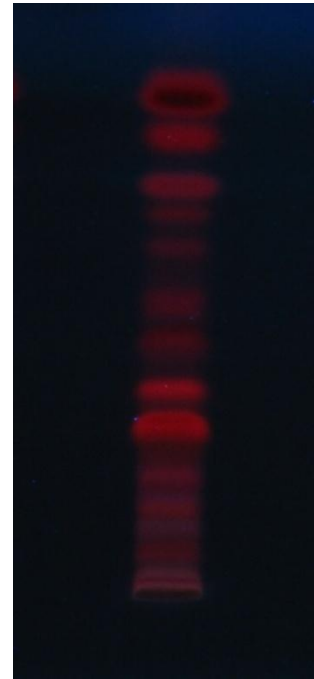


Visible Light (After derivatization)

Fig.-3: HPTLC pic. of Ethanol extract of *Adiantum capillus-veneris* L.(Hansraj) plant:



UV 254nm



UV 366nm



Visible Light (After derivatization)

Reference:

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