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HPTLC finger printing studies and evaluation of Pharmacopoeial Standards for the 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*. 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. The aerial parts of the plant were collected freshly and subjected to macroscopic, microscopic, physico-chemical and 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 propaniods, carbohydrates, carotenoids,alicyclics and flavonoids like rutin, quercetin, quercetin-3-O-glucoside, querciturone, soquercitrin, nicotiflorin, naringin, astragalin, populnin, procyanidin, prodelphinidin, and kaempferol-3-sulfate. The physico-chemical data showed foreign matter %, w/w.-0.48, moisture content %,w/w.-2.856, total ash % w/w.-6.806,acid insoluble ash %,w/w.-3.630 and alcohol, water and hexane soluble extractive values, %,w/v.- 12.23,13.33 and 2.823, pH (1% &10% aq. solution)-6.92 & 6.18 respectively. 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 parameters such as microbial content, heavy metals (As, Cd, Pb and Hg ppm.), Aflatoxins (B1.B2,G1 and G2 ppb.) were detected to be within the permissible limits. The study will be useful for the identification and authentication of the plant in dry form as well as in fresh form. The evaluated phytochemical and 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., pharmacological characters, physico-chemical analysis, TLC/HPTLC research studies, Quality Assurance, Quality Control parameters.

I. INTRODUCTION

Adiantum capillus-veneris L. called in 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 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. 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.

Adiantum capillus-veneris L. (Hansraj) have been extensively used in traditional system of medicine for centuries. 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 Ekhlasi, 2012).

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. 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.). 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:

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. 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 Ekhlasi–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). From *A. edgeworthii*, neohop–12–ene, hop–22 (29)– ene and 2,6–di–tert–butyl p–cresol were isolated (Shiojima and Ageta 1994; Ji *et al*. 2008; Ageta *et al*. 1968). 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). From A. caudatum, important steroids like b–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–oxofern–9 (11)– ene, 3b–acetoxy–21 a9–H–hop–22 (29) ene, 22, 29n– Epoxy–30–norhopane–13b–ol (Mukherjee *et al*. 2001, 2003; Reddy *et al*. 2001). 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). 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 and physico-

chemical parameters viz., ash contained , acid insoluble contained % values and water and alcohol extractive values %, volatile oil %, pH, Loss on drying , detection of heavy metals, aflatoxins and pesticide residue etc. (Sagar *et al.*, 2020; Meena *et al.*,2017)

II. 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.

Methods

Pharmacognostic Studies: For pharmocognostical studies microtome sections were taken for general observations. Leaf clearing, quantitative microscopy for determining stomatal number, stomatal index, palisade ratio, vein islet ratio and vein termination were carried out as per the standard procedure. (Sass ,1940).

Quantitative Microscopy: The cleared materials were washed thoroughly and stained with safranin for quantitative microscopic studies.

Maceration Study: Shade dried and coarsely powdered plant was treated with Jeffrey's reagent for a few hours. The action of the macerating fluid was stopped before the complete separation of all cells. Then the macerated tissue was carefully washed in distilled water to remove as much of the acid as possible and then transferred to 50% alcohol for study. Slides were made by placing small quantities of cells in water on a slide. The excess water was evaporated, mounted in glycerine and observed through microscope (Evans *et al.*,2001).

III. 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.-1a. Arial parts, Fig.-1b. Leaf parts, Fig.-1c. Herbarium sheet of *Adiantum capillus-veneris* L.** respectively

Microscopic Features:

Upper epidermis with thick walled heavily cutinized appear in the T.S. of the stem. Next to the epidermis hypodermis is present followed by the ground tissues made up of parenchymatous cells with prominent air space cells. Meristeles are present in the ground tissue. Cortical cells are parenchymatous in nature and full of starch grains; stele having single layered endodermis which is followed by pericycle; Phloem surrounds the triarch xylem.

Powder Microscopy (Maceration Study)

The drug's macerate shows the fragments of lignified fibers, fiber vessels, epidermal cells and trichomes.

Powder analysis:

The drug is purple to black in colour and have aromatic smell. It shows the tetrahedral spores, cork cells, xylem vessels and multicellular trichomes. The sporangium appeared with incomplete heavily thickened annulus having 18-25 cells.

Quantitative (Microscopy Study)

Upper epidermis with thick walled heavily cutinized appear in the T.S. of the stem. Next to the epidermis hypodermis is present followed by the ground tissues made up of parenchymatous cells with prominent air space cells. Meristeles are present in the ground tissue. Cortical cells are parenchymatous in nature and full of starch grains; stele having single layered endodermis

which is followed by pericycle; Phloem surrounds the triarch xylem. Shown respectively in **Fig.2**-: T.S of *Adiantum capillus-veneris* L. showing prominent stele and vascular bundles (A,B-C); D showing the photosynthetic parenchymatous cells.

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)

The physico-chemical standards for the dry powder of the whole plant (80 mesh) are given in Table-1. average values - Foreign matter %, w/w.-(0.48), moisture content %,w/w.-(2.856), Total ash contained, w/w,%-(6.806 %) and acid in-soluble ash contained, w/w,% (3.630 %) indicate the presence of inorganic materials. The alcohol soluble extractive w/v, % value was (12.23 %) and water soluble extractive value, w/w,% was (13.33 %), hexane soluble extractive value, w/w,% was (2.823 %) which might be due to the presence of polar organic bio-active phyto-chemical constituents and inorganic constituents respectively. The loss on drying / moisture content obtained in the drug was 2.856 % which shows the amount of moisture content present in the drug. The pH of 1% &10% aq. solution was obtained (6.92 & 6.18 respectively).

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 F_{254} (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^{0}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-2,Fig.-3 respectively.

Apply *Ethanol* extract on precoated aluminium TLC plate of silica gel 60 F_{254} 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 atR_f0.10, 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^{0}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-3,Fig-4 respectively.

Microbial Load Analysis: The microbial load and pathogens studies are shown in Table-4.

Heavy Metal Analysis : The medicinal plants materials are generally contaminated with arsenic and heavy metals due to environmental pollution. These components even in trace amounts are dangerous and can damage the important human organs such as kidney, liver and heart (Mukherjee, 2008). The amount of various heavy metals found in the plant material is given in Table-5. The heavy metal contents viz. lead, cadmium, mercury and arsenic as per WHO guidelines were found within the permissible limits viz. 10, 0.3, 1 and 3 ppm. respectively. The plant is hence considered non-pollutant in the environment and it cannot cause any illness.

Analysis of Aflatoxins: The aflatoxin can be acute toxic, carcinogenic, mutagenic, teratogenic and immune suppressive to the human being if these are found in the plant above the prescribed limits (Felix and Mello, 1997). The various aflatoxins found in the plant material are given in Table-6. The aflatoxins B1, B2, G1 and G2 ppb. were found below the detecting limit so the toxic effect of the plant may be considered as nil and hence, the plant is safe for use.

Analysis of Pesticide Residues: The various pesticidal residues of the plant were tested and found nil. The results are shown in Table-7. So the plant may be considered as pesticide resistant and plants are quite safe for humans.

IV. 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 phytochemacal 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: Physico-Chemical Parameters of *Adiantum capillus-veneris* L.(Hansraj) plant:

S. No.	Parameters Analysed	Batch I	Batch II	Batch III
1.	Foreign matter (%, w/w)	0.48	0.48	0.48
2.	Moisture Content/ Loss on drying at 105^{o}C (%,w/w)	9.30	9.38	9.44
3.	Ash contained value (%, w/w) Total ash Acid insoluble ash	6.70% 3.52%	6.82% 3.66%	6.90% 3.71%
4.	Extractive value (%,w/v)			

	Alcohol Soluble - Water Soluble - Hexane Soluble -	12.11% 13.55% 2.74%	12.25% 13.67% 2.81%	12.34% 13.78% 13.78%
5.	pH values 1% aqueous solution - 10% aqueous solution -	 6.92 6.17	 6.91 6.18	 6.91 6.18

Table-2 : 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-3 : 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)	

Table-4 : Analysis of Microbial Load of *Adiantum capillus-veneris* L.(Hansraj) plant:

S. No.	Parameter Analyzed	Results	WHO Limit
1	Total Bacterial Count	300 cfu/gm	10^5cfu/gm
2	Total Fungal Count	100 cfu/gm	10^3cfu/gm
3	Escherichia coli	Absent	Absent
4	Salmonella typhai Spp	Absent	Absent
5	Staphylococcus aurous	Absent	Absent

Table-5 : Estimation of Heavy Metal of *Adiantum capillus-veneris* L.(Hansraj) plant:

S. No.	Parameter Analyzed	Results	WHO Limit
1	Lead	Not detected	10 ppm
2	Cadmium	Not detected	0.3 ppm
3	Mercury	0.0153 ppm	1 ppm
4	Arsenic	0.0036 ppm	3 ppm

Where ppm : parts per million

Table-6 : Estimation of Aflatoxins of *Adiantum capillus-veneris* L.(Hansraj) plant:

S. No.	Parameter Analyzed	Results	WHO Limit
1	Aflatoxin, B1	Below Detectable Limit	0.5 ppb
2	Aflatoxin, B2	Below Detectable Limit	0.1 ppb
3	Aflatoxin,G1	Below Detectable Limit	0.5 ppb
4	Aflatoxin, G2	Below Detectable Limit	0.1 ppb

Where ppb: parts per billion

Table-7 : Estimation of Pesticide Residues of *Adiantum capillus-veneris* L.(Hansraj) plant:

S.N0.	Parameter Analyzed	Results	WHO Limit (mg/kg)
1	DDT (all isomers, sum of ρ, ρ'-DDT, α, ρ' DDT, ρ, ρ'-DDE and ρ, ρ'-TDE (DDD expressed as DDT)	Not detected	1.0

2	HCH (sum of all isomers)	Not detected	0.3
3	Endosulphan (all isomers)	Not detected	3.0
4	Azinphos methyl	Not detected	1.0
5	Alachlor	Not detected	0.02
6	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	Not detected	0.05
7	Chlordane (cis & tans)	Not detected	0.05
8	Chlorfenvinphos	Not detected	0.5
9	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	Not detected	0.05
10	Endrin	Not detected	0.05
11	Ethion	Not detected	2.0
12	Chlorpyrifos	Not detected	0.2
13	Chlorpyrifos-methyl	Not detected	0.1
14	Parathion methyl	Not detected	0.2
15	Malathion	Not detected	1.0
16	Parathion	Not detected	0.5
17	Diazinon	Not detected	0.5
18	Dichlorvos	Not detected	1.0
19	Methidathion	Not detected	0.2
20	Phosalone	Not detected	0.1
21	Fenvalerate	Not detected	1.5
22	Cypermethrin (including other mixtures of constituent isomers sum of isomers)	Not detected	1.0
23	Fenitrothion	Not detected	0.5
24	Deltamethrin	Not detected	0.5
25	Permethrin (sum of isomers)	Not detected	1.0
26	Pirimiphos methyl	Not detected	4.0

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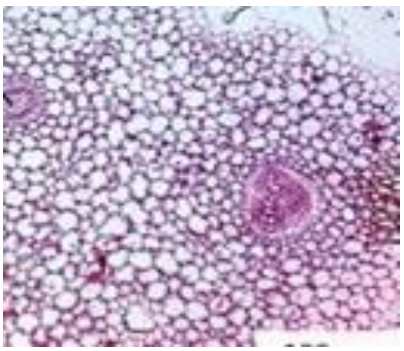
Fingers :

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

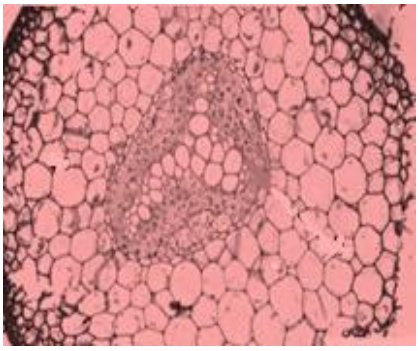
Fig.-1b. Leaf parts of *Adiantum capillus-veneris* L.

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

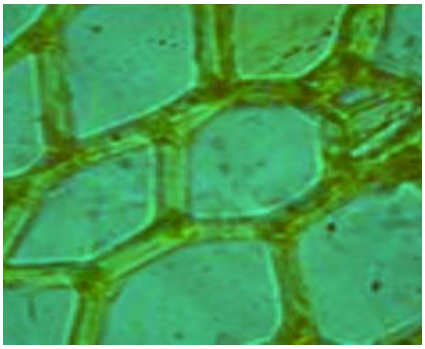
(A)



(B)



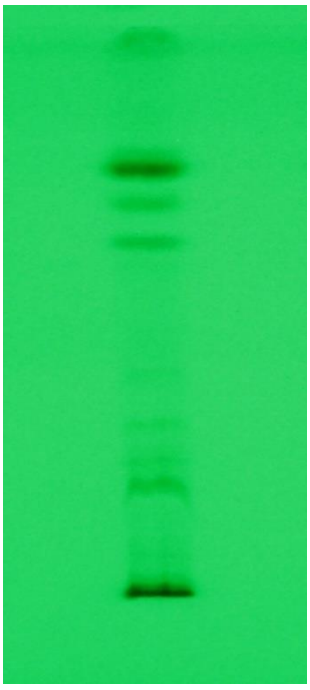
(C)



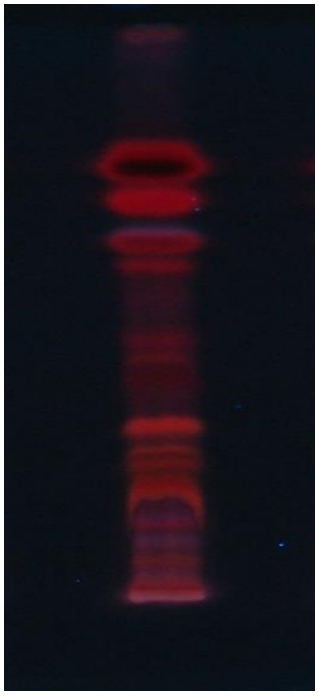
(D)

Fig.2-: T.S of *Adiantum capillus veneris* L. showing prominent stele and vascular bundles (A,B-C); D showing the photosynthetic parenchymatous cells.

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



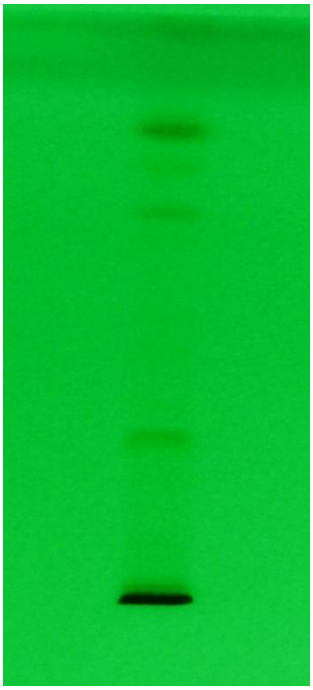
UV 254nm



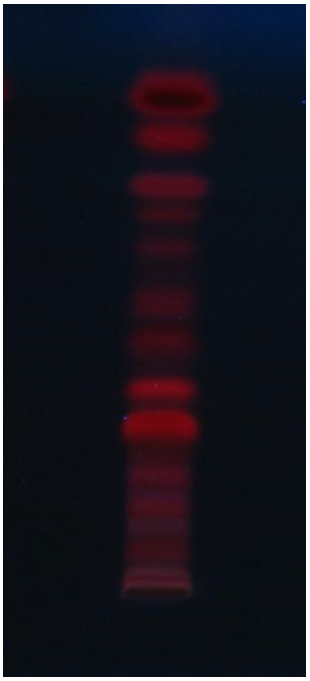
UV 366nm

Visible Light (After derivatization)

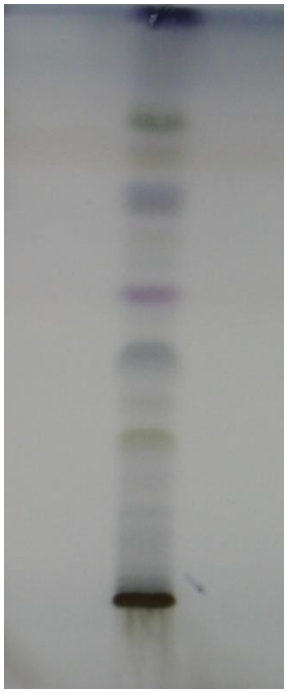
Fig.-4: HPTLC pic. of Ethanol extract of *Adiantum capillus-veneris* L.(Hansraj) plant:



UV 254nm



UV 366nm



Visible Light (After derivatization)

V. REFERENCES

[1] P. K. Sagar, R. Murugeswaran R. Meena, S. Mageswari and P.M.D. Sri, S.Khair, "Standardization and HPTLC. Fingerprinting study of Poly Herbal Unani Formulation -Habb-e-Sara Khas." International Journal of Traditional and Complementary Medicine,Vol.5, Issue.,21, pp.1-13,2020. https://escipub.com/ijtcm-2019-07-1808/

[2] S.D. Yumkham, M. Elangham, R. Nangmaithem, P.D. Naerem, P.K. Singh, "Maiden hair ferns (Adientum L. Pheridaceae-Vittarioideae of North East India: diversity, phytochemistry and utilization," Springer Science + Business Media B-V part of Springer nature, Genet Resource Crop.Eval.2018. https://doi.org/10.1007/s10722-018-0612-y.

[3] R. Meena, S. Mageswari, P.M.D. Sri, S. Sadaf, "Pharmacognostical Studies of Stachytarpheta jamaicensis (L.) Vahl. (Whole plant)." Hippocratic Journal of Unani Medicine,Vol.12, Issue.,4, pp.1-18, 2017. https://ccrum.res.in/ViewData/Multiple?mid=1600

[4] T. Sengottuvel, N. Kannappan, T. Sivakkumar, "Phytochemical and GC–MS analysis of bioactive compounds present in the Adiantum incisum Forssk," Der Pharma Chem,Vol. 7,Issue.,8,pp. 137–142,2015. http://derpharmachemica.com/archive . html. Accessed 16 Sept 2016.

[5] Herbal remedies using maidenhair fern (n.d). Retrieved 10th January 2014, from http://www.ageless.co.za/maidenhair_fern.htm

[6] Family. polypodiaceae (n.d.). Retrieved 20th January 2014, from http://stuartxchange.com/Alambrillo.html

[7] Ansari, K. Ekhlasi, "Adiantum capillus-veneris. L: Phytochemical Constituents, Traditional Uses and Pharmacological Properties: A Review," Journal of advanced scientific research,2012, 21/10/2013, from scienceage.

[8] R. Ansari, K. Ekhlasi–Kazaj, "Adiantum capillus–veneris L.: phytochemical constituents, traditional uses and pharmacological properties:

a review," Journal of Advance Science and Research, Vol. 3,Issue.,4,pp.15–20,2012

[9] C. Pan, Y.G. Chen, X.Y. Maa, J.H. Jiang, F.He, Y.Zhang, "Phytochemical constituents and pharmacological activities of plants from the genus Adiantum: a review," Trop. J. Pharm. Res., Vol.10,Issue.,5,pp.681–692,2011.

[10] W.Y. Kang, Z.Q. Ji, J.M. Wang, "Composition of the essential oil of Adiantum flabellulatum," J. Chem. Nat. Compd.,Vol. 45,Issue.,4,pp.575–577,2009.

[11] K.Y. Chong, H.T.W., Tan, R.T. Corlett, "A checklist of the total vascular plant flora of Singapore: native, naturalized and cultivated species," Singapore Raffles Museum of Biodiversity Research, National University of Singapore,2009. http:// lkcnhm.nus.edu

[12] Z.Q. Ji, G.D. He, W.Y. Kang, "HS–SPME–GC–MS analysis of the essential oil in common Adiantum edgeworthii,". Zhongguo Yaofang, Vol.19,pp.2359–2360,2008.

[13] M. Singh, N. Singh, P.B. Khare, A.K.S. Rawat, "Antimicrobial activity of some important Adiantum species used traditionally in indigenous systems of medicine," J. Ethnopharmacol,Vol. 115,pp.327–329,2008.

[14] P.K. Mukerjee, "Quality control of Herbal Drugs - An approach to evaluation of Botanicals, Business Horizons, Pharmaceutical publishers, New Delhi," 2008.

[15] M. Shaffer-Fehre, "A revised handbook to the flora of Ceylon, Vol. XV, Part., A: ferns and fern-allies. Science Publishers Inc, New York",2006.

[16] Anonymous, "Official Methods of Analysis," International, Horwitz W, Latimer G W, 18th Edition. AOAC. International, Maryland, 2005.

[17] S. Hayat, A. Rahman, M.I. Choudhary, K.M. Khan, H.H. Latif, E Bayer,"Two new triterpenes from fern Adiantum incisum," Z Naturforsch, Vol. 57b,pp.233–238,2002.

[18] W.C. Evans, Trease and Evans, "Pharmacognosy," 14th Edition, WB Saunders Company Ltd., London,2001.

[19] S.K. Borthakur, P. Deka, K.K. Nath,"Illustrated manual of Ferns of Assam," Bishen Singh Mahendra Pal Singh, Dehra Dun,2001.

[20] K. Tsuzuki, A. Ohashi, Y. Arai, K. Masuda, A. Takano, K. Shiojima et al., "Terpenoids from Adiantum caudatum," J. Phytochem,Vol. 58,Issue.,2,pp.363–367,2001.

[21] K.S. Mukherjee, G. Brahmachari, D. Chatterjee, P. Mukherjee, "Triterpene from A. lunulatum," Journal of Indian Chemical Society, Vol. 78,pp.267–268,2001.

[22] V.L.N. Reddy, V. Ravikanth, T.P. Rao, P.V. Diwan, Y. Venkateswarlu, "A new triterpenoid from the fern Adiantum lunulatum and evaluation of antibacterial activity," Phytochem ,Vol. 56,pp.173–175,2001.

[23] T. Nakane ,Y. Arai, K. Masuda, Y. Ishizaki, H. Ageta, K. Shiojima K, "Pharmaceutical society of Japan fern constituents: six new triterpenoid alcohols from Adiantum capillus– veneris," J. Chem Pharm Bull, Vol. 47,Issue.,4,pp.543–547,1999.

[24] T. Nakane, Y.Arai, K. Masuda, Y. Ishizaki, H. Ageta, & K. Shiojima, "Fern constituents: six new triterpenoid alcohols from Adiantum capillus-veneris," Chemical and Pharmaceutical bulletin – Tokyo,Vol.47,pp.543-547,1999.

[25] Anonymous, Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, pp. 25-28,1998.

[26] Anonymous, Official Analytical Methods of the American Spice Trade Association, Inc. 4th edn. New Jersey,pp.149-152,1997.

[27] D. Felix, J.P. Mello, "Handbook of plant and fungal toxicants," CRC Press Inc, USA, pp. 273-274,1997.

[28] H. Wagner,Bladt, “Plant Drug Analysis - A Thin Layer Chromatography Atlas,” Springer-Verlag, 2nd Edition, Germany,1996.

[29] E. Stahl, “Thin Layer Chromatography- A Laboratory Handbook,” George Allen and Unwin Ltd., London,1996.

[30] K.A. Wilson, “Alien ferns in Hawaii,” J. Pacif Science,Vol.50,Issue.,2,pp.127–141,1996. https://www.cabi.org/isc/abstract. Accessed 18 Sept 2016

[31] K. Shiojima, H. Ageta,“Fern constituents: triterpenoids isolated from the leaves of Adiantum edgeworthii Structures of 19–hydroxyadiantone and fern–9(11)–en–25–oic acid,” Chem Pharm Bull, Vol. 42,pp.45–47,1994.

[32] Anonymous, “Research guidelines for evaluating the safety and efficacy of herbal medicines,” World Health Organization, Regional Office for Western Pacific, Manila,1993.

[33] M. Gupta, A. Bagchi, S.K. Roy, A.B. Ray,“Chemical constituents of a member of Adiantum caudatum complex,” Journal of Indian Chemical Society, Vol. 67,pp.86–88,1990.

[34] S.C. Saxena, R.S.A. Yadav, “new plant extract to suppress the population of yellow fever and dengue vector Aedesaegyptii L,” Current Science, Vol. 52,pp.713-715,1983.

[35] F. Imperato, “Kaempferol 3–sulfate in the fern Adiantum capillus–veneris,” Phytochem,Vol. 21,pp.2158–2159,1982.

[36] M.K. Kshirsagar, A.R. Mehta, “Ferns in Gujarat State (India) for presence of antibacterial substances of ferns,” J. Planta Med., Vol. 22,pp.386–390,1972.

[37] Y. Akabori, M. Hasegawa,1969. “Flavonoid pattern in the Pteridaceae II. Flavonoid constituents in the fronds of Adiantum capillus–veneris and A. cuneatum.” Shokubutsugaku Zasshi, Vol. 82,pp.294–297,1969.

[38] D.A. Johnson, “Plant Micro technique” Mc. Graw Hill Book Company Inc., New York and London,1940..

[39] J.E. Sass, “Elements of Botanical Micro Technique,” 1st Edition, McGraw Hill Book Company, New York, USA, pp. 222, 1940.

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