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RESEARCH ARTICLE

PHARMACOPOEIAL AND HPTLC FINGERPRINT STUDIES OF DARCHINI – ANIMMUNITY BOOSTER UNANI SINGLE DRUG

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Abstract

Corona virus disease 2019 (COVID-19) is threatening the worldwide population by causing symptoms like fever, cough, shortness of breathand tirednessetc.A huge population of world is suffering from this disease and no specific vaccine for this pandemic disease has been developed. According to the guidelines of Ministry of AYUSH, this disease can be prevented by taking immunity boosters, as immune system plays a vital role in defence against any disease. In Unani system of medicines, many drugs of plant origin are mentioned in classical literature for strengthening and increasing the immunity of humans. Darchini is one of the potent immune boosters and it is believed that consumption of Darchini decoction with honey strengthens the immune system.The Ministry of AYUSH, Govt. of India in its Advisory has considered Darchini as one of the important ingredient of AYUSH Joshanda/ Kwath/ Kudineer.Since, the drug Darchini is being given to COVID-19 patients as prophylaxis regime, it has become necessary to authenticate and develop its pharmacopoeial standards so that quality raw material can be provided to needy mass. The present study is aimed to develop identity, purity and strength of drug using pharmacognostical, physico-chemical and quality control methods.

Introduction:-

Corona virus disease 2019 (COVID-19) was identified in China during the month of December 2019, and later on by middle of March 2020 it was declared as a pandemic by the World Health Organization (WHO)[1].The common symptoms of COVID-19 disease are fever, cough, sore throat, shortness of breath etc. Currently, no vaccines or specific medicines are available for thetreatment of this disease[2]. In light of the outbreak, the Ministry of AYUSH has considered Unani single drug Darchini one of the important ingredient of AYUSH Joshandagiven to the COVID-19 patients.Scientifically, it is known as Cinnamomum zeylancium.In Unani literature detailed description of Darchinilike Mahiyat (Morphology), Geographical distribution, Mijaz (Temperament), Actions (Afaal), Therapeutic Uses (Istemal) etc. has been mentioned[3].

Therapeutic uses of Darchini as per Classical literature:

Unani Classical Literature reveals that Darchini has various therapeutic actions likeMunaffis-e-Balgham (Expectorant), Daf-e-Tap (Antipyretic), Daf-e-Taffun (Antiseptic), Musaffi (Blood purifier), Katil-e-Jarasim

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(Antibacterial),Muqawwi-e-Aza-Eraesa (Tonic for principal Organs) and used in respiratory problems likeZeequnnafs (Asthma), Nazla (Catarrh), Sual (Cough), Zukam (Coryza),Khushunat-e- Halaq (Sore throat)Bohut-us-Saut (Hoarseness of voice)andHumma (fever) due to above mentioned actions[4,5,6]. Various other medicinal properties viz. lowering of blood cholesterol, diabetes etc. have also been reported[7].The phyto-constituents present in Darchini are eugenol, ethyl cinnamate, methyl chavicol, linalool, cinnamaldehyde and beta-caryophyllene which are responsible for treatment of these ailments[8]. It contains polyphenols, which are natural antioxidants that help in regulating blood sugar levels. The antiviral & antiseptic properties of Darchinihave also been reported. It is used as important ingredient in preparation of many Unani formulations[9].

Keeping in view of its various medicinal properties, the present study is aimed to develop microscopic, powder microscopy, physico-chemical, quality control standards and HPTLC fingerprints of Darchinifor laying down the pharmacopoeial standards which will be helpful to provide quality raw material to manufacturers of Unani medicines for management of COVID-19 disease.

Material and Methods:-

Authentication:

The sample of drug Darchini (Stem Bark) was procured from local raw drug dealers of Hyderabad and authenticated at National Research Institute of Unani Medicine for Skin Disorders, Hyderabad by Dr. Aslam Siddiqui, Research Officer (Pharmacognosy) [10]. The collected sample was dried under shade and stored at ambient temperature for evaluation of Pharmacognostical, Physico-chemical and Quality controlstandards.

Pharmacognostic study:

Compound microscope, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Microphotographs were taken using a microscope attached with camera. Dried stem bark were taken for microscopic studies, transverse sections were prepared and stained as per standard procedure and powder microscopy was performed[11, 12].

Physico-chemical study:

Physico-chemical study was carried out under following parameters such as foreign matter (%), loss on drying (%), total ash (%) at 450 ^{0}C, acid-insoluble ash (%) at 550 ^{0}C, volatile oil content (%), aqueous, alcohol and hexane extractive matter (%) were carried out as per IPC approved standard methods[13].

Preparation of Extracts:

Drug sample (2 g) was extracted with 20 ml of respective solvents (chloroform and alcohol) by refluxing on a water bath for 30 min and filtered using Whatman Filterpaper no. 1, concentrate up to 5 ml and thin layer chromatography studies were carried out[14].

HPTLC studies:

HPTLC was performed on 5 cm × 5 cm Precoated Aluminium Sheets of Silica Gel 60 F_{254} (Merck). Samples solution of about 10µl were applied as 10 mm width bands using Automatic TLC applicator system of the DESAGA Sarstedt Gruppe (Germany). A Linear ascending development withtoluene: ethyl acetate (9:1 v/v) and toluene: ethyl acetate: methanol (7:2:1 v/v/v) as mobile phase was carried out in a twin trough glass chamber previously saturated with mobile phase vapour for 20 min. at room temperature (25 ± 2°C). The development of solvent distance was 80 mm. After development plates were air- dried. TLC plate was scanned by densitometer of DESAGA Sarstedt Gruppe (Germany) at 366, 254 and 580 nm wavelength and operated by Pro Quant 1.06 version software[15].

Quality control parameters:

Quality control parameters viz. Microbial contamination, Heavy Metals, Aflatoxins, and Pesticide residues were analyzed as per WHO methods[16, 17].

Results And Discussion:-

Pharmacognostic Standards:

Macroscopic features:

The Bark pieces are about 0.5 mm thick brittle; occurs as single or double; closely packed compound guills; upto a metre or more in length and about 1 cm in diameter; outer surface; dull yellowish-brown; marked with pale wavy

longitudinal lines with occasional small scars or holes; inner surface darker in colour; striated with longitudinally elongated reticulation; fracture splintery.**(Fig.1)**

Microscopic features:
T.S. of bark shows pericyclic sclerenchyma; 3 or 4 rows of diametric cells; multiple vessels; sometimes tangentially elongated; inner and radial walls often being thicker than the outer; some containing starch 27 grains; small groups of pericylic fibres embedded at intervals in the sclerenchyma; phloem of tangential bands of sieve tissue alternating with parenchyma; and containing axially elongated secreting cells containing volatile oil or mucilage; phloem fibres with very thick walls; upto 30µm in diameter; isolated or in short tangential rows; sieve tubes narrow with transverse sieve plates; collapsed in outer periphery; medullary rays of isodiametric cells; cortical parenchyma and medullary rays containing small starch grains below 10µm in diameter; minute acicular crystals of calcium oxalate present**. (Fig.2-4)**

Powder Microscopy:
Powder microscopic study shows the drug is light reddish brown in colour fragrant odour, sweet and pungent taste and aromatic smell with sensation of warmth. Epidermal cells, parenchymatous cells and scalariform vessels were also observed**(Fig.5-8).**

Physico-chemical Standards:
The physico-chemical standards for the dry powder of the Stem bark of Darchini (80mesh) are given in **Table 1**. The drug contains negligible amount of silicates; shown by the acid insoluble ash (0.41%), but contains considerable amount of inorganic materials; shown by the higher ash value (4.66%). The extractive values with different solvents viz. n-hexane 2.15%, alcohol9.48% and water10.21% showed that the plant contains mainly the polar compounds soluble in alcohol and water. It also contains considerable amount of volatile oil viz. 1.01%.

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**.** The heavy metal contents viz. lead, cadmium, mercury and arsenic were analysed using Atomic Absorption Spectrophotometer and were found within the permissible limits viz. 10, 0.3, 1 and 3 ppm respectively as per WHO guidelines and the results are shown in **Table 2**. Darchini (Stem Bark) is hence considered non-pollutant drug in the environment.

Microbial Load Analysis:
The microbial load viz. TBC, TFC, E.Coliand other pathogens were analyzed as per the standard methods and the results are shown in **Table 3.**

Analysis of Aflatoxins:
The aflatoxins can be acute toxic, carcinogenic, mutagenic, teratogenic and immunosuppressive to the human being if these are found in the plant material above prescribed limits[18]**.** The aflatoxins B_1, B_2, G_1 and G_2 were analysed using HPLC and found within permissible limit as shown in **Table 4.**The toxic effect of the drug sample may be considered as nil and hence is safe to use.

Analysis of Pesticide Residues:
The various pesticidal residues α - HCH, β - HCH, γ - HCH, δ -HCH, op-DDT, pp-DDT, op-DDE, α- Endosulfan, β – Endosulfan, op-DDD and pp-DDD etc. were tested in the drug sample using GC-MS-MS technique and found within permissible limits. The results are shown in **Table 5.**The drug sample may be considered as pesticide resistant.

Thin layer Chromatography:
The thin layer chromatographic studies of chloroform and ethanol extracts were carried out using solvent systems toluene: ethyl acetate (9:1) and toluene: ethyl acetate: methanol (7:2:1) respectively and the results are tabulated in **Table 6.**

Conclusion:-

The evaluated standards will be highly useful to authenticate the drug for providing quality raw material to manufacturers of Unani medicine. From the studies of microbial load, heavy metals, aflatoxins and pesticide residues it can be concluded that the drug Darchini (Stem Bark) is free from microbial contamination, non-pollutantin the environment, non-toxic, pesticide resistant, safe to use and can be consideredas important immunity booster for prevention of COVID-19 disease.

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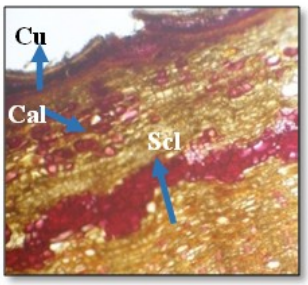
MacroscopicFeature of Darchini (Stem bark)

Fig:-1

Microscopic Features of Darchini (Stem bark)



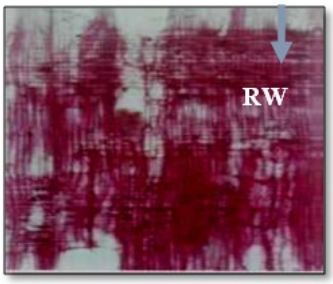


Fig:-2 **Fig:-** 3

Cu: Cuticle**; Cal:** Calcium OxalateCrystals**; RW:** Radial walls
Scl: Sclerenchyma

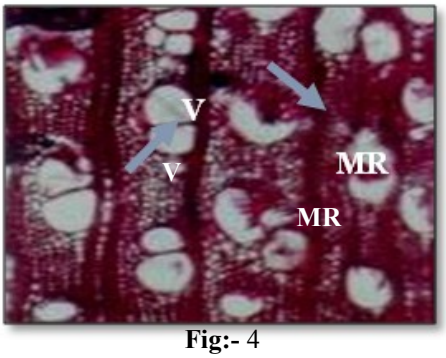


Fig:- 4

V: Vessels**; MR:** Medullary rays

Powder Microscopic Features of Darchini (Stem bark)

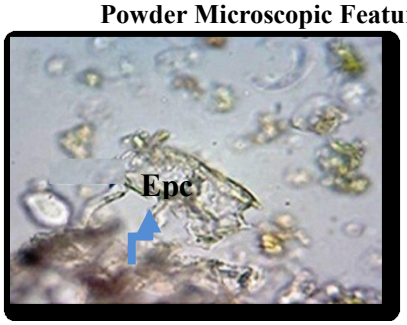


Fig:-5 **Epc:** Epidermal cells

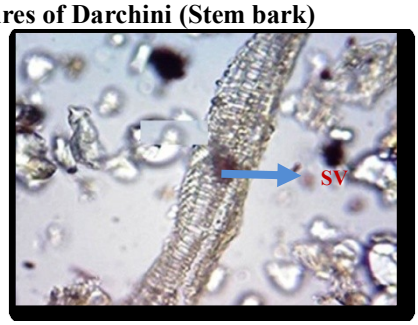


Fig:-6 SV: Scalariform vessels

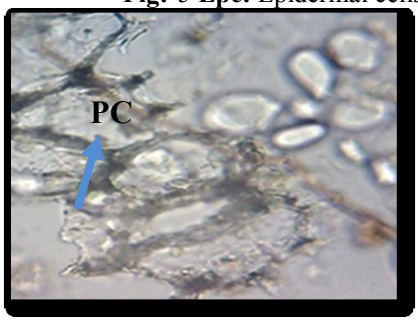


Fig:-7 **PC:** Parenchymatous cells

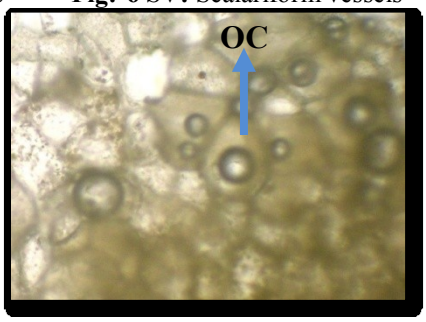
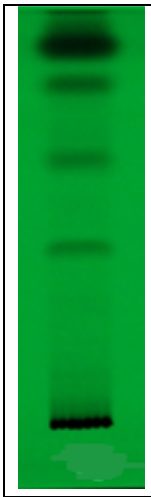
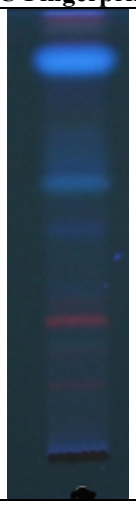


Fig:-8 **OC:** Oil cells

TLC/HPTLC Fingerprints:





UV 254nm	**UV 366nm**
Solvent System: Toluene: Ethyl acetate: Methanol (7:2:1)	
Fig 9.: HPTLC Fingerprints of DarchiniAlcohol extract	

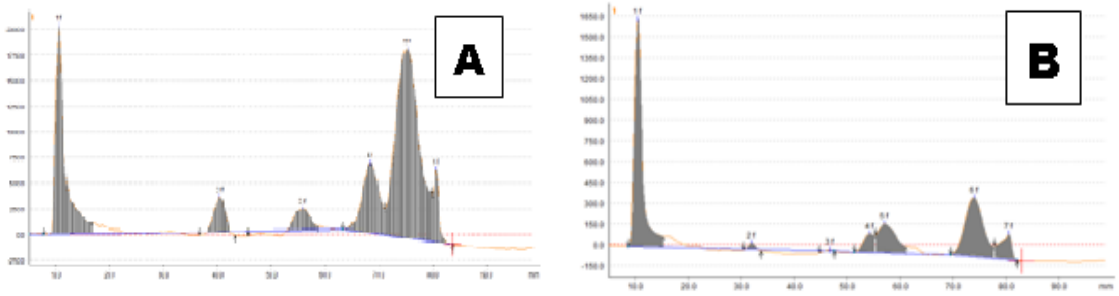
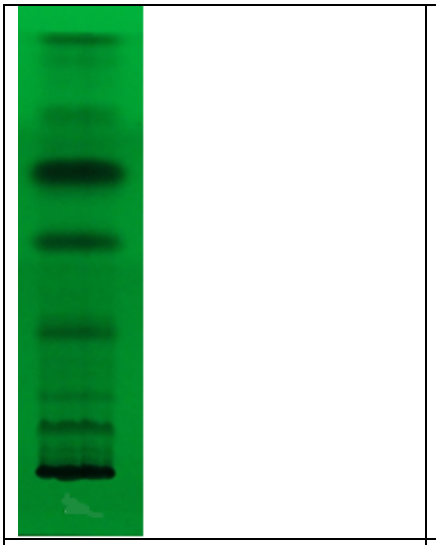


Fig10:- Densitogram of Alcohol extract of Darchini at A) UV 254nm and B) UV 366nm



UV 254nm	UV 366nm	Expose to iodine vapours
Solvent System: Toluene: Ethyl acetate(9:1)		
Fig.11: HPTLC Fingerprints of DarchiniChloroform extract		

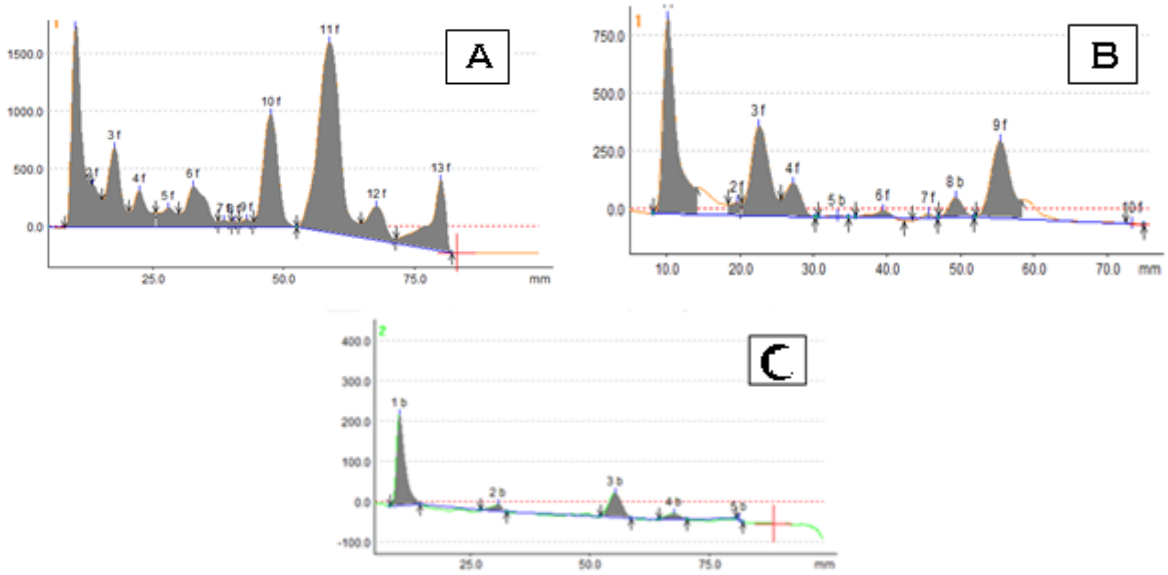


Fig.12:- Densitogram of Chloroform extract of Darchini at A) UV 254nm, B) UV 366nm, and C) Iodine vapour

Table 1:- Physico chemical parameters.

S.No.	Parameter	Result (N=∑/3)
1.	Foreign Matter (%w/w)	0.25
2.	Loss in wt. on drying at 105 ^{o}C (%w/w)	9.85
3.	Total ash (%w/w)	4.66
4.	Acid insoluble ash (%w/w)	0.41
5.	Alcohol soluble extractive (%w/w)	9.48
6.	Water soluble extractive (%w/w)	10.21
7.	Hexane soluble extractive (%w/w)	2.15
8.	Volatile oil (%w/w)	1.01

Table 2:- Qualitative test for Heavy Metals.

S.No.	Parameter	Result	Permissible limitas per WHO & ASU Pharmacopoeia's
1.	Mercury (Hg)	Not detected	1.0 ppm
2.	Lead (Pb)	Not detected	10 ppm
3.	Cadmium (Cd)	Not detected	0.3 ppm
4.	Arsenic (As)	Not detected	3.0 ppm

Table 3:- Microbial contamination**.**

S.No.	Parameter	Result	Permissible limitas per WHO & ASU Pharmacopoeia's
1.	Total microbial plate count:	3×10^{2}cfu/g	10^{7}cfu/g
2.	Total Yeast &Mould count	< 10cfu/g	10^{4}cfu/g
3.	Escherichia coli:	Absent	10^{2}cfu/g
4.	Salmonella spp.:	Absent	None
5.	Pseudomonas aeruginosa	Absent	None
6.	Staphylococcus aureus	Absent	None

Table 4:- Pesticides residue analysis.

S.No	Pesticides residue analysis	LOQ (mg/Kg)/ PPM	Test results (mg/Kg)/ PPM	Permissible limits as per WHO & ASU Pharmacopoeia's (mg/Kg)/ PPM
1.	Alachlor	0.01	BLOQ <0.01	0.02
2.	Aldrin and Dieldrin (sum of)	0.01	BLOQ <0.01	0.05
3.	Azinphos-methyl	0.01	BLOQ <0.01	1.00
4.	Bromopropylate	0.01	BLOQ <0.01	3.00
5.	Chlordane (sum of cis-, trans - and oxychlordane)	0.01	BLOQ <0.01	0.05
6.	Chlorfenvinphos	0.01	BLOQ <0.01	0.50
7.	Chlorpyrifos	0.01	BLOQ <0.01	0.20
8.	Chlorpyrifos-methyl	0.01	BLOQ <0.01	0.10
9.	Cypermethrin (and isomers)	0.01	BLOQ <0.01	1.00
10.	DDT (sum of p,p´-DDT, o,p´-DDT, p,p-DDE and p,p´-TDE)	0.01	BLOQ <0.01	1.00
11.	Deltamethrin	0.01	BLOQ <0.01	0.50
12.	Diazinon	0.01	BLOQ <0.01	0.50
13.	Dichlorvos	0.01	BLOQ <0.01	1.00
14.	Dithiocarbamates (as CS2)	0.01	BLOQ <0.01	2.00
15.	Endosulfan (sum of isomers and endosulfan sulphate)	0.01	BLOQ <0.01	3.00
16.	Endrin	0.01	BLOQ <0.01	0.05

17.	Ethion	0.01	BLOQ <0.01	2.00
18.	Fenitrothion	0.01	BLOQ <0.01	0.50
19.	Fenvalerate	0.01	BLOQ <0.01	1.50
20.	Fonofos	-	ABSENT	0.05
21.	Heptachlor (sum of heptachlor and heptachlorepoxide)	0.01	BLOQ <0.01	0.05
22.	Hexachlorobenzene	0.01	BLOQ <0.01	0.10
23.	Hexachlorocyclohexane isomers (other than γ)	0.01	BLOQ <0.01	0.30
24.	Lindane (γ-hexachlorocyclohexane)	0.01	BLOQ <0.01	0.60
25.	Malathion	0.01	BLOQ <0.01	1.00
26.	Methidathion	0.01	BLOQ <0.01	0.20
27.	Parathion	0.01	BLOQ <0.01	0.50
28.	Parathion-methyl	0.01	BLOQ <0.01	0.20
29.	Permethrin	0.01	BLOQ <0.01	1.00
30.	Phosalone	0.01	BLOQ <0.01	0.10
31.	Piperonylbutoxide	0.01	BLOQ <0.01	3.00
32.	Pirimiphos-methyl	0.01	BLOQ <0.01	4.00
33.	Pyrethrins (sum of)	0.01	BLOQ <0.01	3.00
34.	Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	0.01	BLOQ <0.01	1.00

BLOQ: Below Limit of Quantification; PPM: Parts Per Million

Table 5:- Aflatoxin contamination.

Parameter	**Result**	**Permissible limit**
B1	Nil	Less than 2 ppb
B1+B2+G1+G2	Nil	Less than 5 ppb

TABLE 6:- Thin layer chromatography in various ranges.

Extracts	**Solvent Systems**	**R_f values**		
		254 nm	**366 nm**	**After Derivtizaiton**
Alcohol Extract	Toluene: Ethyl acetate: Methanol (7:2:1)	0.45 0.71 0.88 0.92(All black)	0.14 (red) 0.29 (pink) 0.32 (red) 0.35 (pink) 0.40(light blue) 0.54 (light blue) 0.64(blue) 0.92 (blue)	No spots were appeared
Chloroform Extract	Toluene: Ethyl acetate (9:1)	0.11 0.17 0.32 0.54 0.78 0.80 0.97(All black)	0.17(blue) 0.25 (blue) 0.30(dark blue) 0.38(red) 0.42(red) 0.50(blue) 0.54(red) 0.57(blue) 0.64(lightblue) 0.72(blue)	0.17 0.30 0.64 0.82 (All yellowish brown)

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