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## Pharmacognostical and phytochemical studies on some immunity booster herbs used in Unani system of medicine

**Sonali Sajwan, RK Negi, Asim Ali Khan, AS Khan, SA Ansari, Md. W Ahmed, Jitendra Kumar and RP Meena**

**Abstract**

Unani Medicine offers a number of drugs of plant origin to strengthen and enhance the body's immune system. Having a strong immunity has always been considered important to stay healthy, but it has become even more important in the present scenario, when the whole world is dealing with highly contagious new strains of Corona virus. Strong immunity is the body's first line of defence which protects it from disease causing virus, bacteria and germs. Unani Medicines play a vital role and hold great importance to improve body's immune health. Present study deals with the scientific evaluation of some immunity booster Unani Medicinal plants (Berg-e-Rehan, Zanjabeel, Fofil Siyah, Gilo and Haldi) for their pharmacognostical characters, physico-chemical parameters such as ash values, extractives values, thin layer chromatography (TLC), HPTLC, heavy metal estimation, aflatoxin and microbial contamination.

**Keywords:** Immunity, pharmacognostical, physicochemical, HPTLC, aflatoxin, microbial contamination

**Introduction**

In Unani system of Medicines immunity (Quwat-e-Mudafe'at) is a defence system within the body to protect the host from invading pathogens. In other words it is the ability of the body to neutralize & eliminate the pathogenic micro-organisms & their toxic products, thus enabling people to increase their immunity for control over and to improve health<sup>[1]</sup>. It is not directed against any particular disease but is intended to strengthen the whole body to fight with the disease<sup>2</sup>. Unani medicine offers a variety of Immuno-stimulant drugs of plant origin which enhance the body's immunity and enable the body's resistance power more efficiently in order to prevent the body from various kinds of infections, diseases etc. Hence such drugs can be safely used to deal with highly contagious new strains of corona virus by increasing our immunity.

In Unani system of medicine more emphasis is given to strengthen the immunity of the patient in order to make body's resistance more strong. Survey of classical Unani literature reveals that a large number of immuno-stimulant drugs were being used by great Unani physicians including Hippocrates (460-377 B.C.), Galen (131-201 A.D.), Rhazes (850-925 A.D.), Avicenna (980-1037 A.D.), Ibu Baitar etc.

In Unani system of medicines stimulation of immune responses to alleviate the diseases has been of prime interest over many years and the concept of helping the Quwwat-e- Mudabbera in overcoming the disease pathology.

However, the quality, safety and efficacy of these immuno-stimulant drugs will not only preserve the traditional heritage but also promote the use of traditional medicine in the healthcare.

In order to establish the quality, safety and efficacy, five Unani herbal drugs, viz Gilo (*Tinospora cordifolia*), Berg-e-Rehan (*Ocimum sanctum*), Fofil Siyah (*Piper nigrum*), Zanjabeel (*Zinziber officinale*), Haldi (*Curcuma longa*) were subjected to identification of ingredients, analysis for marco and microscopical studies, physico-chemical parameters, microbial contamination, aflatoxin, heavy metal analysis and high performance thin layer chromatographic studies<sup>[3, 4]</sup>.

**Materials and Method:**

Herbal drugs (Table-1) were resourced from PLIM Herbal garden, local market of Delhi and Ghaziabad. All the ingredients were identified by Assistant Research Officer (Pharmacognosy) using pharmacognostical methods.

The macroscopy, microscopy and powder study were carried out using standard methods [5]. Free hand sections of the single drugs were taken and its photographs were taken using digital camera attached with the microscope. The physico-chemical studies of the drug were carried out in accordance

with the standard pharmacopoeial methods and for HPTLC Profile CAMAG Linomat V applicator was used and Photographs were taken with the help of CAMAG Reprstar 3 photo-documentation system [6].

**Table 1:** List of studied Unani Single Drugs [7]

S. N.	Unani Name	Botanical Name	Part Used
1	Gilo	<i>Tinospora cordifolia</i>	Stem
2	Berg-e-Rehan	<i>Ocimum sanctum</i> L.	Leaves
3	Filfil Siyah	<i>Piper nigrum</i> L.	Fruit
4.	Zanjabeel	<i>Zinziber officinale</i> Rose.	Rhizome
5	Zard Chob	<i>Curcuma longa</i>	Rhizome

## Result and Discussions

The macroscopic, microscopic and powder microscopic features of studies single drug are tabulated in Table 2-4 and

the results of physic-chemical,  $R_f$  values, heavy metals, microbial contamination, aflatoxins and therapeutic uses of the studies single drugs are tabulated in Table 5-10.

**Table 2:** Macroscopic features (Fig. 1-5)

Name of the Drug	Macroscopic features
Gilo	Cylindrical soft wooded stem, 5mm. To25mm. Diameter. Outer surface greyish brown, longitudinally wrinkled and warty and covered with lenticels. Fracture fibrous; odour none and taste very bitter. Fig. 1
Berg-e-rehan	Elliptic oblong, entire serrate leaves with round or subcordate base, upto 6cm. long and 2cm. broad. Upper surface downy, bright Green in colour. Lower surface of lighter colour with prominent pinnately reticulate veins with minute gland dots. odour aromatic and taste slightly pungent. Fig. 2
Filfil Siyah	Fruit grayish- black to black, hard, wrinkled 0.4-0.5 cm in diameter, odour aromatic and taste pungent. Fig. 3
Zanjabeel	Rhizome laterally compressed bearing short, flattish, ovate, oblong, oblique, branches on upper side each having at its apex a depressed scar, pieces about 5- 15 cm long 1.5- 6.5 cm wide and 1- 1.5 cm thick, externally buff colored showing longitudinally striations and occasionally loose fibers; fracture short, smooth, transverse surface exhibiting narrow cortex; a well marked endodermis and a wide stele showing numerous scattered fibro- vascular bundles and yellow secreting cells; odour agreeable and aromatic; taste agreeable and pungent. Fig. 4
Zard Chob	Rhizome large; 3-5x2-4cm; dark yellow inside; greyish brown outside; spicy smell, thick, cylindrical, branched. Fig. 5

**Table 3:** Microscopic features (Fig 6-12)

Name of the Drug	Microscopic features
Gilo	Microscopy of stem shows 2-3 layers of cork followed by 4-5 layered phellogen. Cortex is wide parenchymatous zone containing large columnar type cells filled with mucilage. group of sclerieds consisting of 2-8 cells found in this region. Outer zone of Cortex consists of 2-4 rows of elongated chlorenchymatous cells, cortical cells situated towards inner side filled with plenty of oval shaped starch grains. Several mucilage canals found scattered in the cortex along with tannin containing cells. Fig. 6-7
Berg-e-rehan	Upper and lower epidermis in surface view with diacytic stomata and trichomes. Simple and glandular type of trichomes are present. Epidermis with underlying palisade cells. Fig. 12
Filfil Siyah	T.S. shows following characters: <ul style="list-style-type: none"> <li>• Pericarp consists of external epicarp, a large mesocarp and single layered endocarp.</li> <li>• Epicarp consisting of an outer layer of tangentially elongated cells having dark brownish contents. Non- glandular trichomes are found on the surface of epicarp.</li> <li>• Beneath the epicarp, stone cells are found. Stone cells are also found in the endocarp.</li> <li>• Mesocarp is paranchymatous, containing oil globules and starch grains (simple and compound type).</li> <li>• Testa is represented by a single layer of yellow coloured cells.</li> <li>• Inner perisperm cells are radially elongated, containing starch grains and oleoresins.</li> </ul> Pitted and helical thickenings of trachieds seen. Fig. 10-11
Zanjabeel	T.S. shows following characters: <ul style="list-style-type: none"> <li>• Cork-outer zone of irregularly arranged cells and inner zone of radially arranged cells.</li> </ul> Cortex with thin walled paranchymatous cells having intercellular spaces. These cells containing starch grains, oval in shape mostly 5-15-30-60 $\mu$ long and about 25 $\mu$ wide and numerous ideoplasts. Endodermis slightly thick walled and free from starch. Reticulate or spiral vessels up to 70 $\mu$ in diameter; a group of phloem cells unligified, thick- walled; septate fibers up to 30 $\mu$ wide and 600 $\mu$ long. Numerous ideoblast, about 8- 20 $\mu$ wide, similar those of cortex and associated with vascular bundles, also present, Oleoresin cells are present. Fig. 8
Zard Chob	T.S of the rhizome is more or less circular in outline. The outermost layer is the periderm which consists of 5-6 layers of tangentially elongated cells. This is followed by a broad cortex made of thin walled parenchymatous cells with intercellular spaces. Some cells contain deposition of orange red substance oleoresin. Some others contain yellowish oil globules which almost fill the cells. There is a single layered endodermis composed of thin walled rectangular cells. Close to the endodermis many compactly arranged conjoint collateral vascular bundles are present. A number of small bundles are also Fig. 9

**Table 4:** Powder study

Name of the Drug	Microscopic features
Gilo	Numerous oval shaped starch grains; Prismatic crystals.
Berg-e-rehan	Simple unicellular type of trichome, glandular; Trichome, fragment of epidermis with diacytic stomata.
Filfil Siyah	Stone cells; Vessels with spiral thickenings.
Zanjabeel	Numerous oval shaped starch grains; Fragments of vessel (Spiral).
Zard Chob	No Starch grains; Oil cells; vessels with pitted and spiral thickenings trichomes.

**Macroscopic Features**



**Fig 1:** Gilo

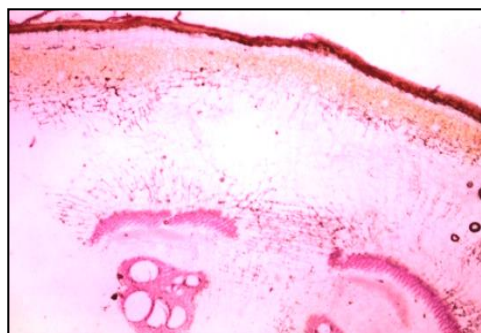


**Fig 5:** Zanjabeel



**Fig 2:** Berg-e-Rehan

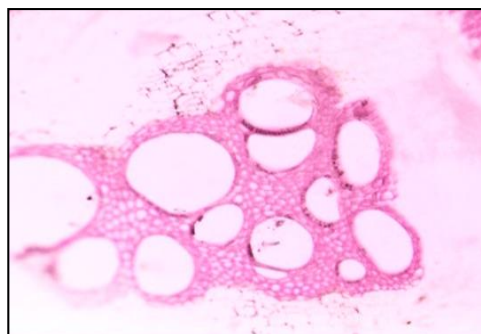
**Microscopic Features**



**Fig 6:** Gilo 4 X



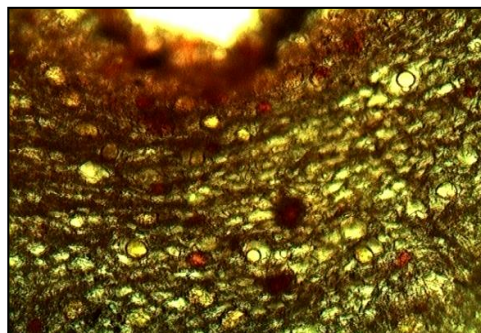
**Fig 3:** Filfil Siyah



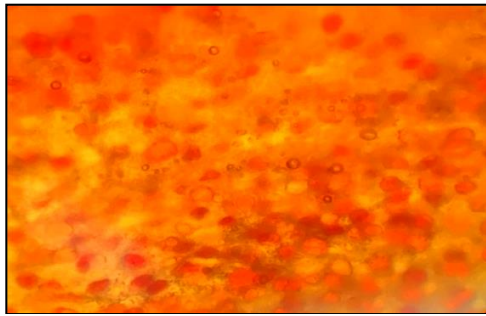
**Fig 7:** Gilo Vascular bundle. 10X



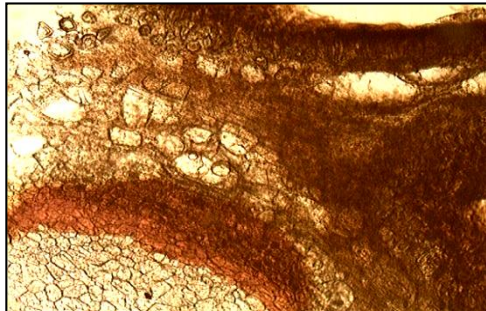
**Fig 4:** Zard Chob



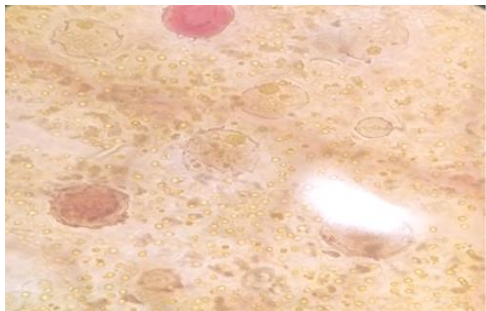
**Fig 8:** Zanjabeel TS 10 x



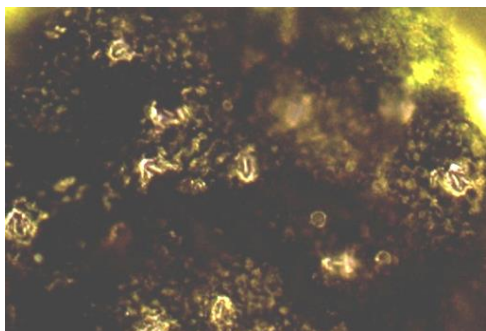
**Fig 9:** Zard chob T.S. 10X



**Fig 10:** Filfil Siyah 10 x

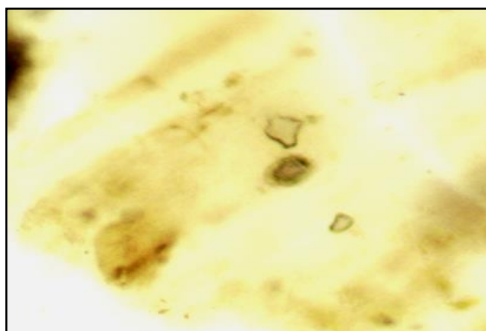


**Fig 11:** Berg-e-Rehan with glandular trichomes and stomata 20x

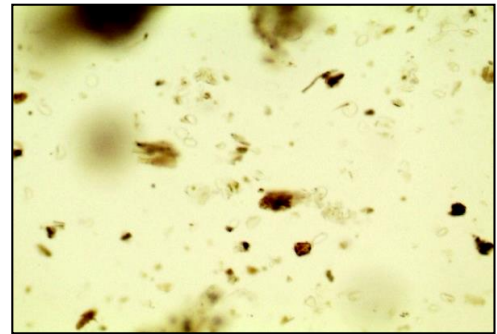


**Fig 12:** Berg-e-Rehan Epidermis with diacytic stomata 20x

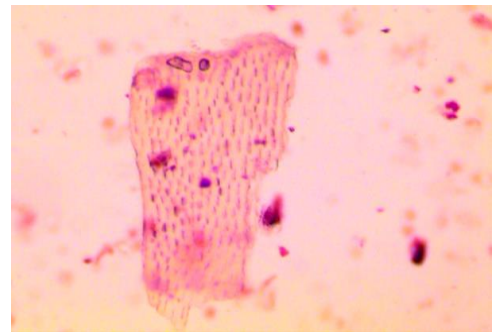
**Powder Microscopy Features**



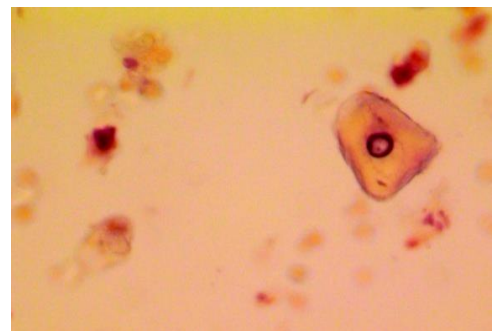
**Fig 13:** Gilo powder prismatic crystals 20x



**Fig 14:** Gilo powder oval shaped starch grains 20X



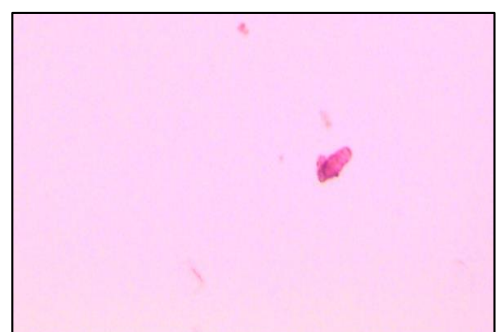
**Fig 15:** Gilo powder bordered pitted tracheidal fibre 20x



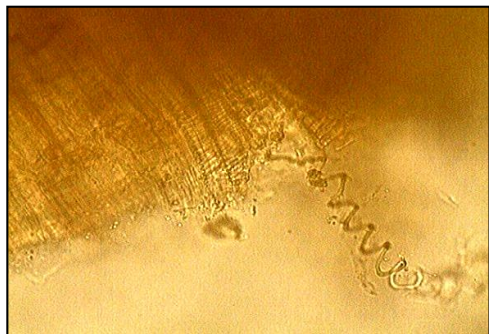
**Fig 16:** Gilo powder showing stone cells 20x



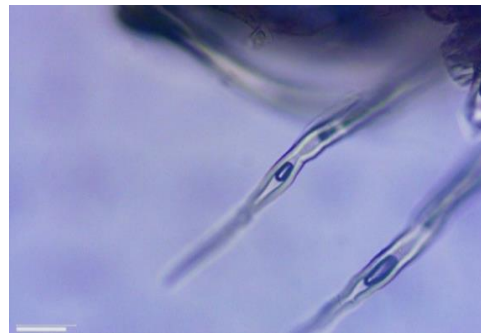
**Fig 17:** Berg-e-Rehan Simple Trichomes 20x



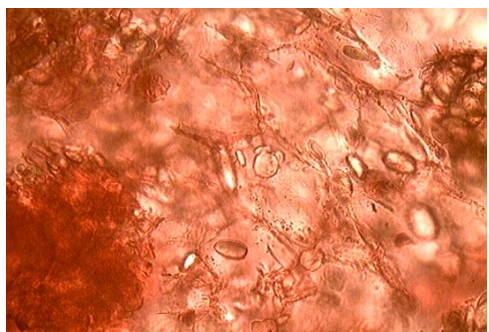
**Fig 18:** Berg-e-Rehan Glandular Trichomes 20x



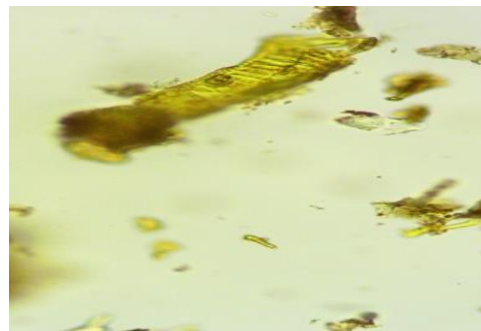
**Fig 19:** Filfil Siyah powder Vessels 20x



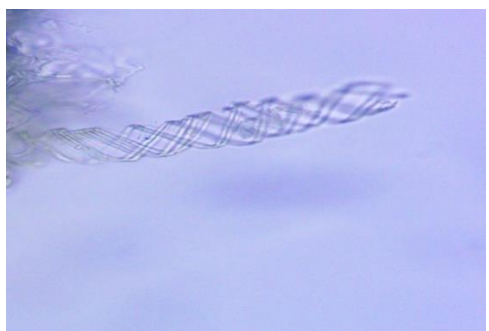
**Fig 22:** Zard Chob trichomes 20x



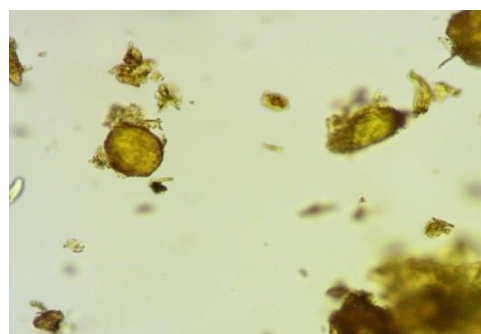
**Fig 20:** Zanjabeel powder starch grains and with spiral thickenings parenchyma cells.20x



**Fig 23:** Zard Chob Powder pitted vessel 20x



**Fig 21:** Zard Chob Powder spiral vessel 20x



**Fig 24:** Zard Chob oleoresin cells 20x

**Table 5:** Physicochemical Parameters

Parameters studies	Results of Studied drugs				
	Gilo	Berg-e-Rehan	Filfil Siyah	Zanjbeel	Zard Chob
Foreign matter (% w/w)	NIL	NIL	NIL	NIL	NIL
Total ash (% w/w)	14.70–15.60	5.64–6.40	4.30 – 4.55	5.15 – 5.40	8.30 – 8.55
Acid insoluble ash (% w/w)	1.45– 1.90	2.90– 3.35	0.52 – 0.67	0.70 – 0.90	0.60 – 0.75
Alcohol soluble extractive (% w/w)	5.25 – 6.10	4.45– 4.87	7.25 – 7.65	4.40 – 4.65	9.50 – 9.85
Water soluble extractive (% w/w)	15.8 – 16.50	7.20– 7.75	7.70 – 7.95	12.20 – 12.45	13.35 – 13.60

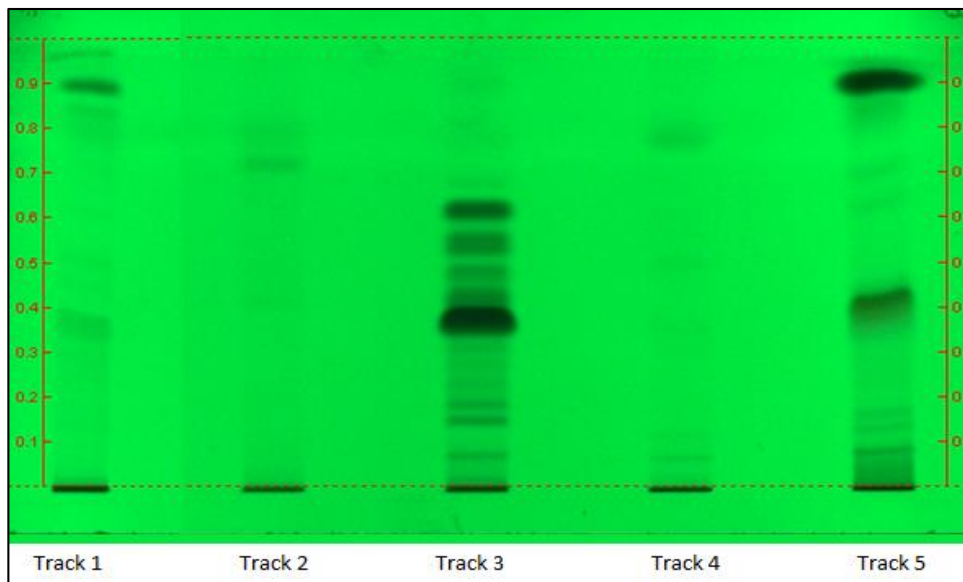
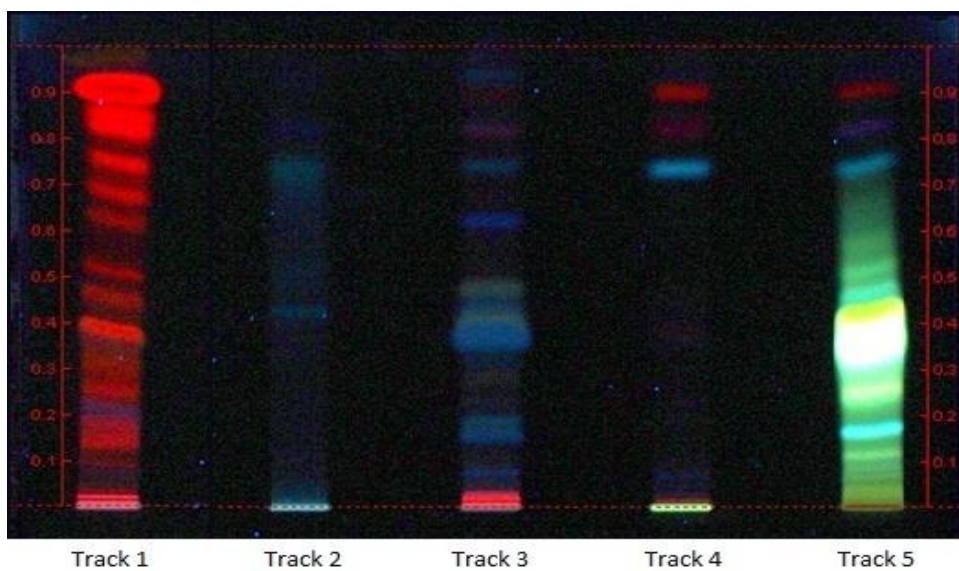
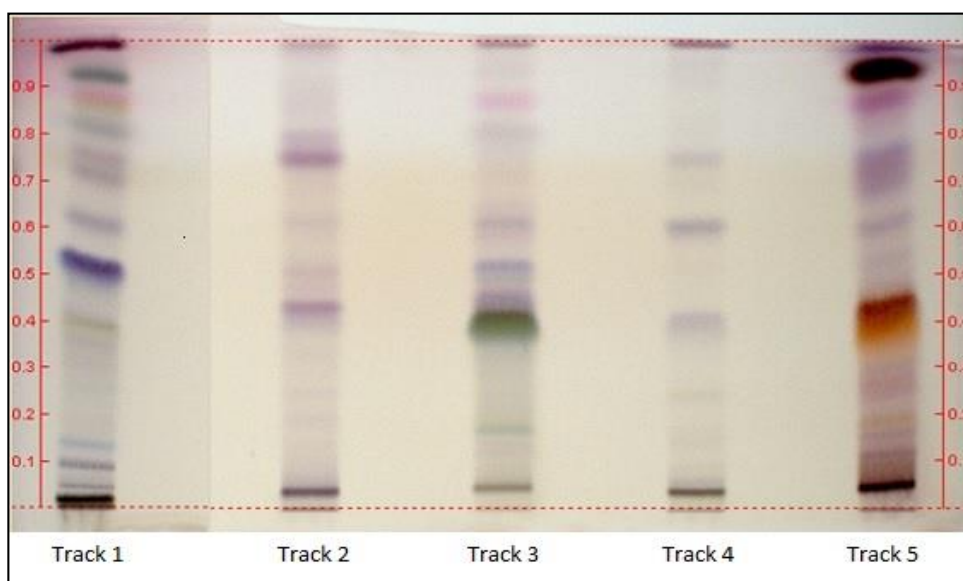
### High Performance Thin Layer Chromatography (HPTLC) Analysis:

#### Sample preparation

The drug samples (2g each) were soaked in 20 ml alcohol separately for 18 hours and refluxed for 10 minutes on water bath and filtered through Whatman No.1 filter paper. The filtrates were concentrated and made up to 10ml in volumetric flasks. HPTLC analysis was carried out as per the standard methods.

HPTLC fingerprinting was performed on 20 cm × 10 cm TLC plates pre-coated with 0.25 μm thin layers of silica gel 60 F<sub>254</sub>

(Merck). The Alcohol extracts (5 μl each) of the samples were applied as 10 mm wide bands on the plate by using CAMAG Linomat 5 applicator. Linear ascending development to a distance of 80 mm using *Toluene: Ethyl acetate (7: 3 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. The plate was air dried and visualized under UV light at λ 254 nm, 366 nm and after derivatization with Anisaldehyde sulphuric acid under visible light. Photographs were taken with the help of CAMAG Reprstar 3 photo-documentation system. The R<sub>f</sub> values of the spots are given in the Table-06.

**HPTLC Fingerprints of studied drugs****Fig 25:** UV-254 nm**Fig 26:** UV-366 nm**Fig 27:** After derivatization with Anisaldehyde sulphuric acid reagent Solvent system: *Toluene: Ethyl acetate (7: 3 v/v)* **Track 1.** Barg-e-Rehan; **Track 2.** Zanjabeel; **Track 3.** Filfil Siyah; **Track 4** Gilo; **Track 5.** Zard Chob

**Table 6:** Rf Values of Alcohol Extract of drugs

Solvent system Toluene : Ethyl acetate (7.0 : 3.0 v/v)	Rf Values		
	254 nm	366 nm	After Derivatization with anisaldehyde sulphuric acid reagent
Barg-e-Rehan ( <i>Ocimum sanctum</i> L.) (Track 1)	0.37(grey), 0.84(grey), 0.90(dark grey)	0.14,0.25,0.31,0.39,0.46,0.51,0.62,0.68,0.75,0.83(all red) and 0.90(dark red).	0.13(blue), 0.39(light violet), 0.52(dark violet), 0.61(violet), 0.71(violet), 0.75(violet), 0.81(light green), 0.88(pink), 0.92(dark green)
ZanjabeelKhushk ( <i>Zingiber officinale</i> Roscoe.) (Track 2)	0.71(grey)	0.43(light blue),0.74(light blue)	0.43(purple),0.50(light purple), 0.61 (light violet), 0.75(purple)
Filfil Siyah ( <i>Piper nigrum</i> L) (Track 3)	0.14(grey), 0.18(grey), 0.38(dark grey), 0.48(grey), 0.55(grey), 0.62(grey)	0.17(blue),0.39(blue), 0.48(green), 0.63(purple), 0.74(blue), 0.82(red)	0.17(green), 0.40(dark green), 0.52(violet), 0.61(purple), 0.80(light purple), 0.87(pink)
Gilo <i>Tinospora cordifolia</i> (Willd) Miers (Track 4)	0.11(grey), 0.77(grey)	0.74(blue), 0.82(light red), 0.91(red)	0.24, 0.41, 0.60,0.75(all violet)
Zard Chob ( <i>Curcuma longa</i> L.) (Track 5)	0.13(grey), 0.16(grey), 0.41(grey), 0.63(grey), 0.70(grey), 0.91(grey)	0.16(fluorescent blue), 0.25(fluorescent light green), 0.36(fluorescent white), 0.74(blue), 0.91(red)	0.44(orange), 0.60(violet),0.73(violet), 0.88(purple), 0.93(dark purple)

**Safety evaluation**

Safety evaluation such as microbial load estimation, aflatoxin contamination, and heavy metal were analyzed and the results are tabulated in the tables 3-5 [8].

**Table 7:** Heavy metal analysis

S. No.	Name of the Drug	Parameter analyzed			
		Lead (Pb)	Cadmium (Cd)	Arsenic (As)	Mercury (Hg)
1	Gilo	ND	ND	ND	ND
2	Berg-e-Rehan	ND	ND	ND	ND
3	Filfil Siyah	ND	ND	ND	ND
4	Zanjabeel	ND	ND	ND	ND
5	Zard Chob	ND	ND	ND	ND
WHO permissible limit		10 ppm	0.3 ppm	3.0 ppm	1.0 ppm

**Table 8:** Microbial contamination

S. No.	Name of the Drug	Parameter analyzed			
		Total Bacterial load	Salmonella spp.	Escherichia coli	Total Fungal count
1	Gilo	Nil	Nil	Nil	Nil
2	Berg-e-Rehan	Nil	Nil	Nil	Nil
3	Filfil Siyah	Nil	Nil	Nil	Nil
4	Zanjabeel	Nil	Nil	Nil	Nil
5	Zard Chob	Nil	Nil	Nil	Nil
WHO permissible limit		Not more than 10 <sup>5</sup> /g	Nil	Nil	Not more than 10 <sup>3</sup> /g

**Table 9:** Aflatoxin

S. No.	Name of the Drug	Parameter analyzed			
		B1	B2	G1	G2
1	Gilo	Nil	Nil	Nil	Nil
2	Berg-e-Rehan	Nil	Nil	Nil	Nil
3	Filfil Siyah	Nil	Nil	Nil	Nil
4	Zanjabeel	Nil	Nil	Nil	Nil
5	Haldi	Nil	Nil	Nil	Nil
WHO permissible limit		0.50 ppm	0.10 ppm	0.50 ppm	0.10 ppm

**Table 10:** Therapeutic Uses [9-13]

Drug	Therapeutic Uses
Gilo	Humma (Fever), Hikka (Itching), Damameel (Boils), Waja-ul-Mafasil (Arthritis)
Berg-e-Rehan	Humma (Fever), Zo'f-e-Qalb (Cardiac asthenia), Khafqan (Palpitation), Nazla Zukam (Common Cold), Su'al (Cough), Ishal (Diarrhoea)
Filfil Siyah	Nafkh-e-shikam (flatulence in stomach), Zof-e-Hazm (weakness of stomach), Zof-e-Bah (Sexual debility), Ehtebas-e-Boul (anuria)
Zanjabeel	Falij (Paralysis), Laqwa (Facial Palsy), Su'e-Hazm (Dyspepsia), Muqawwi-e-Meda (Stomachic), Zo'f-e-Kabid (hepatic debility)

Zard Chob	Su'al (Cough), Zeeq-un-Nafas (bronchial asthma), Tap (fever), Zarba-wa-Saqta (trauma & injury), Istisqa (ascites), Yarqan Suddi (obstructive jaundice), Nafs-ud-Dam (haemoptysis), Fasaad-e-Dam (blood impurities), Kharish (pruritus)
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### Conclusion

Pharmacognostic methods confirms the identity of the drugs of plant origin and chemical methods of quality testing such as physico-chemical parameters, microbial contamination, aflatoxins, heavy metal analysis and high performance thin layer chromatographic studies could be used to establish the quality, safety and efficacy of the plant material. In future this type of research work will definitely helpful for identification and authentication of herbal drugs.

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