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CHROMATOGRAPHIC AND PHARMACOGNOSTIC CHARACTERISATION OF IMPORTANT DRUGS (UNANI HERBAL) FOR THEIR IDENTIFICATION

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ABSTRACT: Standardization and quality control are the key factors in regulating the therapeutic efficacy of herbal drugs. There are various standard pharmacognostic and chemical methods for checking an identity and the quality of single herbal drugs. Present communication highlights macro and micro-morphological characteristics, powder studies, histochemical tests, micro-chemical tests, physico-chemical constants, chromatographic profile and therapeutic uses; which can be useful for checking genuineness of herbal drugs.

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KEYWORDS: Herbal drug, Chromatographic profile, Pharmacognostic, Chemical methods.

1 INTRODUCTION

Herbal drugs are the major component of most of the formulation and used either single or in combination with other ingredients (Plant, animal, mineral and metal drugs) after simple or complex process of pharmacy (Anonymous, 1981)[1]. However, the quality, safety and efficacy of these preparations remains always an issue of great concern while considering the genuineness and presence or absence of ingredients in them (Rai, et al., 2011)[2]. There are various standards pharmacognostic (macro and microscopic evaluation), chemical methods and instrumental methods for identification of ingredients and quality testing.

Present communication provide the macro and micro morphological, powder, histochemical, micro-chemical, chromatographic profile and therapeutical studies of four Unani herbal drugs, viz Zeera Siyah (*Carum carvi*), Filfil Siyah (*Piper nigrum*), Zanjabeel (*Zinziber officinale*), Berg-e-Sudab (*Ruta graveolens*).

2 MATERIALS AND METHODS

2.1 DRUG AND CHEMICALS

Herbal drugs (Table-1) were resourced from Delhi and Ghaziabad market. All the ingredients were

identified by Botanist using pharmacopoeial standards (Johnson, 1940)[3]. 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 and DESAGA photo-documentation system (Wagner, et al., 1984; Stahl, 1996)[4,5].

Thin layer chromatography was done by using 5 g powdered drug which was extracted in 60 ml of absolute alcohol under reflux on water bath for 10 min. Filtered and concentrated the filtrate up to 4 ml. The obtained extract was applied on a pre-coated silica gel plate and developed in Toluene: ethyl acetate (70:30) system in developing chamber. The plate was dried and sprayed with Vanillin Sulphuric acid reagent and again the plate was dried and kept in an oven for heating at 105 °C for 10 minutes, Rf values of the spots are then measured.

3 RESULTS AND DISCUSSION

Table 1 describes the part used for the present study. The macro morphological features are summarised in table 2 while table 3 show the micro-morphological features.

Table 1 Unani drugs under study-(Anonymous, 2000, 2006)[6,7].

S. N.	Unani Name	Botanical/ Mineral Name (Anonymous, 2000).	Part Used
1	Zeera Siyah	<i>Carum carvi Linn.</i>	Fruit
2	Berg-e-Sudab	<i>Ruta graveolens Linn.</i>	Leaves
3	Filfil Siyah	<i>Piper nigrum Linn.</i>	Fruit
4.	Zanjabeel	<i>Zinziber officinale Rose.</i>	Rhizome

Table 2 Macro morphological Features- (Anonymous 2005)[8]

Zeera Siyah (<i>Carum carvi</i>),	Filfil Siyah (<i>Piper nigrum</i>),	Zanjabeel (<i>Zinziber officinale</i>),	Berg-e-Sudab (<i>Ruta graveolens</i>)
Fruit light brown, slightly curved, elongated, mericarps, usually separate, free from the pedicel; carpophores up to 7mm long 2mm broad, plano-convex, narrow, tapering to each end, arcuate, glabrous, brown with five very narrow, yellowish-brown primary ridges; endosperm, orthospermous, odour and taste aromatic and characteristic.	Fruit grayish- black to black, hard, wrinkled 0.4-0.5 cm in diameter, odor aromatic and taste pungent	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; odor agreeable and aromatic; taste agreeable and pungent.	Strong –scented; green leaves, about 5.0-7.5mm long and 2.0-2.5mm broad in size, alternate, smooth, gland dotted leaves are two-three times pinnately divided spatulate or oblong in shape and covered with bloom, odor strongly aromatic and taste slightly bitter.

Table 3 Micro morphological features

Zeera Siyah (<i>Carum carvi</i>),	Filfil Siyah (<i>Piper nigrum</i>),	Zanjabeel (<i>Zinziber officinale</i>),	Berg-e-Sudab (<i>Ruta graveolens</i>)
T.S. shows the following characters: <ul style="list-style-type: none"> • Pericarp having outer epidermis, epidermal cells polygonal in shape covered with cuticle, trichomes absent. • Four vittae four dorsal and two commissural. Volatile oil in cavities. • Mesocarp paranchymatous, costae five in each mericarp with vascular strand consisting of an 	T.S. shows following characters: Pericarp consists of external epicarp, a large mesocarp and single layered endocarp. Epicarp consisting of an outer layer of tangentially elongated cells having dark brownish contents. Non- glandular trichomes are found on the surface of epicarp. Beneath the epicarp, stone cells are found. Stone cells are also found in the endocarp.	T.S. shows following characters: Cork-outer zone of irregularly arranged cells and inner zone of radially arranged cells. Cortex with thin walled paranchymatous cells having intercellular spaces. These cells containing starch grains, oval in shape mostly 5-15-30-60 μ long and about 25 μ wide and numerous ideoplasts. Endodermis slightly thick walled and free from starch.	T.S. shows the following characters: <ul style="list-style-type: none"> • Upper epidermal cells covered with thick cuticle. Epidermal cells are rectangular in shape. • Beneath the epidermis, palisade cells are found. These cells are radially elongated containing chloroplast. • The spongy paranchymatous cells are 4- 5 layers. These cells are polygonal in shape and are loosely arranged, containing starch grains.

<p>inner group of small vessels and fibres, outer group of pitted sclerenchyma with small group of phloem on each lateral surface; on the outer margin of each vascular strand a small schizogenous canal.</p> <ul style="list-style-type: none"> • Endocarp - elongated sub-rectangular cells. Endosperm thick walled, containing much fixed oil and numerous small aleurone grains up to 10μ in diameter, micro rosette crystals are present 	<p>Mesocarp is paranchymatous, containing oil globules and starch grains (simple and compound type). Testa is represented by a single layer of yellow coloured cells. Inner perisperm cells are radially elongated, containing starch grains and oleoresins. Pitted and helical thickenings of trachieds seen.</p>	<p>Reticulate or spiral vessels up to 70μ in diameter; a group of phloem cells unligified, thick-walled; septate fibers up to 30μ wide and 600μ long. Numerous ideoblast, about 8- 20μ wide, similar those of cortex and associated with vascular bundles, also present, Oleoresin cells are present.</p>	<ul style="list-style-type: none"> • Lower epidermal cells are smaller in size and stomata present on the lower epidermis. • Vascular bundles are found in the spongy paranchyama. Rosette of crystals found.
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Table 4 Powder study-

<i>Zeera Siyah (Carum carvi),</i>	<i>Filfil Siyah (Piper nigrum),</i>	<i>Zanjabeel (Zinziber officinale),</i>	<i>Berg-e-Sudab (Ruta graveolens)</i>
Groups of pitted sclerieds. Endosperms with oil globules, aleurone grains and micro grains. Numerous fragments of vittae.	Stone cells. Vessels with spiral Thickenings	Numerous oval shaped starch grains. Fragments of vessel (Spiral).	Stomata present. Vessel with spiral thickenings. Paranchymatous cells. Oil canals.

Table 5 Histochemical tests

	<i>Zeera Siyah (Carum carvi),</i>	<i>Filfil Siyah (Piper nigrum),</i>	<i>Zanjabeel (Zinziber officinale),</i>	<i>Berg-e-Sudab (Ruta graveolens)</i>
Starch-		Gives a blue color reaction with iodine.	Gives a blue color reaction with iodine. Oleo-resin- The cells containing resinous substance are stained reddish with Sudan III. Suberin-After several hours' maceration of the sections in conc. KOH, Suberin and also lignin becomes yellow.	
Calcium oxalate Crystals	The test shows the presence of calcium oxalate in the few cells.			The test shows the presence of calcium oxalate in the few cells.
Oil	Abundant cells containing oil globules are present	Abundant cells containing oil globules are		

	which appear red with Sudan III.	present which appear red with Sudan III.		
Aleurone grains	The appearance of yellow color shows the presence of aleurone grains.			

Table 6 Micro chemical tests colour reaction of drug powder with different acid/chemical reagents

S. N.	Acid/chemical Reagent	Zeera Siyah (<i>Carum carvi</i>)	Filfil Siyah (<i>Piper nigrum</i>)	Zanjabeel (<i>Zinziber officinale</i>)	Berg-e-Sudab (<i>Ruta graveolens</i>)
1.	Conc. Sulphuric Acid	Dark chocolate brown	Reddish brown	Black	Green black
2.	Conc. Hydrochloric Acid	Light yellowish brown		Reddish brown	Dark green
3.	Conc. Nitric Acid	Light brown	Yellowish brown	Orange	Orange yellow
4.	Glacial Acetic Acid	Light yellowish brown	Yellowish brown	No change	No change
5.	Picric Acid	-	Yellow	No change	No change
6.	Iodine Solution	Orange	Black	Bluish black	Bluish black
7.	Ferric chloride Solution (aq.)	Coffee brown	Light yellow with blackish tinge	Bluish green	Bright green
8.	Sodium hydroxide Solution (5%)	Dark yellow	Brown with bluish tinge	Dark brown	Brown
9.	Potassium hydroxide Solution (5%)	Yellowish brown		Dark brown	Brown
10.	Powder as such	Dark coffee brown	Grey	Yellowish brown	

Table-7 TLC fingerprinting data on herbal drugs

Drug	Rf value
Berg-e-Sudab	0.38, 0.45, 0.65, 0.78, 0.88.
Zeera siyah	0.38, 0.54, 0.65, 0.69.
Filfil Siyah	0.38, 0.45, 0.54, 0.65, 0.69, 0.82,
Zanjabeel	0.27, 0.31, 0.38, 0.45, 0.54, 0.65, 0.69, 0.74, 0.89

Table 8 Therapeutic Uses (Anonymous 2007, 2009)[9-11]

Drug	Therapeutic Uses
Berg-e-Sudab	Bahaq(white patches in skin),Bars(Leucoderma),Nafakh--e-shikam(flatulence in stomach),Waj-ul-meda(stomach ache)
Zeera siyah	Zof-e-meda(weakness of the stomach),Nafakh-e-shikam(flatulence in stomach),Su-e-Hazm(Dyspepsia)
Filfil Siyah	Nafkh-e-shikam(flatulence in stomach), Zof-e-Hazm(weakness of stomach),Fasaad-e-Hazm
Zanjabeel	Zof-e-meda(weakness of stomach),Nafakh-e-shikam(flatulence in stomach),Su-e-Hazm(Dyspepsia),Faliz Laqwa(Hemiplegia)

4 CONCLUSION

Pharmacognostic methods confirms the identity of the drug of plant origin, but chemical methods of quality testing such as TLC, HPTLC are frequently used for detecting and identifying most of the plant materials in herbal preparation. These fingerprints are unique and characteristics to individual drug and lead to establish the identity of drug and detection of adulterants.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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