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Research Article

PHARMACOGNOSTIC EVALUATION OF HERBOMINERAL FORMULATION *(ALG-06)* USED IN VITILIGO

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ABSTRACT

Standardization of phytopharmaceuticals are much needed to authenticate by natural or others scientific means therefore, in this research we performed pharmacognostic studies of four herbs *Trigonella foenum graecum L.*(1), *Azadirachta indica L.*(2), *Psoralea corylifolia L.*(3), *Punica granatum L.*(4) and two minerals *red ochre* (5) *and purified sulphur* (6) for herbomineral formulation *(ALG-06)* to cure vitiligo. Fresh or dried seeds of all these herbs as well as their powdered samples were studied according to micro morphological, anatomical and histological aspects by using various chemical reagents. The common characteristic features of the sample herbs were parenchyma and sclerenchymatous cells were present in *Azadirachta indica L.*, *Punica granatum L.* and *Trigonella foenum graecum L.*, oil cells were seen in *Azadirachta indica L.* (large thin walled) and *Psoralea corylifolia L.* (medium thick walled), Ca oxalate crystals prism like were present in *Azadiracta indica L.* and both types prism and cluster were seen in *Punica granatum L.* along with abundant tannin containing cells while thickly lignified cells, fusiform, mucilage and color containing cells were present in both *Azadirachta indica L.* and *Trigonella foenum graecum L.* respectively. Mineral drugs after cleaning and purification were organoleptically standardized through sensory method (color, odor and taste).

Keywords: Vitiligo, *ALG -06*, Pharmacognostic standardization, *Trigonella foenum graecum L, Azadirachta indica L., Psoralea corylifolia L., Punica granatum L.,* Red ochre and purified sulphur

INTRODUCTION

Vitiligo is a specific, often inheritable and acquired disorder characterized by well-circumscribed milky white cutaneous macules devoid of identifiable melanocytes [1]. It has a prevalence of approximately 1% [2]. Extensive research has been done on this disease but reason of onset is still obscure.

In phyto pharmaceutical use standardization of herbal drugs has prim importance because these drugs usually have great diversity in their morphology, climatic and geographical conditions. Ontogenic and heredity characteristics are the major factors which are responsible to produce dynamic changes in plant constituents. In consequence of these factors morphological alteration may also occurs which probably hindered in identification. Therefore recognition of herbal drugs needed to standardize for phytophamaceutical manufacturing either by sensory, microanatomical and physicochemical methods at preliminary stage. In this research, we have prepared such formulation which contains herbs and minerals in combination named as herbomineral formulation (*ALG-06*) to devoid hazardous effects of many treatments which was previously used to cure vitiligo. The aim of this study is to detect the presence of different tissues in the powder drugs of (*ALG-06*) formulation and also determine their patterns of variation in tissue characteristics as well as arrangements of cellular structures which help in the identification of plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.

Trigonella foenum graecum L. **(1)** belongs to the family Fabaceae. Seeds employed as an anti-diabetic, hypocholesterolemic and anti-inflammatory agent. The active constituents contain saponin, coumarins, flavonoids, alkaloids, amino acid, fatty acids, carbohydrates and minerals (calcium, iron, potassium). It is also a good source of both water and fat soluble vitamins i.e. Vitamin C, B and A [3]. It may also protect the gastrointestinal tract from ulcers, stimulate immune system and have antipyretic effect [4].

Azadiracta indica L. **(2)** belongs to the family Meliaceae. The word Azadiracta derived from the Persian Azadirakt meaning 'noble tree'. The active constituents contain alkaloids azadirachtin, resins, meliotannic acid and benzoic acid, glucose, hydrocarbon and proteins. The seed is stimulant and applied externally in rheumatism, antiseptic, insecticides, applied to boils, ulcers and eczema. It is also used in leprosy and sprains [3,5].

Psoralea corylifolia L. **(3)** belongs to the family Fabaceae also known as babchi seeds which contain coumarin, psoralen and isopsoralen. Bavachinin isolated from seeds have anti-inflammatory, antipyretic and analgesics properties. Seeds powder and paste are used in indigenous medicine as laxative, aphrodisiac, anthelmintics, diuretics, stomachic stimulant and diaphoretic in febrile condition [6,7].

Punica granatum L. **(4)** belongs to the family Punicaceae. The fruits have multiple seeds ovary and contain rich phytochemical agents. The active constituent are anthocyanins, flavonoids, alkaloids, tannins, triterpenes and phytosterols. Seeds of plants use as carminative, nutrient, appetite stimulant, astringent, digestive and diuretics [3].

Microscopic determination of *Azadirachta indica L.* and *Punica granatum L.* seeds were done by first time ever.

Red ochre **(5)** In Urdu it is called Geru and English is kaolin and china clay has peculiar strong smell. The medicinal preparation of red oxide is for glutinizing, antidiarrheal and antihemorrhagic agent for bleeding from internal organs [8]. ***Sulphur*** **(6):** Brimstone is a sublimed form and it is commonly called sulphur while the cleaned variety is being used in dry formulation in Unani medicines known as Amlasaar Musaffa. It is mostly occur in shinny yellow color and found free in beds of gypsum and in a state of sublimation in region of extinct volcanoes. It is prepared by roasting, fusion or sublimation for medicinal use [9].

MATERIAL AND METHOD

Plant Materials: The seeds of plants *Psoralea corylifolia L., Punica granatum L., Trigonella foenum graecum L.,* and minerals sulphur and red ocher were purchased from Herbal Market Sadder Karachi, while *Azadiracta indica L.* seeds were collected from trees grown in University of Karachi campus and identification of all herbs and minerals was kindly carried out by Professor Dr. Ghazala H. Rizwani, Department of Pharmacognosy and voucher specimens No.0034,0035, 0036,.0037,0038,0039 had been deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. Macroscopic characters of the seeds of all the herbs (1-4) were noted i.e. color, size, shape, odor, taste, texture and fracture while in the minerals (5 and 6) only color, odor, taste and texture were observed after cleaning. The powder microscopy of all these four herbs was performed as described by [12,14]. For such microscopic evaluation various chemical reagents were used such as chloral hydrate 10%, iodine solution 5% and aqueous glycerin 50%

were used, while the histological examination as described by [13,15]. Herbs (1, 3 and 4) were taken in a dried form after pretreatments for the microscopic observation and determination of their cellular arrangements. In the herbs except *Azadiracta indica* (2) seeds, remaining seeds of three herbs were subjected to boiling at 100°C for specified period of time 1 (2-3min), 3 (8-10min) and 4 (8-9hrs) respectively prior to sliced fine sections.

RESULTS AND DISCUSSION

The pharmacognostic evaluation of (1-4) herbs and (5-6) minerals of herbomineral formulation (*ALG-06*) are as follows.

***Trigonella foenum graecum L.* (1)**

***Organoleptic evaluation*:** The drug *Trigonella foenum graecum L.*(1) consists of dried seeds, which are rhomboidal in shape with a deep olive yellow color which are compressed truncate at both ends. The seed is 3 to 5mm length and 1.5 to 2mm breadth. The funicular point on the lateral side and a V shaped narrow furrow starts just from the depression nearly at the center of the lateral side. This depression lodges both hilum and microphyle. The testa is smooth and hard. The average weight of 100 seeds is 1.369g. Powder of seeds is yellow in color, bitter in taste with pungent and agreeable odor and fracture difficult to break. ***Microscopic studies*:** It reveals the presence of elongated palisade cells, several layers of thin walled parenchyma cells which may be irregular in size or irregular intracellular spaces, polygonal regular thick walled cell with lumen containing yellowish brown pigment of epidermis, aleurons grains containing cells of radical and cotyledon, oil containing cells and oval to round aleuron grains. Single layer of endospermic cells are also visible in abundance, which contains aleuron grains [4].

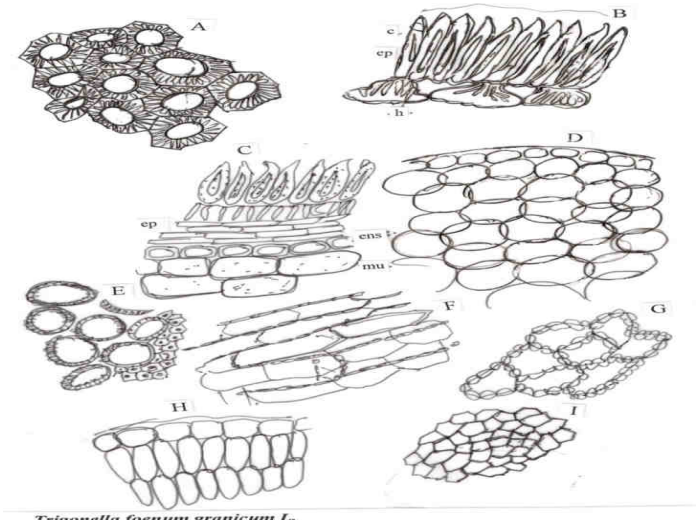

Fig. 1: A-I Powder microscopy of seed *Trigonella foenum graenum L.* (1)

A: Hypodermis of the testa in surface view, **B**: Cuticle, epidermis and hypodermis of the testa in surface view from below, **C**: Part of the seed in sectional view showing the epidermis, hypodermis and Parenchymatous layers, of the testa and the outer most layer and the mucilage cells, **D**: Epidermis and Parenchymatous cells of the cotyledons in sectional view, **E**: Epidermis and hypodermis of the testa in surface view from above, **F**: Layers of the parenchyma of the testa, **G**: Outermost layer of the endosperm, **H**: Undifferentiated parenchyma of the cotyledons, **I**: A single layer of the parenchyma of the testa in surface view.

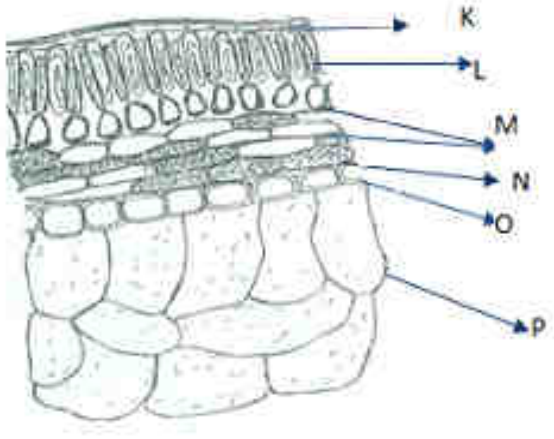

Fig. 2: K-P T.S of seed *Trigonella foecum graecum L.* (1)

(K) Stomata **(L)** Hypodermis based on palisade **(M)** Mesodermal cells **(N)** Endodermis layer of starch grains**(O)** Layer of lignified cells**(P)** multiple layer of mucilage cells.

***Azadiracta indica L.* (2)**

***Organoleptic evaluation*:** Crimson colored oval seeds of *Azadiracta indica L.* had three dimensional in appearance with sharp on one end and slightly depressed laterally and was rounded on the other. Externally abraded surface with three main edges seed occupies almost oval in shape 1-3 cm is approximate length while breadth of seed is 1.5-2 cm and 0.5-1.5cm thickness. The average weight of 100 seeds is 15.1g. Taste was extremely bitter and odor was intense herbaceous oily internal side of the seed was smooth, shiny having a

shrieked yellow cotyledons with reddish brown covering which smaller in all size respect. (i.e. length, breadth and thickness)

***Microscopic studies*:** Pairs of annular vessels group of phloem fibers and two types of stone cells. Oil cells was wider in size and most of them filled with oil residues hard bast also seen abundant with numerous calcium oxalate crystals specially rosette type. Two types of sclereids was also very prominent in the powder one was fusiform sclereids and small-elongated oval shaped sclereids.

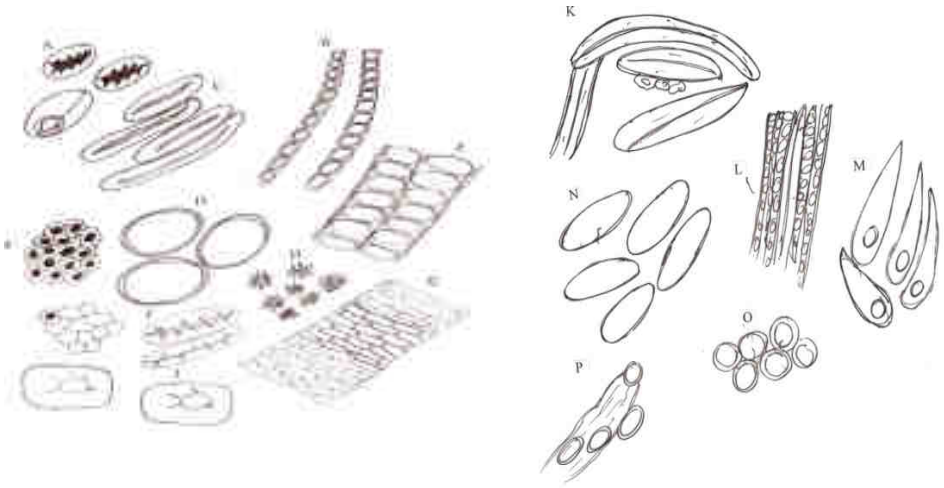

Fig. 3: A- P Powder microscopy of seed *Azadiracta indica L.* (2)

A: Thick lignified stone cells, **B**: Group of phloem fiber, **C:** Elongated small lumen thin walled stone cells, **D:** Oil cells, **E:** Pair of annular vessels, **F:** Sclerenchymatous cell or hard bast fillers, **G:** Thread like fragment, **H**: Rosette calcium oxalate crystals, **I**: Thick large stone cells, **J:** Large lumen stone cells. **K**: Fusiform sclereids, **L**: Part of vascular tissues, **M:** Small pitted sclereids, **N:** Thin phloem fiber overview, **O**: Group of oil cell, **P:** Part of fiber having occasional oil cells.

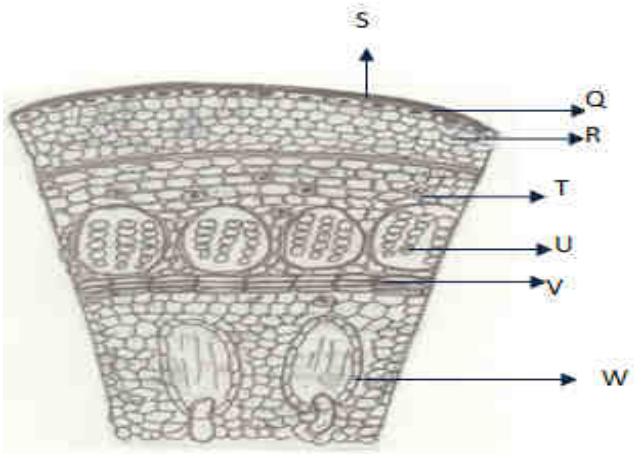

Fig. 4: Q-W T.S of seed *Azadiracta indica L.* (2)

(**Q**) Cuticle (**R**) thickly lignified cells (**S**) Stomata (**T**) Tracheids (**U**) Vascular bundle (**V**) Parenchyma fiber (**W**) Embryo.

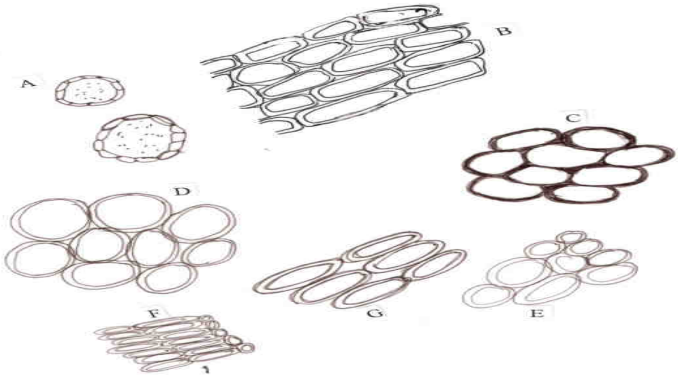

Fig. 5: A-G Powder microscopy of seed *Psoralea corylifolia L.* (3)

A: Oil cells, **B**: Sclerenchymatous cells of testa in surface view, **C**: Sclerenchymatous cells, **D**: Parenchymatous cells of cotyledons, **E**: Parenchymatous cells **F**: Epidermal cells of testa. **G:** Epidermal cells of inner layer.

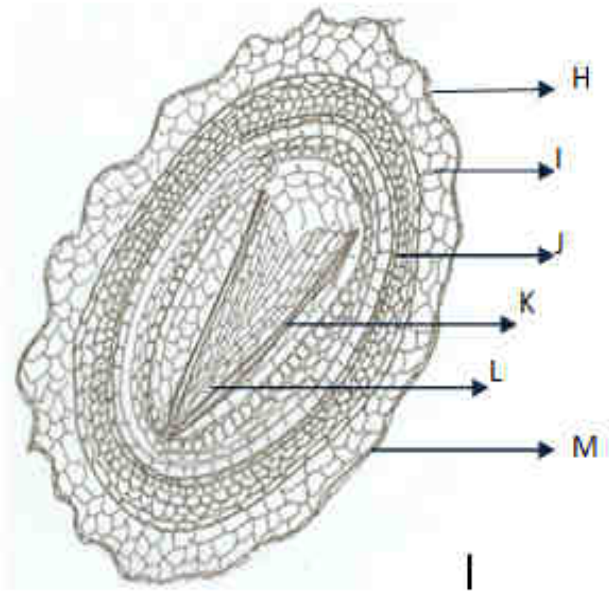

Fig. 6: H-M T.S of seed *Psoralea corylifolia L.* (3)

(**H**)Cuticle **(I)** Epidermis layer of hypodermal cell **(J)** Outer endosperm **(K)** Embryonic arch **(L)** Inner endosperm **(M)** Stomata.

***Psoralea corylifolia L.* (3)**

Organoleptic evaluation: Grayish black *Psoralea corylifolia L.* seeds dull starchy appearance with porous surface and slight depression also seen in the middle region, about 0.5-1cm length and almost same width internally, it is multi layered with light brown reddish brown septum. A prominent micropyle on top and has slightly bitter oily taste while its odor is similar with coconut smell, shape of seeds are oblong reniform and flattened. The interior of seed contained cotyledons and small embryo. The average weight of 100 seeds is 1.8g.The powdered drug of seed is grayish black in color with aromatics odor similar to coconuts oil.

***Microscopic studies*:** The powdered herb reveals that it contains sclerenchymatous cells of testa. Parenchymatous cells of cotyledons. Oil cells and epidermal cells of testa are also frequently visible in the powder.

***Punica-granatum L.* (4)**

Organoleptic evaluation: Seeds of drugs *Punica granatum L.* were sticky, fruity and slightly shiny due to the dried fruit juice on it. Peeled of clear seeds were porous, glossy and had a deep depression which bifurcation the base into two shapes i.e. spatheolate and taste was sweet and sour with characteristic fruity odor, size of the seed was 4-9 mm, 1.6-2.2 mm and 1.2- 1.5 mm thickness with very hard fracture. The average weight of 100 seeds was 2.29g along with dried juicy pulp. Powder of the seeds was reddish brown in color and lumpy, sweet sour in taste with pleasant fruity odor.

Microscopic studies: Prism and cluster of calcium oxalate crystals. Parenchyma with starch grains, fragments of thinner walled and thick walled cork in Surface view, elongated sclereids were also seen abundant along with phloem parenchyma, cells contained tannins and other pigments also observed.

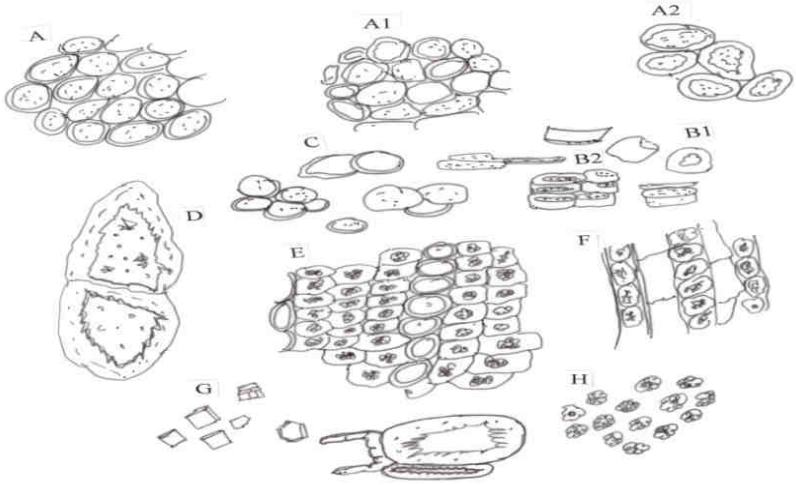

Fig. 7: A-H Powder microscopy of seed *Punica granatum L.* (4)

A: Fragments of thinner walled cork in surface view, **A1:** Fragment of cork tissues sectional view, **A2:** Thick and larger fragment of cork, **B1**: Walled cork in surface view, **B2**: Thick walled cork in sectional view, **C**. Thinner walled cork in sectional view, **D**: Sclereids, **E:** Phloem parenchyma with calcium oxalate cluster crystals and a medullary rays **F:** Part of phloem in radial longitudinally section, **G:** Prisms of calcium oxalate, **H:** Cluster crystals of calcium oxalate.

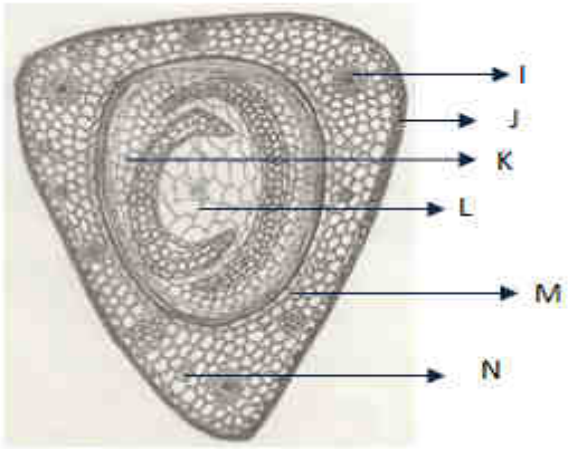

Fig. 8: I-N T.S of seed *Punica granatum L.* (4).

(I) Colateral Vascular bundle **(J)** Hard cuticle **(K)** Endospermic cells **(L)** Pair of embryonic cells **(M)** Cortical zone **(N)** Ca oxalate (prism & cluster)

Red Ochre (5)

Organoleptic Evaluation: It was reddish rust color almost crystalline masses with many margin surfaces were slightly bright powder of red ochre was odorless and almost insipid with adsorbing characteristics.

Fig. 9: Raw and refined powders of drug *red ochre* (5)

Sulphur (6)

Organoleptic Evaluation: Yellow shiny crystals having very prominent striations, sometimes pores were also seen. Refined drug had bitter astringent taste with peculiar strong odor. While crude form easily differentiated by light yellow.

Fig. 10: Raw and refined powder of drug *sulphur* (6)

CONCLUSION

A large number of populations in the world rely on medicinal plants for their health care needs [11]. In this regard most of the well-known herbs used in their specific stages and form are not reported. The pharmacognostic features of entire plants or their specific parts in the literature, as even most of the general formulations required standard protocol for their establishment as a drug in pharmaceutical manufacturing. Therefore any new formulation which is prepared against any disease condition it is necessary to undergone the standardization procedure in a real sense. According to the World Health Organization (WHO) the first step for identification and purification of herbal drugs is the pharmacognostic (macroscopic and microscopic) studies which are essential for any phytopharmaceutical products used for standard formulation [10].

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