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# Chemotaxonomic Standardization of Herbal Drugs Milk Thistle and Globe Thistle

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Chemotaxonomic standardization is critically important to the quality, safety and efficacy of herbal drugs throughout the world and particularly in Pakistan where 80 % population rely on folk medicine for primary health care. In present account an attempt was made to authentify herbal drugs *Silybum marianum* (Milk thistle) and *Echinops echinatus* (Globe thistle) by using multidimensional identification techniques. Milk thistle and globe thistle have been confused with each other due to folk nomenclature problem which probably existed due to their capitulum resemblance of same tribe *Cardueae* of family Asteraceae. In this paper the taxonomic evidences for authentication were based on detailed studies of morphology scanning electron microscopy of pollen and leaf epidermal characteristics where as chemical standards were thin layer chromatography, ultraviolet and infrared analysis. In *Silybum marianum*, flavonoid agylocone revealed the presence of one major and two minor amounts of phenolic acid and *Echinops echinatus* can be distinguished due to the presence of three phenolic acid of small amount, when viewed under 366 nm UV light. It is concluded that these techniques have been used to authenticate problematic herbal drugs in order to maintain WHO standards for globalization of alternative system of medicine.

**Key Words: Chemotaxonomic, Standardization, Milk thistle, Globe thistle.**

## INTRODUCTION

The use of herbal medicine in most developing countries is being practical as normative basis for the maintenance of good health. The World Health Organization (WHO) estimates that 4 billion people (80 %) of the world population use herbal medicine for primary health care[1]. Herbal medicine is a major component in different systems of traditional medicine and is a common element in ayurvedic, homeopathic, naturopathic, traditional oriental

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and Native American Indian medicine. The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka, Thailand, African countries and is becoming popular in Western Europe. An estimate of about 600 medicinal plants are in Pakistan of which 200 medicinal plants are utilized for herbal industry[2].

A survey of the naturally available plant wealth of Pakistan shows that medicinal plants grow in abundance in Northern Pakistan such as Hazara, Malakand, Kurram Agency, Murree Hills, Northern Areas and Baluchistan or cultivated on farmlands in Punjab, Sindh, Baluchistan and North West Frontier Province (NWFP). According to the surveys carried out by the Pakistan Forest Institute, 500 tons of medicinal plants are produced in Hazara and Malakand, 16 tons in Murree Hills and about 24 tons in Northern areas. These plants are collected from the wild, dried processed and sold in the local markets as well as exported to other countries. Pakistan obtains more than 80 % of its medicaments from higher plants[3].

In spite of all natural wealth available, Pakistan imports a substantial amount of medicinal herbs from abroad. The practice of traditional medicine (TM), Tibb (Unani System of Medicine) and homeopathy in Pakistan is regulated through the Unani, Ayurvedic and Homeopathic Act of 1965, whereas the correct identification, manufacture, sale, distribution, import, export, quality, safety, efficacy, *etc.* of the medicine of these systems are not regulated through any strict legal or administrative measures. The major problems in the manufacture of herbal drugs and their wider acceptance in Pakistan are the adulteration and misidentification. Herbalists and drug manufacturing industries confronted with a constant problem of adulteration and correct identification of genuine drug. Certain plants devoid of any activity look very similar to the authentic plant and so substituted intentionally or by mistake. The situation is further complicated in the absence of any quality control system for industry. Some of the manufacturers may have their own in-house standards and specifications of the quality of drugs they produce while the others mostly still practice Organoleptic testing like sight, smell, taste and touch etc. to identify herbal drug material.

In the absence of detailed botanical know-how and the number of plants or their parts use as medicine increased gradually, there arose some confusion in their correct identification and nomenclature. In this way genuine plant drugs are adulterated with closely related species. Plants are usually referred to in published as well as folk literature by their vernacular or common names rather than botanical names. Pakistan with rich plant wealth and having traditional system of medicines faces difficulty in establishing authentic botanical identity of a large number of drug plants. The main difficulty is in keeping the botanical identity of drug plant in ancient Unani literature and traditional systems which arise due to the use of local name of the drug

plants and nomenclatural controversy attributed to more than one plant species, short, incomplete and ambiguous plant description. The botanical sources of large number of traditional medicine found therapeutically effective in indigenous system are still unknown or doubtful. Many workers[4-7] have stressed the need for authentic botanical identification of herbal drugs used in the Unani system of medicine, in order to maintain their quality and efficacy in accordance with WHO guide lines.

Practically the phenomena of misidentification and adulteration related with impurities or complete absence of genuine drug or it may be an addition of lower grade or spoiled drug with genuine one. For instance as in the case of *Echinops echinatus* (Globe thistle) having local and Tibb name Ountkatara is found through out the Pakistan. The vernacular name Ountkatara has been erroneously used for an entire different plant species *Silybum marianum* (Milk thistle) in the monthly magazine National Health (Qaumi Sehat) Lahore of Qarshi herbal industries in its February and October (1999) issues. Similarly Ministry of Food Agriculture and livestock (MINFAL) and National Agriculture Research Center (NARC) Islamabad published a booklet entitled "Cultivation of Untkatara". *Silybum marianum* instead of *Echinops echinatus* which would lead to the manufacture of in genuine drug[8].

*Silybum marianum* is a native of Mediterranean region[9] while *Echinops echinatus* (Untkatara) is indigenous of Eurasia and Africa and its distribution extends from Asia through Afghanistan, Pakistan, India up to Japan. This plant has been used and well known in Tibb since long and its local name Ountkatarais mentioned in many herbals and floras. The vernacular name Ountkatara (*Echinops echinatus*) has been mentioned by several writers[10-13]. However no local name exists for *Silybum marianum* in herbals or of floras, *etc*. The purpose of the present study is to use the techniques with multiple approaches for authentication of problematical medicinal herbs milk thistle and globe thistle to maintain WHO standards in its efforts of primary health care for global community.

## EXPERIMENTAL

Detailed morphological (macro and microscopic) examination was carried out by binocular dissecting microscope, Model Kyowa SZF (0.75x-3.4x) using eye piece, WF 10 × 10/20. The description of plant species were also compared by using different floras[14-18]. Organoleptography confined to the inspection of organoleptic markers such as shape, colour, texture and odour of the herbal drug.

The questionnaire was devised to document medicinal data on collection, storage, processing, marketing, preparation and dosage and toxicity of the herbal drugs, milk thistle and globe thistle. The research work was unique in that the emphasis was on men, women, herbal doctors (Hakims) and

herbs sellers (Pansars). The Medicinal herb data sheet was incorporated into the research work as a means of obtaining detailed information. In total of 75 medicine men, 100 men, 36 women and 39 herb sellers were interviewed from Karachi, Quetta, Lahore, Rawalpindi and Peshawar cities of Pakistan.

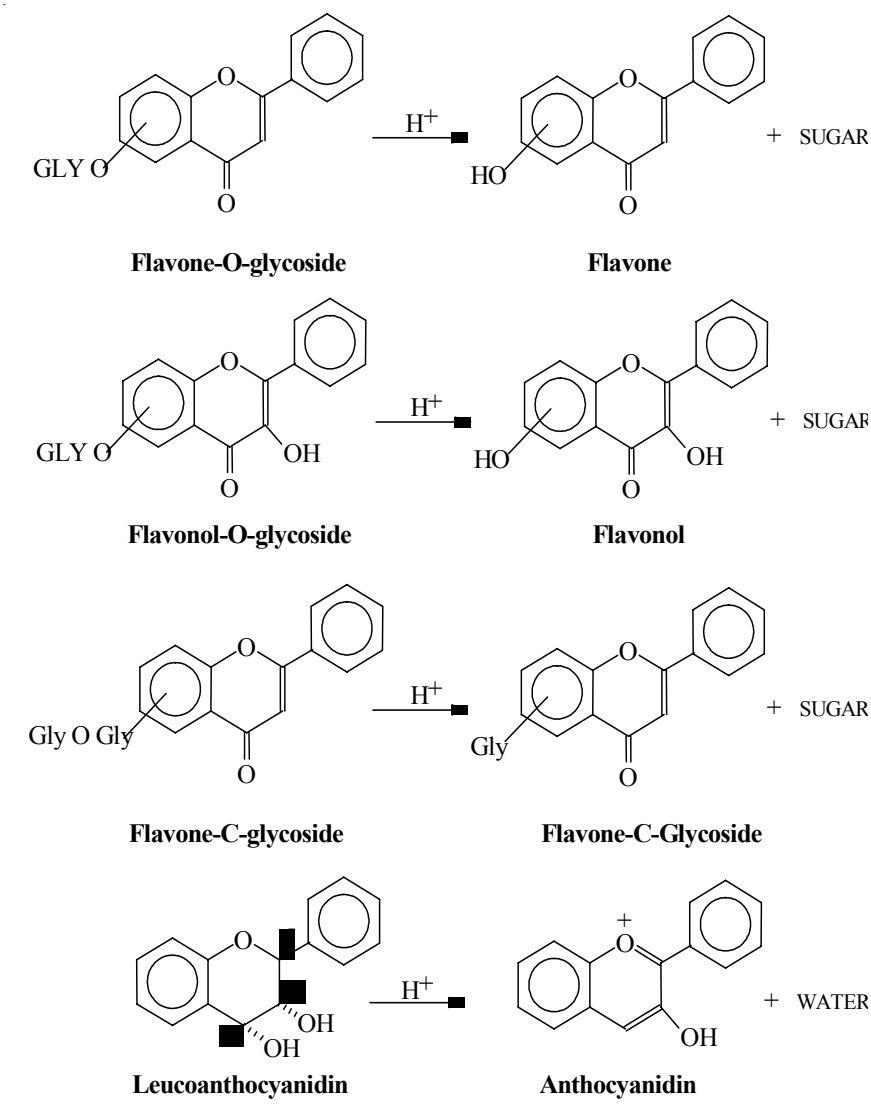
Microscopic examination focuses on scanning electron microscopy (SEM), light microscopy (LM) of pollen and leaf epidermal anatomy. Pollen samples were obtained from herbarium specimens housed in Quaid-i-Azam University, Islamabad and from fresh specimens collected from the field. The list of the voucher specimen is deposited in the herbarium. The pollen grains were prepared for scanning microscopy and light microscopy by the standard method, described by Erdtman[19]. For light microscopy the pollen grains were mounted in stained glycerin jelly[20] and observations were made with a Nikon Labophot microscope (Model 187831 Japan), under (E40, 0.65) and oil immersion (E100, 1.25), using 10X eyepiece. For SEM studies, pollen grains suspended in a drop of 45 % acetic acid were directly transferred with a fine pipette to aluminum stub using double adhesive cello tape and coated with gold in a sputtering chamber (SPI-Module Sputter Coater). The SEM examination was carried out on a Jeol-JSM 5910, scanning electron microscope. The measurements of pollen are based on 25-30 readings from each specimen. Shape of pollen in polar and equatorial view, polar and equatorial diameter, P/E ratio, spines, number of spines between colpi, length of spine, length and width of colpi and exine thickness were studied. The terminology for pollen was used[21,22].

Fresh leaves were used for anatomical studies. Leaf samples were prepared according to the modified method[23,24]. The fresh leaves were placed in a tube filled with 88 % lactic acid kept hot in boiling water bath for about 15 to 20 min. Lactic acid softens the tissues of leaf due to which it's peeling off is made possible. Slides of both abaxial and adaxial surface of leaf were prepared and mounted in clean 88 % lactic acid. Micro-histological photographs of both surfaces were taken by Nikon FX-35 Camera equipped with photomicrograph system (Japan).

**Chemo-Taxonomic methods:** This investigation confined to acid hydrolysis, chemo-profiling (TLC-Fingerprinting) UV and IR analysis.

**Acid hydrolysis:** A very important class of secondary metabolites flavonoids is considered one of the most reliable chemotaxonomic markers[25]. For the extraction of flavonoid aglycones, 5 g dried powder material (roots of *Echinops echinatus* and seeds of *Silybum marianum*) is treated with 2 N HCl (200 mL) and heated for 1 h in a water bath (Model, Memmert-91126-FRG, Germany) at about 100 ºC. By this treatment normally all flavonoids-O-glycosides are converted to flavonoids aglycones, leucoanthocyanidin to anthocyanidins where as the C-glycosides remain unaffected (**Scheme-I**).

After cooling, the flavonoid aglycones are extracted with diethyl ether from the aqueous phase. A second series of extraction by *n*-butanol quantitatively removes the anthocyanidins.




**Scheme-I**

**TLC Finger printing:** The technique of TLC finger printing consists of applying the flavonoid sample on commercially available pre-coated polyamide $F_{254}$ plates (Merck, Germany). For analytical work pre-coated aluminum backed TLC plates which are transparent to UV lights were

used. These plates are loaded with the herbal extract for its analysis. The plates are then developed in TLC tank (Size 20 cm × 20 cm Camag Switzerland) with solvent system. The solvent system used in both the directions is toluene:methanol:methyl ethyl ketone 4:3:3[26]. After drying, the fully developed TLC plates are viewed under 366 nm UV light. This is a very reliable and reproducible method of authentication of a particular herbal drug.

**Digital photography under UV, IR and visible lights:** Digital camera (Sony, DSC-W50) was used in photography of herbal part of the drugs under short wavelength UV light (UVGL-58 Lamp, 254/365 nm), IR lights and visible lights. This high resolution photography in UV, IR and visible lights provide authentic approach toward the authentication of doubtful and problematic plant species and herbal products.

## RESULTS AND DISCUSSION

### Multidimensional identification profile of *Silybum marianum*

**English name (s):** Milk thistle, Holly thistle; **Local name:** Shafa-e-Jigar; **Tib name:** Shafa-e-Jigar; **Family:** *Asteraceae*; **Distribution in Pakistan:** Peshawar, Kohat, Hazara, Havelian, Mansera, Abbotabad, Lahore, Poonch, Kotli, Haripur, Swat, Saidu Sharif, Mianwali, Attock, Islamabad and Rawalpindi; **Distribution in World:** Mediterranean regions of Europe, North Africa, Middle East, Scotland, North America, New Zealand, Chile, USA, Argentina, South Africa, India, China and Canada; **Occurrence and conservation status:** It occurs in fertile lands of improve pastures that have been over grazed and poorly managed. Weedy in its native range. Found in dense stands along road sides, moist and waste areas.

**Description:** Milk thistle is winter annual herb. Through out its native range. The main stem is stout, ridged and branching and the overall plant size can range from 2-6 feet tall. A distinguishing characteristic of milk thistle is the white patches or marbling found along the veins of the dark green leaves. The broad leaves are deeply lobed and basal leaves can be 50 cm long and 25 cm wide. The leaf margins are yellow and tipped with woody spines 4.5 cm long. The leaves are alternate and clasping to the stem. The stem leaves are smaller and not quite as lobed. Each stem ends in a solitary composite flower head, about two inches in diameter, consisting of purple disc flowers. The flower head of milk thistle differs from other thistles with the presence of broad leathery bracts that are also tipped with stiff spines 8.5 cm to 5 cm long. The seeds are heavy, 3.5 cm long, flat, smooth and shiny and the colour ranges from black to brown mottled. The seeds do have a tuft of minutely barbed bristles, which is deciduous and falls off in a ring when the seeds mature.

**Flowering period:** Flowering is from March to April; **Voucher No.:** ISL-MZ-37; **Palynology:** The pollen is monad, tricolporate and echinate. The shape of pollen in polar view is semi-angular and in equatorial view is prolate. Polar diameter with spines is 52.2 µm (51-54 µm) and equatorial diameter is 36 µm (20-44 µm). P/E ratio is 1.45 µm. Spines are apiculate broad based and tapering at the end. Number of spines between calpi are 5-8 and length of spine is 4.5 µm (4-5 µm). Length of colpi is 12.25 µm (1.5-3 µm) and width of colpi is 12.16 µm (10.5-15 µm). Exine thickness is 2.12 µm (1.5-3 µm).

**Leaf epidermal anatomy:** Epidermal cells with thick walled structure. The cells are mostly flattened, polygonal or somewhat irregular shape. Stomata are anomoctetracytic and abundantly distributed on surface. Trichomes were not observed on the surface; **Part used:** Seeds; **Folk medicinal uses:** Liver diseases, chronic hepatitis A, B & C and jaundice; **Preparation and dosage:** Seeds are collected, dried under shade and roasted in vegetable oil. Roasted seeds are ground to obtain powder. Half tea spoon of this powder is taken thrice a day for a month to treat hepatitis A, B & C and other liver complaints. This phytotherapy is effective for chronic hepatitis and jaundice; **Toxicity:** Non-toxic; **Marketing status:** In Pakistan still it is not marketed.

**Organoleptography (Seeds):** Milk thistle has black shiny seeds, crowned with feathery tufts like those of dandelion seeds. Seeds are 0.4-0.6 cm in length and 0.2-0.4 cm in diameter. 100 seeds of equal size (0.5 × 0.2 cm) are about 4 g. Texture of seed surface is smooth and glaucous. Colour of the seed is light brownish with black lines. Upper portion contain tuft of whitish hair. Seeds are slightly oval in shape. General appearance of seed is light blackish and hard in texture. Odourless with slightly bitter in taste. Internal texture of seed is soft and whitish in colour; **Finger printing:** TLC of the flavonoid aglycones extract on polyamide and developed in 4:3:3 solvent system reveals the presence of one major and two minor amounts of phenolic acids when viewed under 366 nm UV light.

**Multidimensional identification profile of *Echinops echinatus***

**English name:** Globe thistle; **Local name (s):** Ount katara, Lay; **Tib name:** Ount katara; **Family:** *Asteraceae (Compositae)*; **Distribution in Pakistan:** Salt Range, Mianwali, Attock, Jhelum, Rawalpindi, Islamabad, NWFP, Sind, Baluchistan; **Distribution in World:** Native to Europe, East to Central Asia and South to the Mountains of Tropical Africa and India; **Occurrence and conservation status:** It is xerophytic weed growing in desert places, in the plains and also in the dry foot hill areas and often rocky habitats. It is abundantly available in restricted habitats in Northern Pakistan and Northern areas especially Chilas and Gilgit.

**Description:** An erect, much branched, armed annual, 30-60 cm tall; branches ascending from the base; leaves 7-12 cm long, oblong, sessile, pinnately divided, lobes triangular or oblong, spinous; spines often 4 cm; flowers heads forming a white ball 2.5-4.0 cm in diameter, with many stout spines, each head contains one flower; outer bracts 6-8, lanceolate, smooth, inner 8 mm long, united, tips bristly, hardening round the silky achene; pappus of short bristles; corolla tubular, slender, deeply cut into 5-segment; style arm thick.

**Flowering period:** April to July; **Voucher No.:** ISL-MZ-67; **Palynology:** The pollen is monad, tricolporate and with minute or rudimentary spines. The shape of pollen in polar view is semi lobate and in equatorial view is prolate. Polar diameter 27.2 µm (25.5-29 µm) and equatorial diameter is 45.75 µm (39.75-49.5 µm). P/E ratio is 0.59 µm. Length of colpi is 2.15 µm (2-2.5 µm) and width of colpi is 7.31 µm (7-7.5 µm). Exine thickness is 3.05 µm (2.5-4.5 µm).

**Leaf epidermal anatomy:** The epidermal cells are of thick smooth walled structure. The cells are mostly tubular, flat or elongated, pentagonal, hexagonal or quadrangular. The stomata are anomocytic and not common on the surface. Trichomes are large and bicelled. Trichomes are generally tapering at the end and base oval shaped; **Part used:** Root and root bark; **Folk medicinal uses:** Sexual debility, delivery, stomach diseases and jaundice.

**Preparation and dosage:** Root and root bark is dried under shade for a week and then crushed to obtain powder. 2-3 g powder is taken daily with milk at night for a month. It is recommended for sexual debility in male. It is also used to treat jaundice. The root bark of the plant and the seeds of dates in equal amount are crushed into powder and a table spoon once a day is useful to cure stomach diseases. 15 g root of Ount Katara is wrapped in a clean cloth and is dipped in 0.5 L milk, 1 L water, 25 g dates and boiled. When only milk remains, 1-2 table spoons of sugar is added to the milk. This milk is used as an aphrodisiac tonic. The powdered root is made into paste and is applied on the belly of pregnant woman at the time of child birth. It is considered helpful for easy delivery of the baby. **Toxicity:** Non-toxic; **Marketing status:** It is widely available in herbal markets throughout the Pakistan. Only aerial parts are available in markets. Dried aerial parts are 30 rupees per Kg.

**Organoleptography (roots):** Dried root pieces are 7-10 cm in length and 1.5-2.5 cm in diameter. 5 pieces of equal size (8 cm × 2 cm) is 25 g. Roots texture is hard, surface colour is light brownish, containing slight ridges with waxy appearance. Root bark is with brownish broken ridges. Internally root texture is hard, bright and whitish in colour. Middle of root internally is soft and look like honey comb, its appearance is like snow.

Roots are odourless and light in taste; **Finger printing:** TLC of root extract reveals the presence of one major amount of flavonoid aglycone, one phenolic acid of significant amount and three phenolic acids of small amount when viewed under 366 nm UV light.

**Macroscopic and microscopic authentication**

The major methods employed in the authentication of herbal materials are macroscopic and microscopic examination and chromatography. In addition, some pharmacopoeial monographs include chemical identification tests. Macroscopic examination involves the comparison of morphological characters that are visible with the naked eye or under low magnification with description of the plant or botanical drug in floras, herbals or monographs. Characters such as size, shape and colour of leaves (or leaf fragments), flowers or fruits are commonly used in macroscopic identification. Microscopic examination focuses on anatomical and palynological structures in the plant material which are visible only under microscope. Features such as trichomes (hairs) shape and structure, the arrangement of stomata in the epidermis, the presence or absence of compounds such as mucilage, starch or lignin or the presence of tissues with characteristic cells, palynological features such as sculpturing, shape of pollen in polar and equatorial view, polar and equatorial diameter, spines, number of spines between colpi, length of spine, length and width of colpi and exine thickness might be used as microscopic studies of herbal drugs.

Authentication of botanical material is a critical step in the use of these materials for both research purposes and commercial preparation. Microscopic evaluation and comparison of authenticated and unauthenticated samples of whole, cut or powdered plant material is a cost effective and accurate means of identifying herbal ingredients. Microscopy can be a useful tool for the detection of botanical and non-botanical adulterants such as pharmaceutical drugs, microbial contaminants and inorganic materials. Advances in microscopic technology and improvements in light, fluorescence, phase contrast and scanning electron microscopy have improved the accuracy and capabilities for botanical authentication. Organoleptic analysis, used in combination with advanced microscopic equipment provides further accuracy for botanical authentication[5].

Enigmatic situation of problematical medicinal plants is chaotic for herbal industry. Numerous diverse problems are confronted to taxonomists in the identification of such medicinal plants. Problems in authentication of genuine drugs arise due to several reasons such as folk classification of medication which grew up in communities without the influence of science and proper documentation. The existence of several common names for the same plant species in different areas. Another problem is the superficial resemblance of plant species with in the same tribe or family such as family

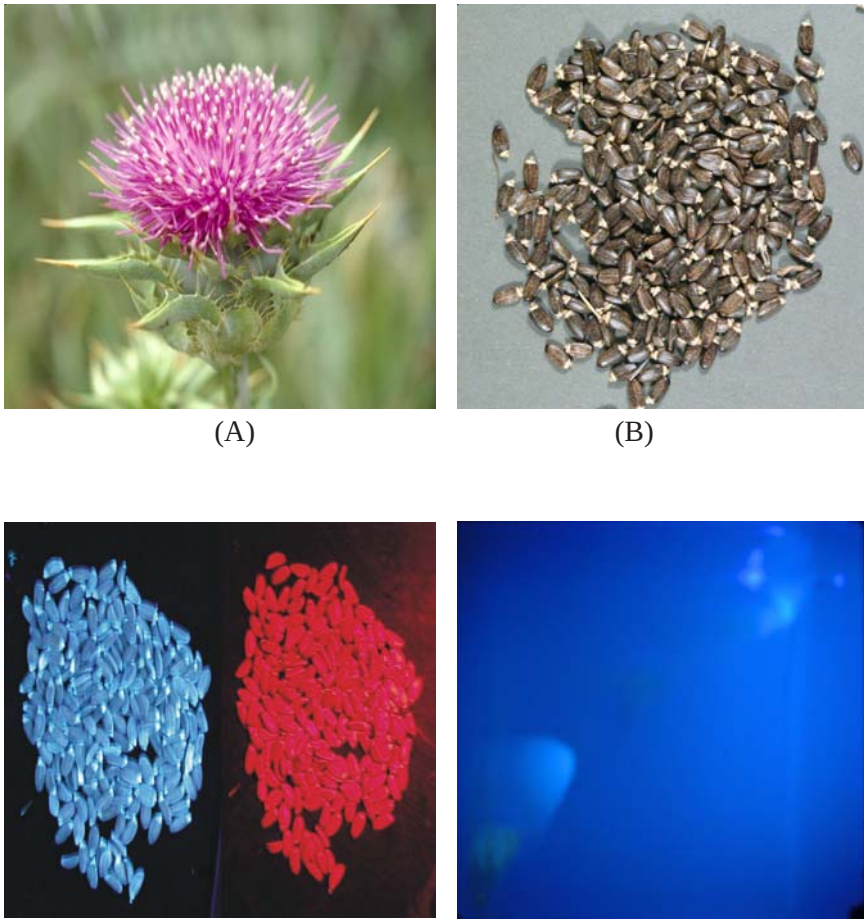
*Asteraceae*[27]. It was stated that the species of *Anthemus* and *Cotula* were utilized instead of *Matricaria chamomella*. Problem of adulteration of medicinal plant arose due to the potential use of different species for similar ailment such as *Boerhaavia procumbens* (Ponernava) and *Trianthema portulcastrum*, both are used for jaundice. The vernacular name 'itset' is used for both species in various areas in Pakistan[28].

*Silybum marianum* and *Echinops echinatus* have been confused with each other due to folk nomenclatural problem which probably existed due to their superficial resemblance in their capitulum and spiny nature of plant body since both the species are in the same tribe *Cardueae*. Although taxonomically both the species belong to different genera. It is essential to mention the fact that herbal drug Ount Katara is *Echinops echinatus*[8]. Erdtman[8] proved that the common name Ount Katara existed in ancient literature for *Echinops echinatus* long time before the introduction of *Silybum marianum* as an alien plant. *Silybum marianum* was not mentioned in ancient herbals and pharmacopeias in Pakistan. Its migration route in Pakistan is still unknown, since it is only mentioned by Stewart Catalogue of vascular plants[29]. There is no other report exists either in floras or herbals.

However both the species *Silybum marianum* and *Echinops echinatus* are being utilized as herbal medicine in Pakistan. The roots of *Echinops echinatus* are used as aphrodisiac tonic and the seeds of *Silybum marianum* are utilized in liver complaints for the treatment of Jaundice. However the root of *Echinops echinatus* is used for the treatment of Jaundice. Hence in the present studies a complete descriptive profile based on multiple taxonomic approaches for the authentication of the species is developed to avoid any further adulteration and misconception related to their vernacular or Tibb name. *Silybum marianum* can be distinguished from *Echinops echinatus* due to the presence of white spots on the leaves. In case of *Silybum marianum* the capitulum is provided with broad leathery bracts, while such bracts are absent in *Echinops echinatus*.

Some of the anatomical features are so diagnostic that they are now commonly used in routine identification. Among the many taxonomically important features, stomata, their arrangement of the surrounding epidermal cells, if they are distinct from the normal epidermal cells is the most valuable. *Silybum marianum* can be distinguished due to annomotetracytic stomata as compared to *Echinops echinatus* which has annomocytic stomata. Similarly, *Echinops echinatus* is characterized due to the presence of bicelled hairs while no such hairs have been observed in *Silybum marianum* (Plate 2 and 4). Particularly valuable taxonomic evidences have been obtained form the study of pollen morphology. The pollen shape of *Silybum marianum* in polar view is semi angular to prolate in equatorial view, while pollen shape in *Echinops echinatus* is semi lobate in polar view is highly diagnostic

feature this species from the rest of the taxa of the Cardueae[30]. Analysis of flowering material shows that pollen grain of *Echinops echinatus* can be distinguished due to semi lobate pollen in polar view while in *Silybum marianum* pollen grain are semi angular in polar view. Exine sculpturing was observed through scanning electron microscopy to characterized the pollen grains of herbal drugs milk thistle and globe thistle (Plate 2 and 4, respectively). In *Echinops echinatus* exine sculpturing is scabrate with minute or rudimentary spines sparingly scattered on the surface. While *Silybum marianum* has well developed sexine spine.

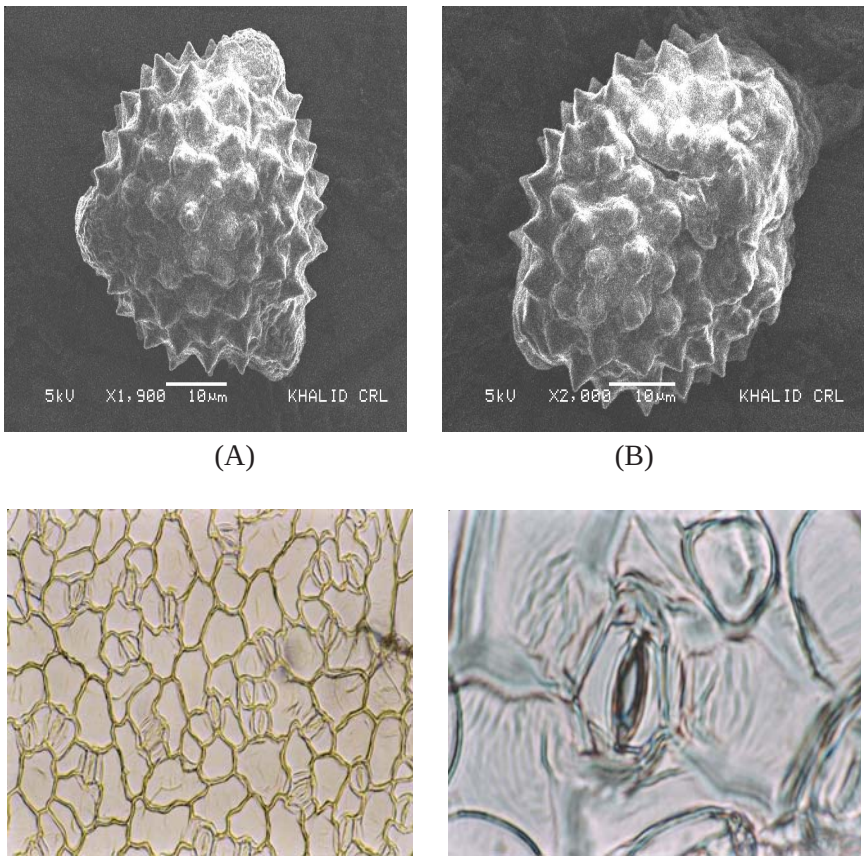


(A) (B)

(C) (D)

A = *Silybum marianum* Flower; B = Seeds; C = Seeds under UV & IR; D = TLC Finger prints of Seeds

**Plate-1:** Photographic key to authentication of *Silybum marianum*




(A) (B)

(C) (D)

Scanning micrographs: *Silybum marianum*; A = Polar view, B = Equatorial view, C = Light micrograph of adaxial surface of leaf (200x), D = Stomata at Adaxial surface (1000x)

**Plate-2:** Photographic key to authentication of *Silybum marianum*

## Chemical authentication

The chromatography is a Greek word meaning colour writing. In early days, it was limited to the separation of colour compounds but now can also be used for separation and purification of colourless compounds. A number of chromatographic techniques are available which are all based on some principal for separation of constituents of mixture, by distribution between a stationary and a mobile phase. TLC is widely implied in herbal authentication and the majority of pharmacopoeia's monograph for herbs include TLC, identification tests. TLC separates a mixture of compound plate coated with an adsorbent such as silica gel, polyamide, cellulose, *etc*. By Co-TLC unknown compound can be easily identified with an authentic

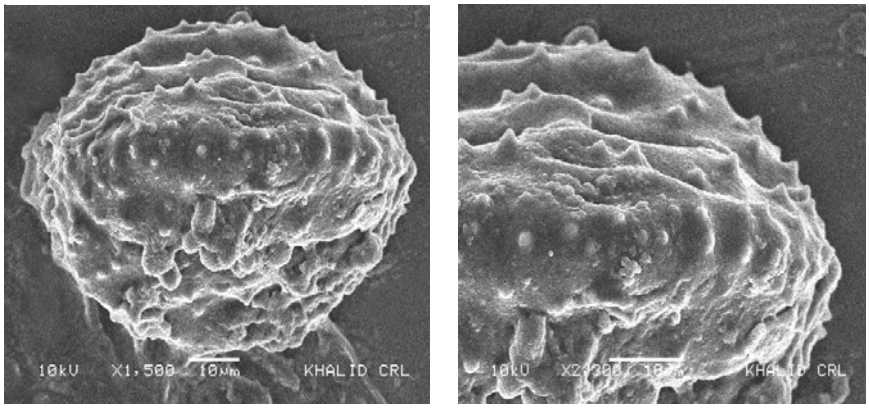
(A) (B)

(C) (D)

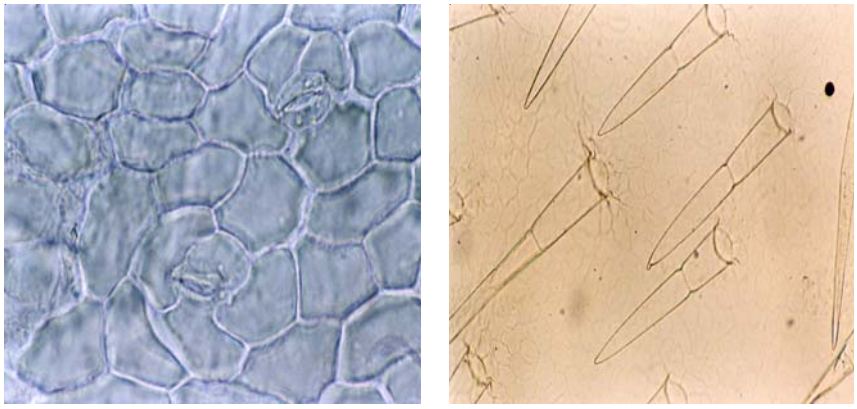
A = *Echinops echinatus* Flower; B = Roots; C = Roots under UV & IR; D = TLC Finger prints of roots

**Plate-3:** Photographic key to authentication of *Echinops echinatus*

compound. HPLC is another type of advance chromatographic techniques which is widely used for the authentication and analysis of herbal material. Milk thistle and globe thisle resemble each other by the presence of similar capitulum and common nomenclature. In this case TLC proved to be an excellent technique for the differentiation of these problematic medicinal species. In case of *Silybum marianum* the TLC appearance of one major and two minor phenolic acids are observed where as in case of *Echinops echinatus* there are one major flavonoid aglycone, one phenolic acid of significant amount and three phenolic acids of small amount clearly distinguished these two problematic species.




(A) (B)



(C) (D)

Scanning micrographs: *Echinops echinatus*; A = Polar view, B = Exine sculpturing pattern, C = Stomata at Adaxial surface (400x), D = Bicelled trichome (200x)

**Plate-4:** Photographic key to authentication of *Echinops echinatus*

To date no reference is available about the use of infrared light for the purpose of authentication of a plant species. However several workers[31,32] have reported the use of infrared light for the blooming of houseplants. In a recent report of Walker and Doyle[33], the infrared photography is of interest to the amateur and commercial photographer and to scientists and technologists because it produces images that are not possible with conventional photography. In its practice, there is not much difference between infrared and normal photography. The same cameras and light sources can usually be used, together with the same processing solutions. Infrared photography, however, is usually only attempted by skilled photographers, scientists and technicians with a particular purpose in mind. The peculiarities of infrared

photography lie in its ability to record what the eye cannot see. The infrared photography has been used in forest survey to distinguish between stands of coniferous and deciduous trees. Thus, the use of infrared photography is a unique and reliable method of authentication of plant species whenever a doubt about the identity of a plant species is in question.

**Photography of plant species under ultraviolet light:** Like infrared, no reference is available about the use of ultraviolet light for the authentication of plant species. However, Weber[34] has posted his work on the web about photographing and comparing the appearance of a number of ornamental flowers under visible and ultraviolet light. He also admits that compilation in identification of plant species do arise which can be resolved by the use of photography under different lights. UV fluorescence may be a common trait to most flowers, but might be of temporary occurrence for parts of the flower. Therefore, viewing of plant species under UV light can provide a authentic approach towards authentication of doubtful and problematic plant species.

## Conclusion

It is not that all adulterations are intentional malpractice as stated in many literatures. With our experience it is noted that the herbal drugs are adulterated unintentionally also. Suppliers are illiterate and not aware about their spurious supply. Major reasons are name confusion, non-availability and lack of knowledge about authentic plant. Even scientific community and traditional physicians are unaware of it. Nowadays, herbal drug industries follow high quality standards using modern techniques and instruments to maintain their quality. In conclusion, the microscopic and chemical accounts of herbal drugs milk thistle with comparative analysis of globe thistle provide an important tool to authenticate these drugs for export and import and used in phytomedicines. Authentication/quality control studies according to WHO standards can offer a wide range of expertise which can be applied to conserving the medicinal plants and clarifying plant names in trade.

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