

Full Length Research Paper

Medico-botanical and chemical standardization of pharmaceutically important plant of *Tricholepis chaetolepis* (Boiss) Rech. f.

Mir Ajab Khan¹, Mushtaq Ahmad¹, Muhammad Zafar¹, Shazia Sultana¹, Sarfaraz Khan Marwat¹, Shabnum Shaheen², Muhammad Khan Leghari³, Gul Jan⁴, Farooq Ahmad⁵ and Abdul Nazir

¹Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

²Department of Botany, Lahore College for Women University, Lahore, Pakistan.

³Pakistan Science Foundation, Ministry of Science and Technology, Islamabad, Pakistan.

⁴Department of Botany, Hazara University, Mansehra, Pakistan.

⁵Department of Botany, University of Agriculture Faisalabad, Pakistan.

Accepted 28 February, 2011

Medico-botanical and chemical standardization of Barham Dandi (*Tricholepis chaetolepis* (Boiss) Rech. f.), a pharmaceutical herbal drug and its adulterants has been carried out. The study includes parameters such as macro-micromorphology of species, pollen (SEM), organoleptography, fluorescence analysis (UV and IR) and certain physico-chemical aspects that is flavonoids finger printing by 2 D thin layer chromatography. The study revealed that by using physico-chemical and taxonomic markers, one can detect the adulteration in herbal raw material for pharmaceutical industries for safe and effective drug preparation.

Key words: *Tricholepis chaetolepis*, medico-botanical, chemical, standardization, pharmaceutical.

INTRODUCTION

The drug "Barham Dandi", botanically the *Tricholepis chaetolepis* (Boiss). Rech. f. of family Asteraceae occupies a pivotal role in the Unani (Greek), Ayurvedic (Indian) and indigenous system of medicine (Pakistan) since long. In Hindi and Urdu it is called Barham Dandi and in English this plant is called yellow thistle. It is found growing throughout South Asia, China and Thailand. It is growing in sandy, arid and semi arid soils of India and Pakistan (Ahmad et al., 2010). *T. chaetolepis* is annual herb densely leaves and with acute sessile leaves more or less all parts of the plant have pharmaceutical value, the aerial parts however occupy a pivotal role in the indigenous system of medicine and are known for their therapeutic and pharmaceutical properties such as antipyretic, nerve tonic and skin infections (Chopra et al., 1956; Nadkarni, 1976). There are number morphologically closely related specie of family Asteraceae are traded throughout the world for same pharmaceutical

purposes as *T. chaetolepis*. However, two most widely used and traded species instead of *T. chaetolepis* includes *Oligochaeta ramosa* (Roxb.) Wagenitz and *Acroptilon repens* (L.) DC. Prodr are also known as Barham Dandi. It is interesting to note that *Acroptilon repens* and *Oligochaeta ramosa* have quite different pharmaceutical application than *T. chaetolepis*.

Review of this literature shows that no work appears to have been done so far regarding its standardization based on medico botanical and chemical characterization. Therefore, the present study is taken up to standardize the genuine source of drug Barham Dandi (*T. chaetolepis*) which could be immense help to check adulteration or substitution, if any before the preparation of pharmaceutical products in industry.

MATERIALS AND METHODS

Market samples were procured from authorized dealers during dealers at herbal shops in Lahore, Islamabad, Quetta, Peshawar and Karachi. Fresh specimens were also collected from Quetta, Bhakkar, Mianwali and Salt range. The specimens were preserved by routine

*Corresponding author. E-mail: mushtaqflora@hotmail.com.

methods and deposit in Herbarium of Pakistan (Isl), Quaid-i-Azam University Islamabad with voucher specimens numbers. Morpho-palynological (LM and SEM) screening for macro and microscopic structures were carried out according to the method described by various workers (Ronald, 2000; Ahmad et al., 2008, 2009, 2010; Zafar et al., 2010). Phytochemical and pharmacognosic method used for standardization includes organoleptography, UR and IR analysis and thin layer chromatography for finger printing of flavonoids (Hasan et al., 2007). Micro and macrophotography of plant, market samples and pollen were carried out for visual observation and authentication.

RESULTS AND DISCUSSION

Barham dandi (*T. chaetolepis*) is sold in Indo-Pak subcontinent herbal markets, is an important traditional medicine. The commercial source of this drug is the dried aerial parts of *T. chaetolepis* (Boiss) Rech. f. This species is very rare in Pakistan and is found only in Balochistan and Kashmir (Ali, 1986). The specimen had also been collected during our expedition to Province Balouchistan during June 2007. It is collected 5 km before Hana Lake while traveling from Quetta. Sometime it is imported from Iran but in Pakistani herbal shops, it is sold under the name of Barham dandi by morphologically similar other species such as *Acroptilon repens* and *Oligochaeta ramosa*. Dried aerial parts of these two species are adulterer in the name of genuine Barham dandi (*T. chaetolepis*). This drug is also confused in classical herbals (text written) by ancient physician of the Unani tibb such as Mohit and Azam, Yadgar and Razi, Makhzan-ul-Advigah, Makhzan-ul-Mufradat e.t.c (Ahmad and Khan, 1998). This drug is usually referred to by their vernacular names. As a result, the botanical identity of this plant based drug has remained doubtful even today in common practices by markets and herbal industries. The name Barham Dandi is attributed to more than one plant species such as *Argemone maxicana*, *T. chaetolepis*, *Lamprachaenium microaphalatum*, *Echinops echinatus*, and *Echinops glabbariana* (Ahmad et al., 2005). Due to this confusion, it creates problems to the herbalists, industries and end users to identify genuine drug. Further research is needed to know the active ingredients of all species included in the drug Barham Dandi (Tables 1 to 3).

Market observation

In common practices the industries and herbalists used *A. repens* and *O. ramosa* instead of *T. chaetolepis*. Because in the Akbari Mandi Lahore which is the biggest herbal market in Pakistan is the main supply source of this drug to industries and herbal shops. During the market survey, it was found that the Akbari mandi (Lahore), Qissa Khawani Market (Peshawar) and even herbal shops at Quetta contain *A. repens* under the name of Barham Dandi. The original Barham Dandi was not

procured as market sample throughout the country. As mentioned earlier *T. chaetolepis*, the authentic source of drug Barham Dandi was collected only from Hanna Oruk near Quetta (Balochistan). During the field surveys at Balochistan, it was interesting to note that *A. repens* was abundantly distributed which is the main supply source to the herbal markets in the country. Dried aerial parts of this species are very difficult to differentiate from genuine drug. The morpho-palynological and thin layer chromatography (TLC) fingerprints helps in this regard to solve the problems of identification of genuine drug.

Morpho-palynology

Morphologically the *T. chaetolepis* is annual herb, 20 to 60 cm long, rigid with densely spinose, narrow-linear, apiculate-cartilaginous sessile leaves (Plate 1 A). While the *A. repens* is smaller in size, 16 to 35 cm long with simple elliptic-linear sessile leaves (Plate 2 A). The *O. ramosa* is quite different species which is smaller in size than *T. chaetolepis* and *A. repens*. *O. ramosa* is 10 to 30 cm long, procumbent sub-erect with radical linear-lanceolate pinnatifid leaves (Plate 3A). Organoleptographic analysis of market samples of *T. chaetolepis* revealed the presence of minute spines on aerial parts and whitish hairs (Plate 1 B). While market samples of *A. repens* contain no spines and whitish hairs (Plate 2 B). In case of *O. ramosa* the branches are hollow containing suture lines externally (Plate 3 B).

Similarly micropalynologically (SEM and LM) the *T. chaetolepis* contain the psilate pollen with reticulate sculpturing having large muri and small lumina (Plates 1 E and F). This species can be distinguished palynologically from *A. repens* which have echinate pollen where muri appear as rudimentary spines and lumina almost occupying the same area as muri (Plates 2 E and F). The pollen of *O. ramosa* are echinate with short or rudimentary spines which are broad at the base but not sharp at the tip (Plates 3 D and E). The pollen of this species are quite different from *T. chaetolepis* but shows somewhat resemblance with pollen of *A. repens*. Quantitative characterization of pollen shows that the *T. chaetolepis* pollen are smaller in size than *A. repens* and *O. ramosa*. The polar and equatorial diameter of the pollen of *T. chaetolepis* is 12.5 and 14 μm while *A. repens* is 39 and 34.5 μm and *O. ramosa* is 21.5 and 23.5 μm respectively. Hence pollen size is helpful as taxonomic character to distinguish these three species.

TLC finger prints

On the basis of TLC finger prints the *T. chaetolepis* can be distinguished from *A. repens* and *O. ramosa* by the presence of one feeble flavonol, two minor phenolic acids and one aurone (Plate 1 D). While in case of *A. repens*

Table 1. *T. chaetolepis* (Boiss) Rech. f. *Syn: Jurinea chaetolepis* Boiss.

English name	Yellow thistle
Local name	Barham Dandi
Tib name	Barham Dandi
Family	Asteraceae
Distribution in Pakistan	Chitral, Zirat, Gilgit, Urak, Hanna (Balouchistan), Oruk.
Distribution in world	Afghanistan, Bhutan, Burma, China, India, Nepal, Iran and Thailand.
Occurrence and conservation status	Very rare found in stony soils.
Description	Annual herb, 20-60 cm long; rigid, densely leafy; leaves numerous, narrow-linear, acute, apiculate-cartilaginous sessile, 10-20 x 1 mm across, appressed; involucre 20mm long; cylindrical base attenuate or rotundate; glabrous, bracts multiseriate, exterior bracts setiformis, inner linear-subulate, membranous, corolla 20 mm long; rose color (light); tube filiform, limb and tube subequal, lacinae 6 mm long; Achene 6 x 2 mm across, thick, compressed, light, brown longitudinally striate, margin light; helium sublateral; pappus light, off white; multiradiatus, scabrid, middle one 12-14 mm long, 5 hairs 16-18 mm long (Plate 1A).
Flowering period	June-July
Voucher No.	ISL-MZ-25
Palynology	Tricolporate, psilate, spheroidal in polar view and prolate in equatorial view. Sculpturing reticulate, with large muri and small lumina. Polar diameter is 12.5 μ m (11-14 μ m) and equatorial diameter is 14 μ m. P/E ratio 0.892 μ m. Colpi length 2.75 μ m and width is 3 μ m and exine thickness is 0.5 μ m (Plate 1 E,F).
Part used	Aerial parts
Pharmaceutical uses	Skin diseases, nerve tonic, skin grains, fever.
Preparation and dosage	Aerial parts are dried under shade and grounded into powder. 1/2 tea spoon thrice a day for 15 days is recommended to treat skin scabies, skin grains and blood purification. 250 mg powder drug is taken twice a day with cow milk for fever and mental weakness.
Toxicity	Non toxic but excessive use may cause dryness.
Marketing status	Marketed under the name of Barham Dani but genuine herbal drug was not found at any herbal shops.
Organoleptography (Aerial parts)	Dried aerial parts contain branches with capitula. Aerial parts are covered with spine which are apiculate and yellowish in color leaves are sessile, minute and modified to spines at the tip. Size of broken branches varies 5-16 cm. Stem cylindrical, all parts covered with whitish hairs. Branches bears 2-5 capitula in the form of bunch. Ray florets are dark purplish in color. Capitula is 2-3.5 cm in size, pappus light off white and Achenes covered with whitish glaucous hairs (Plate 1 B).
Finger printing	TLC of aerial parts extract reveals the presence of smaller amount of flavonol, two minor amounts of phenolic acids and one aurone when viewed under 366 nm UV light (Plate 1D).

Table 2. *A. repens* (L.) DC. Prodr, Syn: *Centaurea repens* L.

English name (s)	Russian Knapweed
Local name	Naryan Band / Naran Ban
Tib name	Naryan Band
Family	Asteraceae
Distribution in Pakistan	Urak, Quetta, Loralai, Ziarat, Kurram Agency, Chitral, Ladak and Kalat.
Distribution in world	Arizona, Colorado, New Mexico, Utah, California, Nevada, South Russia, Iran and North America.
Occurrence and conservation status	Common on the heavier, often saline soils of bottomlands, as well as sub irrigated slopes and flats. The species is also competitive in hayfields, pastures, grain fields, and along roads or irrigation ditches.
Description	Plant erect, branched from the base, 16- 35 cm long, sparsely tomentose. Leaves simple, sessile, elliptic to linear, lower and medium serrate, upper entire, up to 10-15 x 2-4 mm; Involucre – ovoid, bracts 4-5 seriate 5-15 x 2-4 mm, outer short and broadly oblong, inner longer and oblong; Involucral bracts membranous in basal portion, median involucral bracts apiculate apical margin hairy narrowly triangular, and villous. Achene 3-4 x 2 mm oblong – obovoid, faintly ribbed, apex truncate, hilum basal, corolla 1- 1.5 cm long, pink, upper portion campanulate; Pappus 8-10 mm; caducous, pluriserial, with flattened barbellate hairs, becoming plumose at distal ends; common near gravels, river terraces, edge of salt lake, steppe, waste field 800- 1650 ft (Plate 2A).
Flowering period	May-July.
Voucher No.	ISL MZ 23
Palynology	Pollen monad, tricolporate, echinate, spheroidal in polar view and prolate in equatorial view. Polar diameter 39 μ m (37-39 μ m) and equatorial view 34.5 μ m (32-37 μ m), length of colpi 34.5 μ m (32-37 μ m), width of colpi 5.5 μ m, spine length 1.1 μ m, and number of spine between colpi is 5-6. Exine thickness 4 μ m. Reticulate sculpturing. Muri appears as rudimentary spines. Lumina almost occupying the same area as Muri (Plate 2 E and F).
Part Used	Aerial parts
Pharmaceutical uses	Blood purification, stomach pain, fever, dysentery.
Preparation and dosage	50 gm of dried aerial parts are boiled in 2 liters of water. Two teaspoons of this decoction is taken thrice a day for fever, stomach pain and blood purification. Flowers and leaves are dried and ground to powder. 20 gm of this powder is soaked in 50ml of water and 1 teaspoon is taken twice a day for stomach disorder in children. 25 gm of dried leaves of the plant are ground and mixed in 15 gm of wheat flour and 50ml of water. This paste is applied externally on belly for dysentery and pain.
Toxicity	Non toxic
Marketing status	Traded commonly throughout the country under the name of Barham Dandi.
Organoleptography (Aerial parts)	Dried aerial parts are broken; flowers and branches are traded in herbal markets. Flowers are pinkish and branches are light greenish in color. Branches are 10-20 cm, entire involucre ovoid, achene 2-4 mm, pappus 6-9 mm, stem greenish with linear narrow leaves which are elongate spineless aerial parts. Capitulum size is 1-2.7 cm, stem is angular capitulum covering is scarious / papery. Ray florets are purplish in color. Aerial parts are not covered with hairs. Non aromatic with characteristic herbal taste (Plate 2B).
Finger printing	One flavonol + one phenolic acid both in feeble amounts.

Table 3. *O. ramosa* (Roxb.) Wagenitz

Local name	Barham Dandi
Family	Asteraceae
Distribution in Pakistan	Kohat, Jhelum, Bhakkar, Taunk, Karachi, Sind, Balouchistan.
Distribution in world	Pakistan, Afghanistan, India, china, Nepal.
Occurrence and conservation status	Not common. Found in stony and dried sandy soils. Very rare.
Description	Annual herb; branches many, from the base, 10 -30–(50) cm long, proeumbent, suberect, stem scabrid; solid, stiff, ribbed, tomentose, slender sparsely foliate. Leaves radical, linear or linear – lanceolate, 4-7 x 0.5-1 cms across, pinnatifid, lobe oblong – linear or linear 3- 10 x 1-3 mm; cauline leaves linear, linear-lanceolate and pinnatifid, 3-4 x 1-10 mm, all leaves scabrid to pilose, apex spiny-cuspidate, margin spiny, spine 1-2 mm long. Heads solitary terminal oblong obovoid, pedicillate 5-25 x 3-25 mm across; Involucral bracts 4-5 seriate, ovate-lanceolate with diverted stiff spine at the apex, coriaceous yellow, 3-10 x 2-3 mm, tomentose above; Flowers all tubular, tube 10-15 mm long, scabrid brown near the apex. Achene 5-6 x 2 mm, oblong-obovoid gray with pointed end, of achene white, areole on right and pappus 10 mm long, setae 3-4, pappus inner 12-15 mm long (Plate 3A).
Flowering period	March – May
Voucher No.	ISL-MZ-24
Palynology	Pollen are monod, tricolporate, echinate, spheroidal in polar view and prolate in equatorial view. Polar diameter 21.5 μ m (20-23 μ m). Equatorial diameter 23.5 μ m (21-26 μ m), length of colpi 5.5 μ m, width of colpi 4.5 μ m, exine thickness 2.5 μ m. Pollen spines are short or rudimentary, spines are broad at the base and tip is not sharpened. Colpi long and well developed (Plate 3E and F).
Part used	Aerial parts.
Pharmaceutical uses	Tonic, cough, malaria, expulsion of intestinal worms.
Preparation and dosage	Aerial parts are collected, dried under shade and grounded into powder. 2 g thrice a day is taken with water for 20 days to treat cough and malaria. Extract obtained from the fresh aerial parts are recommended for expulsion of intestinal worms.
Toxicity	Non toxic.
Marketing status	Not marketed.
Organoleptography (Aerial parts)	Aerial parts consists of broken bunches and capitula. Branches are hollow and center suture lines on stem. Leaves are sessile, leaf lamina is irregular in shape, leaves are little bit broader and sessile. Capitulum contains large size spines flowers tubular scabrid and branches in color. Aerial parts are light greenish in color. Branches and flowers are non aromatic and taste less (Plate 3 B).
Finger printing	TLC of aerial parts extract reveals the presence of major amounts of phenolic acid and one aurone when viewed under 366 nm UV light (Plate 3 D).

there is one flavonole and one phenolic acid of feeble amount (Plate 2D). The *O. ramosa* can be distinguished by the presence of one major phenolic acid and one aurone (Plate 3C).

Conclusion

Based on our knowledge, experience of indigenous flora,

ancient literature, morphol-palynological characterization and TLC fingerprints in case of genuine Barham Dandi, it is concluded that the authentic source of this drug is *T. chaetolepis* rather than *A. repens* and *O. ramosa*. It is concluded that medico-botanical and chemical aspects, the drug *T. chaetolepis* is authenticated and certain diagnostic characters are established which would help to check if the drug is adulterated or substituted. The study may be applied by any pharmaceutical industry for global

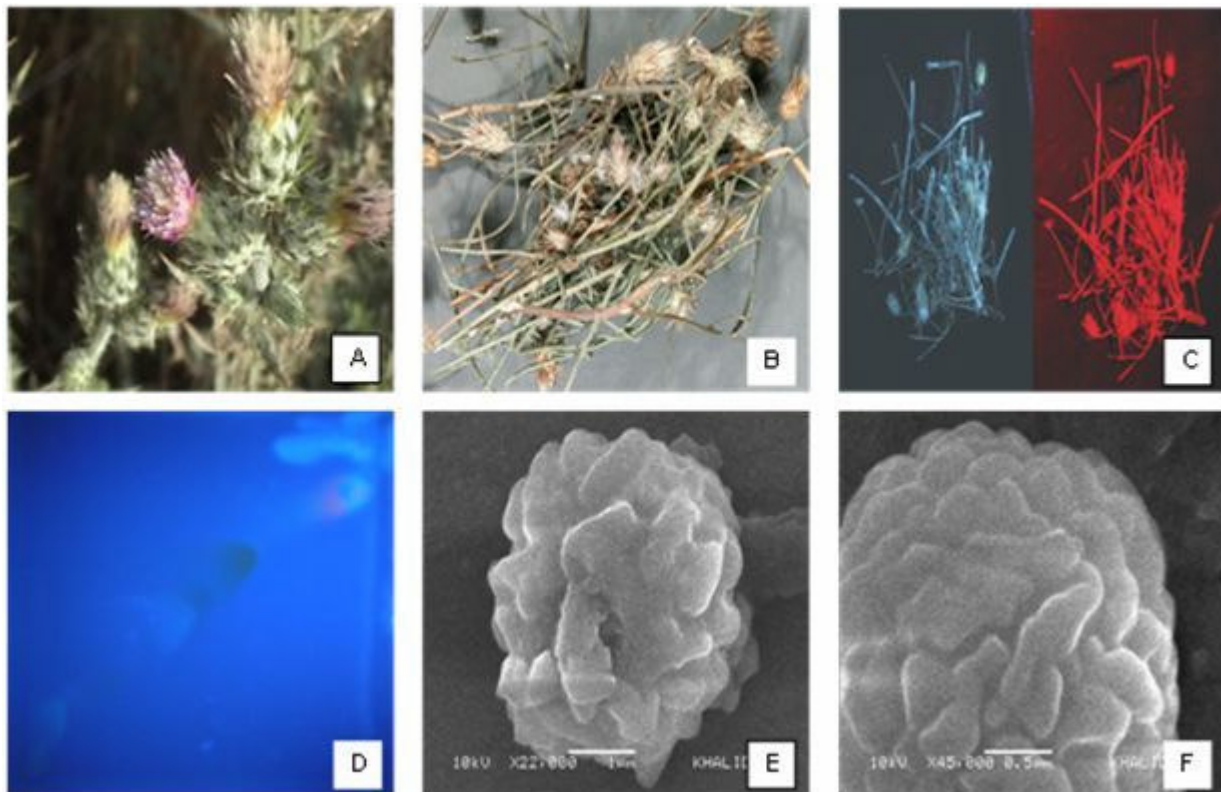


Plate 1. *T. chaetolepis*, A: Flower branch, B: Aerial parts, C: Roots under UV and IR, D: TLC Finger prints, E: Polar view of pollen (SEM), F: Pollen sculpturing (SEM).

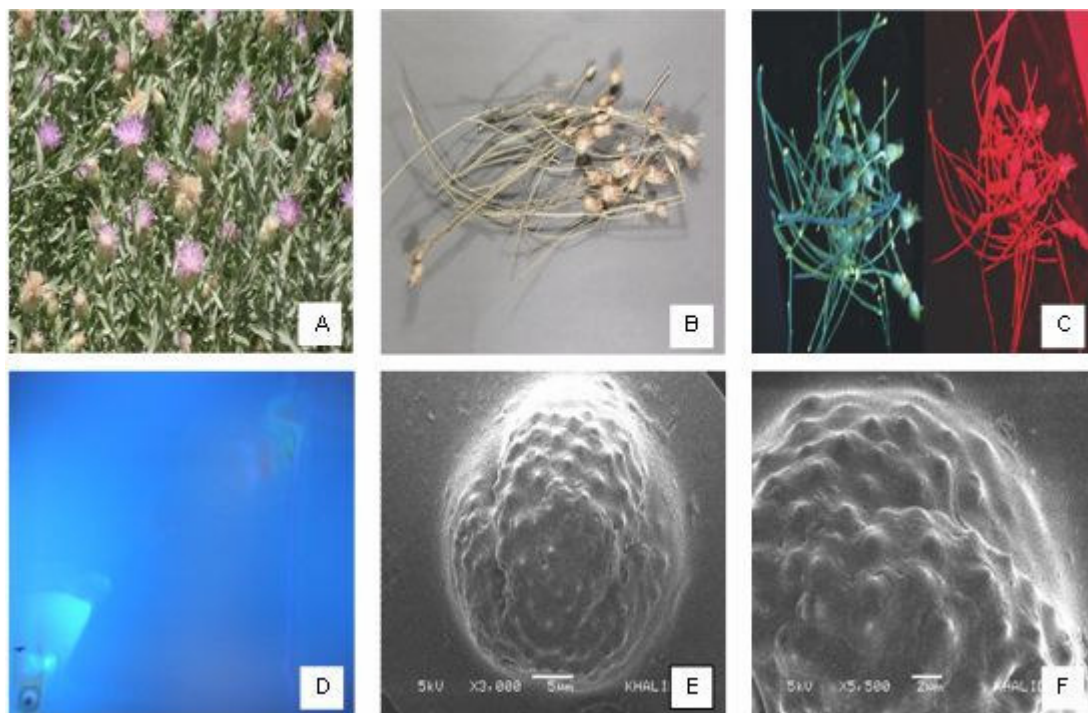


Plate 2. *A. repens*, A: Flower branch, B: Aerial Parts, C: Roots under UV and IR, D: TLC Finger prints, E: Polar view of pollen (SEM), F: Pollen sculpturing (SEM).



Plate 3. *O. ramosa*, A: Flower branch, B: Aerial Parts, C: Roots under UV and IR, D: TLC Finger prints, E. Polar view of pollen (SEM), F: Pollen sculpturing (SEM).

interest to standardize herbal products.

REFERENCES

- Ahmad I, Khan NA (1998). Methodology for the identification of controversial and Unestablished drugs of Unani repository, In: Proceedings of the national seminar on research methodology in Unani Medicine, Department of History of Medicine and Science, Jamia Hamdard, New Delhi, pp. 69-80.
- Ahmad M, Khan MA, Hasan A, Zafar M, Sultana S (2008). Chemotaxonomic standardization of herbal drugs Milk thistle and Globe thistle. *Asian J. Chem.*, 6(20): 4443-4459.
- Ahmad M, Khan MA, Ahmad M, Zafar M, Zulqarnain (2009). Morpho-palynological and leaf epidermal anatomy of weeds of district Tank, NWFP Pakistan. *Pak. J. Weed Sci.*, 15(4): 309-320.
- Ahmad M, Khan M, Zafar M, Arshad Z, Sultana S, Abbasi BH, Din US. (2010). Use of chemotaxonomic markers for misidentified medicinal plants used in traditional medicines. *J. Med. Plant Res.*, 4(13): 1244-1252
- Ali SI (1986). Under exploited economic plants of Pakistan. *J. Arid Environ.*, 11: 17-25.
- Chopra RN, Nayar SL, Chopra IC (1956). In *Glossary of Indian medicinal plants*, Vol. I. Council of Scientific and Industrial Research, New Delhi, P. 197.
- Hasan A, Khan MA, Ahmad M (2007). Authenticity of Folk Medicinal Plants of Pakistan. Quaid-i-Azam University Islamabad Pakistan. 1:1-165. Website: <http://www.qau.edu.pk/pakplantsbook/index.htm>
- Nadkarni KM (1976). *Indian Materia Medica*, Popular Prakashan Publications. Ltd., Bombay, Vol. 2.
- Ronald OK (2000). *Pollen and spores*. 2nd Ed. American Association of Stratigraphic Palynologists, pp. 13-21.
- Zafar M, Khan MA, Ahmad M, Sultana S, Qureshi R, Tareen RB (2010). Authentication of misidentified crude herbal drugs marketed in Pakistan. *J. Med. Plants Res.*, 4(15): 1584-1593.