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TAXONOMIC, PHARMACOGNOSTIC AND PHYSICOCHEMICAL AUTHENTICATION OF *COLCHICUM LUTEUM* BAKER (SURANJANTALKH) FROM ITS COMMERCIAL ADULTERANT

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Abstract

The main objective of current study is to elucidate taxonomic, pharmacognostic and physicochemical behavior of *Colchicum luteum* (Suranjantalkh) for its proper identification and authentication from its cheap and tasteless adulterant. *Colchicum luteum* is one of the most rare and hence expensive medicinal plants. It is an active part of many unani formulations due to presence of an alkaloid colchicine which is claimed to be effective in arthritis, gout, rheumatism and internal injuries. In order to overcome demand of its corm, suppliers and herb sellers adultered bulbs of a monocotyledon plant *Narcissus tazetta.* This type of study reveals to be helpful in differentiating plants on basis of leaf epidermal anatomy, palynology, phamacognosy and physicochemical values. It is an important step in field of herbal medicine to provide pure and original medicinal plants to yield their maximum effectiveness.

Key words: *Colchicum luteum,* Authentication, Physicochemical, Standardization, Taxonomy.

Introduction

Medicinal plants are frequently used in herbal industries for manufacture of packed medicines and taken in form of raw herbs by indigenous people (Shinwari *et al.*, 2014). They considered herbal medicines as moderate and balanced source to live safe and healthier life. Medicinal plants have a broad-spectrum compound of therapeutic importance (Shinwari *et al.*, 2015). A large variety of traditionally used medicines is collected in bulk from wild without any regulation (Shabbir *et al.*, 2003). It is necessary to gather this living resource with wisdom and proper care to maintain its long-term survival. Plant collectors leave no vegetative part for its regeneration in next season. Due to over exploitation, such valuable resources become rare in their natural habitat. Hamilton (2004) estimated that globally 10,000 medicinal plant species are being threatened. Medicinal plants in Swat and Chitral regions of Pakistan are threatened at rate of 24.5% and 22.1% respectively (Gilani & Khan, 2003; Shinwari, 2010). It was reported by Shinwari & Gilani (2003) that improper collection of rhizomes results in depletion of medicinal reservoir in specific area. Their less availability in spite of their growing demand leads a break in demand and supply, which consequently results in adulteration and substitution of genuine crude drug. This practice results in poor quality of medicinal plant in herbal stores, which is dreadful. A study was carried out by Adams *et al.* (2013) on substitution of tuberous roots of *Cyperus rotundus* with *Aconitum heterophyllum* due to depletion of original medicinal source. Major problem which herbal industry is facing now days is lack of standardization. Evaluation of medicinal plants helps to control adulteration and substitution. A step is taken in this study to distinguish and authenticate corm of *Colchicum luteum* with bulb of *Narcissus tazetta* which is commonly adultered in herbal stores to overcome demand and raises economic benefits of herb sellers.

Colchicum has its place in tribe Colchiceae, family Colchicaceae and a member of order Liliales (Dahlgren *et al.*, 1985) whereas *Narcissus tazetta* stands in Asparagales order. *Colchicum luteum* is commonly known as Suranjantalkh (Shinwari *et al.*, 2003). It is a monocot plant and collected in bulk from meadows in Kashmir. Monocotyledons occupy important position among plant groups with reference to staple food, horticulture and generation of metabolites for pharmaceutical industries. The official part in *Colchicum luteum* is its corm that is actually modified and thickened form of stem. It contains alkaloid known as colchicine having several medicinal benefits. It is used in gout and arthritis (Siddiqui *et al*., 2002). Corms of *Colchicum* also acts as a blood purifier (Ahmad *et al.*, 2006). In all medicinal systems, colchicine is utilized to treat internal injuries. Authentication of morphological similar medicinal plants is very helpful as irrelevant use of medicinal plant is not effective in any sort of ailments.

Materials and Methods

Field trips were arranged in accordance to flowering periods of medicinal plants *i.e.* from late January to early March, 2012. The official part of plant was collected from field, dried and ground to make a powder utilized in pharmacognostic and physicochemical analysis.

Morphological evaluation: Morphological data was recorded both of herbarium specimens as well as fresh specimen during several field trips. Field photographs of plants in their natural habitat were captured. For detailed quantitative studies, hard scale as well as binocular dissecting microscope (SZF model Kyowa, Japan) of magnifying mirrors of 5X, 10X, 20X were used. The morphological studies were compiled in assistance of different floras (Nasir & Ali, 1979; 1980).

Leaf epidermal evaluation: Anatomical studies were carried out by preparing slides by modified method of Ahmad *et al*. (2010). Place central portion of 4-5 mature leaves of each plant in a test tube having 4 ml concentrated nitric acid and 2 g potassium chloride. Add 1 ml distilled

water and heat test tube at a boiling temperature until leaves become unstiffen. Pour the test tube mixture in Petri dish and wash 2-3 times with tap water. Take glass slide and place leaf on it with great care .While preparing abaxial surface, the leaf was placed at adaxial surface upwards and vice versa. Epidermis is peeled by sharp razor and debris was removed by camel hair brush. Bleach drops were poured on the epidermis for 45 seconds to get rid of chloroplast traces and washed epidermis again. Finally1-2 drops of lactic acid are added on slide and put cover slip on it. Observe slide under light Meiji microscope and set proper mirror magnifications for detailed study. Descriptive terms used for leaf anatomy were in harmony with Metcalfe (1960). Photographs of both surfaces were taken by CCD Digital camera (Model: DK 5000) equipped with Leica light microscope (Model: DM 1000). Magnifiers of 40X and 100X were used to obtain the finer details of both abaxial and adaxial surfaces, which were helpful to identify and differentiate epidermal cells.

Pollen evaluation (LM and SEM): Using dissecting microscope pollen material of plants having mature anthers is removed using forceps and needle. Erdtman method (1969) was followed for slide preparation. According to which a drop of acetic acid is added on a slide and pollen material is shifted to slide. Crushed it with needle until debris was separated from broken anthers. Remove debris using needle from slide. Pour 1-2 drops of glycerin jelly on slide and used paraffin wax to seal slide. At least 12-15 slides were prepared for each plant. Take each slide and study in detail under microscope.

For SEM bloomed flowers were exposed under Meiji MX 5200H binocular dissecting microscope. Pollen grains were separated by means of dissecting needle and shifted to metallic stubs which were further gold coated by Gold sputter JFC-15000. Detailed structure was observed and photographs were captured using JSM-6490LA. Terminologies used to express characters were in accordance with Moore *et al.* (1991) and Punt *et al.* (2007).

Organoleptic evaluation: Dried plant specimens of pharmacopoeia quality that must be available to deal as an authentic reference are taken and studied by help of sensory organs. Taste, odor, color, texture and size of plant parts i.e., stem, leaves, flower, fruit and seed were observed and recorded.

Pharmacognostic evaluation

Fluorescence analysis and solubility tests: Fluorescence analysis and solubility tests were carried out to distinguish genuine medicinal plant from its adulterants (Afaq *et al*., 1998; Abid *et al*., 2005). For this purpose, reagents of almost ten types including acids, alkalis and alcohols both in concentrated and dilute form of Merck brand were used.Weigh1 gram each of powdered herbal drug and their adulterant. Weighed powder with 5 ml chemical reagent were taken in test tube to check fluorescence analysis and solubility behavior of powdered material both in cold and hot state. Color change of drug and its adulterant were matched with Nippon color scheme.

Physicochemical evaluation

Determination of total ash: Ash was investigated by following procedure recommended by Gupta (2003). Weigh 3 grams of each powdered plant in pre cleaned labeled silica crucible. It was incinerated in muffle furnace for 5-6 hours at continuous temperature of 450-500^{o}C until a constant weight is obtained. Put silica crucible in desiccators for cooling. Reweigh immediately and note difference in weight.

Determination of acid insoluble ash: Ahmad & Sharma (2001) introduced method for acid insoluble, water soluble and insoluble ash. According to which add 25 ml HCL to the crucible containing ash and boil it for 5 minutes. Use watch glass to cover it. Take 5 ml of water in watch glass, rinse it and add to crucible. Insoluble residues were collected on ash less filter paper; wash it with hot water until it became neutral. Put insoluble matters into crucible again, heat it to get a constant weight. Finally place it in desiccators for cooling and then weigh residue immediately. Percentage of acid insoluble acid is calculated from air dried drug.

Determination of water-soluble ash and water insoluble ash: Add 25 ml water to crucible containing ash and boil for 5 minutes. Insoluble residues were collected on ash less filter paper. Wash it with hot water until it became neutral. Place it again in crucible; heat it to gain a constant weight. Finally place it in desiccators for 30 minutes .On cooling reweigh and subtracted it from weight of air-dried drug, which is actually weight of water- soluble ash. However, weight of insoluble water ash is calculated with reference to air-dried drug.

Determination of moisture content: Moisture content was determined by following method given by Mukherjee (2002).Weigh 1 gram of powdered medicinal plant (W1) in Petri dish and oven dried at 100-105 ◦c for one hour. Put it out from oven, placed in desiccators to cool. Reweigh oven-dried drug (W2). Difference in weight showed moisture content.

Results and Discussion

Colchicum luteum corms are ovoid, fleshy, arranged in vertical position having brown sheaths and a central longitudinal groove. In addition to corms all members of Colchicaceae family have sheathing leaves, parallel venation and fruit is capsule having alkaloid colchicine in their corms and seeds (Dahlgren *et al*., 1985). Among Amaryllidaceae Narcissus is a small genus consists of 65 species and ranks among economically significant ornamental plants (Hanks, 2002) (Table 1). *Narcissus tazetta* is propagated by bulb, grown in winter and dormant in summer. It has linear leaves and blossoms in clusters from winter to spring in Mediterranean areas. Morphologically both plants have entirely different corms. Organoleptographic evaluation suggests that corm of *Colchicum luteum* has bitter taste which justifies Ellwood *et al*. (1971) which noticed corms have unpleasant and acrid taste. Bulbs of *Narcissus tazetta* are tasteless and if added in powder of *Colchicum luteum* does not show any characteristic taste and odor (Table 2, Figs. 1 and 2 (a, b).

Table 1. General description of authentic source (*Colchicum luteum*) and its adulterant (*Narcissus tazetta*).

Sr.#	Plant name /Family	Trade part	Common names	Distributional range	Habit and habitat	Flowering/ Fruiting period	Price/Kg (PKR)
1.	*Colchicum luteum* Baker. Colchicaceae	Corms	**English name:** Golden collyrium, Yellow colchicum, Autumn crocus, Meadow Saffron **Local name:** Suranjantalkh, Phanphor, Ziarguly **Trade name:** Suranjantalkh.	In world distributed in Himalays, India, Afghanistan, Turkestan In Pakistan grown in Margalla hills, Murree, Mansehra, Swat, Chitral, Kashmir, Dir, Gilgit	It is an annual perennial medicinal plant of rocky and clayey soil at an altitude of 900-2700 m	Mid-February till April /April-June	1200-1400
2.	*Narcissus tazetta* L. Amaryllidaceae	Bulbs	**English name:** Bunch flowered narcissus, Daffodils, Chinese sacred lily **Local name:** Nargis, **Trade name:** Barg-e-nargis	In world found in Africa, Europe, Asia and Mediterranean areas. In Pakistan found in Islamabad, Lahore, Sind, Peshawar	An ornamental Plant grown in damp areas, fields and meadows	November-February / March-April	80-90

Table 2. Taxonomic investigations (Botanical and organoleptic description).

Sr. #	Plant name	Botanical description	Organoleptic description
1.	*Colchicum luteum*	A small perennial herb attaining height of 2-10 inches. Corms light brown colored with a longitudinal groove on one side, mostly oblong, 2-3 cm× 1.5-2 cm. Leaves broad linear shape with dimensions 12-18 cm× 0.8-2 cm Simple, dark green on upper and whitish green on lower side, margins entire having obtuse tips. Flower yellow, bisexual, funnel shaped ;Tepals 6 in number, 2.5-3 cm long, lanceolate to oblanceolate, gathered together at base to form a 8- 9 cm tube. Stamens 6; filaments 4-4.5 mm long, anthers linear, yellow. Gynoecium has superior ovary with 3 thread shaped styles, greater in length from stamens, little stigma, trilocular ovary with enormous ovules. Fruit capsule, ovoid, 2.5-3cm long. Seeds indefinite, spherical, brown colored	Dried plant constitutes corms, stem, leaves, flower and fruit. Corm is brown colored with a deep lengthwise groove like shell of snail. Stem soft, delicate, pressed, a papery brown covering which can easily be separated. Internally stem is pale yellow, rough 0.5 cm wide. Leaves soft, lanceolate having brownish green color. Small mustard yellow flower, tepals blackish yellow, and reproductive organs appear to pale yellow in color. Tasteless and herbaceous odor is recorded
2.	*Narcissus tazetta*	A monocot perennial plant grown from a light brown bulb, ovoid, 2-4 cm in diameter, thick and fleshy. Leaves 4-8, having V shaped origin, erect, margin entire, unsharpened tip, flat having indistinct parallel venation. Leaf size 12-48 cm×2-13.5 cm. A scape appears between middle of leaves having cluster of flowers shaped like open cups. Scape apex is enveloped by membranous sheath called spathe. Flowers stalked, pedicels are of unequal length ranging 1.2-4.5 cm. Petals 6, cream colored, petals pointed, located underside of sepals. Next to sepals a central cup shaped corona of golden yellow color arises formed by 6 flaps. Corona covers inside reproductive parts of flower i.e., 6 stamens adhered to corona; being in two sets of three each; one inserted at upper side and one at lower side of corona wall. Style short 18-22 mm long bearing trilobed stigma, ovary inferior. Fruit capsule, ovoid, green colored, seeds black, shiny, somewhat spherical, 3-4 mm	*Narcissus tazetta* has bulb, long leaves and cluster of flowers at the tip of central leaf which became scape now. Dried bulb resembles with small sized onion and has brown color. Leaf blade turned into orange yellow color. Its size is reduced width wise and leathery. Leaf is odorless and tasteless. Sepals color changed and become pale yellow or cream color and central cup shaped corona becomes light yellow. Fruit capsule is straw colored having shiny, irregular black seeds

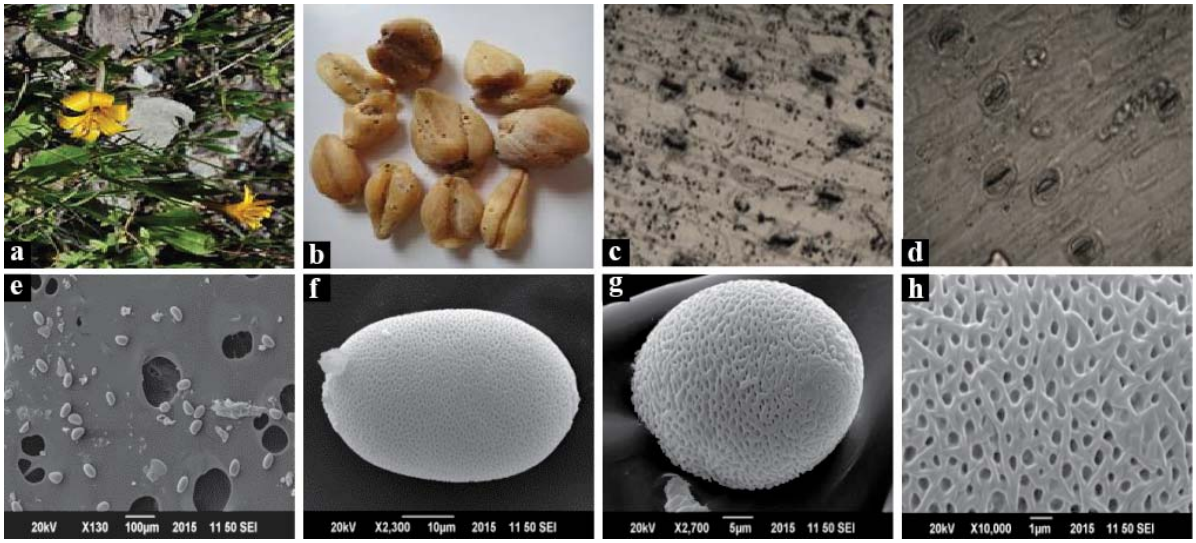

Fig. 1. *Colchicum luteum* field photograph (1a), dried corms (1b), Abaxial leaf surface (1c), Adaxial leaf surface (1d), SEM Pollen view (1e), Equatorial view (1f, g), Tectum sculpturing (1h).

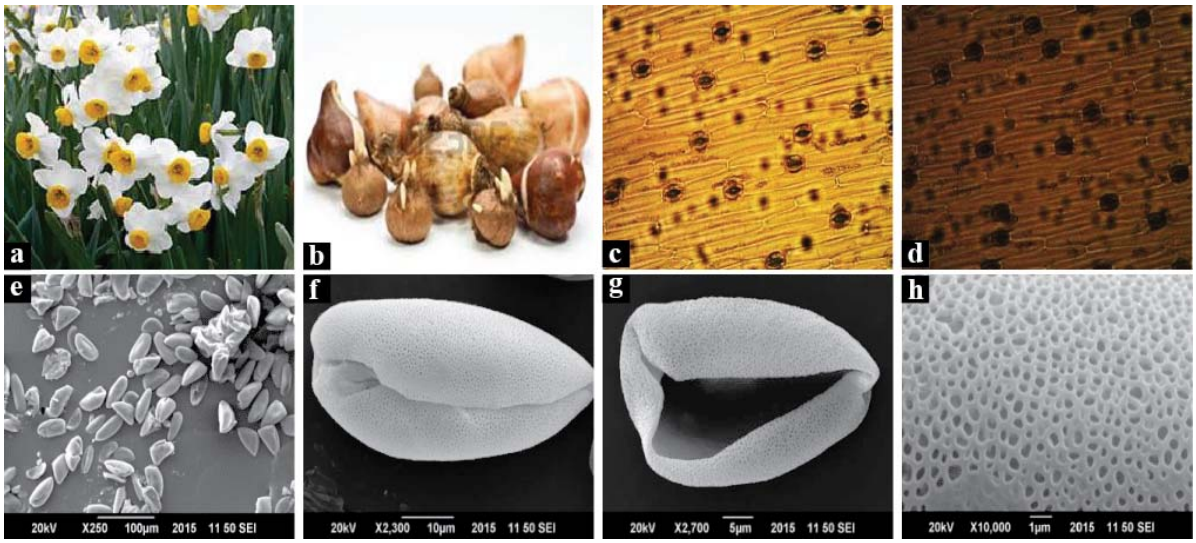

Fig. 2. *Narcissus tazetta* field photograph (2a), dried corms (2b), Abaxial leaf surface (2c), Adaxial leaf surface (2d), SEM Pollen view (2e), Equatorial view (2f, g), Tectum sculpturing (2h).

Anatomical evaluation: Epidermal evaluation of leaf including type of stomatal complex and trichomes are helpful tools in identification of medicinal plants utilized commercially (Ogunkunle & Oladele, 1997). Leaf epidermal cells in *Colchicum luteum* are long and rectangular and are in agreement with Sevgi & Kucuker (2011) who studied detailed anatomy in *Colchicum boissieri* whereas *Narcissus tazetta* has tubular and rectangular epidermal cells. The trichomes are absent in foliage epidermal anatomy of both plants. Detailed anatomical studies suggest being monocotyledons epidermal cells have straight walls and stomata are anomocytic. Stomatal index of abaxial and adaxial surface is almost same which depicts monocotyledons trend having same stomatal number on both epidermal surfaces (Edeoga *et al*., 2009) (Table 3; Figs. 1 and 2 (c, d).

Pollen evaluation: Pollen study serves as a diagnostic tool to distinguish genuine material from its adulterants. Rickett (2007) mentioned that pollen characters like aperture type and sculpturing pattern are source of additional information about systematics. Pollen evaluation using SEM depicts clear differences between both plants as *Colchicum luteum* pollen are circular in polar whereas oblate in equatorial view and are dipolar which is characteristic of Colchicaceae. Dusen and Sumbul (2013) concluded similar findings while studying pollen details of eight species of colchicum taxa. In *Narcissus tazetta* a characteristic feature is observed which is its monocolporate pollen and colpi is lengthened and parallel to its entire length. Datta *et al*. (2003) also concluded that single colpus is prolonged to longer axis and pollens are ellipsoidal. Presence of such characteristic makes it entirely different from pollen of *Colchicum luteum*. Reticulate sculpturing is a common characteristic of both pollen types (Table 4; Figs. 1 and 2 (e-h).

Table 3. Leaf epidermal evaluation.

Sr.#	(a) Qualitative features of epidermal cells:							
	Plant name	**Surface**	**Shape of epidermal cells**	**Margin of epidermal cells**	**Stomata P/A**	**Type of stomata**	**Trichomes P/A**	**Type of trichomes**
1.	*Colchicum luteum*	Ab	Rectangular	Straight	Present	Anomocytic	Absent	-
		Ad	Rectangular	Straight	Present	Anomocytic	Absent	-
2.	*Narcissus tazetta*	Ab	Tubular / rectangular	Straight	Present	Anomocytic	Absent	-
		Ad	Tubular / rectangular	Straight	Present	Anomocytic	Absent	-

Sr. #	(b) Quantitative features of epidermal cells:					
	Plant name	**Surface**	**Leaf epidermis length × width (µm)**	**Stomatal complex Length × width (µm)**	**Trichomes Length × width (µm)**	**Stomatal index**
1.	*Colchicum luteum*	Ab	127.5 (125-130) × 14.9 (13.7-16.2)	17.5 (15-20) × 16.2 (15-17.5)	Absent	26.9
		Ad	107 (98-119.5) × 12.3 (9.5-15)	14 (11.5-15.7) × 15 (12.7-16.7)	Absent	23.7
2.	*Narcissus tazetta*	Ab	100 (96-104) × 15 (13.7-16.2)	22.5 (21.2-23.7) × 21.2 (20-22.5)	Absent	21.98
		Ad	87.7 (83-92.5) × 13.7 (12.5-15)	20.5 (19.7-21.2) × 19.5 (18.5-20.5)	Absent	19.45

Table 4. Pollen evaluation.

Sr.#	(a) Qualitative features:						
	Plant name	**Type of pollen**	**Shape in polar view**	**Shape in equatorial view**	**Exine ornamentation**	**Colpi P/A**	**Pore P/A**
1.	*Colchicum luteum*	Diporate	Circular	Oblate	Reticulate	Absent	Present
2.	*Narcissus tazetta*	Monocolporate	Prolate-spheroidal	Ellipsoidal	Reticulate	Present	Absent

Sr. #	(b) Quantitative features:							
	Plant name	**Polar diameter (µm)**	**Equatorial diameter (µm)**	**P/E ratio**	**Length of colpi (µm)**	**Width of colpi (µm)**	**Pore diameter (µm)**	**Exine thickness (µm)**
1.	*Colchicum luteum*	28.7 (27.5-30) × 25.6 (25-26.2)	50 (45-55) × 25.4 (23.7-27.5)	0.57			7.4 (7.3-7.5) × 6.5 (6.4-6.6)	1.2(1-1.5)
2.	*Narcissus tazetta*	25.2 (23.2-27.2) × 19.9 (18.7-21.2)	48.3 (42.4-54.3)× 28.5 (25.3-31.8)	0.52	16.5(16-17)	4.7 (4.5-5)	_	1.8 (1.5-2.25)

Table 5. Fluorescence analysis and solubility tests by cold and hot methods of medicinal plant powder (*Colchicum luteum*).

Sr.#	Medicinal plant powder and reagent used	Method cold/hot	Visible light	UV light	Filter paper under visible light	Filter paper under UV light	Solubility
1.	Corm powder+ 5% KOH	Cold	Copper bell	Marsh green	Yellowish orange	Light orange	Freely soluble
		Hot	Copper	Brownish green	Light golden	Yellow orange	Freely soluble
2.	Corm powder+ 10% aq. $FeCl_3$	Cold	Light brown	Greenish brown	Orange brown	Golden grain	Freely soluble
		Hot	Copper	Dark brown	Deep orange	Deep orange	Freely soluble
3.	Corm powder+ dH_2O	Cold	White asparagus	Amber light	White asparagus	Silk knot	Freely soluble
		Hot	Filtered light	Amber light	White asparagus	Hearth yellow	Freely soluble
4.	Corm powder + H_2SO_4	Cold	Hearth yellow	Amber light	Black	Brownish black	Sparingly soluble
		Hot	Black	Blackish brown	Black	Black	Sparingly soluble
5.	Corm powder + $CHCl_3$	Cold	Badge	Glinted white	Colorless	Colorless	Sparingly soluble
		Hot	Light pink	Spring cut	White	Light yellow	Soluble
6.	Corm powder + C_2H_5OH	Cold	White asparagus	Plum frost	White	White	Soluble
		Hot	Filtered light	Plum frost	White	White	Soluble
7.	Corm powder +CH_3COOH	Cold	Comfy tan	Greenish blue	Colorless	Caramel cream	Sparingly soluble
		Hot	Amber light	Spiny green	White	Pinkish blue	Soluble
8.	Corm powder + C_6H_6	Cold	Creamy white	Bluish white	Colorless	White	Freely soluble
		Hot	White	Bluish white	White	Light blue	Soluble

Table 6. Fluorescence analysis and solubility tests by cold and hot methods of medicinal plant powder (*Narcissus tazetta*).

Sr.#	Medicinal plant powder and reagent used	Method cold/hot	Visible light	UV light	Filter paper under visible light	Filter paper under UV light	Solubility
1.	Bulb powder+ 5% KOH	Cold	Light orange	Light green	Light golden	Spiny green	Partially soluble
		Hot	Deep orange	Spiny green	Golden brown	Spiny green	Partially soluble
2.	Bulb powder+ 10% aq. $FeCl_3$	Cold	Light golden	Celery seed	Golden brown	Dirty yellow	Partially soluble
		Hot	Light brown	Light brown	Brown	Dark orange	Slightly soluble
3.	Bulb powder+ dH_2O	Cold	Creamy white	White	Colorless	Off white	Partially soluble
		Hot	Milky white	Milky green	Colorless	Caramel cream	Partially soluble
4.	Bulb powder + H_2SO_4	Cold	White	Sky blue	Light brown	Celery seed	Partially soluble
		Hot	Deep blue	Dirty purple	Black	Blackish brown	Partially soluble
5.	Bulb powder +$CHCl_3$	Cold	White asparagus	Whitish green	Colorless	Colorless	Partially soluble
		Hot	Filtered light	Bluish yellow	Pale yellow	Light blue	Partially soluble
6.	Bulb powder +C_2H_5OH	Cold	White asparagus	Plum frost	White	Colorless	Partially soluble
		Hot	Filtered light	Faded violet	White	Caramelcream	Slightly soluble
7.	Bulb powder+CH_3COOH	Cold	Peach	Whitish green	Colorless	Light pink	Soluble
		Hot	Amber light	Whitish green	Pale yellow	Light pink	Slightly soluble
8.	Bulb powder + C_6H_6	Cold	Rust	Sea green	Hearth yellow	Whitish green	Partially soluble
		Hot	Light golden	Milky green	White	White	Slightly soluble

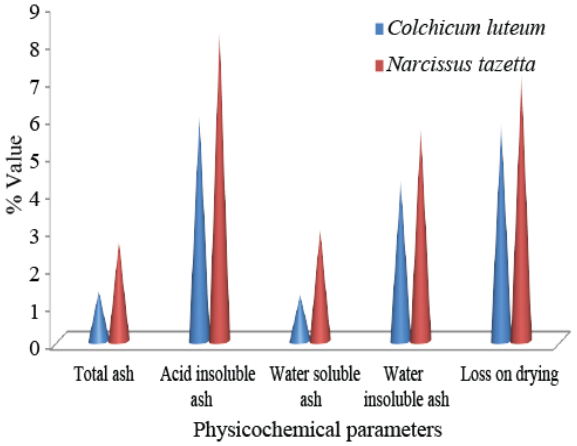

Fig. 3. Physicochemical evaluation of *Colchicum luteum* and *Narcissus tazetta.*

Pharmacognostic evaluation: *Colchicum luteum* has colchicine in its corm that is dissolved in different reagents carried out for fluorescence analysis and solubility tests. Powdered corm and tuber of both plants are added in different reagents to check their color variation in day and UV light and solubility. Results of *Colchicum luteum* are in agreement with Colchicine response in color and solubility with reagents as it is readily soluble in water, chloroform, methanol and ethanol (Akram *et al*., 2012). It shows violet color when treated with HNO_3. Powder of *Narcissus tazetta* shows entirely different colors and solubility behavior which clearly depicts that both powders does not show affinity in their chemical composition and hence in their tendency towards different chemicals (Tables 5 and 6).

Physicochemical evaluation: Physicochemical parameters (Sanmugarajah *et al*., 2013) are important to check adulteration in medicinal plants. Loss on drying in *Colchicum luteum* is less 5.8%, which shows it has less risk of microbial attacks as compared to *Narcissus tazetta* with 7.1% moisture content. Ash value, acid insoluble ash, water soluble ash and water insoluble ash of *Colchicum luteum* is 1.33, 6, 1.24 and 4.28% whereas *Narcissus tazetta* has 2.65, 8.24, 2.97 and 5.66% respectively (Fig. 3). Physicochemical values are in accordance with standard Indian pharmacopeia that has proven their distinctiveness (Anon., 1995).

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