

REPORT

Pharmacognostic and physicochemical screening of *Euphorbia nivulia* Buch.-Ham.

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Abstract: *Euphorbia nivulia* Buch.-Ham. (Euphorbiaceae) is commonly known as Indian Spurge Tree in English, and “Saj Thor” or “Jhanami booti” in local language. The plant is used traditionally in the treatment of various diseases like inflammation, fever, worm infection, asthma, cough, wounds and diabetes. In current study fresh as well as dried aerial parts of the plant and cut sections were examined, both macroscopically and microscopically. The study also deals with fluorescence analysis and phytochemical characteristics and other WHO recommended methods for standardization. WHO guidelines on quality control for medicinal plants materials were used for pharmacognostical evaluation of *E. nivulia*, phytochemical screening helps in determining the predominant classes of active constituents responsible for the activity. The present work will be helpful in identification of the fresh and dried samples of aerial parts pharmacognostically and anatomically. These studies will serve as a reference for correct identification and may be helpful in checking any type of adulteration. These observations will also help in differentiating this species from closely related species of the same genus and family.

Keywords: *Euphorbia nivulia*, pharmacognostic, macroscopic, microscopic, physicochemical.

INTRODUCTION

Euphorbia is a large genus comprised of more than 2000 species throughout the world, out of these about 195 species are found in India (Aditya, 2010). The genus consists of herbs, shrubs and trees which occur in diverse habitats (Basak *et al.*, 2009). *Euphorbia nivulia* Buch.-Ham. is of interest to natural products researchers due to its diverse biological activities. There is limited literature on the biological activities of *Euphorbia nivulia* (Badgujar & Mahajan, 2011). Northern and central India is habitat of the plant where it is planted as hedge plant often in dry areas and wild in arid soils. Species of this family are widely distributed in tropical Asia, Africa, Australia and Europe. The plant is indigenous to India, Myanmar and Pakistan (Radcliffe-Smith 2011). Other imported reported species of genus *Euphorbia* are: *Euphorbia indica* Lamk; *Euphorbia milii* Desmoul, *Euphorbia neriifolia* auct. Non Linn., *Euphorbia pulcherrima* Willd. and *Euphorbia tirucalli* Linn. Chemically, it contains tetracyclic triterpenes and three ingol diterpenes (Ravikanth *et al.*, 2003). Lectin, a high molecular weight glycoprotein (Inamdar *et al.*, 1988), and Nivulia-II and Nivulian-III, two other glycoproteins were isolated from the latex (Badgujar and Mahajan, 2010). The latex also contains alkaloids, cynogenic glycosides,

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phenolic compounds, tannins and terpenes (Mahajan and Badgujr, 2008). It contains citric, tartaric and mallic acids, euphol, nerifloiol, fat, albuminoids, hydrolytic enzymes, etc. Juices of leaves, bark, root, stem or latex is used traditionally for medicinal purpose. Leaf juice is used as a purgative and diuretic while leaf paste neem oil is applied in rheumatism. Plant latex possesses purgative properties and is used against worms (Pullaiah, 2006) also used in diseases like jaundice and enlargement of liver. Boro community of Assam uses leaf juice in pains and boils (Basumatary *et al.*, 2004). Latex is reported for bronchodilation (Savithrama *et al.*, 2007) and fleshy stem is recommended in cough (Mahajan and Badgujr, 2008). Latex of leaf and root is used in skin and ear disorders, worm infection, swelling and retention of urine (Britto *et al.*, 2010). Stem is applied to bone fractures and latex possesses antiseptic properties (Kumar and Chaturvedi, 2010).

MATERIALS AND METHODS

Plant material

Aerial parts (leaves, branches, stem and flowers) of fresh, well grown *Euphorbia nivulia* plant (10kg) were collected during the months of March and April 2015 from Hasil Pur Road and adjoining areas of Bahawalpur region, Pakistan and authenticated by taxonomist, Department of

Botany, The Islamia University of Bahawalpur. Voucher specimen (EN-AP-05-12-041) was deposited in the herbarium of Pharmacology Research Lab, Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan. The collected plant parts were chopped into pieces and spread on filter paper under shade for drying at room temperature for forty days; these were powdered by an electric grinder, and sieved through No.60 mesh. The powder was kept in closed amber colour glass bottles for microscopy. The fresh material was used for organoleptic evaluation, while the dried form was used in the physicochemical analysis and other parameters.

Solvents and chemicals

All chemicals and solvents used were of analytical grade (Merck and B.D.H.) and were purchased from local market.

Extraction

The powdered plant material (1kg) was macerated in 70% ethanol at room temperature for 10 days. The mixture was filtered three times with muslin cloth separately and then further filtration was done by Whatman Grade-1 filter paper. The filtrate was then evaporated under reduced pressure (-760mm Hg) and controlled temperature on the rotary evaporator (Heidolph Laborota 4000-efficient Germany and Buchi Rotavapor R-200). A thick and semisolid, dark brown gummy mass was obtained which was then placed in oven (Memmert Beschichtung Loading Model 100-800). The dried material was weighed, labeled and then stored at 4°C in refrigerator in air tight container. The condensed extract was used for further experiments. Macroscopic evaluation of the fresh aerial parts was performed using method of Brain and Turner, 1975. Microscopic evaluation was carried out with the powdered drug. This was treated with various reagents such as chloral hydrate solution 10%, glycerin 50% and iodine solution 5%. Small quantity of fine powder was placed on glass slide and 2 drops of mounting media was added; with the help of fine needle it was thoroughly mixed. Glass cover slip was placed on slide and with the help of fine blotting paper slide and also the sides of cover slips were cleaned. The tissues and their organization was observed with binocular microscope initially at low power (eye piece = 10 x and objective = 5x) and then at high power (eye piece = 18 x and objective 10x, 40x and 100x). A standard work was used for comparison to identify the observed microscopic structures (Jackson and Snowdown 1990; Youngken 1950). Photographs of the specimens were made with the help of digital camera (Cannon digital IXUS 85 IS). Fresh plant parts (leaf, stem and flower) were used for free hand section cutting. For transverse section, the plant stem slice was cut into thin sections with razor blade and submerged into water in a glass petri dish. Thin and uniformed sections were separated. For transverse section of leaf, midrib was placed into potato block and sections

were cut with razor blade by hand and placed into water in a glass petri dish. Thin and uniform sections were separated carefully with the help of microscope. For transverse section of flower, the slices of flower holding pedicle were placed into potato blocks and sections were cut. The selected sections of three organs (stem, leaf and flower) were immediately transferred to a watch glass containing 10% ethanol, then after that these sections were transferred to series of ethanol grades in ascending order, i.e., 20%, 30%, 40% and 50% respectively. Each cut section(s) was placed in each grade for 2 minutes. Then these sections were shifted to a petri dish containing 1-2 drops of safranin for 5 minutes duration. Afterward these sections were relocated in petri dishes that contain 60%, 70% and 80% ethanol, for 2 minutes. Light green (1 drop) was added and allowed to stand for 3-5 minute; then placing these sections in 90% and 100% ethanol for 2 minutes in each grade. In the last placed these in clove oil for one minute. Each specimen was shifted to glass slide and covered with cover slip after adding one drop of Canada balsam. Binocular microscope was used to observe the cellular structures (Ruzin, 1999). Safranin solution was used for staining of thick walled tissues like xylem, collenchyma cells, while light green was used for staining of thin walled tissues like phloem, parenchyma cells.

Preliminary phytochemical screening

Preliminary qualitative phytochemical screening of the crude extract to identify phyto constituents like alkaloids, glycosides, flavonoids, tannins, saponins and phenols, was carried out by using standard conventional procedures (Brain and Turner, 1975).

Physico-chemical analysis

According to the WHO guidelines on quality control standard methods for medicinal plant materials percentage of moisture content or loss on drying, total ash value, acid insoluble and water soluble ash value, extractive values and swelling and foaming index were examined.

Fluorescence analysis

Fluorescence analysis was performed on dried powder of the aerial parts (branches, stem, leaves) as such and after treating with water, NaOH, HCl, H₂SO₄, HNO₃, picric acid, acetic acid, methanol and ethanol, using ordinary and ultra violet light (254 and 366 nm wavelength).

RESULTS

Organoleptic and macroscopic evaluation

The plant is 3-9 m in height. Stem is hard with straight often whorled branches along with straight paired, stipulary spines often blackish. Leaves are 8.5-21.5 cm long and 4-5cm wide. The fresh leaves are dark green in colour with herbaceous odour; simple, fleshy, succulent, having characteristic taste. Leaves are obovate oblong or



Plate 1: *Euphorbia nivulia*



Plate 2: Crowded and rosette formation of leaves



Plate 3: Stem with black spines



Plate 4: Fresh leaves

spathulate, glabrous and are crowded at the end of branches. However, the dried leaves are grey brownish in colour, tasteless but characteristic odour, with broken crumpled and papery fracture; their texture is thin and papery. Both dried and fresh leaves have cuneate shape, their apex is sub-acute but base is acute. Leaves have throughout entire, even and smooth margins. Venation is pinnate. Lamina is entire and flat. Only the mid vein is visible in normal conditions. Secondary and tertiary veins are not visible due to their fleshy nature (plate 1-5).

Microscopic evaluation

Powder microscopy (aerial parts)

The fine powder was mounted in water, chloral hydrate, glycerin and iodine. The observed features revealed that the powder of aerial parts contains fragments of epidermal tissues composed of thick walled polygonal cells. Stomata are anomocytic type but infrequent. Calcium oxalate crystals are numerous idioblastic, rosette, square, prismatic and needle shape. Starch granules are both simple and compound. Annular vessels are well arranged showing spits on their secondary walls and spiral vessels.

There are spongy and xylem parenchyma cells. The cell walls of the fairly abundant (xylem) fibres are thickened or lignified or only moderately thickened and slightly lignified. Trichomes are glandular, unicellate, or multicellular with blunt tip, which are found scattered and attached to fragments of epidermis (plate 6-11).

Transverse section of leaf

TS of leaf showed epidermis, which is composed of single layered thick rectangular cells and is covered with cuticle. Cells of ground tissues are thin and spherical to irregular in shape. Laticifers are distributed around the vascular bundle. Lamina is differentiated into mesophyll, upper and lower epidermis. On surface view both the epidermal cell appear polygonal in shape. Mesophyll tissue consists of thin walled parenchyma cells. Mesophyll tissues are composed of palisade cells which consists of two or three layered zone of radially elongated cells. Spongy mesophyll cells are wider. There are wide air chambers in spongy mesophyll cells. Midrib is composed of epidermis, collenchyma and spongy parenchyma cells. Vascular bundles (VB) are covered with endodermis and

Table 1: Phytochemical screening

Test	Observation	Inference
Alkaloids		
Hager's test	Yellow ppt	Alkaloids present
Mayer's test	Creamy ppt	Alkaloids present
Wagner's test	Reddish brown ppt	Alkaloids present
Glycosides		
Keller-Killani test	Brown ring appeared at junction	Glycosides present
Tannins		
FeCl ₃ test	Blackish color	Tannins present
Flavonoids		
Test with Alkal Solution	Dark yellow color	Flavonoids present
Saponins		
Froth test	Froth formation	Saponins present
Phenolic Contents		
FeCl ₃ Test	Blackish color	Phenolic contents present
Carbohydrates		
Fehling's solution	Brick red color on boiling	Carbohydrates present

Table 2: Fluorescence analysis of powder

Sr.#	Powdered crude drug+ reagent	Ordinary light	UV light	
			254 nm	366 nm
1	Powder as such	Light yellow	Brownish grey	Fluorescent yellow
2	Powder +H ₂ O	Brownish yellow	Yellowish green	Yellow
3	Powder +1N NaOH	Yellowish brown	Grey	Light green
4	Powder +HCl	Brownish yellow	Blackish brown	Dark brown
5	Powder +H ₂ SO ₄	Brownish black	Black	Black
6	Powder +Picric acid	Bright yellow	Green	Brownish green
7	Powder + Acetic acid	Buff yellow	Light brown	Yellow with bright particles
8	Powder+ HNO ₃	Reddish brown	Dark brown	Greyish black
9	Powder+ Methanol	Light green	Greyish green	Light green
10	Powder+ Ethanol	Light green	Light green	Fluorescent green

are prominent towards the ventral side. The vascular bundles are composed of non lignified phloem (green colour) and lignified xylem (pink colour). Between loosely arranged spongy cells, calcium oxalate crystals were seen. Starch granules are scattered in spongy parenchyma (plate16-19).

Transverse section of stem

TS of the young stem showed normal dicot structure. Epidermis is thick; the cells are small and arranged compactly. Cortical region is found as multilayered cells and has been crashed due to massive secondary growth. Below epidermis, there is a layer of collenchymas, which are thick walled but still living. Secondary growth is found extensive; secondary phloem is distributed in distinct patches. The pith is large. Vascular bundles are composed of (pink) xylem and (green) phloem. The central region of pith is rich in laticifers which secrete white thick milky latex (plate 13-15).

Transverse section of flower

T S of flower showed the outermost layer is epidermis. Below epidermis ground tissues are composed of parenchyma cells. Inside ground tissues laticifers are present. Around central pith, vascular bundle are present, composed of xylem and phloem (plate 12).

Preliminary phytochemical screening

The results for presence of different phytochemicals like glycosides, alkaloids, saponins, flavonoids, phenols, tannins and carbohydrates are shown in table 1.

Physico-chemical analysis

The moisture content or loss on drying at 105°C was found to be 7%. The percentage of extractive values was found to be 16% and 12% in water and ethanol. Acid insoluble ash, water soluble ash and total ash value was found 1.06%, 17.97% and 20.51% respectively. Swelling index was found 5 ml while foaming index was less than 100 cm. Fluorescence Analysis The results of fluorescence analysis have been shown in table 2.

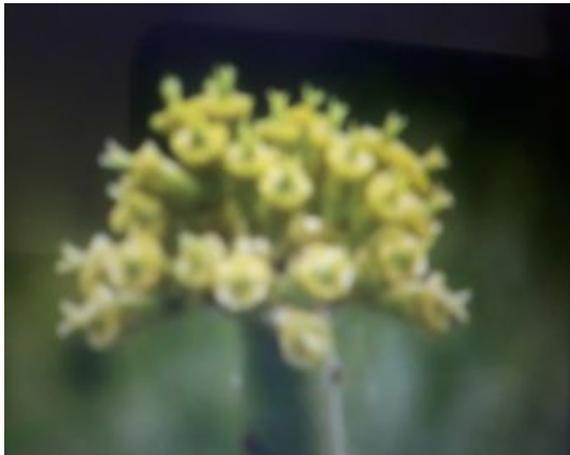


Plate 5: Flowers (fully blossomed)



Plate 6: Pitted vessel

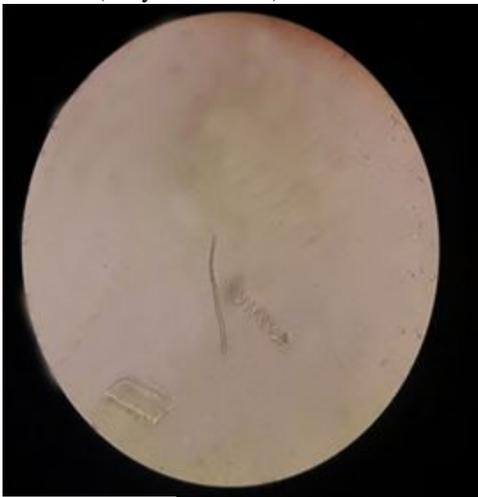


Plate 7: Vessel and fibre

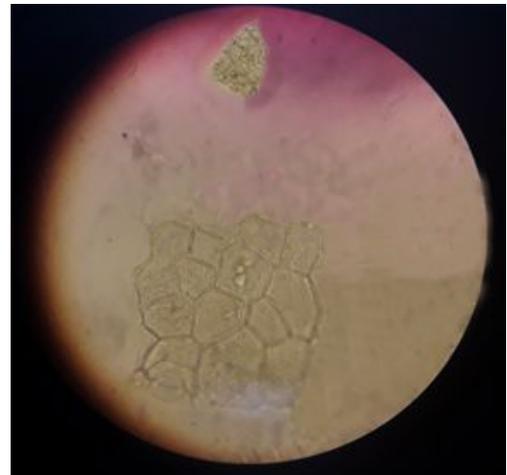


Plate 8: Parenchyma cells



Plate 9: Fibres



Plate 10: Trichomes



Plate 11: Calcium oxalate crystals

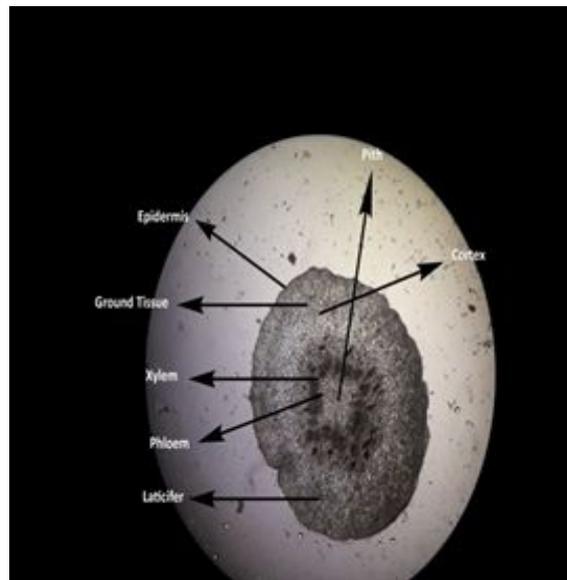


Plate 12: TS Flower (Pedicel)



Plate 13: TS Stem (Bark & Wood)

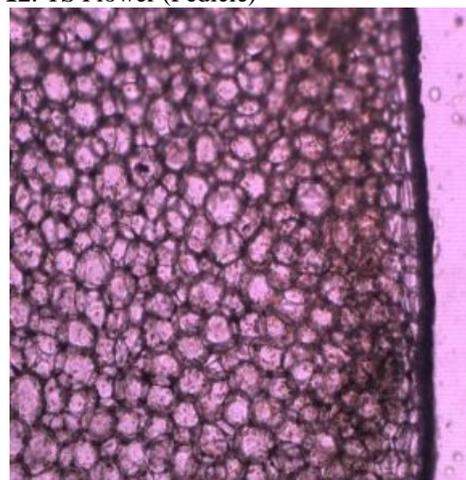


Plate 14: TS Stem (Parenchyma & latex cells)

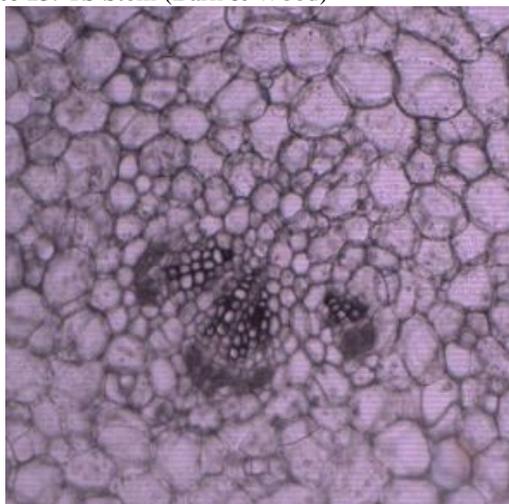


Plate 15: TS Stem (Vascular Bundle)

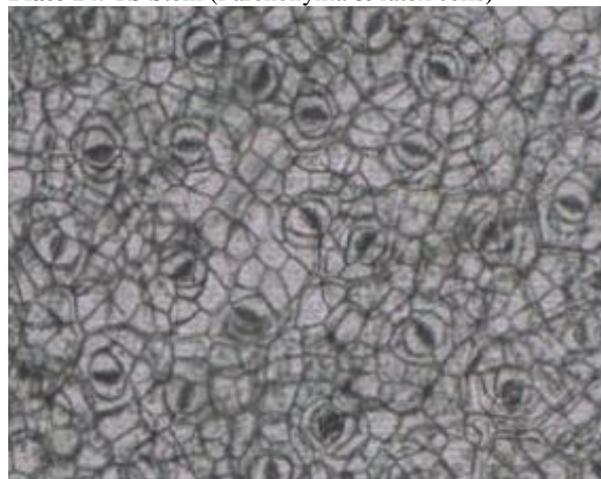


Plate 16: TS Leaf (Upper Epidermis)

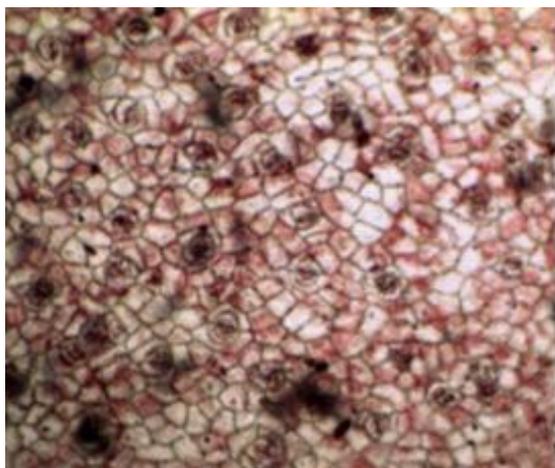


Plate 17: TS Leaf (Upper Epidermis)



Plate 18: TS Leaf (Lower Epidermis)

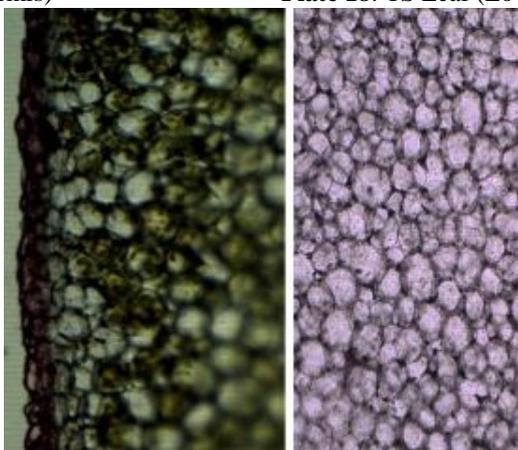


Plate 19: TS Leaf (Hypodermis with cuticle)

DISCUSSION

Although nowadays different sophisticated modern research techniques and tools are available for the evaluation of the plant drugs but macroscopic and microscopic methods are still one of the most simplest, reliable, precise and economical methods to confirm the correct identity of the plant source. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a plant is the first criteria to establish the identity and also the degree of purity of material and should be performed out before any tests are conducted. Organoleptic /macroscopical evaluation is a qualitative test based on the study of morphological characters of crude drugs and serves as diagnostic tool. In current research work a study was conducted on the aerial parts of a medicinal plant *E. nivulia*. The microscopic studies of the powder and transverse sections showed the presence of various histological structures like epidermal cells, stomata, starch granules, vessels of different types, fibres, vascular bundles, laticifers and unicellular blunted and glandular trichomes. The use of different stains or reagents helps to differentiate various cellular structures on the basis of

their chemical composition (Mali & Panchal, 2017). Fluorescence is an important phenomenon exhibited by various chemical components present in plant sample. If the constituents themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different chemical reagents; thus some crude drugs may be assessed qualitatively in this way and it is an important parameter for pharmacognostical evaluation of a drug sample (Janchen & Issaq, 1988). Fluorescence analysis showed different characteristic fluorescent colours in various inorganic and organic chemical reagents. Fluorescent phenomenon of the powder determines the purity and quality of plant sample. Various chemical constituents present in the powder sample exhibited fluorescence at visible day light and U.V (Mukherjee, 2002). The histoanatomical observations of various aerial parts thus will provide useful information for quality control of the crude drug. Powder, qualitative, fluorescence and transverse section standards provide valuable information to authenticate the natural product of a plant kingdom. Results of preliminary phytochemical screening showed the presence of various bioactive compounds in the aerial parts of the plant which may have diversified therapeutic value for curing ailments. Primary

phytochemical screening showed the presence of a variety of secondary metabolites which are known to have therapeutic value for curing ailments; for example, saponins, flavonoids, tannins, alkaloids and phenols have anti-inflammatory activities whereas flavonoids, glycosides, alkaloids, tannins and phenols have hypoglycemic and hepatoprotective properties (Orhan *et al.*, 2007; Sharma *et al.*, 2008). The physicochemical parameters are helpful in finding the purity and quality of the drug. The results suggest that the powder of the plant has higher aqueous extractive value followed by alcohol. Water soluble extractive value indicates sugars, inorganic compounds and acids; and alcohol soluble extractive value shows the presence of polar components like glycosides, flavonoids, steroids and phenols etc. Loss on drying was 7%. Moisture contents greatly affect stability of the natural products. To prevent chemical decomposition and microbial contamination low moisture content is needed. Due to presence of mucilage swelling index was in the range of 5 ml; while foaming index was less than 100, i.e., insignificant. By estimating ash value quality and also the purity of powdered crude drugs can be determined. On incineration crude drug normally leaves an ash consisting of carbonates, silicates and phosphates of calcium, potassium, magnesium and sodium. Total ash determines that how much care is required in preparation of a crude drug. Silica or calcium oxalate in the crude drug can be determined by acid insoluble ash test (Genest *et al.*, 1963). Ash value is also significant for detection of the nature of adulterant added to the drug with an intention of adulteration; for determination of impurities, authenticity, quality and purity of the test sample (Wallis, 1984). Ash value usually represents inorganic salts which are present in the drug sample and residue remaining after incineration (WHO, 1998). Total ash value of leaves indicates impurities or the inorganic composition or earthy materials presence in the plant material (Sharma, 2013)

CONCLUSION

Detailed standardized work on pharmacognostic evaluation for this plant has yet not been reported in literature. Aerial parts powder subjected for macroscopic, microscopic pharmacognostic analysis provides important information which may be helpful in the authentication of the crude drug and also to check adulteration for quality control of raw material. The pharmacognostic parameters observed in present study, being reported for the first time adds to the existing knowledge of *E. nivulia* and be quite useful for the identification, standardization, development and preparation of the crude drug's formulation and inclusion in various pharmacopoeias to be utilized as a potential therapeutic agent for treating various diseases. The current observation will also be helpful in differentiating the leaves of this species from closely related species of same genus and family.

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