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MICROSCOPICAL EVALUATION & PROXIMATE ANALYSIS SESBANIA GRANDIFLORA A FOLK LORE PLANT

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ABSTRACT: Sesbania Grandiflora is a folk lore plant the great medicinal value and evaluated microscopically for the presence of histological characters and proximate analysis. Microscopical characters identify by Rotary microtome technics and proximate analysis is carried out using standard methods. Microscopical characters show the presence of epidermis, anisocytic stomata, phloem, xylem, veins, vein islets, ground tissue &trichomes, glandular trichomes not found in the leaves. The proximate analysis shows Moisture content, Dry mater content, Total ash value, Acid insoluble ash value and Water soluble ash value respectively. Sesbania Grandiflora shows further the presence of Stomatal number is 140/mm^2, petiole appears more or less in tetragonal outline with two adaxial thick, short wings like humps. Present study reveals that diagnostic characters help full for identification genuineness which play key role in authentication of plant and used medicinally health care needs of livestock and human beings. Therefore, these diagnostic characters are help full for detecting adulterants, can also serve as active constituents of herbal medicines and useful in the treatment of various diseases in future.

Key words: Microscopical characters, proximate analysis, Rotary microtome, Sesbania Grandiflora

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INTRODUCTION

S. Grandiflora is found in plains to 500 m. It is often planted as prop for piper betel it is distributed in tropical Africa, Asia, Indonesia and India. The Genus Sesbania has More than 50 species which are distributed in warm and wet regions. Some of the species are cultivated and ornamental. The leaf is reported to contain 8.4 g protein, 1.4 g fat, 2.2g fiber, 1,130 Ca, 80 mg.P, 3.9mg Fe, 9,000 IU vit.A,and 169 mg ascorbic acid. 1684 mg Ca, 258 mg P, 21 mg Na, 2,005 mg K, 25,679 µg β-carotene, 242 mg ascorbic acid. It is believed to have Plants are the ridiculous sources of all the elements important for human beings. Like all living things, plants requisite obtain certain elements from their environment in order to endure their biological functions necessary for survival. Some elements are essential for growth for structure formation, as components of biologically active molecules while others have some other beneficial effects. Metals and minerals present in biological system play a significant role in the metabolism of plants and humans (Galan et al 2005).

Sesbania Grandiflora is A perusal of the literature reveals that the following uses have been reported from the various parts of the plant, they are laxative, diuretic, emetic, emmenagogue, febrifuge agati is a folk remedy for bruises, catarrh, dysentery, eyes, fevers, headaches, smallpox and stomatitis (Hari et al 2014). Bark, leaves, gums and flowers are considered medicinal. The astringent bark was used in treating smallpox and other eruptive fevers. Presence of active constituents important for treating various diseases (Heghedűş-Mîndruet al 2014).The inorganic compounds are essential in trace amounts to play an important role in nutrition (JothiKarumari et al 2014).It is believed that great mainstream of Microscopical characters are evaluated for the quality, purity their by helpful for its genuineness.The screening of the actual Microscopical characters of plant origin and assessment of authentication (Malhotra et al 1998) Keeping this in view the present study aims to evaluate Microscopical characters collected from Anantapuramu region by using rotary microtome and proximate analysis using standard methods.

MATERIALS AND METHODS

Collection and Identification of the Plant, The medicinal plant were collected from the place Raghavendra institute of pharmaceutical education and research, Anantapuramu district, India. The plant is identified referring various floras available in the Department of P. G. Studies and Research in Pharmacognosy.

Collection of Specimen

The plant specimens for the proposed study were collected from Sesbania grandiflora (L) Pers. Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid – 5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual additional of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. de waxing of the sections was by customary procedure (Mathad 2016). The sections were stained with Toluidine blue as per the method published by O'Brien et al. (Muhammad Zafar 2010). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cyto chemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green IKI (for Starch).For studying the Stomatal morphology, venation pattern and trichomes distribution, Para dermal section (section taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid(SayemaArefin et al 2015) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab Photo2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Shute et al 1964).

Proximate Analysis

The fresh leaves were shade dried for about 15-20 days and they were powdered with electronic blending machine. The powdered materials were stored in air tight containers for future use.

Ashing of Plant Materials

10 gm of plant material is taken in a crucible and heated for 2 hours at 450^0 C on muffle furnace. And the collected ash measure for its percentage

Acid Digestion of Plant Materials

1 gm of ash (sample) by weight was taken in a beaker and 3:3 ml of conc. Sulphuric acid and perchloric acid is added. The samples are heated to get clear solutions, subsequently it was cooled by adding distilled water and the samples were filtered with whatman filter paper no 42,Heat the whatman paper in muffle furnaceand the collected ash calculate percentage. Proximate Analysis (Soetan et al 2010; Tamilarasi et al 1968).

Proximate analysis of a substance constitutes different classes of nutrients present in samples such as moisture content, dry matter, Ash value, acid insoluble value, water soluble ash value etc.

Determination of Total Moisture Content

10g of fresh sample heated on hot air oven at 250^{0}C temperature until it free from water moisture content and cooled. The weight of the dry sample was taken and the percentage of it was calculated with reference to the fresh sample.

Determination of Dry matter content

10g of fresh sample heated on hot air oven at 250^{0}C temperature until it free from moisture content and then cooled .The weight of dry sample was taken and the percentage of it was calculated with reference to the fresh sample.

Determination of Total ash value

10g of dry powder was taken in silica crucible and heated on a muffle furnace at 450^{0}C temperature until it free from carbon and then cooled .The weight of ash was taken and the percentage of it were calculated with reference to the air-dried sample.

Determination of Acid insoluble ash value

The total ash obtained was boiled for 5 minutes with 25 ml of 2 N HCL, filtered and the insoluble matter was collected on ash less filter paper. Then it was washed with hot water, ignited in silica crucible for 15 minutes at temperature 3800C not exceeding more than450^{0}C, cooled and weighed the obtained residue. The percentage of acid insoluble ash was calculated with reference to the air-dried sample

Determination of Water soluble ash value

The total ash obtained was boiled for 5 minutes with 25 ml of water for few minutes, filtered and the insoluble matter was collected on ash less filter paper. Then it was washed with hot water, ignited in silica crucible for 15 minutes at temperature not exceeding 450^{0}C cooled and weighed the obtained residue. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the airdried sample. Finally, the percentage of water soluble ash was calculated with reference to the air-dried sample.

RESULTS

In the present study selected plant were evaluated for the presence of Microscopical characters and proximate analysis. The Microscopical characters are shown in the figures and proximate analysis such as moisture content; dry matter, total ash value etc are tabulated in Table No1.in our study leaflets are dorsiventral. The Midrib is Plano convex is sectional view with flat adaxial side and broadly conical abaxial side it is 450 µm thick in vertical plane 400 µm wide in horizontal plane. The xylem elements are thick walled wide angular Meta xylem elements are nearly 30 µm wide. Phloem hand is 80 µm wide (fig 1.2). The lamina is bifacial with smooth and even surfaces. It is 200 µm thick. The palisade zone is 60 µm in height (fig 2.1); the adaxial and abaxial epidermal layers are stomatiferous. The stomata are predominantly anisocytic one small and two large subsidiary cells. The guard cells are elliptic measuring 20x12 µm in size. Stomatal number is 140/mm^2 (fig 2.1). The vein islets are distinct; they are polygonal ranging from squarish to hexagonal in outline (fig 3.2). The petiolule is circular (fig. 4.1). It has prominent epidermal layer comprising of thick walled cells with hemi spherical outer walls. The petiolule is 700 µm in diameter. Petiole appears more or less in tetragonal outline with two adaxial thick, short wings like humps (fig 4.1). It is 1.75 mm in vertical plane and 1.55 mm in horizontal plane. The powder preparation of the leaf shows stomata which are either anisocytic or paracytic type. The guard cells are Elliptic and measure 20 µm long and 15 µm wide (fig 5.1). Epidermal trichomes are absent circular striations are not evident. Proximate analysis shows the Moisture content, dry matter, Total ash, Water soluble ash and Acid insoluble ash values respectively in the plant (table 01).

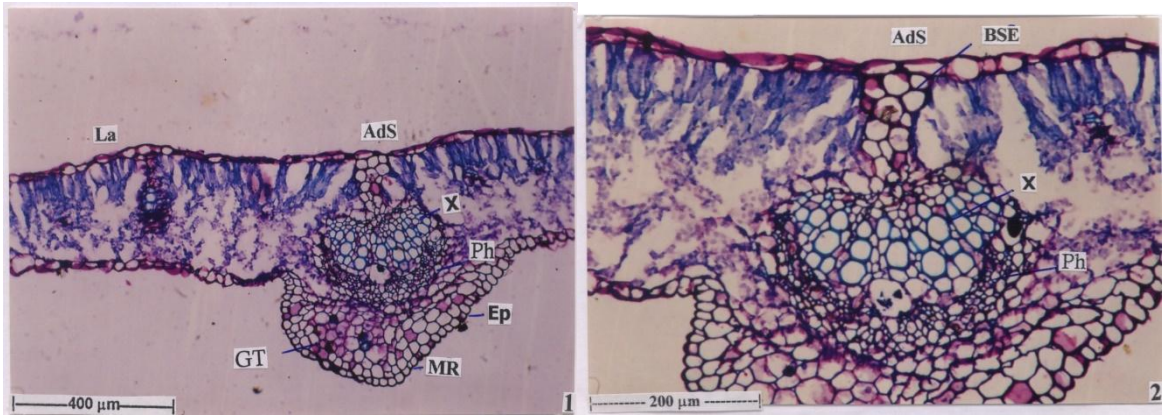
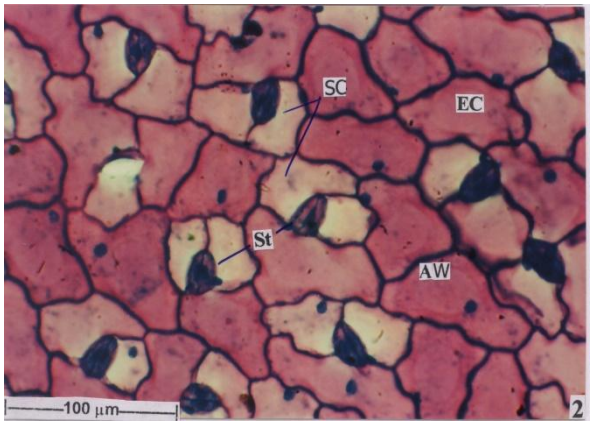


Fig. 1.1TS of leaflet through midrib and lamina. Fig. 1.2 TS of midrib – enlarged



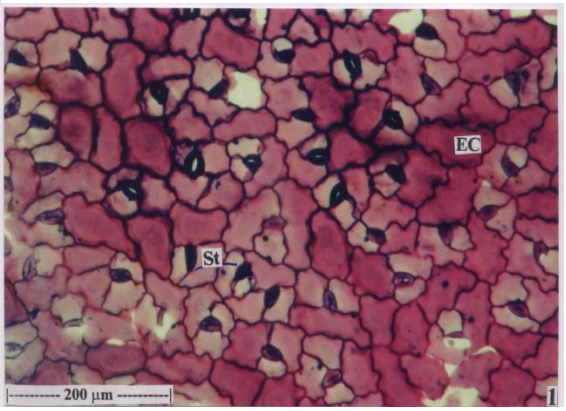
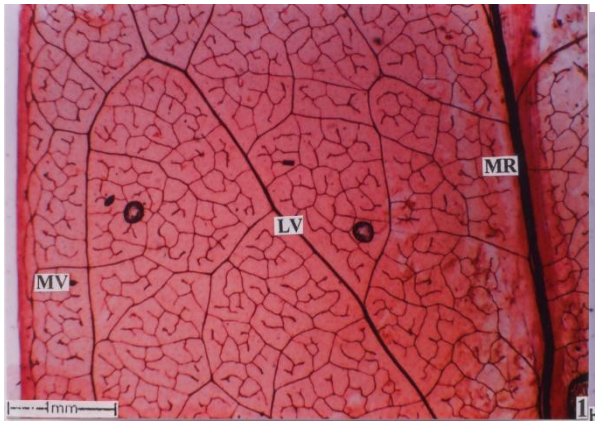
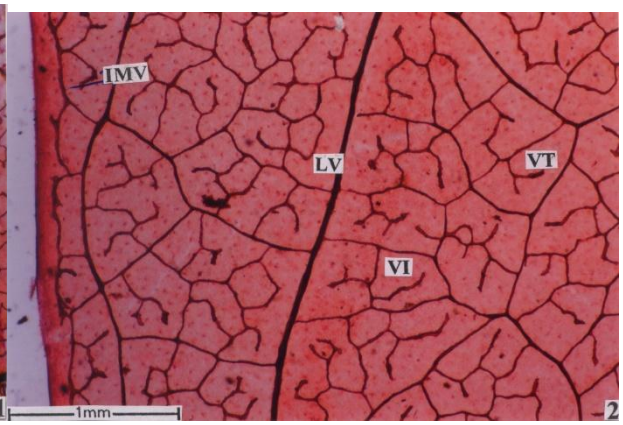
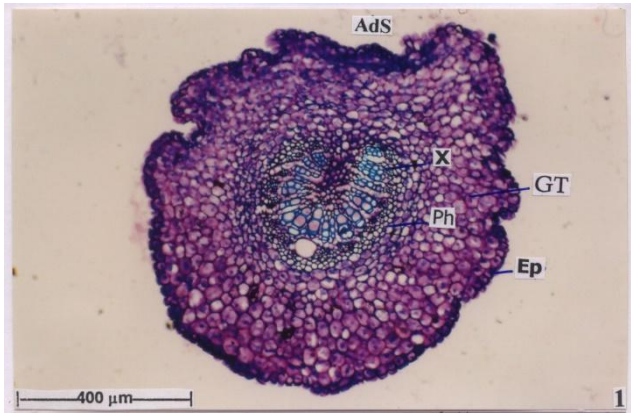


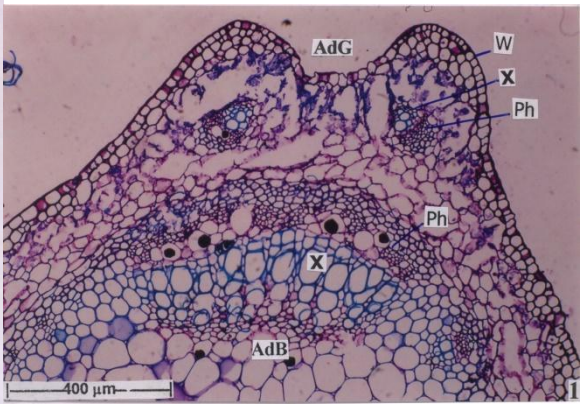
Fig. 2.1 Stomata & Epidermal cells enlarged Fig. 2.2 Stomata and epidermal cells



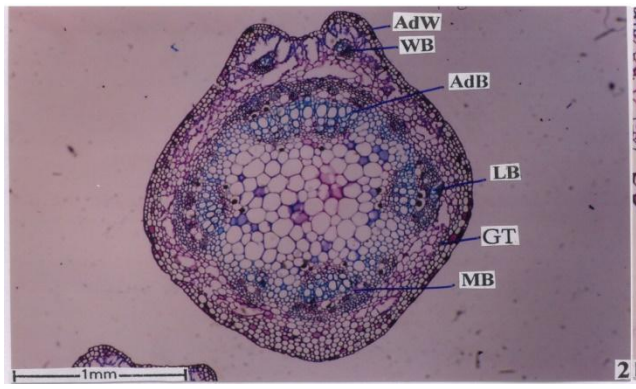


3.Venation pattern under low magnification **3.Vein islets and vein terminations – enlarged**





4.1 TS of petiolule, showing gross sectional outline **Fig. 4.2 TS of petiole, showing outline and vascular**



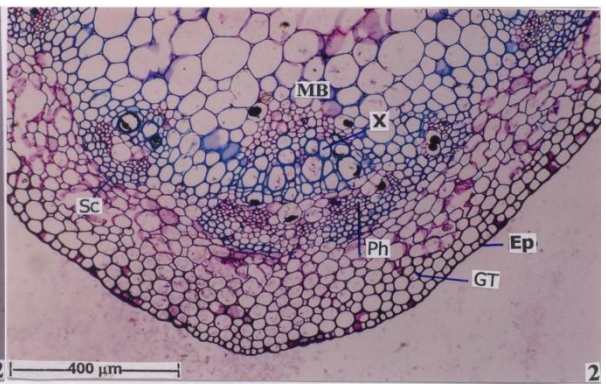


Fig. 5.1 TS of Petiole Upper portion Fig. 5.2 TS of Petiole Lower portion

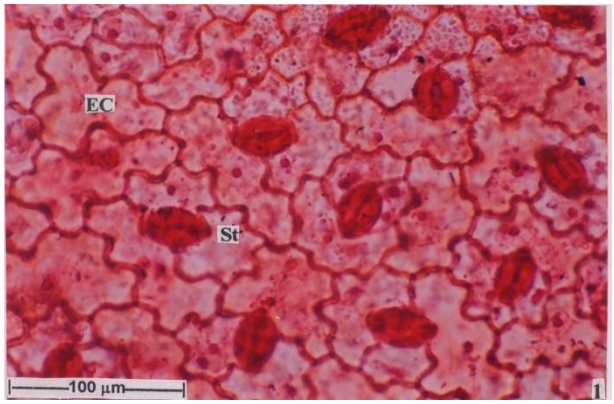


Fig. 6.1 Powder Microscopy Fragments Stomatal Epidermis as seen in the powder

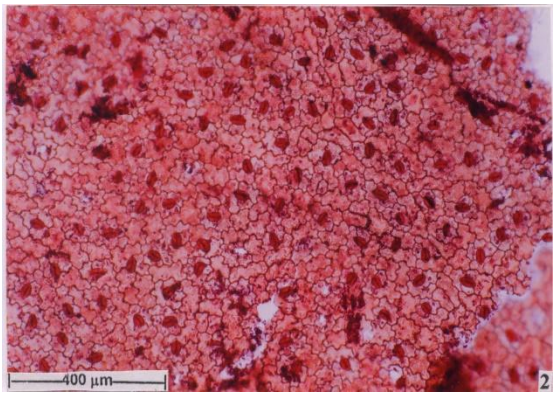


Fig. 6.2 Powder Microscopy Stomata Distributed

Table 1: Proximate analysis of selected plants in (%) w/w.

Plant name	Moisture Content %	Dry weight Content %	Total Ash %	Acid Insoluble Content %	Water Soluble Ash %
Sesbania Grandilora	86.23	13.77	17.23	9.17	11.89

DISCUSSIONS

The results of the present study shows theleaflets are dorsiventral. The leaf is scientific name Sesbania Grandiflora, family fabaceae containing anisocytic stomata also known as crusiferous stomata. Anisocytic means small opening pore covered with kidney shaped guard cell that kidney shaped guard further covered with epidermal cells one epidermal cell smaller and other two epidermal cellsare big. The trace element and proximate analysis plays an important role in exploring new potential drugs from medicinal plants as it provides nutritional and phyto constituent values. The Midrib is Plano convex is sectional view with flat adaxial side and broadly conical abaxial side it is 450 µm thick in vertical plane 400 µm wide in horizontal plane. The xylem elements are thick walled wide angular Meta xylem elements are nearly 30 µm wide xylem helpful for supply of water to the leaf their by important for many chemical reaction. Phloem hand is 80 µm wide (fig1.2). The lamina is bifacial with smooth and even surfaces. It is 200 µm thick. The palisade zone is 60 µm in height (fig2.1); the adaxial and abaxial epidermal layers are stomatiferous. The stomata are predominantly anisocytic one small and two large subsidiary cells. The guard cells are elliptic measuring 20x12 µm in size. Stomatal number is 140/mm^2 (fig2.1). The vein islets are distinct; they are polygonal ranging from squarish to hexagonal in outline (fig3.2). The petiolule is circular (fig. 4.1). It has prominent epidermal layer comprising of thick walled cells with hemi spherical outer walls. The petiolule is 700 µm in diameter. Petiole appears more or less in tetragonal outline with two adaxial thick, short wings like humps (fig4.1). It is 1.75 mm in vertical plane and 1.55 mm in horizontal plane. The powder preparation of the leaf shows stomata which are either anisocytic or paracytic type. The guard cells are Elliptic and measure 20 µm long and 15 µm wide (fig5.1). Epidermal trichomes are absent circular striations are not evident. Proximate analysis shows the Moisture content, dry matter, Total ash, Water soluble ash and Acid insoluble ash values respectively in the plant (table1). Concentration of element content in the Sesbania Grandiflora varies due to different climatic conditions like soil, water and temperatures. The trace element and proximate analysis plays an important role in exploring new potential drugs from medicinal plants as it provides nutritional and Phyto constituent values. Potassium K2+ act as Cofactor that functions in protein synthesis, activation of enzymes, major solute functioning in water balance and thus affecting osmosis, operation of stomata in plants. Potassium required for glycogenesis, cellular enzymatic reactions, and cell membrane function in humans (Tan et al 2006). Magnesium Mg2+ is an important component of chlorophyll in plants. It activates many enzymes involved in photosynthesis, respiration and are involved in the synthesis of DNA and RNA of plants. Magnesium is absorbed in the intestine and then transported through the blood to cells and tissues of humans. It is also a constituent of bones, teeth, enzyme cofactor kinesis' etc (Thimmiah et al 2006).Calcium Ca2+ is highly related to cell wall stability and membrane integrity in plants. It is also required for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability in humans. Iron Fe2+ is a important component of cytochromes, electron transport, activates some enzymes, plays a role in chlorophyll synthesis.

Fe is required for making Hb. Biologically important compounds of iron are hemoglobin, myoglobin, cytochromes, catalases and peroxidase (Uzama et al 2012). Zinc Zn2+ activates formation of chlorophyll, activates some enzymes, and plays a role in formation of auxin, chloroplasts and starch. Carbonic anhydrase is present in erythrocytes, kidney tubules; gastrointestinal mucosa and glandular epithelium of humans are Zn dependent enzymes. Manganese Mn2+ helps in formation of amino acids, activates some enzymes, coenzyme activity, required for water-splitting step of photosynthesis, chlorophyll synthesis, Mn is a part of enzymes involved in urea formation, pyruvate metabolism and the galactotransferase of connective tissue biosynthesis in humans.To ensure the safety and efficacy of herbal medicines used, standardization and the development of the processing aspects of phyto-medicines are very important. The proximate analysis of plant parts, foods, feeding stuffs and other compounds reveals the presence of protein, carbohydrate, fat, crude fibre and moisture in them. The concentration of elements in helpful for conclusion that the plants will have specific roles in the treatment of different diseases.

CONCLUSION

The present study was an effort to know the biological properties of medicinal plants by trace element and proximate analysis. From the study it can be concluded that the plant contains important trace elements and the percentage of all the trace elements within permissible level which directly influence the quality of secondary metabolites. The proximate analysis such as moisture content, dry matter, total ash, acid insoluble ash and water soluble ash value shows the quality of plants. The data obtained from this study can be used to discover novel herbal drugs to treat various diseases of animals as well as human beings. Further studies are needed in this direction to explore more pharmacological actions of these plants.

Conflict of interest

Statement we declare that we have no conflict of interest.

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