Research Article

Pharmacological, Micromorphological Studies on *Solanum mauritianum* Scop. (Solanaceae): A Search

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ABSTRACT

Bug weed (*Solanum mauritianum* Scop.) is an evergreen woody species of Solanaceae. It belongs to South America. Ripe fruits are used by the local people as vegetable during famine periods and also employed to cure different ailments. The present investigation was designed to evaluate the pharmacological, micromorphological studies on *Solanum mauritianum* Scop. to evaluate its potentialities. The phytochemical constituents in the different solvent extracts include alkaloids, saponins, tannins, flavonoides and proteins. Alkaloids form the major compounds in chloroform, petroleum ether and ethanol extracts. Microscopic study showed distinct morphological characteristics in the stem and leaves. The other components evaluated are moisture, total ash, acid-insoluble ash, sulphated ash, alcohol soluble extractive and water soluble extractive. The study scientifically validates the use of the species in traditional medicine. Further studies are warranted to isolate, quantify and fractionate the major phytochemical – alkaloids and to prove its medicinal value.

Keywords: *Solanum mauritianum*, solvent extracts, phytochemicals, alkaloids, micromorphological features.

INTRODUCTION

Plant based drugs have been in use from prehistoric period in the form ayurvedic, homeopathic and unnani medicine. Herbals are significant among the traditional and modern system of medicine. Medicinal plants application has been increased through phytochemical researches and also the negative impacts of allopathic drugs including the cost. Indigenous people in most part of the world mainly depend on the traditional methods of treatments using natural products. 80 % of the population in developing countries is still use folklore medicines from plant resources[1]. The medicinal properties of herbals are because of diverse potent phytochemicals and nutrients. Thus, they form the raw materials for pharmaceutical companies. These compounds singly or synergestically produces physiological action on the human body[2]. Information related with these secondary biomolecules discloses new sources of lead molecules for curing infectious diseases to cancer[3].

The major phytochemicals are alkaloids, flavonoides, tannins, cyanogenics, glycosides, polyphenolic compounds, saponins, lignins and lignans. Phytochemicals exhibits wide range of biological properties due to their antioxidant potentialities[4].

Solanaceae represent an important economically and medicinally group in angiosperms. *Solanum*, represents the hyper-diverse type member of this family comprising appx. 2000 species distributed in the tropical, sub-tropical and a few in temperate zones[5]. For decades, many of the *Solanum* species were used by the different ethnic groups in dried or fresh forms for treatment of various ailments and also as vegetables.

Bug weed (*Solanum mauritianum* Scop.) is a fast-growing, soft, evergreen woody shrub of South American origin. The plant body is wooly studded throughout with soft white hairs. The leaves are large with pale green velvety upper surface, while the lower surface is white and tomentose. Flowers are lilac in terminal clusters and fruits are yellowish round berries contain abundant seeds. Bug weed causes threat to plantations and other indigenous species. Further, its hairs are skin and respiratory irritants, and the unripe berries are toxic.

MATERIALS AND METHODS

Plant Material

Solanum mauritianum Scop., the plant sample used for this study was collected from the wild habitat of Munnar hills, Idukki district, Kerala (Figs. 1 a and b). Identity was confirmed by referring floras and authenticated by matching with herbarium of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, (JNTBGRI) Palode, Trivandrum and the herbarium was deposited in the department herbarium (UCT 1279).

Micromorphological Study

Free hand sectioning was carried systematically. The sections made were passed sequentially through alcohol solutions in the order: 30, 50, 70, 95% and absolute alcohol, allowing them for 5 min in each. The dehydrated samples were cleared of their natural wax by passing them through different proportions of alcohol and chloroform series in the following ratios (3:1; 1:1; 1:3) v/v for 10 minute in each, and as the chloroform gradually

replaced the alcohol, the process was repeated from the pure chloroform and down the series again within same time interval. These were rehydrated in alcohol solution starting with absolute then 95, 70, 50, 30 and stained with 1% Alcian blue for 2 min, washed off with water before counter-staining with 1% safranin for 2 minutes. The stain was washed off and placed on clean glass slide with a drop of glycerol and covered with clean cover slip[6]. These slides were viewed with the light microscope and microphotographs were taken.

Figure 1a & b: Plant material *Solanum mauritianum* Scop. showing flowers and fruits

Preparation of leaf samples

Fresh, healthy leaves were washed thoroughly 2-3 times with running tap water and were air dried under shade. After complete drying, the sample was subjected to hot continuous extraction by soxhlet method using non polar to polar solvents such as chloroform, petroleum ether, ethyl acetate, ethanol and water sequentially. The filtrate was collected in sterile bottles and was stored by refrigeration at 5° C until use.

Preliminary Phytochemical Analysis

This was carried out according to the methods described by Khandelwal[7]. Qualitative phytochemicals analysis of the different solvent extracts of the *S. mauritianum* in terms of alkaloid, saponin, tannins, phenols, flavonoids and protein were made as shown below.

Test for alkaloids

0.1mg of the extract was added 10ml methanol. After that 2ml filtrate were taken and added 1% HCL and add 6 drops of Mayer's reagent/Wagners reagent/Dragendorffs reagent. It produced cream/brown/red/orange precipitate indicate the presence of alkaloids.

Test for saponins

Approximate 0.5ml extract was taken and added 5ml distilled water. Frothing persistence suggest the presence of saponins.

Test for tannins

0.1mg taken was added 10ml distilled water. Take 2ml filtrate and added 2ml $FeCl_3$. Then bluish black precipitate indicates the presence of tannins.

Test for flavonoids

0.1mg extract was taken and added 10ml ethanol. Take 2ml filtrate add conc. HCl and magnesium ribbon. Pink/ red colour indicates the presence of flavonoids.

Test for protein

Take 2ml of the extract and add few drops of Millons reagent and mix thoroughly and heat. White precipitate is formed and the precipitate turns brick red after boiling.

Test for steroids

The extracts were refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in the Chloroform.

Salkowski test

To the test extract solution add few drops of conc.H_2SO_4, shaken and allowed to stand, lower layer turned red indicating the presence of steroids.

Anthraquinones glycosides

Borntrager's test

Crude extract was mixed with 5ml of 10% H_2SO_4 and was boiled for 5 minute. Filtered while hot, cooled; the filtrate was shaken gently with equal volume of benzene. Benzene layer was separated and then treated with half of its volume solution of ammonia (10%) and was allowed to separate. The ammoniacal layer acquired rose pink colour due to the presence of anthraquinones

Phenolic compounds

0.1mg extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. Dark green colour suggests the presence of phenolics.

Determination of foreign matter

100g of the powdered sample of the crude drug was weighed accurately. It was then spread in a thin layer on a white tile uniformly without overlapping and the inspection was done with naked eyes or with the help of a

6X lens. The foreign matter was then separated manually as completely as possible. The portions of this sorted foreign matter were weighed and the content of each group in g / 100 g of air-dried sample was calculated.

Determination of loss on drying

A glass-Stoppard, shallow weighing bottle was weighed, accurately 2 g of the specified sample was weighed and then transferred to the bottle and the bottle containing the content was again accurately weighed. The sample was then distributed as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10mm. The loaded bottles were placed in the drying chamber (hot air oven). Eventually the sample was then dried to constant weight for the specified time and at the temperature (over 110°C). After the drying was completed the bottle was promptly removed and allowed to cool at room temperature in desiccators before weighing. Finally the weight of the bottle along with the contents was weighed and the percentage loss in weight was calculated.

Determination of ash value total ash

Accurately 2g of the ground, air-dried material was weighed and placed in a previously ignited and tarred silica crucible. The powder sample was then spread evenly and then was incinerated to a constant weight by gradually increasing the temperature to 500-600 °C until it was white, indicating the absence of carbon. The crucible was then cooled in a desiccator and finally weighed. The content of total ash in terms of percentage w/w of air dried material was calculated.

Acid insoluble ash

The crucible containing the total ash obtained after incineration was further boiled gently for 5 min with 25 ml of hydrochloric acid (~70 g/l) and was then covered with a watch glass. The watch glass was then rinsed with 5ml of hot water and this liquid was then added to the crucible. The insoluble matter was then collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was then transferred to the crucible, dried on a hot-plate and then was ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 min, and then weighed without delay. The content of acid-insoluble ash was calculated in mg per gram of air-dried material.

Water soluble ash

The crucible containing total ash, 25 ml of water was added and boiled for 5 minute. The insoluble matter was then collected on an ash less filter paper. It was then washed with hot water and finally ignited in a crucible for 15 min at a temperature not exceeding 450°C. The percentage of water soluble ash in mg per gram of air-dried material was reported.

Extractive Value

5g of coarsely powdered air-dried material was accurately weighed and placed in a glass-stoppered conical flask. Powder was then macerated with 100ml of the solvent (water/ethanol) concerned for 6 hours, shaking frequently, and then was allowed to stand for 18 h.

It was then filtered rapidly taking care not to lose any solvent; 25 ml of this filtrate was transferred to a tarred flat-bottomed dish and was evaporated to dryness on a water-bath.

It was followed by drying at 105°C for 6 h, cooled in a desiccator for 30 min and was weighed without delay. The content of extractable matter in mg per g of air-dried material was then calculated.

RESULTS AND DISCUSSION

Herbals are source of bioactive phytochemicals or nutrients. Studies carried out during the last several decades have shown that these phytochemicals have pertaining role in minimizing chronic diseases like cancer, diabetes and coronary heart disease.

These includes phytochemicals with disease-preventing functions like dietary fibre, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents.

As the initial part of the study micromorphological features in the species was analyzed.

T.S of mature stem shows epidermis composing of thick-walled, tangentially elongated cells, followed by layers of collenchymas and thin-walled oval or circular parenchymatous cells, stone cells either single or in groups, secondary phloem composed of sieve elements and phloem parenchyma traversed by medullary rays, xylem composed of vessels, tracheids, fibre trachieds, parenchyma and transverse by medullary rays, all elements being lignified, vessels and tracheids with bordered pits, fibers with a few simple pits, xylem parenchyma rectangular or lightly elongated with simple pits and rarely with reticulate thickening, crystals of calcium oxalate as sandy masses and simple starch grains present in secondary cortex, phloem and medullary rays (Figs. 2 a, b, c, d and e).

Figure 2a: Cross section of stem of S.mauritianum Scop.

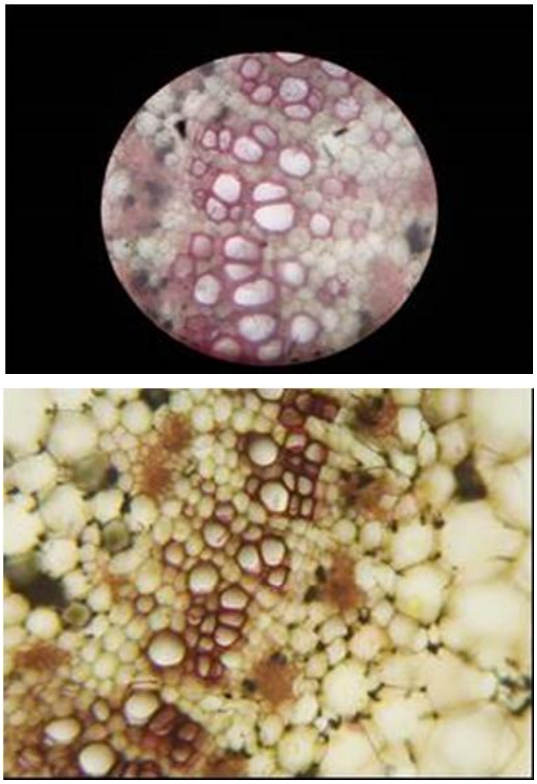


Figure 2b & c: Primary xylem of *S. mauritianum* enlarged.

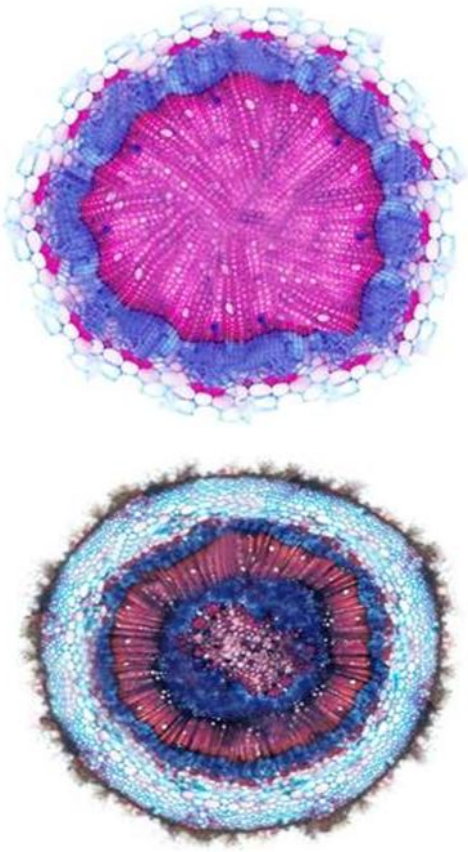


Figure 2d & e: C.S of stem of *S. mauritianum* after secondary growth.

C.S of petiole also displays more or less similar structure to that of stem i.e. a cuticularized epidermis, 2-4 layers of collenchyma and a massive parenchymatous cortex. Stele is U shaped. Epidermis is covered with numerous multiradiate trichomes (Fig. 3).

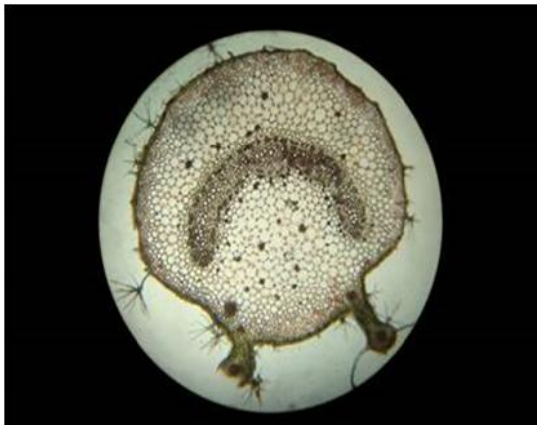


Figure 3: C.S of petiole of *S.mauritianum*.

Leaf shows the typical dorsi-ventral features with velvety trichomes all along the leaf epidermis.

Mesophyll is differentiated in to upper palisade and lower spongy tissues. *S. mauritianum* foliar epidermal study revealed the presence of anisotricytic stomata and stellate trichomes at both the adaxial and abaxial foliar surfaces (Figs. 4 a, b, c, d, e and f).

Adaxial and abaxial surfaces has 23.2 and 25.1% stomatal index respectively. Similarly, the trichome index was 32 and 28% for the adaxial and abaxial surfaces respectively. Stomatal length were more or less similar in the adaxial and abaxial sides i.e., 5.2 ± 0.18 µm with 4.3% coefficient of variation (CV) and width of 3.4 ± 0.09 µm with 5 % CV. Glandular trichomes are shortly pedicellate (3 celled), unisseriated with rounded apical cells and also stellate plurisseriated dendroid stelliform noticed on the stems, pedicels and the external face of the calyx, and scarce in petioles(Fig. 4). Carvalho[8] revealed this type of trichomes as emergencies, meanwhile Siddiqi[9] classified the paleous dentritic trichomes, not as emergencies, since they are constituted of epidermal tissues, whereas emergencies are also formed of subepidermal tissues[9]. In fact, a subepidermal layer was not observed in the paleous trichomes. Maria Almeida Alves[10] analyzed *Solanum cernuum* in terms of morphological, anatomical, macro and micromolecular markers revealed similar trichomes

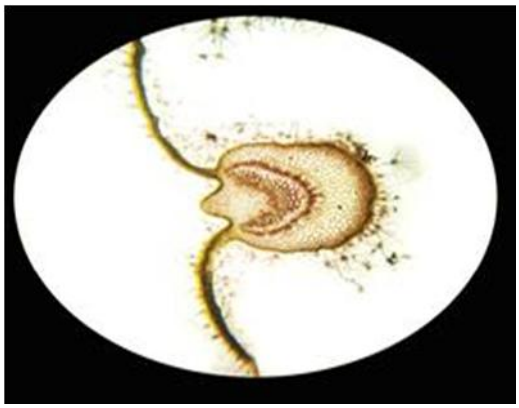
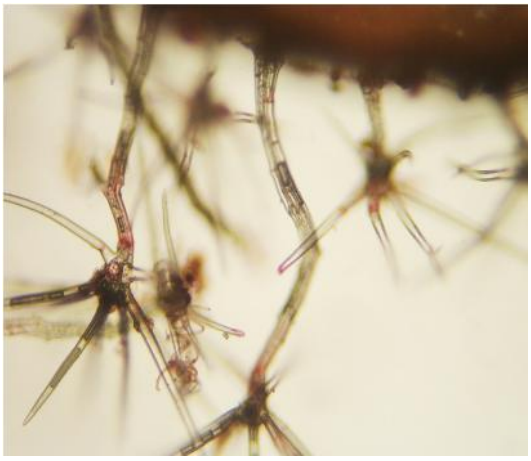


Figure 4a: C.S of leaf of *S.mauritianum*.



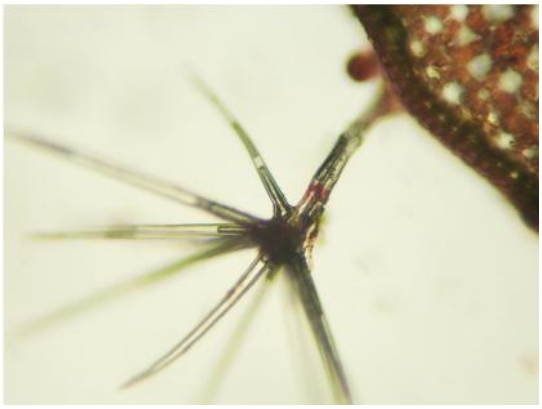


Figure 4b & c: Dendroid hair in *S. mauritianum*.

Figure 4d: Bifurcated hair of *S. mauritianum*.

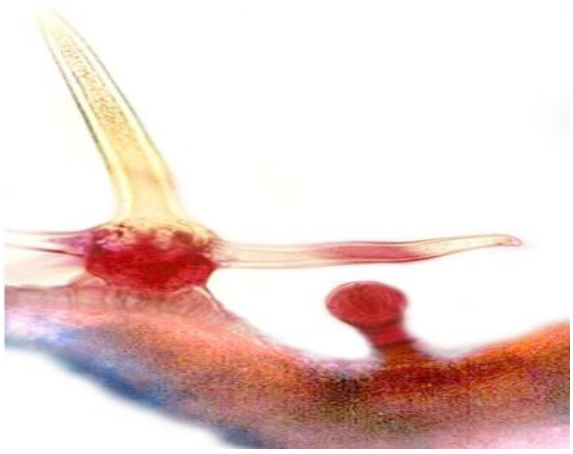


Figure 4e: Glandular hair of *S. mauritianum*.

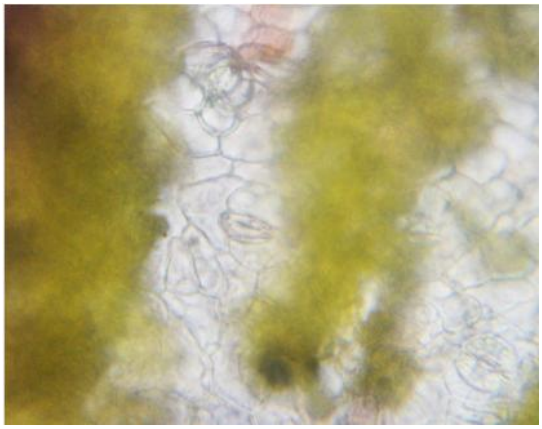


Figure 4f: Stomata of *S. mauritianum*.

Physiochemical Parameters

Physiochemical studies revealed the following parameters such as moisture content (2.9%), total ash (11.7%), foreign matter (1.87%), acid insoluble ash (2.95%) and sulphated ash (9.7%). The extraction values were alcohol soluble fraction was 10.5% and that of water soluble content was 7.9%.

The phytochemical study revealed the presence of alkaloids, flavonoids, glycosides, phenols, steroids, saponins and tannins. Thus, the preliminary phytochemical tests are helpful in finding phytochemical constituents in the plant that may lead to their quantitative estimation and also in locating the source of pharmacologically active secondary metabolites. Interestingly, like other *Solanum* species alkaloids are present in remarkable levels in chloroform, petroleum ether and ethanol extracts as revealed by the qualitative analysis (Table 1).

Phytochemicals like alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bacterial effects[11]. They exhibit marked physiological activity when administered to animals. In the present study, the observed alkaloid content could be responsible for their much expected medicinal values. Saponin are glycosides which have soapy form with precipitating and coagulating properties towards red blood cells[12], like haemolytic activity, cholesterol binding properties and bitterness. Tannins are water soluble polyphenols and function as antimicrobial agent by precipitating proteins. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins[13]. Phytotherapatically, the presence of tannins might have accounted for the bitter taste and have been reported to hasten the healing of wounds and inflamed mucous membrane[14]. Flavonoids are potent water soluble antioxidants and free radical scavengers, which prevent oxidant cell damage have strong anticancer activity[15,16] and anti-inflammatory activities. Similarly, the proteins found to be bioactive against certain ailments[17]. Phytochemical constituents in *Solanum* species account for its medicinal potentialities and can be employed for the treatment of various diseases/ailments such as cough,

liver problem, stomach-ache, skin diseases, inflammation, jaundice, tooth ache etc which substantiates the usage by the local people[18,19].

CONCLUSION

Thus, the present investigation revealed that the leaves of *Solanum mauritianum* have potential source of useful drugs due to the presence of phytochemicals and can be utilized in the treatment of many diseases/ailments and also may be exploited for use in the pharmaceutical and cosmetic industries.

However, further studies required to quantify the alkaloids, its fractionation and structural characterization from the crude extract for proper drug development.

Table 1: Preliminary phytochemical analysis in *Solanum mauritianum* Scoop. using different solvent systems

	Hex	Chloro	Pet. Ether	Ethyl Acetate	Ethanol	Aqueous
Alkaloids	+	+++	+	++	++	+
Flavonoids	-	-	+	-	++	+
Anthraquinones glycosides	-	-	-	+	++	+
Phenols	+	+	+	+	++	-
Steroids	-	+	-	+	-	-
Saponins	+	+	+	+	+	-
Tannins	-	-	-	+	+	-
Protein	-	-	-	-	+	++

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