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Scanning electron microscopic and IR finger printing study as taxonomic character in medicinally important Spiny Nightshade *Solanum virginianum* L.

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ABSTRACT

Electron microscopic characters were widely used for taxonomic circumscription. Scanning electron microscopy (SEM) has been used as an important tool to resolve finer structures of biological specimens for specific identification and characterization. Ultrastructural features obtained from SEM often constitute a valuable source of information in diagnosing already circumscribed taxa and/or in phylogenetic inference. In the present study, pollen, seed and leaf of Solanum virginianum were analysed by scanning electron microscopy. SEM of seed coat showed compactly packed irregular units delimited by prominent ridges along the boundaries which are inconspicuous towards the hilum region. Seed is more or less oval which becomes little extended in the hilar region. Upper leaf surface of Solanum virginianum showed the presence of long multicellular non glandular hairs with bulbous tip, short stalked multicellular glandular hairs and multicellular non glandular stalked hairs with pointed tip whereas, lower leaf surface showed few stellate hairs, long multicellular non glandular hairs with bulbous tip and multicellular non glandular stalked hairs with pointed tip. Stomatal indices for the upper and lower leaf epidermises varied from 3.92 to 8.44 respectively and the vein islet number showed a value of 11.7. Pollen grains are small, globular, trizonocolporate with microechinate ornamentation. Pollen dimorphism was noticed in terms of small and large grains. Large grains are without operculum. The IR fingerprints for the species are 3059, 1020, 1101 and 1317 which are unique in terms of its alkaloid and phenolic content.

Keywords: IR spectrum, Pollen morphology, Scanning Electron Microscopy, Seed surface, *Solanum virginianum*, Stomatal index, Vein islets.

INTRODUCTION

Solanum is a large, cosmopolitan genus, comprises 1,500 species, about 75% of the Solanaceae, but in the absence of a recent critical assessment these figures may well be too high. The genus is mainly tropical but a few are temperate. The greatest diversity of species is to be found in South and Central America; Australia and Africa are less rich but have a significant number of endemics. Eurasia is poor in species numbers. Many of the species in the genus are food plants, ornamentals, weeds or medicinal. It is important that the classification and nomenclature of a genus with such an extensive interaction with man should be understood and stabilized. The genus S*olanum* however, has a certain taxonomic notoriety, derived in part from the large number of species names described, which complicates specimen identification, but also in part from the difficulty of identifying natural species groups. A number of factors are responsible for this and these include the poor definition of the generic limits, the occurrence of suites of attributes in varying combinations throughout the genus, the phenotypic plasticity and genetic variation in many of the species, and the continual reclassification of parts of the genus which has ended in nomenclatural confusion.

Solanum virginianum L. a prickly, diffuse under shrub, somewhat woody at the base; stem somewhat zigzag. Prickles compressed, straight, yellow, often exceeding 1.3 cm long. Leaves 5-10 cm long, ovate or elliptic, sinuate or subpinnatifid, obtuse or subacute, armed on the midrib and nerves with long yellow sharp prickles. Flowers are in extra-axillary few-flowered cymes; corolla white, 2 cm long. Berry 1.3-2 cm diam., yellow or white with green veins, surrounded by the enlarged calyx. Roots are diuretic and expectorant; employed in cough, asthma, chest pain and catarrhal fever. Fruit juice is useful in sore throat and rheumatism. Stem, flowers and fruits are carminative. Paste of the leaves is applied on painful joints to relieve pains. Seeds are given as an expectorant in asthma and cough. Decoction of the plant is useful in gonorrhea. The plant also possesses cardioactive and antipyretic acivities. Crude plant extract caused hypotension which has been attributed to release of histamine by some constituents.

The plant contains sterols, alkaloids and glycosides. The plant also contains quercetin glycoside, apigenin, sitosterol and carpesterol. Fruits contain steroidal glycoalkaloids, solasonine, solamargine, solasurine, solanocarpine, solanine-S and alkaloidal bases, solanidine-S and solasodine. Seeds contain solanocarpine. Dry fruits contain traces of isochlorogenic, neochlorogenic, chlorogenic and, caffeic acid. Quercetin-3-O-β-D-glucopyranosyl-(1-4)-mannopyranoside, apigenin and sitosterol have also been isolated from dry fruits. In the present study, microstructural analysis in leaf, pollen and seed was attempted on *Solanum virginianum*. Infrared spectral analysis was also attempted to know the phytochemical uniqueness of the medicinal herb.

MATERIALS AND METHODS

Solanum virginianum was collected from Vithura of Kerala. The sample was identified and confirmed by comparing with the herbarium at the Department of Botany, University of Calicut. A voucher specimen was prepared and deposited in the herbarium of the Department of Botany, University College, Thiruvananthapuram.

Scanning electron microscopy:
Fresh leaf pieces (10 x 10 mm^2) from *Solanum virginianum* was cut into 3 mm^2 pieces and subsequently fixed in 2.5% glutaraldehyde (prepared in 0.2 M sodium cacodylate buffer, pH 7.2) for 2 h. The samples were then dehydrated in an alcohol-acetone series at room temperature of 25 ± 2 °C. The dehydrated materials were then dried in a critical point drier (EMS-850) using CO_2 as the transition fluid. The dried samples were mounted on the copper stubs, keeping the abaxial leaf surface up by using double-sided sticky tape, and then coated with gold (20 nm thickness) in a sputter coater. The coated samples were examined under scanning electron microscope (JEOL 100 CX II-ASID 4D, Japan) at 15 kV. Images were captured digitally with an Image Slave computer program for Windows.

Stomatal index:
The number of stomata in 30 randomly selected microscopic field areas from six leaves was counted per plant to obtain stomatal and epidermal cell frequency. Leaf area served per stoma was calculated based upon the stomatal frequency per unit area. Stomatal index (SI) was calculated according to the formula of Salisbury [1]: SI = S/E + S X 100 where S is the number of stomata per unit leaf area and E is the number of epidermal cells per unit leaf area. The mean values of stomatal indices were statistically analyzed using one way ANOVA and t test.

Vein islet study:
The fully expanded third pair of leaves from the terminal part of the branch was collected from ten representative plants. Leaves were immersed in 80% ethanol for 48-72 h with several changes of solvent to remove chlorophyll pigments. Leaf samples were then washed and treated with 3-5% NaOH at 60˚ C for 24-36 h. The digested leaf tissue was carefully brushed apart to obtain the leaf skeleton. These were further hardened by treating with saturated chloral hydrate solution for several days, washed, dehydrated and preserved. To study minor venation patterns, small bits were cut from the central part of the leaf skeletons (excluding mid rib and marginal parts), stained with safranin and mounted in euparol. Absolute vein islet numbers were calculated by Gupta[2]; the terminology of Hickey [3] is followed for the description of leaf architecture.

Light and SEM study on pollen:
Anthers from 70% alcohol fixed flower buds are used for pollen acetolysis following the standard methodology of Erdtman [4] Ultrastructure of pollen was analyzed by scanning electron microscope (SEM). A few drops of the pollen/ethanol mixture were placed on a SEM stub, smeared, and allowed to dry. The pollen samples then were

coated with gold, viewed and photographed with SEM. The terminologies used are in accordance with Erdtman [4]; Faegri and Iversen [5] and Walker and Doyle [6].

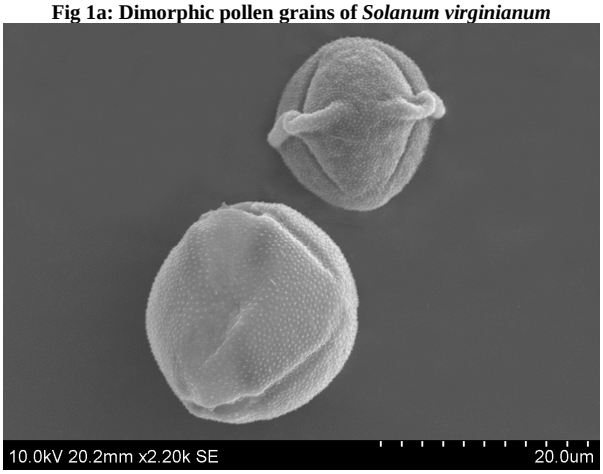
Seed surface studies:
Mature dry seeds (without fixation) were glued to aluminum stubs and coated with gold palladium to a thickness of 40 to 50 nm using a JEOL Finecoat Ion Sputter JFL 1100. The specimens were viewed in a SEM and photographed at different magnifications.

IR spectral studies:
The leaves (approximately 3-4 cm) taken from different *S. virginianum* were pooled as one sample. The samples were immediately dried in an oven for 2days at 60°C. Tablets for FTIR spectroscopy were prepared in an agate mortars, by mixing leaves powder (2 mg) with KBr (1:100 p/p). The absorbance spectra were measured between 300 and 4500cm-1. At least three spectra were obtained for each sample.

RESULTS AND DISCUSSION

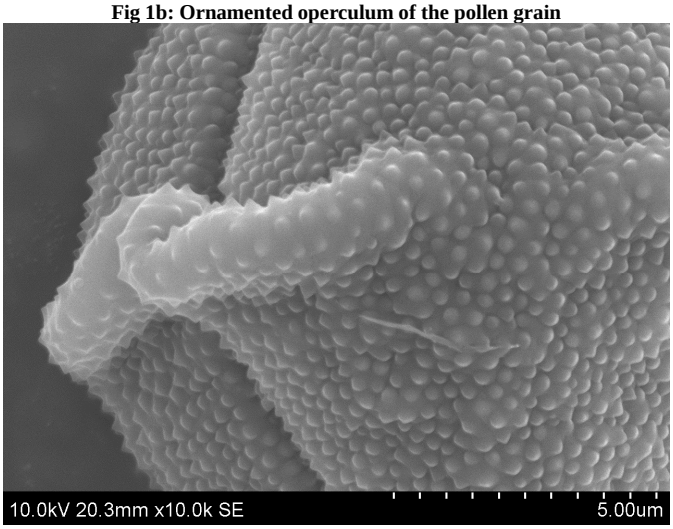
Scanning electron micrographs of the *S. virginianum* pollen revealed dimorphic pollen grains- large and small grains (Fig.1a).

Fig 1a: Dimorphic pollen grains of *Solanum virginianum*



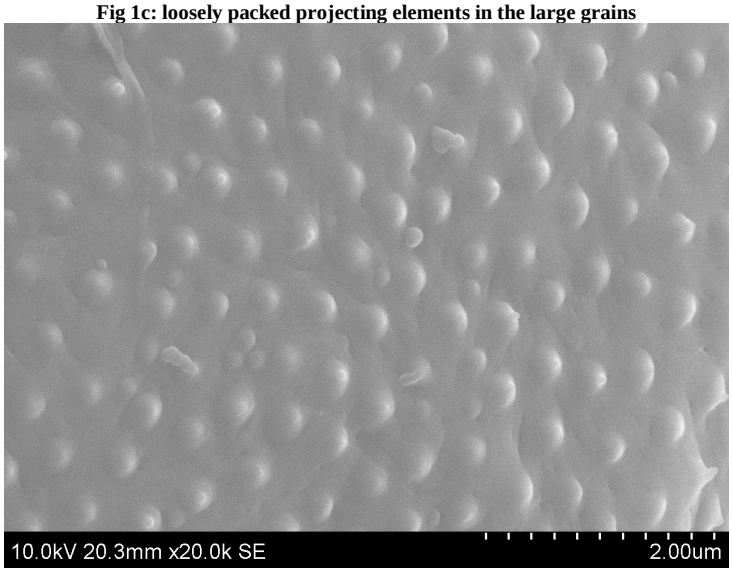
Small grains are globular, trizonocolporate, operculate with finger like operculum at the ora region. Ornamentation is micro echinate. Operculum is also ornamented (Fig.1b). i.e., triangular, obtuse, convex.

Fig 1b: Ornamented operculum of the pollen grain



Large grains are brevicolporate and colporate. Additional bulging was observed towards polar region. Projecting ornamentation elements are loosely packed in the large grains (Fig.1c).

Fig 1c: loosely packed projecting elements in the large grains



The pollen morphological structures indicate the small size of the pollen grain and the occurrence of the little complex ornamentation in species of *S. virginianum* facilitate with the expulsion process of these grains inside of the poricidal anthers. This process is generally executed through vibrations or "buzz pollination" by solitary insects. Similarly, the large pollen grains with complex ornamentation form deposits and block the anthers orifices in the species. This way, a close relationship between the pollen morphology confirmed by acetolysis treatments and the pollination syndrome by "buzz pollination" presented in this research, reinforce the observations and the hypothesis of Thorp [7]. Due to the fact that the pollen grains which are small and with little ornamentation, it is probable that the grain will be expelled more easily from the poricidal anthers during the vibration of the bees. A similar study on *Solanum* species (Solanaceae) by Edmonds [8] emphasized that the pollen grains of the *Solanum* did not represent significant morphological variations. According to this author, the *Solanum* pollen grain morphological variations refer only to the opening type and exine ornamentation level similar to *Hyoscyamus* species. Similar pollen variations are reported in *Papaver* species. The variations presented are due to many factors, such as isolation mechanisms and speciation, and genomic intermediary combinations that can, on the whole, explain the dissimilarities, found in the morphological variations of *S. virginianum.*

Fig 2 a: long glandular trichomes and

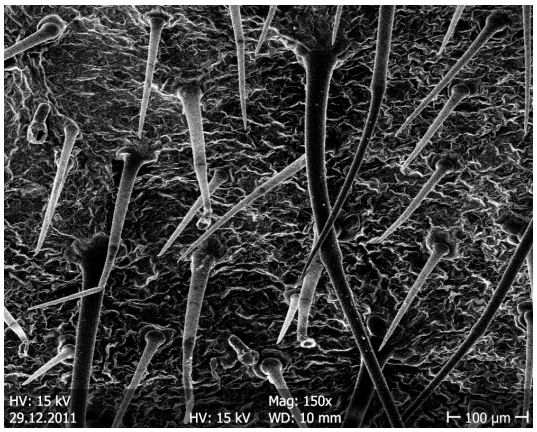
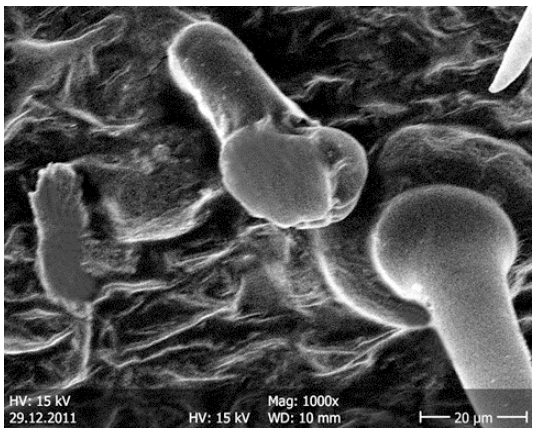


Fig 2 b: short stalked glandular trichomes non glandular trichomes

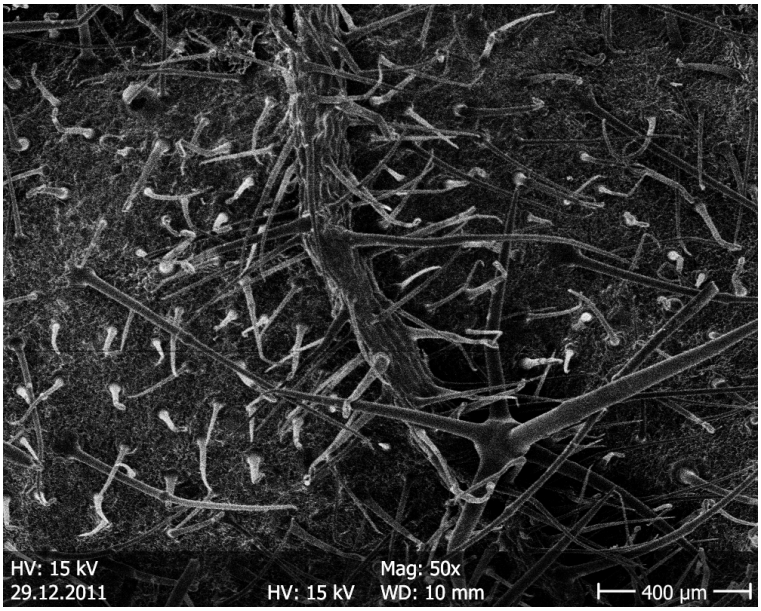


The pollen grains of the *Solanum virginianum* studied showed similarities in their pollen attributes of wall sculpture, aperture, and symmetry with other *Solanum* species. However, the greatest variation observed in pollen morphology lies in the size of their pollen. This observation was unique with taxonomical importance. The pollen shape expressed as the ratio of the length of polar axis to that of equatorial dimension is found to be more or less quinquangular in polar view and elliptic in equatorial view [4].

Epidermal hairs are diverse in *S.virginianum* i.e., upper leaf surface showed the presence of long multicellular glandular hairs with bulbous tip, short stalked multicellular glandular hairs and multicellular non glandular stalked hairs with pointed tip (Fig.2a & 2b).

Lower leaf surface showed few stellate hairs, long multicellular glandular hairs with bulbous tip and multicellular non glandular stalked hairs with pointed tip (Fig.2c).

Fig 2c: Lower leaf surface showing stellate hairs and glandular trichomes



The stellate hair was a star-like hair comprising of uniseriate rays emerging at the base of a central hair protruding at right angles from the hair producing surface.The results are comparable with the adaxial and abaxial surfaces of the leaves of *S. aculeastrum* which also showed numerous glandular and non-glandular trichomes. This is a natural phenomenon in most angiosperms [9].

Glandular trichomes are characterized by having 'heads' (glands) that release, on contact, sticky and/or toxic exudates that may entrap, irritate or potentially kill some pests [10]. These glands contain important secondary metabolites including terpenes, essential oils, flavonoids and lipophilic components[11] [12]. In most species, the source of these secondary metabolites has been attributed to the trichomes [13]. The possession of glandular trichomes is characteristic of the genus *Solanum* and of many other members of Solanaceae, with the exception of *Nicotiana glauca* and *Solandra nitida* [14]. The two types of glandular trichomes identified on the leaves of *S. aculeastrum* might be responsible for the production, accumulation and release of volatile and secondary metabolites such as the saponins and steroid alkaloids reported by Drewes and Van Staden [15]. Although, micro-morphological studies alone do not provide the information required to establish sites of synthesis in cells [11], it is plausible to assume that the therapeutic compounds in *S. aculeastrum* are produced by the glandular trichomes. Stomata are amphistomatic and anisocytic which were more prevalent on the abaxial surface than the adaxial surface. The stomatal indices of both adaxial and abaxial leaf epidermis of *S. virginianum* varied from 3.92 for upper epidermis to 8.44 for the lower. Similarly, the vein islets value was 11.7.

Seed is more or less oval which becomes little extended in the hilar region. SEM of sporoderm of seed showed compactly packed irregular units delimited by prominent ridges along the boundaries which are inconspicuous towards the hilum region (Fig. 3a & 3b).

Fig 3a: Seed surface

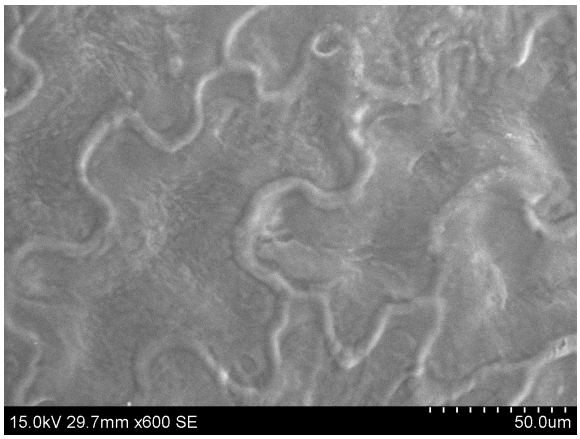
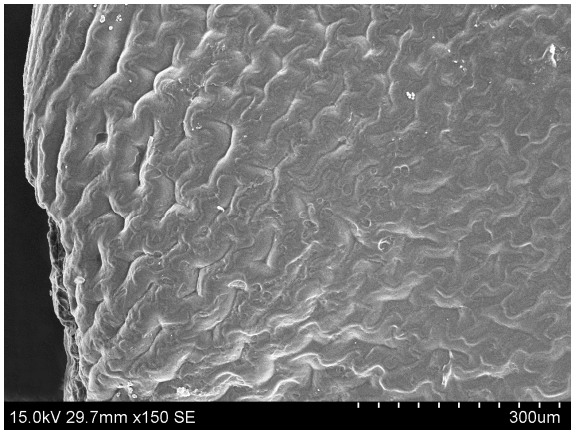
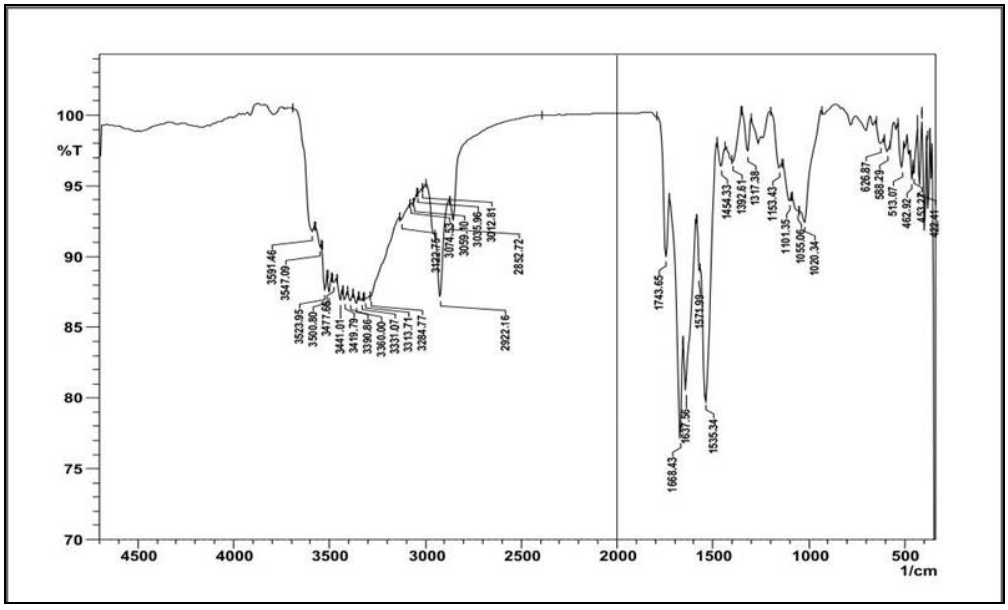


Fig 3b: Seed hilum region



Radial walls are more or less regularly sinuate-undulate, with about 7-12 corners. They are generally zigzagged and their lumens at seed border often line up in vertical with the lumens which are located near the hilum. The morphology of seed coat is usually stable and is little influenced by external environmental conditions whilst the seeds develop and ripen within the fruit. Therefore seed characteristics can provide valuable information in the delimitation and identification of species. In 1983, Edmonds used SEM for describing details of the spermoderm features in *Solanum* section *Solanum,* found all species had very similar features of the thin bands at the lateral walls of the outer epidermal cells of the testa. Lester [16] revealed the "fibrils" in the spermoderm of *S. aethiopicum, S. anguivi* and *S. violaceum*. Later, he used micromorphology of the spermoderm cells for deriving evolutionary relationships of tomato, potato and other *Solanum* [17].

Fig 4a: IR spectrum of *Solanum virginianum*



Total number of infra red peaks was 37. The IR spectra in the sharp absorption peak at 1600 – 1760 cm^{-1} are assigned to C=O stretching vibration in carbonyl compounds which may be characterized by the presence of high

content of terpenoids and flavanoids. The presence of a narrow and sharp peak at ~2925 cm^{-1} and ~2853 cm^{-1} was assigned to C-H and C-H (methoxy compounds) stretching vibration respectively. The presence of diterpenes were further proven with the absorption band of hydroxyl (3500 – 3480 cm^{-1}), ester carbonyl (1270 – 1150 cm^{-1}) and phenyl (1600, 1420 cm^{-1}). By visual recognition, there are no significant difference in the characteristic absorption bands but the intensity of certain wavelength do differ from each others especially at the fingerprint region (1800 – 800 cm^{-1}). The IR fingerprints in the range 3059 – 3122 represents the alkaloids the major drug compounds in *Solanum* species (Fig. 4a).

CONCLUSION

In conclusion, micromorphology of the leaf surface, pollen and seed coat in this study could be used for characterizing this particular species and presence of the spiral secondary thickening and fibrils or hair on the surface might explain the relation and evolution of species . The phylogenetic relationship among other species of the genus, however also deserves further investigation. The IR fingerprints reveal the phytochemicals in the plant which in turn reflect the medicinal value of *S.virginianum*.

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