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A COMPARATIVE STUDY ON THE PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHYSICO-CHEMICAL STUDIES OF SOLANUM MELONGENA L., SOLANUM XANTHOCARPUM L. AND SOLANUM TRILOBATUM L. (SOLANACEAE)

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ABSTRACT

The plant species of the family Solanaceae contain trees, shrubs and herbs with natural compounds. The Solanum genus of this family accommodates diverse groups of flowering plants, which include medicinal plants and food crops. Among medicinal plants, *Solanum melongena* L., *Solanum xanthocarpum* L. and *Solanum trilobatum* L. are commonly used in the Sri Lankan traditional and Ayurveda system of medicine. These three medicinal plants have demonstrated strong efficacy against diseases, especially in respiratory system due to their pharmacologically active secondary metabolites. No chemical and pharmacognostical comparison have been done for Sri Lankan grown species of these three plants. Hence the main objective of this study was to compare the physicochemical, phytochemical and pharmacognostical profiles of *S. melongena*, *S. xanthocarpum* and *S. trilobatum* L. to standardize the three plant materials. Pharmacognostical characters and quality control parameters of ash values, extractive values, heavy metals and qualitative phytochemical analysis were performed according to WHO guidelines. Further analysis of TLC and HPTLC fingerprint patterns of methanolic extract of these plants was also conducted. The results of ash values and extractable values were comparable with the results in Ayurveda pharmacopeia of India and water-soluble ash content of *S. xanthocarpum* (6.4 %) is higher than the *S. melongena* (2.8 %) and *S. trilobatum* (4.3 %). The hot water and hot methanol extractable matter of *S. xanthocarpum* plant is higher (23.8%, 20.4%) than the *S. melongena* (8.8%, 9.7%) and *S. trilobatum* (13.8%, 7.4%) respectively. Phytochemical analysis reveals the presence of saponins, alkaloids, tannins, phenols, flavanoids, terpinoids, cardiac glycosides and steroids. Results of these physico-chemical, phytochemical, pharmacognostical and TLC analysis can be used to compare the specific features among these three species, assess the quality and detection of any adulteration for *S. melongena*, *S. xanthocarpum* and *S. trilobatum*.

KEYWORD: Solanaceae, Pharmacognosy, Physicochemical analysis, Phytochemicals.

INTRODUCTION

The plant species of the family Solanaceae contain trees, shrubs and herbs with valuable natural compounds. Solanum is one of the largest and most hyper diversity genuses of this family which include medicinal plants and food crops (e.g. potatoes, tomatoes, egg plants and chili peppers) with wide range of growing habits in the different parts of the world.[1] This genus contains about 1500-2000 plants species and has been used for medicinal purpose from ancient time. Among those plants *Solanum melongena* L., *Solanum xanthocarpum* L. and *Solanum trilobatum* L. are commonly used in the Sri Lankan traditional system and Ayurveda system of medicine.

Solanum melongena is commonly known as *Elabatu* in Sinhala which is widely used as a food and as a medicine. It is a perennial semi-woody plant having purple colour flowers and white colour mature fruits with green veins.[2] In Sri Lanka, roots of this plant are used as a substitute for *Solanum indicum* (*Vruhati* in Sanskrit). *Vruhati* is commonly found ingredient in formulae of Ayurveda and Sri Lankan traditional system of Medicine. *Solanum xanthocarpum* is an annual herbaceous plant that is commonly known as *Katuwelbatu* in Sinhala with purple colour flowers and yellow colour mature fruits.[3] *Solanum trilobatum* is a small subscandent undershrub with numerous hooked prickles, known as *Wel-tibbatu* in Sinhala. stems of the plant are slender with long divaricate branches. Stems

are glabrous and provided with many flattened, hooked and decurved very sharp prickles. Leaves are simple and alternate and small. Flowers are violet purple in colour and fruit is a berry.[4]

The nutraceutical and pharmaceutical values of the solanum species are high as foods and as medicines. This is due to the presence of bioactive phytoconstituents such as steroidal saponins, steroidal alkaloids, glycoalkaloids, terpenes, flavonoids, lignains, phenolic compounds, etc. Among them, steroidal alkaloids and glycoalkaloids have a special status in traditional and modern systems of medicine possessing a wide range of bioactivities such as antimicrobial, analgesic, hepatoprotective, immunomodulatory, anticancer, etc.[5,6,7]

In Sri Lanka, high demand can be observed for these three species, *S. melongena*, *S. xanthocarpum* and *S. trilobatum* which cultivated for commercial purpose in various parts of the Sri Lanka. These three plants are widely used for the diseases in the respiratory track specially for asthma, cough, rhinitis conditions, bronchitis and other diseases in respiratory system, gastrointestinal system and in blood circulatory system[8]. Preparing standards are need of time due to the increased demand of herbal materials and hence, this study was planned to compare the physicochemical, phytochemical and pharmacognostical profiles of *S. melongena*, *S. xanthocarpum* and *S. trilobatum* L. in order to identified specific characters and develop standard parameters for crude drugs used in herbal drug manufacturing system.

Fig. 1: *Solanum melongena* Plant and Usage part (Dried roots).

Fig. 2: *Solanum xanthocarpum* Plant and Usage part (Dried whole plant).

Fig. 3: *Solanum trilobatum* Plant and Usage part (Dried whole plant).

MATERIALS AND METHODS

Collection and Identification of plant materials

Roots of *S. melongena* and whole plant parts (Panchangaya) of the *S. xanthocarpum* and *S. trilobatum* were collected from Herbal Garden of Institute of Indigenous Medicine, Rajagiriya of Colombo district, Sri Lanka and authenticated by the Curator of National Herbarium of Peradeniya, Sri Lanka and Department of Ayurveda Pharmacology, Pharmaceutics and Community Medicine, Faculty of Indigenous Medicine, University of Colombo, Sri Lanka.

Pharmacognostical study of raw material

Plant parts were macroscopically and microscopically identified according to standard methods mentioned in Ayurveda Pharmacopeia of India (API). All the images presented were taken by the author using a Samsung digital camera (PL 211 Samsung, London, UK).

Physicochemical studies

Physicochemical studies of the dried plant parts were done according to the WHO herbal drug standardization guidelines. Hot and cold-water extractable matter, hot and cold methanol extractable matter, total ash, acid insoluble ash and water-soluble ash were determined.

Preparation of extracts

The air-dried powdered materials (200 g from each) were refluxed with methanol and water in for 6 hrs respectively. Prepared extracts were filtered separately and dried by evaporation using rotary evaporator (Buchi, Rotavapor R-210, Switzerland) and the obtained extracts were kept in the refrigerator for further investigation.

Qualitative phytochemical analysis

Phytochemical analysis of hot water and hot methanol extracts of three plants was done for the presence of alkaloids, phenols, terpinoids, flavonoids, steroids, saponins, cardiac glycosides using standard procedure describe by Goveas[9] and Dahanayake and co-workers[10] with some modifications.

Determination of heavy metals

The Inductively Couple Plasma Spectrometry (ICP-MS) was used to analyze the heavy metals (Pb, Cd, As, Hg) in the selected plant materials.

Development of Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) fingerprint profiles

The fresh plant of *Solanum xanthocarpum*, *Solanum trilobatum* and roots of *Solanum melongena* were washed and dried in shade. The dried plant materials were grinded to fine powder and extractions were carried out by using 5g of powdered plant materials with 100 ml of methanol. Prepared extracts were filtered and dried by evaporation using rotary evaporator. One gram of each methanol extract was dissolved in 5 ml of methanol and spotted (10 µl from each extract) on a pre-coated TLC plate. Then TLC fingerprint profiles of *S. xanthocarpum*, *S. trilobatum* and *S. melongena* were developed by using Dichlomethane: Methanol: Cyclohexane in a ratio of 3:0.5:1.5 v/v. TLC fingerprint profiles of three extracts were scanned using a HPTLC (CAMAG HPTLC (Switzerland) comprising CAMAG Linomat5 applicator, CAMAG TLC scanner3, Wincats software, Version 1.44).

RESULTS AND DISCUSSION

Pharmacognostical study

Macroscopic and microscopic features were recorded for *S. xanthocarpum*, *S. trilobatum* and *S. melongena* plants.

Macroscopical features of *Solanum melongena*

S. melongena is a perennial herb and it is often semi-woody at base. The plant is erect, 30-85 cm high with numerous divaricately spreading branches. Branches contain scattered straight, compressed and yellow colour prickles and younger parts covered with dense stellate tomentum. Leaves are simple, alternate and exstipulate, oblong to oval and usually unequal sided at base. Leaves also contain straight erect prickles and it is few in number. Flowers are bright purple in colour and large. Fruit is a globose berry, 2.5 cm long, surrounded by the much-enlarged calyx. The fruit is yellow or white in colour with green veins and seeds numerous.

Microscopical features of *S. melongena*

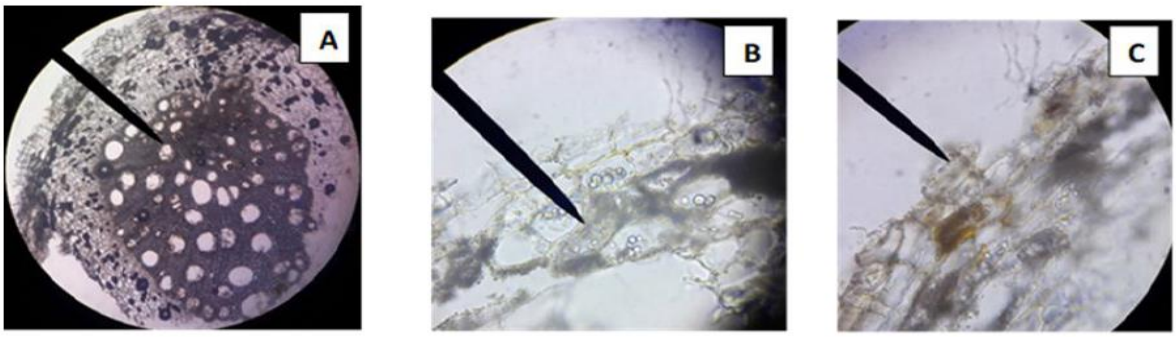

Fig. 4: Microscopical view of *solanum melongena* root, a-diagrammatic section of root (lp), b-starch grains, c-brown colouring matter.

Macroscopical features of *Solanum trilobatum*

Solanum trilobatum is a small subscandent shrub with many hooked prickles. Stems are slender with long divaricated branches with a few stellate hairs on the young shoots. Mature stems are glabrous with numerous flattened hooked and de-curved sharp prickles. Leaves are simple, alternate and small, 1.8—3.7 cm long, rotund-ovate in outline. Leaves are irregularly 3 or 5-lobed, glabrous often with 2 or 3 small, curved prickles on midrib. Flowers are regular and violet purple in colour. The fruit is a globose berry, 0.8 cm long, smooth and scarlet in colour.

Microscopical features of *Solanum trilobatum*

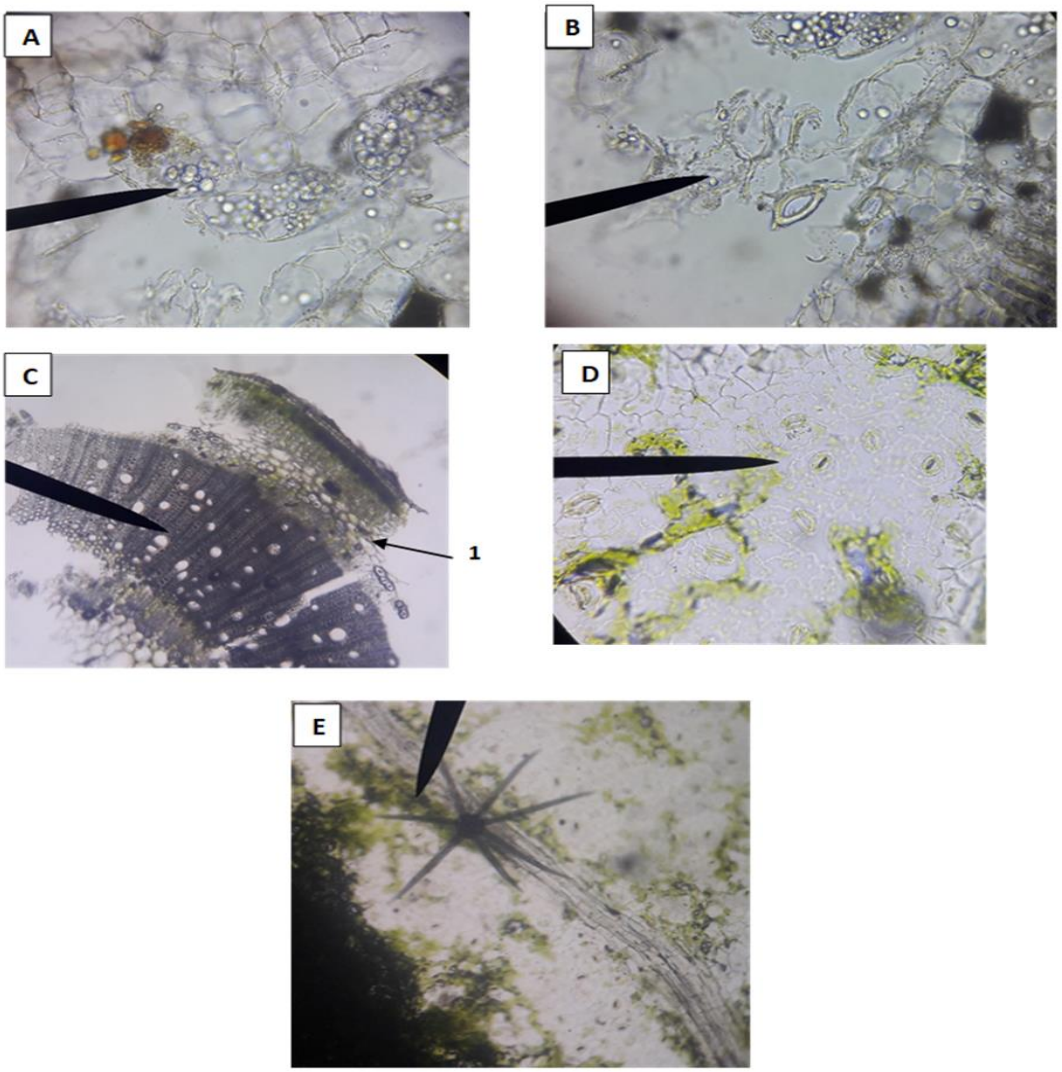

Fig. 5: Microscopical view of *solanum trilobatum* A- root - simple starch grains & colouring matter, B-thick-walled fibers in the root, C- (1) discontinuing pericycle layer of the root, D- anisocytic stomata of leaf, E- multi armed, multicellular covering trichome of leaf.

Macroscopical features of *Solanum. xanthocarpum*

The stems of the plant were herbaceous, prickly with prominent nodes and internodes, green when fresh and young branches were covered with numerous hairs. The mature stems were glabrous and its furrows were more prominent. Leaves are petiolate, exstipulate and ovate to oblong in shape and green in colour. Veins and midribs were full of sharp prickles. The root of the plant was long, cylindrical, tapering and bearing a number of fine longitudinal and few transverse wrinkles. Flower were ebracteate, pedicellate, regular, complete and bluish purple in colour. Fruit was a berry and globular in shape and unripe fruits variegated with green and white strips, ripe fruit shows different yellow and white shades. The seeds of the fruit were circular, flat, numerous, embedded in a fleshy mesocarp and the taste was bitter and acrid.

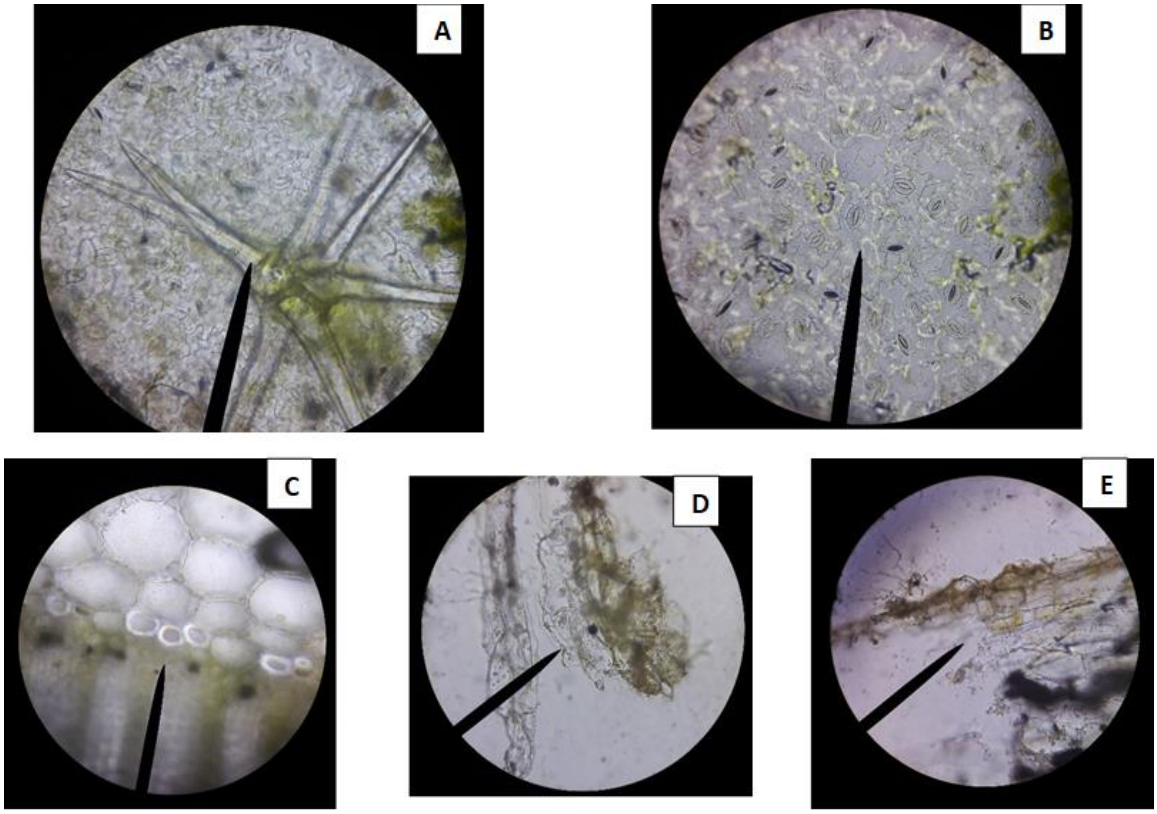

Fig. 6: Microscopical view of *solanum xanthocarpum*a-trichomas-leaf, B-stunted stomata-leaf, C-thick-walled fibers-stem, D-microcrystals-root, E-starch granules and resinous matter- root.

Physico-chemical studies

The results of physico-chemical studies are summarized in table 1 and 2. Total ash, water soluble ash, acid insoluble ash, hot water and cold-water extractive values, hot methanol and cold methanol extractive values were determined for three solanum species.

Determination of Ash values

Table 1: Determination of ash values of *solanum melongena, solanum Xanthocarpum* and *Solanum trilobatum.*

Plant name	Total ash	Water soluble ash content	Acid insoluble ash content
Solanum melongena	5.24 % ± 0.0	2.17 % ± 0.1	0.03% ± 0.01
Solanum xanthocarpum	10.01% ± 0.08	6.36% ± 0.06	0.03% ± 0.01
Solanum trilobatum	9.46 % ± 0.07	4.26 % ± 0.09	0.73 % ± 0.0

Table 2: Extractive values of *solanum melongena, solanum Xanthocarpum* and *Solanum trilobatum*.

Plant	Solvent – water		Solvent – Methanol	
	Cold	Hot	Cold	Hot
Solanum melongena	6.41 % ± 0.15	8.8 % ± 0.5	4.7 % ± 0.0	9.7 % ± 0.1
Solanum xanthocarpum	12.8% ± 0.1	23.8% ± 0.9	3.8% ± 0.1	20.4% ± 0.8
Solanum trilobatum	10.9 % ± 0.3	13.8 % ± 0.9	5.6 % ± 0.2	7.4 % ± 0.3

Table 3: Heavy metal analysis of *solanum melongena, solanum Xanthocarpum* and *Solanum trilobatum.*

Plant	Concentration (mg/kg)			
	Pb	Cd	As	Hg
Solanum melongena	0.13	0.05	Not detected (<0.05)	Not detected (<0.05)
Solanum xanthocarpum	0.17	Not detected (<0.05)	0.05	0.11
Solanum trilobatum	0.30	0.08	0.10	0.07

Qualitative phytochemical analysis

Results of qualitative phytochemical analysis of 3 solanum species were summarized in table 3.

Table 4: Results of phytochemical analysis of *solanum melongena* (sm), *solanum xanthocarpum* (sx) and *solanum trilobatum* (st)

Phyto constituent	SM		SX		ST	
	Hot water extract	Hot methanol extract	Hot water extract	Hot methanol extract	Hot water extract	Hot methanol extract
Saponins	+++	+	+++	-ve	+++	+++
Tannins						
*Folin reagent test	++	++	+++	+++	++	++
*$Fecl_3$ Test	-ve	-ve	+++	+++	++	++
*Pb acetate	++	++	+++	+++	+++	+++
*Vanilline test	-ve	-ve	-ve	-ve	-ve	-ve
Alkaloids						
*Mayer's reagent test	-ve	-ve	-ve	-ve	-ve	-ve
*Picric acid test	-ve	-ve	-ve	-ve	-ve	-ve
*Wagner test	+++	-ve	++	++	++	-ve
*Tannic acid	-ve	-ve	-ve	-ve	-ve	-ve
Steroids	+++	-ve	-ve	-ve	-ve	+++
Cardiac glycosides	++	++	++	++	+++	+++
Flavanoids	+++	+++	++	++	++	-ve
Phenols						
*Folin reagent test	+++	+++	+++	++	++	++
*$Fecl_3$ Test	-ve	-ve	+++	+++	++	++
*Pb acetate	+++	+++	+++	+++	+++	+++
*Vanilline test	-ve	-ve	-ve	-ve	-ve	-ve
Terpenoids	++	+++	+++	+++	++	-ve

+++ - high amounts ++ - moderate amounts + - low amounts

Phytochemical analysis reveals the presence of phenols, saponins, tannins, steroids, flavonoids, terpenoids and cardiac glycosides.

TLC and High Performance Thin Layer Chromatography studies

TLC profile was observed in *Solanum melongena* bearing Rf values of 0.3, 0.6, 0.7 and 0.76; *Solanum xanthocarpum* bearing Rf values of 0.3, 0.6, 0.7, 0.76 and 0.8; *Piper longum* bearing Rf values of 0.2, 0.5, 0.68, 0.8 and 0.9 and *Solanum trilobatum* bearing Rf value of 0.3. 0.6 and 0.7 at 245 nm.

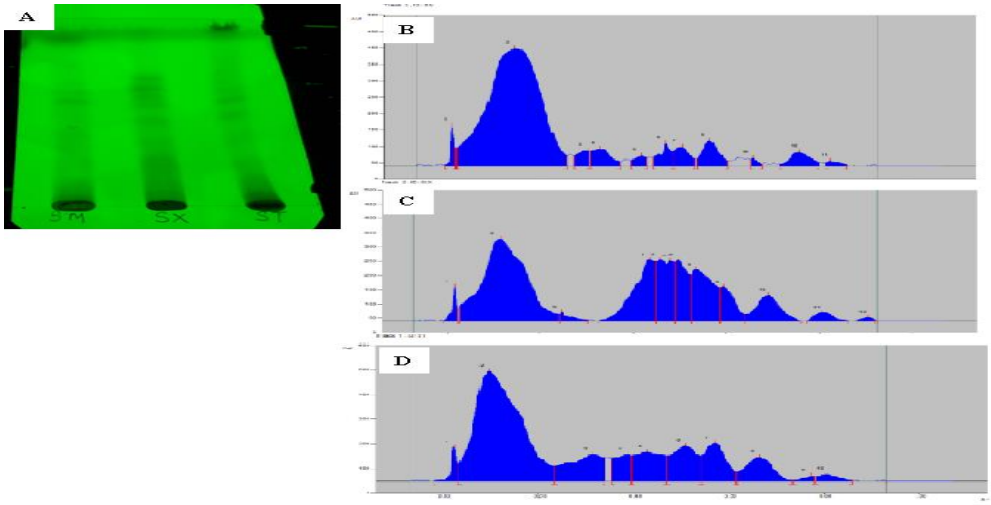

Fig. 7: A: Tlc fingerprint profiles of methanolic extract of *solanum melongena (1), solanum xanthocarpum (2)* and *solanum trilobatum (3)* at 254 nm B: hptlc fingerprint profile of *solanum melongena, C: solanum xanthocarpum* d: *solanum trilobatum.*

DISCUSSION

Solanum melongena, *Solanum xanthocarpum* and *Solanum trilobatum* plants are very useful medicines in Ayurveda and the traditional system of medicine in Sri Lanka which are mainly used for the diseases of the respiratory system. As per Ayurveda Pharmacopea of India and Sri Lanka, these three species are included in many herbal and herbo-mineral pharmaceutical preparations such as decoctions, pills, pastes, medicated oils etc.

Quality assurance of raw materials and herbal medicines is essential to obtain quality and effective drugs in the treatment. Standardization and quality control are the tools that we use to assure the quality of products. In the present scenario the demand for plant based raw medicines is increasing globally in developed as well as developing countries.

Most of the herbal preparations are a mixture of several ingredients. Their cumulative effect increases the efficacy of the drug in curing the diseases of humans. Bioassays play an important role in the process of herbal drug standardization and quality control. Pharmacognostical studies, detection of various physico-chemical parameters and phytochemical parameters are being used to standardize the herbal medicines. At the initiation of the pharmaceutical manufacturing process of the plant-based medicines, pharmacognostical studies are more important to identified genuine plant materials which directly effect on the potency of the final product.

At present, adulteration of plant raw materials with low quality plant materials is a burning problem in herbal drug industry which shows major effects in the commercial use of herbal medicines.[11] This situation also gets worse by the misidentification of herbal materials due to lack of correct knowledge about the medicinal plants. Therefore, an examination to determine macroscopic and microscopic characteristics is the first step for confirming the identity and degree of purity of herbal materials and it should be done before any tests are undertaken.[12]

Identification of the anatomical features of *S. melongena, S. xanthocarpum and S. trilobatum* plants can be used to confirm the botanical identity with Family solanaceae. The importance of pharmacognosy has been broadly discussed in herbal pharmaceutical field in recent years. The pharmacognostical studies include parameters which help in identifying adulteration in fresh plant materials as well as materials in dry powder from. When plants are in dried form or in powder form it fails to show its morphological identity and is easily prone to adulteration. Hence pharmacognostical studies ensure plant identity which will help and prevent adulterations.[13] The roots of *S. melongena* and *S. trilobatum* and whole plant of *S. xanthocarpum* are widely used in Ayurveda and Sri Lankan traditional system of medicine. Hence the results of this macroscopical and microscopical studies will provide references for identification of these 3 Solanum species.

Ash values of herbal raw materials are used to determine the quality and purity of crude materials. It reveals the presence of various impurities like carbonates, oxalate and silicate in the plant materials. The number of inorganic compounds can be detected by analyzing water soluble ash content. Also, silica and contamination with earthy materials can be detected by analyzing the acid insoluble ash content. Moisture contents of the drugs should be within the reference values mentioned in Ayurveda pharmacopeia and minimal level of moisture can discourage the growth of bacteria and fungi during the storage.[14]

The ash values of 3 plant materials of this study were measured according to the WHO guidelines on herbal drug standardization and results were comparable with Ayurveda pharmacopieal reference values.

Estimation of extractable matters determines the amount of active constituents in each amount of plant material with a specific solvent which extracted. The extractions of any raw materials with a particular solvent yield a mixture containing different phytochemicals of the plants. The composition of these phytochemicals depends upon the nature of the drug and the solvent that we used. It also gives a clue whether the crude drug is exhausted or not.[15]

Herbal materials can present health risks due to the presence of toxic materials namely, Hg, As, Cd and Pb which are harmful to humans.[16] According to the WHO recommendations, herbal medicines should be free from heavy metals or within the limits of heavy metals. Heavy metals are deposited in different parts of the plants which enter through the biological cycle of the plant.[17] The concentration of Hg, As, Cd and Pb detected in the *S. melongena* roots, *S. trilobatum* roots and whole plant of *S. xanthocarpum* using the atomic absorption spectrophotometer were found to be within the acceptable limits.

Research on analysis of phytochemicals in these three plant species of Solanaceae family are rich in phytochemicals such as phenols, tannins, flavonoids, alkaloids, terpenoids, etc.[18] The therapeutic potential of these bioactive compounds helps to cure several diseases, especially diseases in the respiratory system.[19] The quantitative analysis of the phytochemicals in hot water and hot methanol extracts of *S. melongena* roots, *S. trilobatum* roots and whole plant of *S. xanthocarpum* showed the presence of the saponins, phenols, flavonoids, tannins, terpinoids and cardiac glycosides. Phytochemical analysis is very important to determine the quality of herbal materials.

Chromatographic analysis methods are widely used in pharmaceutical industries for the correct identification of

raw materials and detecting the quality of the herbal materials.[20] High Performance Thin Lyer Chromatography (HPTLC) is a developed modern adaptation of TLC with advanced separation efficiency and detection limit.[20] Results of HPTLC fingerprint patterns of methanolic extracts of 3 species of Solanum genus showed presence of various phytoconstituents. Further, the peaks demonstrated a similar pattern of all three extracts with changing of peak heights.

CONCLUSION

Pharmacognostical features of these plants will be useful when detecting the adulterants of herbal raw materials. Trichomas are present in the leaves of *Solanum xanthocarpum* and *Solanum trilobatum* show same characters belongs to genus solanum. It also reveals that the structure of the tricoma can be used to identify one plant from another plant of the same genus appropriately.

Results of this physico-chemical, phytochemical and TLC analysis can be used to compare the relevant parameters of these three species and can be used as a reference for setting limits for the quality assurance of *S. melongena*, *S. xanthocarpum* and *S. trilobatum*.

Conflicts of interest

The authors declare no conflict of interest.

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