Morpho-genetic Diversity and Anti-bacterial Activity in Root Extracts of Nine Solanaceous Species

D.T. Jeyakumar, U.H.A.J.De Silva[1], D.R.R.P. Dissanayake[1]
M.D.M.Chamikara[1] and S.D.S.S. Sooriyapathirana[1*]

Postgraduate Institute of Science,
University of Peradeniya,
Sri Lanka

ABSTRACT: *The family Solanaceae is immensely important because it has many species with food and medicinal values. There are nine major Solanaceous spp. Namely S. Melongena L. (Wambatu and Elabatu), S. virginianum L. (Katuwelbatu), S. torvum Sw. (Thibbatu), S. violaceum Ortega (Thiththathibbatu), S. trilobatum L. (Welthibbatu), S. hispidum Pers. (Gonabatu), S. pubescens Willd.(Walthibbatu), S. nigrum sensu Trimen (Small Kalukenweriya) and Datura stramonium L.(Sudu aththana) collected from Uva Province, Sri Lanka, a prominent area of growing these species. Currently these species are underutilized in spite of their medicinal importance. No detailed morpho-genetic and medicinal level characterization of these species has been reported to promote their economic use. Therefore, the present study was conducted to assess the morpho-genetic diversity and the antibacterial activity of these Solanaceous spp. The plant height, canopy width and leaf and fruit morphology were highly variable among these species. DNA barcoding using locus matK revealed a total of eight haplotypes. The polymorphism in matK locus is not diverse enough to set the species limit of the genotypes within S. melongena and to distinguish Thiththathibbatu from Walthibbatu. The antibacterial activity assay using ethanol extracts of roots demonstrated that all these species have inhibitory activity against Gram positive Staphylococcus aureus (NCTC4838) and Gram negative Escherichia coli (JM109).*

Keywords: *matK, Solanum, medicinal plants, antibacterial activity, morphological diversity*

INTRODUCTION

Solanaceae is an economically important family which consists of many species used for medicinal, culinary, cosmetic and ornamental purposes (Mueller *et al*., 2005; Sekara *et al*., 2007). The genus Solanum comprises nearly half of the species classified under family Solanaceae (102 genera and 3000 species). *S. melongena* (brinjal or eggplant) fruits contain carbohydrates, crude fibre, protein, vitamins and minerals (Edem *et al*., 2009; Chinedu *et al*., 2011) which are essential components in human diet. The fruits of eggplants and related species contain very high amount of ascorbic and linoleic acids along with valuable flavonoids, alkaloids, tannins, saponins and numerous phytochemicals (Chen *et al*., 2009; Chinedu *et al*., 2011; Ogundajo *et al*., 2013).

[1]Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Sri Lanka
*Corresponding Author:sunethss09@gmail.com

Medicinal plants possess greater significance in current medical practices and the medicinally valuable Solananeous species are highly important in this context (Nadkarni, 1927). The World Health Organization (WHO) has declared that the monetary value of medicinal plant trade has reached 500 million USD and will be increased up to more than five trillion USD in the upcoming years (WHO, 2013) since herbal medicines are proven as less toxic with fewer side effects compared to synthetic medicines (Pari and Umamaheswari, 2000). Many domesticated Solanaceous species are used for therapeutic purposes and in steroid industry as they are rich in phytochemicals namely diosgenin and solasodine (Gbile and Adesina, 1988). Avicenna, the father of modern medicine, stated that "eggplant is the medicinal vegetable of modern world" (Sekara *et al*., 2007). Nasunin, a flavonoid found in eggplants known to protect cell membranes from damages by acting as an antioxidant (Noda *et al*., 2000) and other flavonoids in eggplants confer hypolipidemic effects (Sudheesh *et al*., 1997). Because of the presence of valuable flavonoids and alkaloids (Trease and Evans, 2011), *Solanum* spp. have been routinely utilized in indigenous medicine to cure numerous ailments including obesity, asthma, allergic rhinitis, nasal catarrh, skin infections, swollen joint-pains, gastro-esophageal reflux, constipation, dyspepsia, diabetes, glaucoma, rheumatism, hyperlipidemia, cold, fever and dizziness (Igwe *et al*., 2003; Odetola *et al*., 2004; Bello *et al*., 2005). The saponins in eggplants can reduce high blood pressure and prevent cancer (Dhellat *et al*., 2006) and also used as adjuvants in vaccines (Ross and Kassum, 2012) along with solasoline, solamargin and diosgenin (Saijo *et al*., 1982). Several studies revealed that Solanaceous species contain many phytochemicals such as tropein, sapagenin, tomatidine, solverbascine, progesterone, 16-progenolone and yamogenin (Segal *et al*., 1977; Adam *et al*., 1979 and 1980; Jain and Sahoo, 1981a and 1981b; Bose and Gosh, 1980). The phenolic compounds present in Solanaceous plants are used in the preparation of sunscreens (Dobson, 2010). Solanaceous species, *S. xanthocarpum*, *S. torvum*, *S. nigrum*, *S. trilobatum* and *S. melongena* possess inhibitory effects against the bacterial pathogens *Psuedomonas aeuroginosa*, *Bacillus subtilis* and *B. typhi* (Raj and Suchithra, 2009; Doss *et al.,* 2009; Sheeba, 2010). *Solanum* spp. are widely used as a source of fodder, fuel, dye and ink (Fontem and Schippers, 2004; Devi *et al*., 2012).

In Sri Lanka, *Solanum* spp. are generally known as '*Batu*' (in *Sinhala*) and '*Kaththiri*' (in Tamil). There are 10 species of *batu* found in Sri Lanka, namely *S. melongena* (*Wambatu*, *Elabatu*), *S. virginianum* (*Katuwelbatu*), *S. torvum* (*Thibbatu*), *S. violaceum* (*Thiththathibbatu*), *S. trilobatum* (*Welthibbatu*), *S. hispidum* (*Gonabatu*), *S. pubescens* (*Walthibbatu*), *S. lasiocarpum* (*Malabatu*) and *S. capsicoides* (*Dehelbatu*) (Dassanayake and Fosberg, 1988). These *Solanum* spp. except *S. lasiocarpum* and *S. capsicoides* are predominantly grown in *Uva* Province, Sri Lanka. The important medicinal values of these Solanaceous species are given in Table 1. The rural livelihood of the *Uva* Province greatly depends on subsistent farming. There is a significant demand for the roots of medicinally important Solanaceous species that are commonly grown in *Uva* Province. However, they are still in the underutilized status due to socio-economic reasons and lack of morphological and biomolecular studies to efficiently characterize different species. However, India has paid significant attention on these Solanaceous species and characterized them for efficient utilization in the herbal medicine market and Ayurvedic medical practices (Ven Murthy *et al*., 2010; Tamboli *et al*., 2015).

If the Solanaceous species found in a certain area are to be exploited economically, they must be morphologically characterized as the first step to document their variation (Yousaf *et al.,* 2008). Then their taxonomic identities can be established using the sequences of DNA barcoding loci such as *matK* (Li and Zhou, 2007; Gao *et al.,* 2008), a quickly evolving coding region in plastid genomes (Fazekas, 2008; Lahaye, 2008).

Although the roots of Solanaceous species are used in Sri Lankan Ayurvedic medical practices and herbal medical preparations, their exact bio-activities have not been characterized. Especially it is imperative to know the antibacterial activity present in the roots to efficiently utilize them in herbal medicine. Therefore, the present study was conducted to assess the morpho-genetic diversity of commonly found Solanaceous species in the *Uva* Province and to document the antibacterial activity of their roots against model pathogenic bacterial strains.

MATERIALS AND METHODS

Sample Collection

A total of eight species in the genus *Solanum* and as an out-group, *Sudu Aththana* (*Datura stramonium*), a Solanaceous species, were collected from the areas *Badulla*, *Bandarawela*, *Welimada*, *Haputale* and *Wellawaya* in *Uva* Province. Two *Wambatu* and four *Elabatu* cultivars were collected from *S. melongena* (Table 1). Henceforth, these eight species and six different cultivars of *S. melongena* are referred to as genotypes. The morphological parameters of shoots, leaves and fruits were recorded using five randomly selected plants/genotype and immature shoots were collected and stored at -80 ºC for DNA extraction.

Table 1. Solanaceous species/genotypes and their important medicinal properties

Species		Medicinal properties/uses
Vernacular name	**Botanical name[$]**	
Gonabatu	***S. hispidum***	-
Katuwelbatu	***S. virginianum*** *S. surattense* *S. xanrhocarpum* (Britto *et al.*, 2011; Abbas *et al.*, 2014)	• A component in Ayurvedic Medical Preparations (AMP) *Dashamoolarishtaya* and *Lagupanchamula* (Jayanthy *et al.*, 2012). • Use to treat itching, fever, cough and cold; make adipose issues thinner; rectify the problems in seminal ejaculation (Singh *et al*., 1996; Devi *et al*., 2012). • Possess larvicidal property against *Anopheles* spp., *Aedes* spp. and *Culex* spp. (Bansal *et al*., 2009). • Has antidiabetic potential (Gupta *et al*., 2005).
** Large Elabatu* ** Long Elabatu* ** Round Elabatu without prickles* ** Round Elabatu with prickles*	***S. melongena***	• A component in AMPs *Dashamoolarishtaya* and *Lagupanchamula* (Jayanthy *et al*., 2012). • Possess anticonvulsant (Adesina, 1985), analgesic, sedative, hypotensive, antipyretic and cardiovascular healing properties (Vohora *et al*., 1984; Ojewole and Adesina, 1983). • Act as anti-inflammatory agents, menopause controllers, antioxidants, free radical scavengers, anti-allergic and antimicrobial substances (De Sousa *et al*., 2007). • Protects cell membrane from damages by acting as an antioxidant (Noda *et al*., 2000) and has hypolipidemic effect (Sudheesh *et al*., 1997).
Sudhu Aththana	***Datura stramonium***	• Possess analgesic, antiasthmatic (Soni *et al*., 2012), antioxidant (Kumar *et al*., 2008) and antibacterial properties (Taye *et al*., 2011).
Small Kalukenweriya	***S. nigrum*** *S. americanum* (Britto *et al*., 2011)	• Act as analgesic and sedative agent with powerful nacrotic properties (Taherpour *et al*., 2013) • Important ingredient to cure tuberculosis (Kaushik *et al*., 2009) • Use to treat mouth ulcers and other skin diseases (Jain, 1968; Edmonds and Chewya, 1997)

		• Possess hepato-protective, diuretic, antipyretic (Jain *et al*., 2011) and anticancer activity on cervical carcinoma (Jian *et al*., 2008) • Has strong antidiabetic activity (Sohrabipour *et al*., 2014)
* *Thibbatu*	***S. torvum***	• Possess antibacterial effect against *P. aeuroginosa*, *B. subtilis* and *B. typhi* (Raj and Suchithra, 2009; Doss *et al*., 2009; Sheeba, 2010), *Klebsiella pneumoniae* (Parameswari*et al*., 2012), *Salmonella typhi* (Ahmed *et al*., 2013), *E. coli* and *S. aureus* (Singh *et al*., 2007; Cuthbertson and Murchie, 2005; Amer *et al*., 2013)
* *Thiththathibbatu*	***S. violaceum*** *S. indicum* (Jayanthy *et al.*, 2012)	• A component in AMPs *Dashamoolarishtaya* and *Lagupanchamula* (Jayanthy *et al.*, 2012) • Use to cure rheumatism, sore throat (Devi *et al*., 2012), inflammation, toothache, ascites, oedema and wound infections (Huang *et al*., 2008). • Possess antiinflammatory, antihypersensitive and wound healing properties (Ma *et al*., 2006; Bahgat *et al*., 2008).
Walthibbatu	***S. pubescens***	-
* *Wambatu hybrid* (eggplants) * *Wambatu local* (eggplants)	***S. melongena***	• Possess no medicinal value, potentially allergenic if included in herbal medical preparations (Rao, 2011, Marasinghe *et al*., 2016)
Welthibbatu	***S. trilobatum***	• A component in AMPs *Dashamoolarishtaya* and *Lagupanchamula* (Jayanthy *et al.*, 2012)

*Use as a vegetable beside medicinal uses. $The frequently used botanical name is given in bold case letters.

Assessment of Morphological Diversity

The plant height and canopy width at maturity of the field grown plants were measured. The colour of the stem was recorded before maturity (prior to onset of bloom) and at maturity according to Vejdemo-Johansson *et al*., (2014). The presence of hairs and prickles on the stem at maturity was also recorded. The petiole length, colour (Vejdemo-Johansson *et al*., 2014) length, breadth, angle of the leaf with stem, the presence of prickles on the leaves and hairs were recorded in five leaves per plant at mature stage as the parameters related to leaf morphology. Similarly, for floral morphological parameters; length and breadth of petals and sepals, flower diameter, stamen length, numbers of prickles in the pedicel and petal colour were recorded. For fruit parameters; orientation of the fruit from the stem, fruit shape, curvature, apex shape, presence of surface hairs, degree of glossiness, colour (Vejdemo-Johansson *et al*., 2014) and colour distribution, length, breadth, weight, stalk length and number of prickles in the stalk were recorded at the same growth stage.

Assessment of Molecular Diversity

DNA Extraction, PCR and Sequencing

Genomic DNA was extracted from young tender leaves using Dneasy® Plant Mini Kit (Qiagen, Solna, Sweden) (Catalog No. 69104) and stored at -20 ºC. The PCR was carried out using *matK* universal barcoding primer pair for plants (forward primer: 5' CGA TCT ATT CAT TCA ATA TTT C 3' and reverse primer: 5' TCTAGCACACGAAAGTCGAAGT3'). DNA amplification was performed in 15 µL reactions containing 1×GoTaq® Green Master Mix (Promega Corporation, Madison, Wisconsin, USA, Catalog No. M7122), 0.2 µM each of forward and reverse primers and 1.0 µL of DNA template (60 ng / µL) employing a Thermal Cycler (Takara, Japan, Catalog No. TP350) under the PCR conditions: initial denaturation of 5 min at 94 °C; 35 cycles of 30 sec at 94 °C; 1.5 min at 48 °C; 2.5 min at 72 °C; and a final extension step of 10 min at 72 °C. The PCR products were visualized in a 2 %

ethidium bromide stained agarose gel and purified using Promega Wizard® SV Gel and PCR Clean-Up System (Catalog No. A9282) to remove excess dNTPs, primers and primer dimers. Forward DNA sequencing of the purified PCR fragments of each Solanaceous spp. was carried out using Sanger Sequencing based ABI 3500 Series Genetic Analyzer (Applied Biosystems®, Catalog No. 4440462).

Assessment of Antibacterial Activity of Root Extractions

The roots were collected from the plants used for morphological measurements and dried under sunlight. Dried root samples were then crushed into fine particles using a food grade grinder and subjected to ethanol extraction using a rotary evaporator. Extracts were dissolved in Dimethylsulfoxide (DMSO) and alcohol was taken out completely. Antibacterial activity of these extracts were tested using agar disc-diffusion method against two model pathogenic species *Escherichia coli* (JM109) and *Staphylococcus aureus* (NCTC 4838).A total volume of 20 ml autoclaved Mueller Hinton Agar (MHA) medium was poured into each sterile petri dish and allowed to solidify. Then 100 µL of each bacterial cell culture was spread on separate petri dishes evenly. Autoclaved Whatman filter paper discs having diameter of 6 mm were moistened with 30 µl of each root extract and placed on the medium of the MHA plate. A control was also maintained with DMSO alone. Eventually the inoculated plates were incubated at 37 $^{\circ}$C for 24 hours. The diameter of the zone of inhibition (DZBI) around each paper disc was recorded as an indicator of antibacterial activity. Each experiment was performed in triplicate.

Data Analysis

The qualitative parameters of the stem, leaves, flowers and fruits were descriptively assessed and recorded. The quantitative parameters of the stem, canopy, leaves, flowers and fruits were subjected to normality testing and GLM procedure using the statistical package SAS 9.1 (SAS Institute, Carry, NC, USA). Pearson's Correlation Coefficients (PCC) were calculated using CORR procedure in SAS among the quantitative morphological parameters of leaves, flowers and fruits separately. The quantitative morphological parameters were subjected to Principal Component (PC) Analysis and the first four calculated PCs were used to perform cluster analysis and dendrogram construction using the algorithms of Average Linkage and Pearson's Distance in Minitab 16 (Minitab Inc., USA). The DNA sequences of the *mat*K region were submitted to GenBank (http://www.ncbi.nlm.nih.gov, ***GenBank Submission ID: 1920215***).The *matK* barcodes were aligned using Clustal Omega Package (http://www.ebi.ac.uk) and a dendrogram was constructed employing Unwieghted Pair-Wise Group Method using Arithmetic Average (UPGMA) of hierarchical cluster analysis in DARwin Software Version 6.0 (Perrier and Jacquemoud-Collet, 2006; Perrier *et al*., 2003) (http://darwin.cirad.fr/). The DZBI data of the antibacterial activity against *E. coli* and *S. aureus* were subjected to ANOVA and mean separation procedure in SAS.

RESULTS AND DISCUSSION

Morphological Diversity

Plant Height and Canopy Width

The mean plant height (PlH) at maturity was significantly highest in the genotypes *Thibbatu, Thiththathibbatu* and *Walthibbatu* (285.0 cm, 268.9 cm and 203.9 cm respectively). The

genotypes *Wambatu local*, *Long Elabatu* and *Round Elabatu* were having the mean PlH in the range of 94.0-95.0cm. *Wambatu hybrid* was significantly shorter than *Wambatu local* (mean PlH of 78.8 cm and 94.9 cm respectively). The genotypes *Katuwelbatu* and *Welthibbatu* had the shortest PlH (37.1 cm and 29.1 cm respectively) out of 14 genotypes studied (Table 2) ($P<0.05$). The mean canopy width (CW) was also significantly higher in genotypes *Thibbatu*, *Thiththathibbatu* and *Walthibbatu*. The genotype *Round Elabatu with prickles* possessed the lowest mean CW (Table 2). The PCC between mean PlH and mean CW was 91.2 % ($P<0.05$). The stem colours before maturity and at maturity were highly variable among the genotypes. A total of 13 genotypes except *Welthibbatu* contained hairs on the stem and the genotypes *Gonabatu*, *Thibbatu* and *Thiththathibbatu* contained prickles in addition to the hairs. There were two distinct genotypes of *Round Elabatu* in which one genotype contained both hairs and prickles whereas the other genotype contained hairs only. The genotype *Welthibbatu* contained prickles only (Table 2).

Table 2. Mean plant height (PlH), mean canopy width (CW) and stem chracteristics

Genotype	Plant (cm)		Stem		
	Mean height	Mean canopy width	Colour before maturity	Colour at maturity	Presence of hairs and prickles
Gonabatu	140.5[b]	149.1[b]	I10	H15	Hairs with prickles
Katuwelbatu	37.1[e]	59.7[d]	G14	G10	Hairs only
Large Elabatu	94.7[c]	92.5[c]	I06	I39	Hairs only
Long Elabatu	106.5[c]	118.5 [c]	I06	I40	Hairs only
Round Elabatu without prickles	94.1[c]	94.4[c]	I06	I40	Hairs only
Round Elabatu with prickles	50.9[e]	36.9[e]	I06	I40	Hairs with prickles
SudhuAththana	154.0[b]	166.2[b]	G15	G10	Hairs only
Small Kalukenweriya	62.1[d]	51.0[d]	G16	H15	Hairs only
Thibbatu	285.0[a]	238.8[a]	G14	E11	Hairs with prickles
Thiththathibbatu	268.9[a]	329.1[a]	F13	G10	Hairs with prickles
Walthibbatu	203.9[a]	253.9[a]	E12	E14	Hairs only
Wambatu hybrid	78.8[d]	85.7[c]	F14	G14	Hairs only
Wambatu local	94.9[c]	93.1[c]	H14	G08	Hairs only
Welthibbatu	29.1[e]	50.7[d]	F14	G07	Prickles only

Colour codes are according to Vejdemo-Johansson *et al*., (2014).
Means denoted by the same letters within the columns are not significantly different at $P<0.05$.

Leaf Morphology

The mean petiole length (PL) was the highest in *Katuwalbatu* and *Sudhu Aththana* (5.7 cm and 5.5 cm respectively) and the lowest in *Small Kalukenweriya* (2.1 cm). The mature leaf colour was visualized as four different classes. The mean leaf length (LL) and the mean leaf breadth (LB) were also significantly different among the genotypes. The mean leaf tip angle (LTA) was also found to be significantly variable among the genotypes. The genotypes

Gonabatu, *Sudhu Aththana*, *Small Kalukenweriya*, *Wambatu* local and *Walthibbatu* were having a LTA close to the size of a right angle. The *Wambatu* hybrid had the LTA of 76.7° compared to that of *Wambatu* local (92.0°) (P<0.05). The genotype *Katuwelbatu* contained significantly higher number of prickles (65.5) and the genotypes *Gonabtu*, *Round Elabatu with prickles*, *Thibbatu*, *Thiththathibbatu* contained some prickles and a total of eight genotypes did not possess prickles. *Gonabatu* and *Walthibbatu* contained many hairs on the leaves whereas the leaves of genotype *Katuwelbatu* did not contain hairs. There were six genotypes with intermediate number of hairs on the leaves (Table 3).

All the quantitative parameters of the leaf morphology were significantly correlated to each other except LB and LTA. The LL and LB were significantly, positively and very highly correlated (PCC of 0.83, P<0.05). The LL, PL and the number of prickles per leaf were significantly and negatively correlated with LTA and the strength of correlation was not very high (PCC of 0.18-0.23%, P<0.0001) (Table4). The leaf shapes of the genotypes are given in Plate 1.

Table 3. Variation in leaf morphology

Genotype	Mean petiole length (cm)	Colour at maturity	Mean length (cm)	Mean breadth (cm)	Mean tip angle°	Mean no of prickles	Abundance of hairs*
Gonabatu	4.6^{b}	G14	27.5^{a}	22.4^{a}	82.7^{b}	5.9^{d}	3
Katuwelbatu	5.7^{a}	F14	12.6^{d}	13.1^{b}	61.5^{c}	65.5^{a}	0
Large Elabatu	4.4^{b}	G14	15.6^{c}	10.7^{d}	74.0^{b}	0.0^{g}	2
Long Elabatu	3.3^{c}	H15	10.7^{e}	8.1^{e}	67.2^{c}	0.0^{g}	2
Round Elabatu without prickles	2.7^{d}	G14	11.4^{e}	7.8^{e}	75.2^{b}	0.0^{g}	2
Round Elabatu with prickles	2.7^{d}	F15	9.3^{f}	7.0^{e}	55.0^{c}	13.0^{b}	2
Sudhu Aththana	5.5^{a}	G14	17.6^{b}	12.1^{c}	88.0^{b}	0.0^{g}	1
Small Kalukenweriya	2.1^{d}	H18	4.5^{g}	2.8^{f}	90.0^{a}	0.0^{g}	0
Thibbatu	3.1^{c}	G14	11.3^{e}	8.7^{e}	73.2^{b}	1.1^{f}	2
Thiththathibbatu	2.8^{d}	H15	9.2^{f}	7.1^{e}	78.7^{b}	4.3^{e}	2
Walthibbatu	5.2^{a}	H15	25.7^{a}	10.4^{e}	45.2^{d}	0.0^{g}	3
Wambatu hybrid	3.7^{c}	G15	12.2^{d}	8.4^{e}	76.7^{b}	0.0^{g}	1
Wambatu local	3.4^{c}	F14	12.2^{a}	8.7^{e}	92.0^{a}	0.0^{g}	1
Welthibbatu	2.3^{d}	G15	4.4^{g}	3.7^{f}	86.3^{b}	8.0^{c}	1

Colour codes are according toVejdemo-Johansson *et al*., (2014). Mean values denoted by the same letters within the column are not significantly different (P<0.05). * 0 (none), 1 (few), 2 (intermediate), 3 (many)

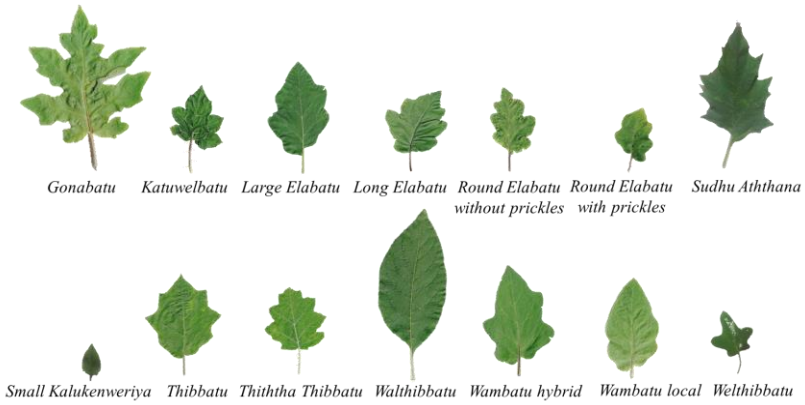

Plate 1. Leaf shapes of the Solanaceous genotypes. (Images are not shown according to the scale, and the size parameters are given in Table 3.)

Table 4. Pearson's Correlation Coefficients (PCC) between quantitative leaf parameters

	Leaf breadth	Leaf petiole length	No. of prickles/leaf	Tip angle
Leaf length	0.83 ****	0.63 ****	-0.05 ****	-0.21 ****
Leaf breadth		0.60 ****	0.20 ****	-0.03
Leaf petiole Length			0.32 ****	-0.18 ****
No. of prickles/leaf				-0.23 ****

***P<0.0001

Flower Morphology

The parameters petal length (PeL), petal breadth (PB), sepal length (SL), sepal breadth (SB), flower diameter (FD), pedicel length (PedL) and stamen length (StL) were significantly variable among the genotypes (Table 5). The genotypes *Gonabatu*, *Katuwelbatu*, one cultivar of *Round Elabatu*, *Thibbatu*, *Wambatulocal* and *Welthibbatu* possessed prickles on the pedicel whereas other eight genotypes did not possess prickles on pedicel. Genotype *Sudhu Aththana* contained the largest flowers (Table 5). The shape and colour variations of the flowers are given in Plate 2 and petal colour is categorically indicated in Table 5. Except the number of prickles in the flower pedicel, all other quantitative parameters of the flowers indicated significantly positive correlations (0.63 - 0.99) (Table 6, P<0.0001).

Table 5. Variation in floral morphology

Genotype	Petal		Sepal		Flower				
	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Diameter (cm)	Pedicel length (cm)	Stamen length (cm)	No. of prickles in the pedicel	Petal colour
Gonabatu	1.6^{b}	0.7^{c}	0.6^{c}	0.3^{b}	2.7^{c}	0.7^{d}	0.9^{c}	0.5^{d}	B31
Katuwelbatu	1.3^{b}	0.6^{c}	0.3^{d}	0.2^{b}	2.3^{b}	1.7^{c}	0.9^{c}	17.2^{a}	B15
Large Elabatu	2.0^{b}	1.4^{b}	0.8^{c}	0.3^{b}	3.3^{b}	1.6^{c}	0.8^{c}	0.0^{e}	F35
Long Elabatu	1.9^{b}	1.2^{b}	0.8^{c}	0.2^{b}	3.2^{b}	1.8^{c}	0.7^{c}	0.0^{e}	F34
Round Elabatu without prickles	1.8^{b}	1.2^{b}	0.7^{c}	0.3^{b}	2.5^{c}	1.7^{c}	1.1^{b}	0.0^{e}	E35
Round Elabatu with prickles	1.1^{b}	0.6^{c}	0.4^{d}	0.2^{b}	2.0^{c}	1.1^{c}	0.5^{c}	2.5^{c}	E35
SudhuAththana	9.3^{a}	5.2^{a}	4.1^{a}	3.0^{a}	6.3^{a}	3.9^{a}	7.6^{a}	0.0^{e}	B32
Small Kalukenweriya	1.0^{b}	0.3^{d}	0.3^{d}	0.2^{b}	1.2^{d}	1.2^{c}	0.7^{c}	0.0^{e}	C12
Thibbatu	1.3^{b}	0.5^{c}	0.4^{d}	0.2^{b}	2.3^{c}	0.9^{d}	0.8^{c}	0.0^{e}	B8
Thiththathibbatu	1.3^{b}	0.6^{c}	0.4^{d}	0.2^{b}	2.1^{c}	1.2^{c}	0.9^{c}	0.5^{d}	G34
Walthibbatu	1.0^{b}	0.4^{d}	0.4^{d}	0.2^{b}	1.5^{d}	0.3^{d}	0.4^{c}	0.0^{e}	F34
Wambatu hybrid	1.8^{b}	0.9^{b}	1.1^{b}	0.4^{b}	3.3^{b}	1.7^{c}	1.2^{b}	0.0^{e}	G36
Wambatu local	2.1^{b}	1.1^{b}	1.2^{b}	0.4^{b}	3.4^{b}	2.4^{b}	1.2^{b}	4.6^{b}	C35
Welthibbatu	1.1^{b}	0.6^{c}	0.5^{d}	0.3^{b}	1.8^{d}	0.4^{d}	0.6^{c}	0.9^{d}	C36

Colour codes are according to Vejdemo-Johansson *et al*., (2014).
Mean values denoted by same letters within a column are not significantly different at P<0.05.

Plate 2. Variation in flower shape of Solanaceous genotypes.

Table 6. Pearson's Correlation Coefficients among quantitative floral parameters

	Petal breadth	Flower diameter	Sepal length	Sepal breadth	Pedicel length	Stamen length	No.of prickles/pedicel
Petal length	0.98 ****	0.83 ****	0.97 ****	0.98 ****	0.71 ****	0.98 ****	0.00
Petal breadth		0.86 ****	0.96 ****	0.94 ****	0.73 ****	0.94 ****	0.00
Flower diameter			0.87 ****	0.74 ****	0.79 ****	0.76 ****	0.00
Sepal length				0.99 ****	0.68 ****	0.95 ****	0.00
Sepal breadth					0.63 ****	0.95 ****	0.07
Pedicel length						0.73 ****	0.25
Stamen length							0.05

***P<0.0001

Fruit Morphology

There were two types of fruit orientation from the stem namely erect and pendent. The fruit shape, curvature and shape of the apex were not highly variable among the genotypes. *Walthibbatu* was the only genotype containing hairs on the fruit surface. *Sudhu Aththana* and *Walthibbatu* had dull appearance of the fruit whereas fruit surface of other genotypes were shiny i.e. with high degree of glossiness. The fruit colour was variable among the genotypes and six genotypes had even colour and eight genotypes had bicolour (Table 7).

Fruit length (FL), fruit breadth (FB) and fruit stalk length (FSL) were significantly variable among the genotypes (P<0.05). The genotypes *Katuwelbatu*, one cultivar of *Round Elabatu, Wambatu local* and *Welthibbatu* contained prickles on the fruit stalk and genotype *Katuwelbatu* had the significantly highest number of prickles on the fruit stalk (15.9, P<0.05) (Table 8). The fruit shapes are given in Plate 3. All measured quantitative fruit parameters had significant and positive correlations with each other. The correlation between FL and FB was 0.44 whereas FL and fruit weight (FW) showed PCC of 0.93(P<0.0001). The FSL was also very highly correlated to the size parameters (0.50 to 0.84 of PCC, P<0.0001). The number of prickles on the stalk had low correlation coefficient with other fruit parameters despite significance (0.04 to 0.20 of PCC, P<0.0001) (Table 9).

The Principal Components calculated based on the quantitative morphological parameters were subjected to cluster analysis and respective dendrogram is shown in Fig.1. All the *Elabatu* genotypes were clustered together at 76.8% of morphological similarly coefficient. *Thiththathibbatu* and *Thibbatu* were clustered together at 89.5% morphological similarly coefficient. However, genotype *Katuwelbatu* was only 41.3% similar to the rest of the genotypes. The out group genotype *Sudhu Aththana* was only exhibiting 12.0% similarly to the rest of the genotypes (Fig. 1).

Table 7. The qualitative parameters of fruit morphology in Solanaceous spp.

Genotype	Orientation	Shape	Curvature	Apex shape	Surface hairs	Glossiness	Colour		Colour distribution
Gonabatu	Erect	Round	None	Round	None	2	G15		Even
Katuwelbatu	Pendant	Round	None	Round	None	2	D6		Even
Large Elabatu	Pendant	Round	None	Round	None	2	E14	B14	Bicolour
Long Elabatu	Pendant	Oblong	Slightly curved	Protruded	None	2	D14	B14	Bicolour
Round Elabatu without prickles	Pendant	Round	None	Round	None	2	E14	B14	Bicolour
Round Elabatu with prickles	Pendant	Round	None	Round	None	2	F14	B09	Bicolour
SudhuAththana	Pendant	Round	None	Round	None	1	E14		Even
Small Kalukenweriya	Pendant	Round	None	Round	None	3	I32		Even
Thibbatu	Erect	Round	None	Round	None	2	F16		Even
Thiththathibbatu	Erect	Round	None	Round	None	3	F13		Bicolour
Walthibbatu	Erect	Round	None	Round	Many	1	F14		Even
Wambatu hybrid	Pendant	Semi-long	Slightly curved	Protruded	None	3	F36	A	Bicolour
Wambatu local	Pendant	Semi-long	Slightly curved	Protruded	None	3	I40	A	Bicolour
Welthibbatu	Pendant	Round	None	Round	None	3	G16		Bicolour

Colour codes are according to Vejdemo-Johansson *et al*., (2014).
Glossiness: 1 (dull), 2 (fairly shiny), 3 (shiny)

Table 8. The quantitative morphological parameters of Solanaceous fruitspp.

Genotype	Length (cm)	Breadth (cm)	Weight (g)	Stalk length (cm)	Mean no. of prickles in stalk
Gonabatu	1.2^{e}	1.2^{d}	1.3^{f}	1.8^{c}	0.0^{d}
Katuwelbatu	2.0^{e}	2.1^{c}	4.6^{e}	1.9^{c}	15.9^{a}
Large Elabatu	3.6^{c}	4.4^{a}	34.6^{c}	4.3^{b}	0.0^{d}
Long Elabatu	4.4^{b}	2.6^{c}	15.0^{d}	4.5^{b}	0.0^{d}
Round Elabatu without prickles	2.4^{d}	2.5^{c}	9.0^{e}	3.8^{b}	0.0^{d}
Round Elabatu with prickles	2.3^{d}	2.3^{c}	6.2^{e}	2.4^{c}	2.1^{b}
Sudhu Aththana	4.8^{b}	4.5^{a}	19.3^{d}	1.9^{c}	0.0^{d}
Small Kalukenweriya	0.9^{f}	0.9^{e}	0.1^{f}	0.8^{d}	0.0^{d}
Thibbatu	1.1^{e}	1.2^{d}	1.4^{f}	1.6^{c}	0.0^{d}
Thiththathibbatu	0.8^{f}	0.8^{e}	0.3^{f}	1.7^{c}	0.0^{d}
Walthibbatu	1.0^{e}	0.9^{e}	0.7^{f}	0.6^{d}	0.0^{d}
Wambatu hybrid	16.3^{a}	3.4^{b}	64.1^{b}	6.6^{a}	0.0^{d}
Wambatu local	16.2^{a}	4.4^{a}	85.3^{a}	7.5^{a}	0.1^{c}
Welthibbatu	0.9^{f}	1.0^{d}	0.6^{f}	2.1^{c}	0.3^{c}

Mean values denoted by the same letters within the column are not significantly different at P<0.05.

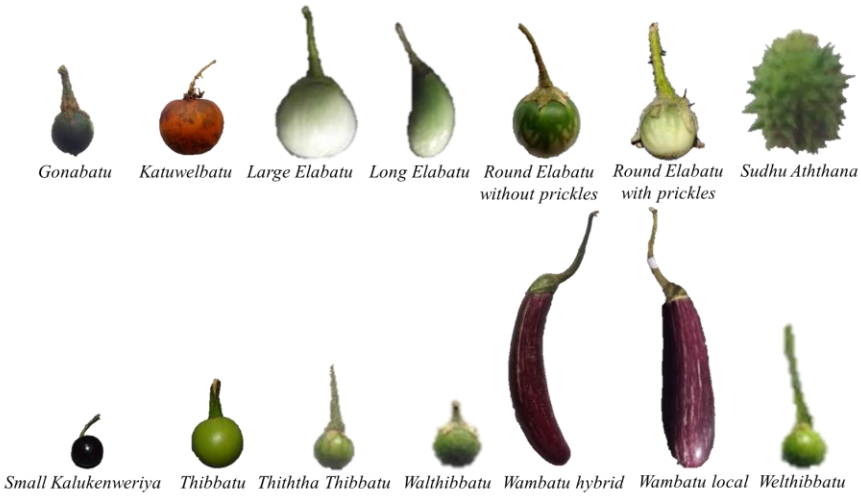

Plate 3. Variation in fruit shape of Solanaceous genotypes.

Table 9. Pearson's Correlation Coefficients (PCC) among quantitative fruit parameters

	Fruit breadth	Fruit weight	Stalk length	No. of prickles in stalk
Fruit length	0.44****	0.93****	0.80****	0.13****
Fruit breadth		0.58****	0.50****	0.04****
Fruit weight			0.84****	0.14****
Stalk length				0.20****

**P<0.0001

Genetic Diversity Analysis

DNA Barcoding

PCR based on the *matK* DNA barcoding primers yielded an amplicon with the length of 936 bp (Fig.2A). The DNA sequence polymorphism within the tested 14 Solanaceous genotypes indicated that there were total of eight haplotypes present and all *Wambatu* and *Elabatu* genotypes shared the same haplotype. The genotypes *Thiththathibbatu* and *Walthibbatu* also shared a common haplotype. It was clear that other six genotypes were having unique *matK* haplotypes. A total of 52 Single Nucleotide Polymorphisms (SNPs) were detected within the *matK* locus among the eight haplotypes. All *Wambatu* and *Elabatu* cultivars (i.e. *S. melongena*) got a unique SNP at 388thbp position which was not present in other species. A total of 42 unique SNPs found in Solanaceous spp. Out of the 52 SNPs, 49 were bi-allelic and three were tri-allelic indicating the quickly evolving species specific nature of *matK* region within Solanaceous spp. The *matK* sequences of the 14 species were submitted to GenBank (www.ncbi.nlm.nih.gov) and the GenBank accession numbers are KX258741, KX258742, KX258743, KX258744, KX258745, KX258746, KX258747, KX258748, KX258749, KX258750, KX258751, KX258752, KX258753 and KX258754 according to the order of genotypes given in Table 1.The haplotype variations of the *matK* region isshown with the colours in Figure 2B. The rooted tree diagram with the evolutionary distances

among the haplotypes is shown in Figure 3. The rooted tree diagram indicated that four *Thibbatu* cultivars are closely related to *S. melongena*.

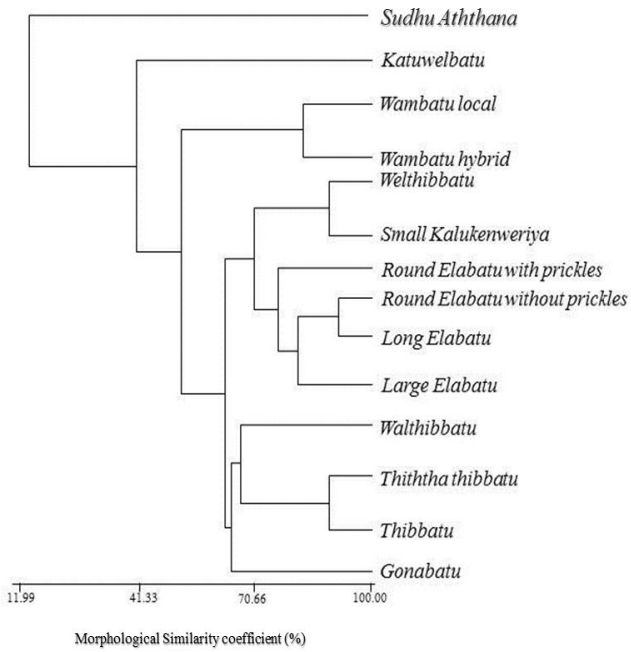

Figure 1. The dendrogram constructed based on the principal components computed using quantitative morphological parameters of flowers, fruits and plants of Solanaceous genotypes.

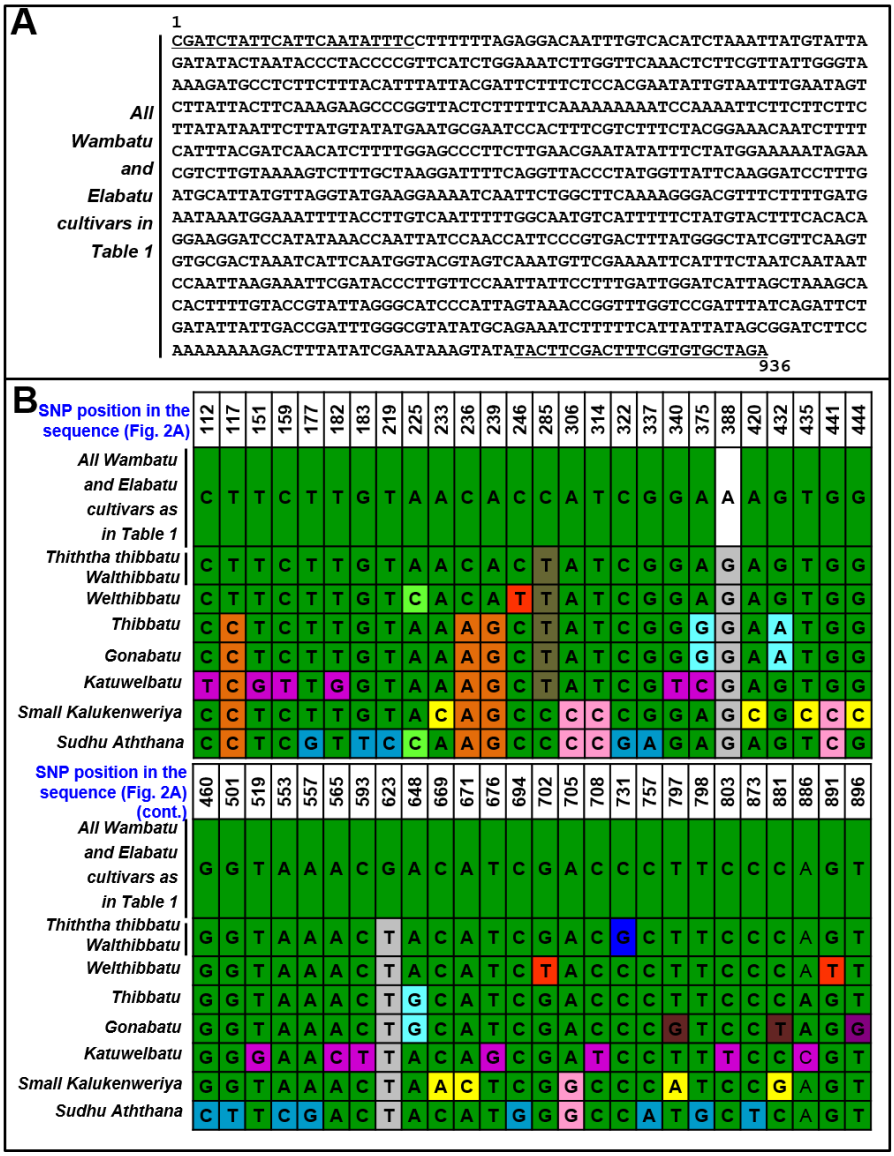

Figure 2. DNA polymorphism in the *matK*locus in Solanaceous species.
A: *matK*DNA sequence of all *Wambatu* and *Elabatu* cultivarslisted in Table 1. The forward and reverse *matK* primers are underlined. The total length of the amplicon is 936 bp. The starting and ending bases are shown with the numbers 1 and 936 respectively. B: The SNP positions and the SNP alleles in the *matK*genomic locus of Solanaceous species. The SNP positions within Fig.2B are shown in vertically aligned numbers and the names of the genotypes/groups are shown in the left. The SNP alleles are shown in front of each genotypes/group and colour shades for cells are provided to show shared and unique alleles.

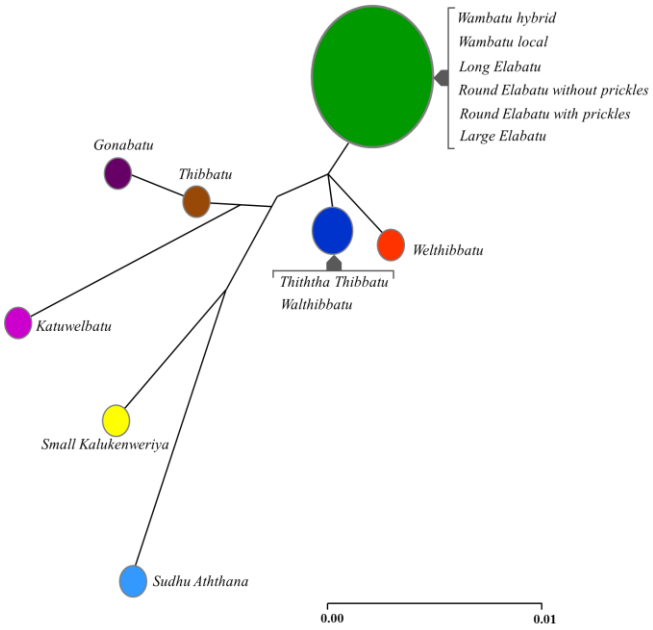

Figure 3. The rooted tree diagram showing genetic diversity of the Solanaceous genotypes based on the DNA sequence polymorphism in *matK*region. Scale bar represent 0.01 of genetic divergence.

Antibacterial Activity of Solanaceous spp.

The antibacterial analysis of the root extracts of Solanaceous genotypes exhibited significantly higher activity against Gram negative *E. coli* and Gram positive *S. aureus*. The solvent DMSO (i.e. negative control) did not exhibit any antibacterial activity (mean DZBI was 0.0 mm) against two bacteria (Table 10). The genotype *Gonabatu* and *Thiththathibbatu* exhibited the significantly highest antibacterial activity (mean DZBIs of 10.4 mm and 10.2 mm respectively) against *E. coli*. Other *Solanum* genotypes also exhibited 7.5 mm to 9.6 mm of mean DZBI against *E. coli*. In general the antibacterial activity was highest against *S. aureus* in comparison to *E. coli*. The highest antibacterial activity on *S. aureus* was shown by the genotype *Welthibbatu* (13.0 mm of mean DZBI) followed by *Gonabatu* (11.5 mm mean DZBI). All the other 12 genotypes showed significantly similar antibacterial activity on *S.aureus* (10.6 mm of mean DZBI for 12 genotypes).

Table 10. Antibacterial activity of the ethanol extracts prepared from the roots of *Solanaceous* spp.

Genotype	Mean diameter of the zone of inhibition (DZBI) (mm)	
	E. coli	***S. aureus***
Gonabatu	10.4^{a}	11.5^{b}
Katuwelbatu	8.2^{c}	10.6^{c}
Large Elabatu	9.4^{b}	10.8^{c}
Long Elabatu	9.6^{b}	11.0^{c}
Round Elabatu without prickles	8.0^{c}	10.8^{c}
Round Elabatu with prickles	7.7^{d}	11.0^{c}
Sudhu Aththana	7.5^{d}	10.4^{c}
Small Kalukenweriya	7.9^{d}	10.7^{c}
Thibbatu	8.7^{c}	10.6^{c}
Thiththathibbatu	10.2^{a}	10.1^{c}
Walthibbatu	7.8^{d}	10.6^{c}
Wambatu hybrid	7.5^{d}	10.2^{c}
Wambatu local	7.9^{d}	10.3^{c}
Welthibbatu	9.0^{b}	13.0^{a}
Control [Dimethylsulfoxide (DMSO) only]	0.0^{e}	0.0^{d}

Means denoted by the same letters within each column are not significantly different at $P<0.05$.

The family Solanaceae is considered as one of the most cosmopolitan families of Angiosperms because it exhibits higher morphological diversity (Jennifer and James, 1997; Bean, 2004, Knapp *et al*., 2004). In the present study, it was observed that, *Solanaceous* species are morphologically diverse and the PC analysis followed by cluster procedure was able to discriminate all the species into unique groups. The species *Sudhu Aththan*a is 12% morphologically similar to the rest simply because it does not belong to the genus *Solanum*. However the other *Solanum* genotypes were also showing a significantly higher diversity and within the genus *Solanum*, *Katuwelbatu* could be considered as the local outgroup. *Welthibbatu* is very similar to *Small Kalukenweriya* than to other *Thibbatu* species. It has been reported that many morphological traits such as fruit size, shape and taste which are associated with the domestication process of eggplants are controlled by one or few genes (Frary *et al*., 2000; Doganlar *et al*., 2002; Van der Knapp and Tanksley, 2003; Cong *et al*., 2008; Xiao *et al*., 2008; Wang *et al*., 2008). Hence assortment and preservation of germplasm exclusively based on morphological features can be uncertain as morphologically dissimilar accessions may differ by only one or two alleles (Tumbilen *et al*., 2011). The morphological traits are also affected by micro climatic conditions although samples were collected from the same province. Therefore molecular methods coupled with morphological analysis are preferred for proper germplasm characterization and conservation (Sifau *et al*., 2014). Morphological features are inadequate to establish a genetic relationship especially for the species like *Solanum*, which are capable of crossing with more distant species (Daunay *et al*., 1999). It is also possible to trace out and scientifically document the changes

in the pathways of domestication process through genetic diversity analysis (Doebley *et al*., 1997) which could be a potential next step of the present study.

The precise identification of species is very important for efficient utilization of medicinally important Solanaceous species in Ayurvedic and herbal medicine industry. Analysis of genetic polymorphism is an efficient tool for the utilization and conservation of plant genetic resources because morphological keys are effective only for a particular stage of life (Sifau *et al*., 2014). The medicinal plants such as ginger, ginseng, bamboo and moringa have been characterized using DNA barcoding loci (Cortese *et al.,* 2010). The barcoding locus *matk* is preferred by many taxonomists and applied biologists because of its high polymorphism. In the present study, *matK* was chosen because it provided greater DNA polymorphism among the Solanaceous spp (Yu *et al.,* 2011).

The *matK* polymorphism among 14 Solanaceous genotypes revealed that *Wambatu* genotypes and *Elabatu* genotypes are all belonging to same species *S. melongena* as reported in Dassanayake and Fosberg, (1988). However according to the morphological diversity assessments these genotypes are distinctly different from each other. It is not logical to think that these genotypes are different species but, some major genes or quantitative trait loci (QTL) would cause the variations in fruit and plant morphology. To efficiently characterize these genotypes which share same *matk* sequence, the underlying genes / QTLs for the fruit and leaf variation must be characterized. However sequencing of other DNA barcoding loci would also provide an absolute judgment to include all *Wambatu* and *Ealabtu* genotypes within the same species. It would also be interesting to see whether these *Elabatu* and *Wambatu* genotypes are crossing with each other creating higher morphological diversity due to potentially higher heterotic effects. The species *Welthibbatu* and *Thiththathibbatu* share the same *matK* locus implying that they are putatively belonged to same species therefore DNA barcoding of other loci is compulsory to establish their species limits.

The word Solanaceae is derived from Latin word 'Solari' which means to 'soothe' referring to its pharmacological properties (Yousaf *et al*., 2008), which are due to alkaloids (Friedman and McDonald, 1997) in which solanin, solasodine, scopolamine, atropine and hyoscyamine are considered as the major ones (Stanker *et al.,* 1994). In Sri Lanka, the roots of *Solanaceous* species are predominantly used in Ayurvedic medical preparations. Out of the 14 species studied *Katuwelbatu*, all *Ealabatu*, *Sudhu Aththana*, *Small Kalukenweriya* and *Walthibbatu* are used for the medicinal purposes. However the species *Gonabatu*, *Thiththathibbatu* and *Welthibbatu* which are considered as medicinally not important species have significant antibacterial activity against model pathogens. All these species have significantly higher antibacterial activity compared to the control and *S. aureus* was affected more by the root extracts than *E. coli.* This is because of the presence of bilayer cell membrane in Gram negative *E. coli* compared to Gram positive *S. aureus* which has a single cell membrane. It is important to document the antibacterial activity against the model pathogens to conduct future assays on specific virulent pathogens. The disc diffusion method can be used to detect the antibacterial activity of series of concentrations to determine the minimum inhibitory concentration for the root extracts of Solanaceous spp.

The morphological variation assessment, *matK* DNA barcodes and antibacterial activity against the model pathogens in combination provide a strong platform to use these Solanaceous species in Ayurvedic and herbal medicinal industries in Sri Lanka.

CONCLUSIONS

The morphological assessment of the commonly grown nine Solanaceous species revealed high diversity. The *matK* based DNA barcoding revealed that all the *Wambatu* and *Elabatu* genotypes share the same haplotype, *Thiththathibbatu* and *Walthibbatu*also share a common haplotype and other six species have unique haplotypes. The significantly highest antibacterial activity was observed in *Gonabatu* and *Thiththathibbatu* against *E.coli* and in *Walthibbatu* against *S. aureus*.

Acknowledgements: *Royal Botanical Gardens, Peradeniya, Department of National Botanic Gardens, Sri Lanka for facilitating sample identification process.*

REFERENCES

Abbas, K., Niaz, U., Hussain, T., Saeed, M.A., Javaid, Z., Idrees, A. and Rasool, S. (2014).Antimicrobial activity of fruits of *Solanum nigrum* and *Solanum xanthocarpum*. Acta Pol. Pharm. *71*, 415 - 421.

Adam, G., Huong, H.T. and Khoi, N.H. (1979). The constituents of the Vietnamese drug plant *Solanum verbascifolium* L. Planta Med. *36*, 238 - 239.

Adam, G., Huong, H.T. and Khoi, N.H. (1980).Solaverbascine - a new 22, 26-epiminocholestane alkaloid from *S. verbascifolium*.Phytochemistry. *19*, 1002 - 1003.

Adesina, S.K. (1985). Constituents of *Solanum dasyphyllum* fruits.Lloydia. *48*, 147.

Ahmed, A., Tayela, E. and Wael, F. (2013). Production of anticandidal cotton textiles treated with oak gall extract. Rev. Argent. Microbiol. *45*, 271 - 276.

Amer, W.M., Abouwarda, A.M., Garfia, E.L., Dawoud, G.T.M. and Abdelmohsen, G. (2013).International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS). *2(6)*, 1282 - 1306.

Bahgat, A., Abdel, A., Raafat, M., Mahdy, A., ELkhatib, A., Ismail, A. and Khayyal, M. (2008).*Solanum indicum* spp. distichum extract is effective against L-NAME induced hypertension inrats. Fundam.Clin.Pharmacol.*22(6)*, 693 - 699.

Bansal, S., Singh, K. and Kumar, S. (2009). Larvicidal activity of the extracts from different parts of the plant *Solanum xanthocarpum* against important mosquito vectors in the arid region. J. Environ. Biol. *2*, 221 - 226.

Bean, A.R. (2004). The taxonomy and ecology of *Solanum*subg.*Leptostemonum* (Dunal) Bitter (*Solanaceae*) in Queensland and far North-Eastern New South Wales, Australia. Austrobaileya. *6(4)*, 639 - 816.

Bello, S.O., Muhammad, B.Y., Gammaniel, K.S., Abdu-Aguye, I., Ahmed, H., Njoku, C.H., Pindiga, U. H. and Salka, A.M. (2005).Preliminary Evaluation of the Toxicity and Some Pharmacological Properties of the Aqueous Crude Extract of *Solanum melongena*. Res. J. Agric. and Biol. Sci. *1(1)*, 1 - 9.

Bose, B. and Ghosh.C. (1980). Studies on the variation of chemical constituents of *S. nigrum* ripe and unripe berries. J. Inst. Chem. *52(2)*, 83 - 84.

Britto, A.J., Gracelin, D.H.S. and Rathna Kumar, P.B.J. (2011). Antimicrobial activity of a few medicinal plants against gram negative bacteria. Int. J. Appl. Biol. Pharm. *2(3)*, 457 - 461.

Chen, R., Feng, L., Li, H.D., Zhang, H. and Yang, F. (2009). Two novel oilgosaccharides from *Solanum nigrum*. Carbohydr. Res. *344*, 1775-1777.

Chinedu, S.N., Olasumbo, A.C., Eboji, O.K., Emiloju, O.C., Arinola, O.K. and Dania, D.I. (2011). Proximate and phytochemical analyses of *Solanum aethiopicum* L. and *Solanum macrocarpon* L. fruits. Res.J.chem.sci. *1(3)*, 2231 - 606.

Cong, B., Barrero, L.S. and Tanksley, S.D. (2008). Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size in tomato domestication. Nat. Genet. *40*, 800 - 804.

Cortese, L.M., Honig, J., Miller, C. and Bonos, S.A. (2010).Genetic diversity of twelve switch grass population using molecular and morphological markers. *Bioenergy Res*. *5(3)*, 262 - 271.

Cuthbertson, A.G.S. and Murchie, A.K. (2005). Economic spray thresholds in need of revision in Northern Irish Bramley orchards. Biological News. *32*, 19.

Dassanayake, M.D. and Fosberg, F.R. (1988). A revised handbook of the flora of Ceylon, Vol 6, CRC Press, Sri Lanka.

Daunay, M.C., Dalmon, A. and Lester, R.N. (1999). Management of a collection of *Solanum* species for eggplant (*Solanum melongena* L.) breeding purposes. pp. 369-383. *In*: Nee, M., Lester, R.N. and Jessop. J.P. (Ed.) *Solanaceae* IV, Advances in Biology and Utilization, Royal Botanic Gardens, Kew.

De Sousa, R.R., Queiroz, K.C., Souza, A.C., Gurgueira, S.A., Augusto, A.C., Miranda, M.A., Peppelenbosch, M.P., Ferreira, C.V. and Aoyama, H. (2007). Phosphoprotein levels, MAPK activities and NF kappaB expression are affected by fisetin, J. Enzyme Inhib. Med. Chem. *22(4)*, 439 - 444.

Devi, S., Gupta, A.K. and Singh, M. (2012). Ethano-Medicinal use of plants belonging to families Fabaceae and Solanaceae, Hamirpur District (H.P).ISSN 2250 - 3153.

Dhellat, J.R., Matouba, E., Maloumbi, M.G., Nzikou, J.M., Dzoudo, M.G., Linder, M., Parmenite, M. and Desobry, S. (2006). Afr. J. Biotechnol.*5*, 987.

Dobson, C.R. (2010). Medicinal Natural Product. A Biosynthetic Approach, Willey and Sons, pp. 925-978.

Doebley, J., Stec, A. and Hubbard, L. (1997). The evolution of apical dominance in maize. Nature. *386*, 485 - 488.

Doganlar, S., Frary, A., Daunay, M.C., Lester, R.N., Tanksley, S.D. (2002). Conversation of gene function in the *Solanaceae* as revealed by comparative mapping of domestication traits in eggplant. Genetics. *161*, 1713 - 1726.

Doss, A., Mubarack, H. and Dhanabalan, R. (2009). Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. Indian J. Sci. Technol. *2(2*). 41 - 43.

Edem, Christoper, A., Dosunmu, Miranda, I., Bassey, Francisca, I., Wilson, Charles, Umoren and Patience. (2009). A comparative assessment of the proximate composition, ascorbic acid and heavy metal content of two species of garden egg (*Solanum gilo* and *Solanum aubergine*). Pakistan J. Nutrition. *8(5)*, 582 - 584.

Edmonds, J.M. and Chewya, J.A. (1997). Black Nightshades*: Solanum nigrum L. and related species.*Promoting the conservation and use of underutilized and neglected crops.International Plant Genetic Resources Institute (IPGRI), Rome, Italy.pp.159.

Fazekas, A.J. (2008). Multiple multi-locus DNA barcodes from the plastid genome discriminate plant species equally well. PLoS ONE.*3*, e2802.

Fontem, D.A. and Schippers, R.R. (2004). *Solanum scabrum* Mill. pp. 493-498. *In*: Grubben G.J.H.and Denton O.A. (Ed.) *In*: Plant Resources of Tropical Africa. PROTA Foundation. Backhuys Publishers., Leiden, Netherlands.

Frary, A., Nesbitt, T.C., Frary, A., Grandillo, S., Van der Knaap, E., Cong, B., Liu, J., Meller, J., Elber, R., Alpert, K. and Tanksley, S. (2000). Fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. Sci. *289*, 85 - 88.

Friedman, M. and McDonald, G.M. (1997). Potato glycol alkaloids: Chemistry, analysis, safety and plant physiology. Crit. Rev. Plant Sci. *16 (1)*, 55 - 132.

Gao, X., Zhu, Y.P., Wu, B.C., Zhao, Y.M., Chen, J.Q. and Hang, Y.Y. (2008). Phylogeny of *Dioscorea* sect.Stenophora based on chloroplast *matK*, *rbcL* and *trnL-F* sequences. J. Syst. Evol.*46*, 315 - 321.

Gbile, Z.O. and Adesina, S.K. (1988). Nigerian *Solanum* species of economic importance. Ann. Missouri Botanical Garden.*75*, 862 - 865.

Gupta, S., Mal, M. and Bhattacharya, P. (2005). Evaluation of hypoglycemic potential of *Solanum xanthocarpum* (*Solanaceae)* fruits in normal and streptozotocin induced diabetic rats. European Bull. Drug. Res. 13.

Huang, W.H., Hsu, C.W. and Fang, J.T. (2008). Central diabetes insipidus following digestion *Solanumindicum L*. concentrated solution. Clin.Toxicol. *46 (4)*, 293 - 296.

Igwe, S.A., Akunyili, D.N. and Ogbogu, C. (2003). Effects of *Solanum melongena* (garden egg) on some visual functions of visually active Igbos of Nigeria, J. Ethnopharmacol. *86(2-3)*, 135 - 138.

Jain, R., Sharma, A., Gupta, S., Sarethy, I.P. and Gabrani, R. (2011). *Solanum nigrum*: current perspectives on therapeutic properties. Altern. Med. Rev. *16 (1)*, 78 - 85.

Jain, S.C and Sahoo, S.L. (1981a). *Solanum verbascifolium* suspension cultures. *Pharmazie. 36*, 714 - 715.

Jain, S.C. and S.L. Sahoo. (1981b). Isolation and characterization of steroidal sapogenins and glycoalkaloids from *Solanum verbascifolium* L. Chem. Pharm Bull. *29*, 1765 - 1767.

Jain, S.K. (1968). Medicinal Plants. Thomson Press Ltd, India, pp. 133-134.

Jayanthy, A., Sulaiman, C.T. and Rema Shree, A.B. (2012). Pharmacognostical and phdytochemical comparison of roots of *Solanum* species used in ayurvedic formulations. Int. Pharmacognosy and Phytoche. Res. *4 (1)*, 28 - 37.

Jennifer, M.E. and James, A.C. (1997). Black nightshades *Solanum nigrum* L. and related species. International Plant Genetic Resources Institute. Rome, Italy.

Jian, L., Qingwang, L., Tao, F. and Kun, L. (2008). Aqueous extract of *Solanum nigrum* inhibit the growth of cervical carcinoma (U14) via modulating immune response of tumor bearing mice and inducing apoptosis of tumor cells. Fitoterapia. *79(7-8)*, 548 - 556.

Kaushik, D., Jogpal, V., Kaushik, P., Lal, S., Saneja, A., Sharma, C. and Aneja, K.R. (2009). Evaluation of activities of *Solanum nigrum* fruit extract. Arch. Appl. Sci. Res. *1(1)*, 43 - 50.

Knapp, S., Bohs, L., Nee, M. and Spooner, D.M. (2004). *Solanaceae*- a model for linking genomics with biodiversity. Comp. Funct. Genom. *5*, 285 - 291.

Kumar, P.S., Sucheta, S., Deepa, V.S., Selvamani, P. and Latha, S. (2008). Antioxidant activity in some selected Indian medicinal plants. Afr. J. Biotechnol. *7*, 1826-1828.

Lahaye, R. (2008). DNA barcoding the floras of biodiversity hotspots.PNAS. *105*, 2923 - 2928.

Li, X.X. and Zhou, Z.K. (2007). The higher-level phylogeny of monocots based on *matK*, *rbcL* and 18S rDNA sequences. Acta Phytotaxon. Sin. *45*, 113 - 133.

Ma, P., Cao, T.T., Gu, G.F., Zhao, X., Du, Y.G. and Zhang, Y. (2006). Inducement effect of synthetic indiosides from *Solanum indicum*L. on apoptosis of human heptocarcinoma cell line Bel-7402 and its mechanism. Chin. J. Cancer. *25(4)*, 438 - 442.

Marasinghe M.M.P.C., Mawalagedara S.M.U.P., Ishan M., Dunuwille S.W.M.B., Janaththani P., Dayananda A.G.M.L.K., Weebadde C.K., Yakandawala D.M.D. and Sooriyapathirana S.D.S.S. (2016). DNA based Authenication of *Solanum melongena* var. insanum (V. Elabatu) roots in herbal medicine market to circumvent the use of noxious adulterant, *Solanum melongena* (V. Eggplant) roots. J. Sci., Eastern University, Sri Lanka. *7*, 35 - 52.

Mueller, L.A., Solow, T.H., Taylor, N., Skwarecki, B., Buels, R., Binns, J., Lin, C., Wright, M.N., Ahrens, R., Wang, Y., Herbst, E.V., Keyder, E.R., Menda, N., Zamir, D. and Tanksley, S.D. (2005). The SOL genomic network.A comparative resource for Solanaceae biology and beyond. Plant Physiol.*138*, 1310 - 1317.

Nadkarni, K.M. (1927). Indian Materia Medica, Bombay.

Noda, Y., Kneyuki, T., Igarashi, K. and Packer, M.L. (2000). Antioxidant activity of nasunin, an anthocyanin in eggplant peels, Toxicol. *148*, 119 - 123.

Odetola, A.A., Iranloye, Y.O. and Akinloye, O. (2004).Hypolipidaemic potentials of *Solanum melongena* and *Solanum gilo*on hypercholesterolemic rabbits. Pakistan J.Nutr. *3(3)*, 180-187.

Ogundajo, A.L., Oladosu, I.A., Ogundawe, Flamini, G. and Owolabi, M.S. (2013). Study on the volatile constituents of *Solanum nigrum* var. *virginicum* L. from Nigeria. Asian J. Plant and Res. *3(1)*, 94 - 98.

Ojewole, J.A.O. and Adesina, S.K. (1983). Cardiovascular and neuromuscular actions of scopoletin isolated from the fruit of *T. tetraptera*. *Taub*. Planta Med, 10,49-99.

Parameswari, K., Aluru, S. and Kishori, B. (2012). In vitro antibacterial activity in the extracts of *Solanum nigrum*, Indian Streams Res. J. *2*, 1 - 4.

Pari, L. and Umamaheswari, J. (2000).Antihyperglycaemic activity of *Musa sapientum* flowers: Effect on lipid peroxidation in alloxan diabetic rats. Phytother. Res. *14*, 136 - 138.

Perrier, X. and Jacquemoud-Collet, J.P. (2006). DARwin software. Available at http://darwin.cirad.fr/

Perrier, X., Flori, A. and Bonnot, F. (2003). Data analysis methods. pp. 43-76. *In*: Hamon, P., Seguin, M., Perrier, X. and Glaszmann, J.C. (Ed.) Genetic diversity of cultivated tropical plants. Enfield, Science Publishers, Montpellier.

Raj, S.K. and Suchitra. (2009). Evaluation of antimicrobial potential of different extracts of *Solanum xanthocarpum* Schard and Wendl. Afr. J. Microbiol. Res. *3*, 97 - 100.

Rao, C.K. (2011). Use of brinjal (*Solanum melongena* L.) in alternative systems of medicine in India.Foundation for Biotechnology Awareness and Education. Bangalore, India.

Ross, J.A. and Kassum, C.M. (2012). Dietary saponins availability, metabolic effects and safety. J. Ann. Rev. Nutrition. *24(3)*, 19 - 30.

Saijo, R., Murakami, K., Nohara, T., Tomimatsu, T., Sato, A. and Matsuoka, K. (1982). Studies on the constituents of *Solanum* plants. II. On the constituents of the immature berries of *S. nigrum* L. Yakugaku Zasshi. *102*, 300 - 305.

Segal, R., Milo-Goldzweig I. and Zaitschek. D.V.(1977). Diosgenin and yamogenin from *S. incanum*. Lloydia. *40*, 604.

Sekara, A., Cebula, S. and Kunicki, E. (2007). Cultivated eggplants-origin, breeding objectives and genetic resources, a review. Ann. Hort. *19(1)*, 97 - 114.

Sheeba, E. (2010). Antibacterial activity of *Solanum surattense* Burm.F. Kathmandu University Journal of Science, Engineering and Technology. *6*, 1 - 4.

Sifau, M.O., Akinpelu, A., Ogunkanmi, L.A., Adekayo, K.O., Oboh, B.O. and Ogundipe, O.T. (2014). Genetic diversity in Nigerian brinjal eggplant (*Solanum melongena L.*) as revealed by random amplified polymorphic DNA (RAPD) markers. Afr. J. Biotech. *21*, 2119 - 2126.

Singh, O., Subharani, K., Singh, N., Devi, N. and Nevidita, L. (2007).Isolation of steroidal Glycosides from Solanum xanthocarpum and studies on their antifungal activities. Nat. Prod. Res. *21*, 585 - 590.

Singh, V.K., Ali, Z.A., Zaide, S.T.H., Siddiqui, M.K. (1996). Ethnomedicinal uses of plants of Gonda district forests of Uttar Pradesh, India. Fitoterpia. *67(2)*, 129 - 139.

Sohrabipour, S., Kharazmi, F., Soltani, N. and Kamlinejad, M. (2014). Biphasic effect of *Solanum nigrum* fruit extract on vascular mesenteric beds in non-diabetic and streptozotocin-induced diabetic rats. Pharmacogn. Res.*6 (2)*, 148 - 152.

Soni, P., Siddiqui, A.A., Dwivedi, J. and Soni, V. (2012).Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree.Asian Pac. J. Trop. Biomed. *12*, 1002 - 1008.

Stanker, L.H., Kamps-Holtzapple, C. and Friedman, M. (1994).Development and characterization of monoclonal antibodies that differentiate between potato and tomato glycoalkaloid and aglycons. Agric. Food Chem.*42*, 2360 - 2366.

Sudheesh, S., Presannakumar, G., Vijayakumar, S. and Vijayalashmi, N.R. (1997).Hypolipidemic effect of flavonoids from *Solanum melongena.*Plant Foods Hum. Nutr.*51*, 321 - 330.

Taherpour, A., Khodaei, M.M., Ameen, B.A.H., Ghaitouli, M., Mahdizadeh, N., Amjadian, H.R. and Larijani, K. (2013).Chemical composition analysis of the essential oil of Solanumnnigrum L. by HS/SPME method and calculation of the biochemical coefficients of the components.Arabian Journal of Chemistry.[Accessed on 24.12.2015].Available at http://dx.doi.org/10.1016/j.arabje.2013.08.015.

Tamboli, E.T., Chester, K. and Ahmad, S. (2015). Quality control aspects of herbs and botanicals in developing countries: *Coleus forskohlii* Briq: a case study. J. Pharm. Bioallied. Sci. *7(4)*, 254 - 259.

Taye, B., Giday, M., Animut, A., Seid, J., (2011).Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. Asian Pac. J. Trop. Biomed. *5*, 370 - 375.

Trease, G.E. and Evans, W.C. (2011). Pharmacognosy. Braillian Tridal, Macmillian Publishers, Canada, pp. 89-95.

Tumbilen, Y., Frary, A., Mutlu, S. and Doganlar, S. (2011).Genetic diversity in Turkish eggplant (*Solanum melongena*) varieties as determined by morphological and molecular analyses. Int. Res. J. Biotech. *2(1)*, 016 - 025.

Van der Knapp, E. and Tanksley, S.D. (2003). The making of a bell pepper – shaped tomato fruit: identification of loci controlling fruit morphology in Yellow Stuff tomato. Theor. Appl. Genet.*107*, 139 - 147.

Vejdemo-Johansson, M., Vejdemo, S. and Ek, C. (2014). Comparing distributions of color words: Pitfalls and metric choices. PLoSONE. *9*, e89184.

Ven Murthy, M.R., Ranjekar, P.K., Ramassamy, C. and Deshpande, M. (2010). Scientific basis for the use of Indian ayurvedic medicinal plants in the treatment of neurodegenerative disorders: ashwagandha. Cent.Nerv. Syst. Agents Med. Chem. *10(3)*, 238 - 246.

Vohora, S.B., Kumar, I. and Khan, M.S.Y. (1984).Effect of alkaloids of *Solanum melongena* on the central nervous system, J. Ethnopharmacol. *11*, 331 - 336.

Wang, J., Gao, T. and Knapp, S. (2008). Ancient Chinese literature reveals pathways of eggplant domestication. Ann. Bot. *102*, 891 - 897.

WHO.(2013). WHO Traditional medicine strategy 2014-2023, World Health Organization.
Xiao, H., Jiang, N., Schaffner, E., Stockinger, E.J. and Van der Knaap, E. (2008).A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. Sci. *319*, 1527 - 1530.

Yousaf, Z., Masood, S., Shinwari, Z.K., Khan, M.A. and Rabani, A. (2008). Evaluation of taxonomic status of medicinal species of the genus *Hyoscyamous, Withania, Atropa* and Datura based on poly acrylamide gel electrophoresis. Pakistan J. Bot. *40(6)*, 2289 - 2297.

Yu, J., Xue, J.H. and Zhou, S.L. (2011). New universal *matK* primers for DNA barcoding of angiosperms. J. Syst. Evol. *49(3)*, 1762 - 1781.