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RESEARCH ARTICLE

Green-Spinach, Red-Spinach, and Tree-Spinach ('Three-Fold Spinach' in Sri Lanka): An Insight into Phylogenetics and Consumer Preference

T. H. I. Gayathree[1], S. I. Karunarathne[1], L. T. Ranaweera[1], H. S. M. Jayarathne[1], S. K. Kannangara[1], A. P. D. T. Ranathunga[1], C. Weebadde[2], S. S. Sooriyapathirana[1,3]*

[1]Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, 20400, Peradeniya, Sri Lanka, [2]Department of Plant, Soil and Microbial Sciences, College of Agriculture and Natural Resources, Michigan State University, East Lansing, 48824, USA, [3]Postgraduate Institute of Science, University of Peradeniya, 20400, Peradeniya, Sri Lanka

ABSTRACT

Three types of spinach, green spinach (GS), red spinach (RS), and tree spinach (TS) are consumed in Sri Lanka. GS, RS, and TS are referred to as *Basella alba*, *Basella rubra*, and *Talinum fruticosum* respectively. However, some taxonomists categorize GS and RS under *B. alba* causing an ambiguity. Due to the poor sanitation, consumers prefer to purchase greenhouse-grown spinach over field grown material. However, the taste parameters of field grown and greenhouse-grown spinach have not been assessed. The objectives of the present study were to resolve the taxonomic ambiguity between GS and RS, identify the evolutionary relationship of TS to other two species and to assess the organoleptic preference on the dishes prepared using greenhouse and field-grown shoot-tops of three spinach. The genomic DNA extracted from GS, RS, and TS, PCR amplified and sequenced for the barcoding markers *rbcL, ITS, matK-trnT and atpB-rbcL.* The sequences obtained along with other reported related sequences were subjected to phylogenetic analysis. A sensory test was carried out using the shoot-tops of three species grown under greenhouse and field conditions. The taste panelists were asked to rank the dishes for preferred levels of color, aroma, texture, bitterness, and overall taste and the data were subjected to the association analysis. The *rbcL* and *ITS* markers separate GS and RS into two well -supported clades, *B. alba* and *B. rubra* respectively. The polymorphisms of *atpB-rbcL* and *matK-trnT* markers support the definition of two species. The monophyly of *B. alba* and *B. rubra* with *T. fruticosum* must be the reason for the same palate in dishes and designation of all three species under "spinach" in Sri Lanka. The taste panel data demonstrated that there is no specific fondness for greenhouse or field grown materials enabling the popularization of greenhouse-grown spinach to answer the safety concerns.

Keywords: *Basella alba*; *Basella rubra*; *Basella*ceae; Portulacaceae; Spinach in Sri Lanka; *Talinum fruticosum*; True spinach

INTRODUCTION

Green leafy vegetables (GLVs) are one of the most important components in our diet. The GLVs provide vitamins, dietary fiber, minerals, carbohydrates and proteins (Slavin and Lloyd, 2012). Spinach is an important GLV that we consume frequently. The name 'spinach' is a versatile term used vaguely in different parts of the world (Acikgoz and Adiloglu, 2018). The 'true spinach' is the species called *Spinacia oleraceae* of family Oleraceae (Deshmukh and Gaikwad, 2014). However, especially in Sri Lanka, people use the term 'spinach' for three GLVs namely *Basella alba* L. and *B. rubra* L. of the Family *Basella*ceae and *Talinum fruticosum* (Jacq.) Wild. of the Family Portulacaceae (Acikgoz and Adiloglu, 2018; Alexandre et al., 2017; Sperling and Bittrich, 1993). Both *Basella* species are found in Sri Lanka where *B. alba* is referred to as Green Spinach (GS), or Green *Nivithi* and *B. rubra* is referred to as Red Spinach (RS) or Red *Nivithi* (Dassanayake and Fosberg, 1995). However, in some other information sources, RS is also identified as *B. alba* (Deshmukh and Gaikwad, 2014; Roy et al., 2010) requiring a taxonomic revision for these two species. GS and RS are also commonly known as Ceylon spinach, Malabar spinach, Indian spinach, Climber spinach, and Vine spinach in various regions of the world complicating the exact nomenclature of these two species (Acikgoz and Adiloglu, 2018; Deshmukh and Gaikwad, 2014; Singh et al., 2016).

***Corresponding author:**
S. S. Sooriyapathirana, Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, 20400, Peradeniya, Sri Lanka. Postgraduate Institute of Science, University of Peradeniya, 20400, Peradeniya, Sri Lanka. **E-mail:** sunethuop@gmail.com

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In Sri Lanka, *T. fruticosum* is referred to as Tree-Spinach (TS) or Tree *Nivithi* because, it is an upright small herbaceous plant (Alexandre et al., 2017; Wood, 2013). The two *Basella* species, GS and RS, are very popular GLVs in Sri Lanka. Although consumed in rural parts as a delicious GLV, TS is still considered as a seasonal soft weed appearing after rainy seasons (Smith, 2004; Njoroge et al., 2004). Interestingly, despite falling under a different family, when cooked, TS possesses the same taste to GS or RS. Although TS is considered as a type of spinach, it shows distinctive variations from the two *Basella* species that are creeping vines which can grow up to 12m if support is available for vining (Sperling and Bittrich, 1993). Moreover, the leaves of the *Basella* vines are fleshy and ovate, and the stems are green and red in GS and RS respectively. In contrast, the leaves of TS are green and spathulate retaining its morphological distinctiveness from GS and RS (Flora and Fauna Web, 2019). The immature and just matured leaves and soft vines/stems (collectively referred to as shoot-tops) are used as the edible parts of *Basella* spp. Similarly, the shoot-tops of TS without flowers and fruits are used as the edible part. The rural people prefer to harvest TS shoot-tops before flowering, and if occasionally flowers are available, they are removed during the cleaning (Kristine et al., 2015).

Basella shoot-tops have a significant phytonutrient profile with a broad spectrum of vitamins, minerals, and antioxidants (Afolabi and Oloyede, 2014; Liao et al., 2015). *Basella* shoot-tops also contain proteins, fat, vitamins A, C, E, K, B9, niacin and thiamine, and minerals such as calcium, iron, magnesium, manganese, and copper (Folarin et al., 2001). Several studies show that *B. rubra* is a rich source of antioxidants such as β-carotene, lutein, zeaxanthine, and carotenoids (Singh et al., 2016; Sonkar et al., 2012). In addition to the dietary fiber present in stems and leaves of the plants, *Basella* spp. are also a rich source of non-starchy polysaccharide; a type of mucilage, which possesses a wide range of beneficial effects (Glassgen et al., 1993). Yanadaiah et al. (2011); have shown that GS possesses a flavonoid named Kaempherol. Further *Basella* leaves are a rich source of iron, which helps to reduce anemia (Bamidele et al., 2010). The leaves of GS and RS are composed of a high amount of mucilage, and it is enriched with a mixture of polysaccharides (Palanuvej et al., 2009) and composed of glucan (de Boer et al., 2005). Toshiyuki et al. (2001) have shown that *Basella* mucilage provides excellent consistency that aids for improvement as cosmetics and medicine for skin diseases. Furthermore many phytochemical studies have reported that *Basella* shoot-tops are enriched with anthocyanins causing purple or violet color in fruits, flowers, stems, and leaves (Glassgen et al., 1993), and several types of phenolic compounds which have high potential in antioxidant activity (Maisuthisakul et al., 2008).

In traditional medicine, GS is used to treat hemorrhages, sexual weaknesses, ulcers, skin diseases, as a laxative for children and pregnant mothers (Shruthi et al., 2012), cold-related infections, cough, and anemia (Rahamatullah et al., 2010). Premakumari et al. (2010) have reported that the methanolic extracts of GS possess antimicrobial activity. A study conducted using the aqueous ethanolic, and petroleum ether extracts of the leaves of RS have exhibited antimicrobial activities (Sen et al., 2010).

TS also has many therapeutic values and high nutritional values. Ezekwe et al. (2001) have shown that TS is a rich source of vitamin C, vitamin E, omega-3 fatty acids, calcium, magnesium, soluble fibers (pectin), potassium, β-carotene, proteins, and dietary fiber. Liang et al. (2011) have reported that polysaccharides of *T. fruticosum* are effective in hepatoprotective activity and as an antioxidant and effective in controlling diabetes mellitus and high cholesterol levels (Joshua et al., 2012).

Because of the health concerns and poor cleanliness of the field-grown products, currently, people prefer to purchase greenhouse-grown GLVs (Gruda, 2005; GPN, 2019; The Food Journal and Food, Nutrition and Science, 2015). There are frequent cases of food poisoning due to contaminated GLVs, and the environmental conditions in the hot and humid climates in tropical countries such as Sri Lanka favors the growth of pests and pathogens (McDonald and Stukenbrock, 2016; Anderson et al., 2004). However, no studies have ever done in Sri Lanka to check the consumer preference on greenhouse-grown GS, RS, and TS over field-grown counterparts. Therefore the objectives of the present study was to resolve the species delimits of GS and RS and the phylogenetic positions of GS, RS and TS within the related worldwide germplasm, and to assess the consumer preference on the dishes prepared using greenhouse and field-grown shoot-tops of the three species.

MATERIALS AND METHODS

Plant material

The stem cuttings of TS, GS and RS were collected from National Agricultural Technology Park at *Gannoruwa* (7.165930 °N; 80.3527804 °E) and established separately in a greenhouse at *Peradeniya* (7.161151°N; 80.353180 °E) and a field at *Nawalapitiya* (7.258172 °N, 80.598116 °E), Sri Lanka. The plants were raised according to general crop recommendations for spinach given in Department of Agriculture, Sri Lanka (DOA, 2018). The plant morphological variation; especially regarding the edible shoot-tops were observed.

Molecular analysis

DNA extraction, PCR and sequencing

The immature leaves were collected from each accession, ground in liquid nitrogen to acquire a fine powder, and stored at -80 °C until processed for DNA extraction. Then the genomic DNA was extracted from young leaves using DNeasy® Plant Mini Kit (Qiagen, Solna, Sweden) according to the manufacturer's instructions. The DNA was stored at -20 °C. The extracted DNA samples were subsequently amplified with four standard universal plant DNA barcoding primer pairs; *rbcL, ITS, atpB-rbcL, and matK-trnT*. (Table 1) The PCR was carried out in a thermal cycler (Takara, Otsu Shiga, Japan) according to the profiles indicated in Table 1. The 30 µl PCR mixtures were prepared containing 1× Go Taq® Green Master Mix (Promega Corporation, Madison, Wisconsin, USA), 1µl of each forward and reverse primers, 7 µl of spermidine and 1 µl of template DNA. The amplified PCR products were visualized and size separated in 2 % agarose gels. The PCR products were purified using QIAquick® PCR Purification Kit (Catalog No: 28104, Qiagen, Hilden, Germany). The purified PCR products of all four markers were cycle sequenced (3×) using the Genetic Analyzer ABI 3500 (Applied Biosystems®).

Assessment of the consumer preference

For the culinary preparation according to the most common recipe for spinach in Sri Lanka; first, 50 g of chopped onions and 10 g of garlic pieces were tempered with 15 ml of coconut oil in a frying pan. Then 1kg of spinach shoot-tops were weighed and cut into about 5 cm pieces and tossed into the frying pan. The 15 g of salt, 5 g of black pepper and 10 g of paprika powder were also added and mixed. Then, the heat was reduced, and 150 ml of coconut milk (first extract) was added whilst stirring. Finally, the dish was allowed to simmer for about another two minutes. The prepared dishes (field-grown and greenhouse-grown GS, RS, and TS) were served to a panel of 30 human-subjects to rank according to the preferred levels of color, aroma, texture, bitterness and overall taste. For each of these parameters, a three-tier scoring system was employed, and panelists were requested to assign three for the highest, two for the medium and one for the least preferred levels of each parameter (Simmen et al., 2004). Consumer preference data were subjected to association analysis using chi square ($\chi 2$) test and Cramer's V coefficient (CVC) calculation available in the FREQ Procedure of the Statistical Package, SAS 9.1 (SAS Institute, Cary, NC, USA).

Phylogenetic analysis

The MEGA software v.7 (Kumar et al., 2016) was used to edit the raw sequence reads and to construct the consensus sequences. To search the phylogenetic position of the study-species, the phylogeny given in Xu et al. (2018) was reconstructed with the sequences generated in this study. Initially, the multiple sequence alignments were constructed separately for *ITS* and *rbcL* markers individually and then the alignments were concatenated in MEGA v.7. The phylogenetic concordance of combined datasets was checked through a partition homogeneity test (ILD) (Planet, 2006). Since we used a non-coding nuclear marker (*ITS*) and coding plastid marker (*rbcL*), the differential evolutionary processes must be accounted in the downstream analysis. Thus, the PartitionFinder 2 (Lanfear et al., 2016) was employed to define the best partition scheme and the best model of evolution for each partition. The data blocks in our alignments were predefined for coding and non-coding

Table 1: The primer details and the PCR profiles of the DNA barcoding markers

DNA marker	Sequence	PCR Profile										References
		Initial denaturation		Denaturation		Primer annealing		Initial extension		Final extension		
		T[a]	Time	T	Time	T	Time	T	Time	T	Time	
rbcL	F-ATGTCACCACAAACAG AGACTAAAGC	98	45 sec	98	10 sec	55	30 sec	72	40 sec	72	10 mins	Levin et al., (2003)
	R- GTAAAATCAAGTC CACCRCG											Kress and Erickson, (2007)
ITS1-4	F-TCCGTAGGTGA ACCTTGCGG	95	3 mins	95	1 min	55	1 min	72	1.30 mins	72	4 mins	White et al., (1990)
	R-TCCTCCGC TTATTGATATGC											
atpB-rbcL spacer	F-GAAGTAGT AGGATTGATTCTC	94	4 mins	94	30 sec	45	30 sec	72	2 mins	72	5 mins	Hoot and Taylor, (2001)
	R-TACAGTTGT CCATGTACCAG											
matK-trnT spacer	F-GCATAAATATAYTC CYGAAARATAAGTGG	95	1 min 30 sec	95	30 sec	48	1 min	68	2 mins	68	20 mins	Wicke and Quandt, (2009)
	R-TGGGTTGCTAACTCAATGG											

[a]Tempertaures given in °C

markers in PartitionFinder 2 platform in CIPRES Science Gateway (Miller et al., 2010). In this platform, the model selection was carried out using (Cavanaugh, 1997) corrected Akaike information criteria (AICc) using hcluster (Lanfear et al., 2014) and K-means algorithms (Frandsen et al., 2015). The phylogeny was constructed in Maximum Likelihood (ML) and Bayesian frameworks for higher statistical accuracy. The ML tree search was implemented using the rapid bootstrap algorithm (Stamatakis et al., 2008) for 1000 iterations in RAxML-VI-HPC workflow (Stamatakis, 2006) using CIPRES supercomputer. In the tree construction, the tree was evaluated using the GTRGAMMA model and related the partition profile for our dataset to conclude a rigorous analysis. All the bipartition values were used to calculate the final bootstrap values. The best tree with highest –log likelihood value was used to draw a single tree topology using all the bipartitions during the analysis. MrBays (Huelsenbeck and Ronquist, 2001) was used in the CIPRES platform to construct the phylogenetic tree in the Bayesian framework. The partition log file that contains the model information and partition criteria was used to assess the different markers separately during the tree- search. The trees were probed in tree spaces using two hot and cold chains of Markov chain Monte Carlo (MCMC) running for 60 million generations. The initial 25% of the trees were discarded as burn-in, and the trees probed after maximum chain convergence were used to draw the final 50% majority rule consensus tree. The chain convergence and tree independent sampling were checked by assessing Effective Sample Size (ESS) in TRACER v1. 4 (Rambaut and Drummond, 2007). Finally, all the trees constructed in the study were visualized and edited using FigTree v1.4.3 (Rambaut, 2014).

RESULTS AND DISCUSSION

Morphological features of the shoot-tops

The morphological structures of the leaf shape, leaf size, stem color, and inflorescence/flower of GS, RS, and TS observed are displayed in Fig. 1. Both GS and RS exhibited succulent, branched, smooth vines which reaches about 12m in length and TS was observed as an erect herb of about 30–100cm in height with swollen roots and succulent stems. GS and TS got green color stems while RS got a reddish purple color stem. Stems of GS and RS showed quadrangular shape of about 1 to 3 cm thickness with prominent nodes and internodes. GS and RS got fleshy, broad, alternate/ovate/heart shaped, chordate base, dark green color leaves which are tapering to a pointed tip (according to the key given in Norton-Brown Herbarium, 2018). In contrast, the leaves of TS got oblanceolate shaped leaves which lush, green in color, fleshy up to about 5- 8 cm length. The leaves of TS were observed around the stem as spirally arranged nearly opposite and crowded at the top of the stem (Norton-Brown Herbarium, 2018).

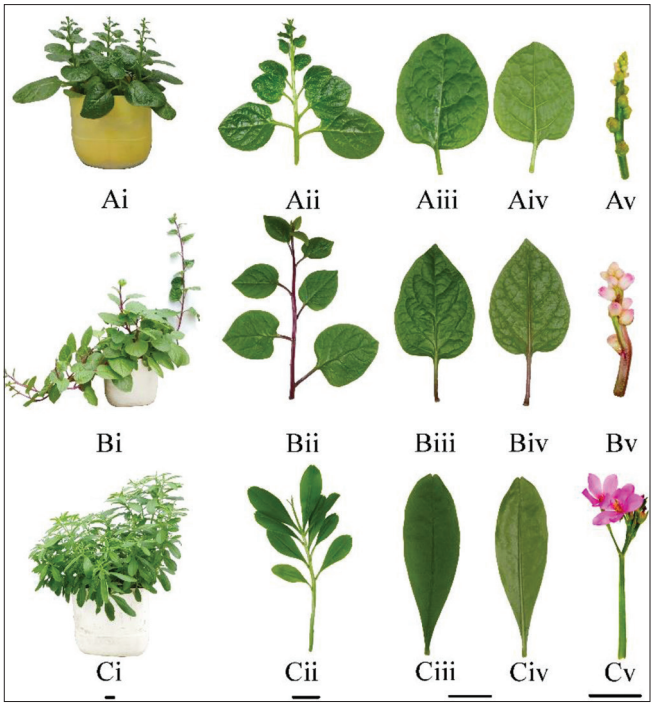


Fig 1. The morphological features of the key plant parts (plant, shoot-top and leaf and inflorescence/flower). A: Green Spinach (*Basella alba*); B: Red Spinach (*Basella rubra*); C: Tree Spinach (*Talinum fruticosum*); i: greenhouse grown potted plants; ii: shoot-tops at edible stage; iii: adaxial view of the leaf; iv: abaxial view of the leaf; v: inflorescence/ flower. Scale bars represent 1 cm separately for pot, shoot-top leaf, and inflorescence/flower images.

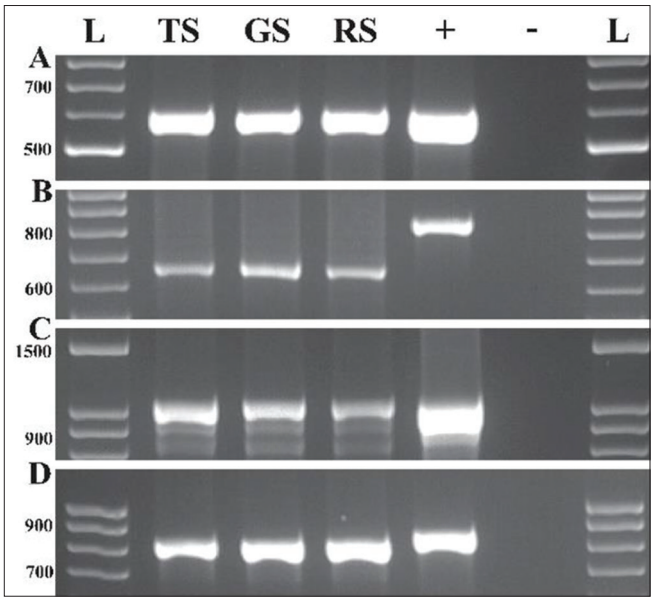


Fig 2. The PCR products size separated on 2% agarose gels. A: *rbcL*; B: *ITS1-4*; C: *atpB-rbcL* spacer; D: *matK-trnT*. L: Ladder. TS: Tree Spinach (*Talinum fruticosum*); GS: Green Spinach (*Basella alba*); RS: Red Spinach (*Basella rubra*). +: Positive Control (rice); -: Negative control.

Species delimits and their phylogenetic positions

The PCR amplicons obtained for the four plant DNA markers are given in Fig. 2. The DNA sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/nuccore) under the accession numbers of *rbcL* (MK598680-MK598685); *ITS1-4* (MK610431-MK610436); *atpB-rbcL* spacer (MK610244-MK610249); *matK-trnT* (MK610250-MK610255).

According to the phylogenetic analysis performed, there was a similar clade structure with higher node support in the trees constructed in both ML and Bayesian frameworks. The Bayesian trees converged maximally after initial 50,000 runs, and the initial 10% of the trees were discarded as burn-in. The ESS is greater than 200 for all the considered priors indicated that the tree search was carried out independently in the tree-space (Stamatakis et al., 2008; Huelsenbeck and Ronquist, 2001; Rambaut and Drummond, 2007).

According to the phylogenetic analyses, the two *Basella* species, GS and RS, assessed in the present study got clustered with the other *B. alba* species in the world (Fig. 3). Thereby the clade structure of the Family Basellaceae was congruent with the previous studies. The species representing the genus *Basella* were separated into two clades (Fig. 3 and 4). The GS and RS were nested separately in the two clades. The locus *ITS* is monomorphic between

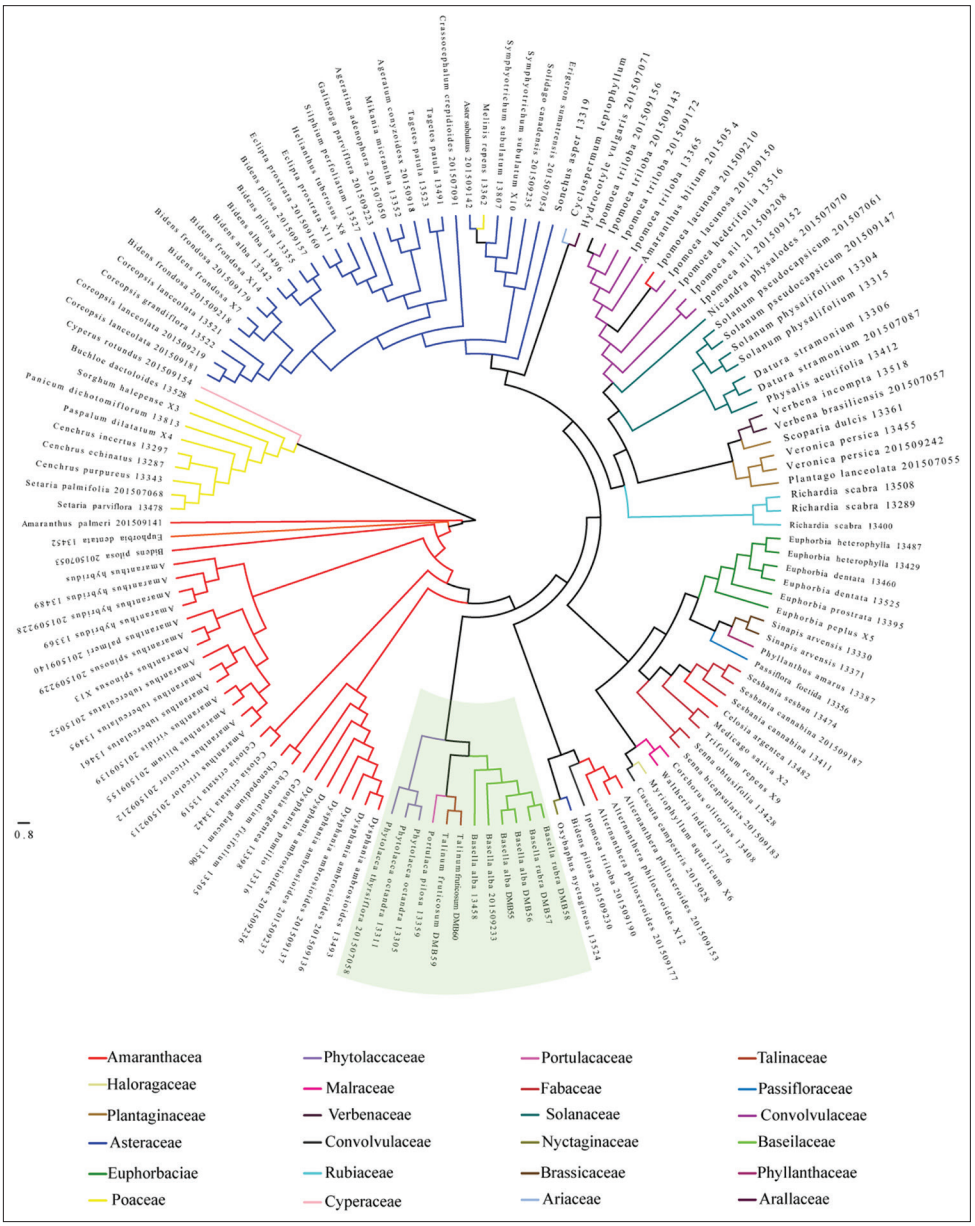


Fig 3. Phylogenetic tree constructed using the combined data of the markers, *rbcL* and *ITS* for the genera *Basella* and *Talinum* with respect to the related Families. The sequences generated in the present study are represented by the voucher numbers DMB55-DMB60. The scale bar indicates the nucleotide substitution per site.

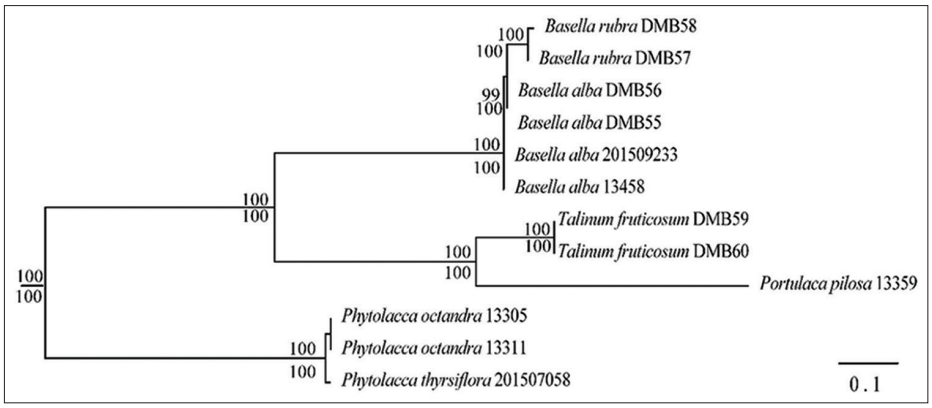


Fig 4. The relevant clade of the best tree given by ML tree-search for *rbcL* and *ITS* loci. The sequences generated in the present study are represented by the voucher numbers DMB55-DMB60. The scale bar indicates the nucleotide substitutions per site. The bootstrap values are given over the nodes and posterior probabilities are given below the nodes.

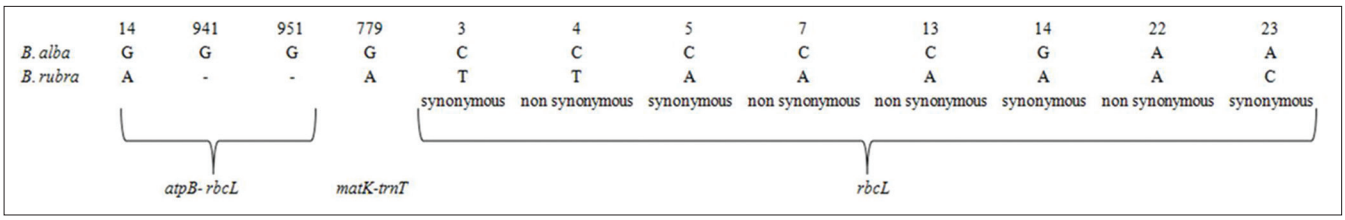


Fig 5. The SNP and INDEL profile generated using sequence polymorphisms observed for the markers assessed for *B. alba* and B. *rubra*. The *ITS* marker is not shown because of the absence of mutations between *B. alba* and B. *rubra* found in Sri Lanka.

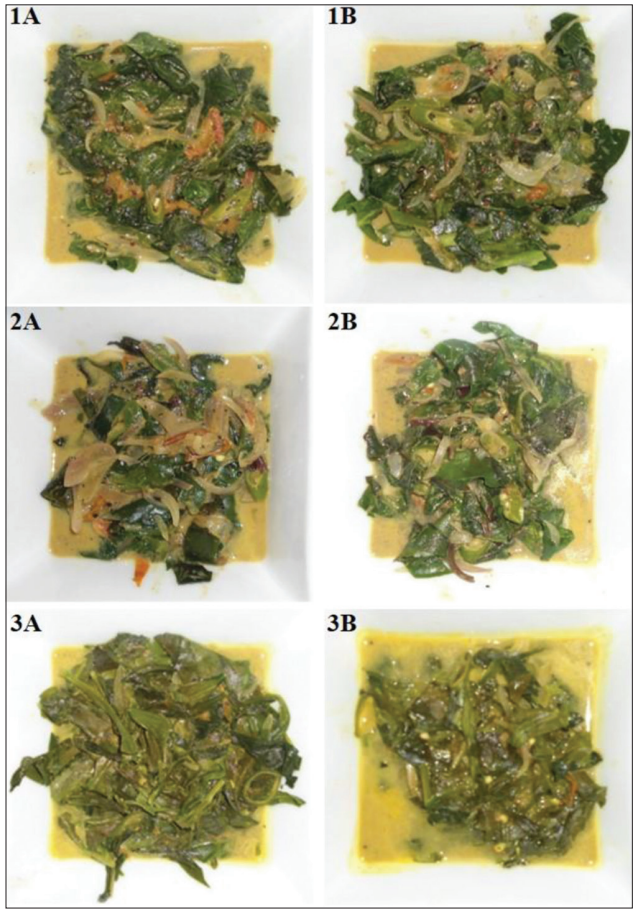
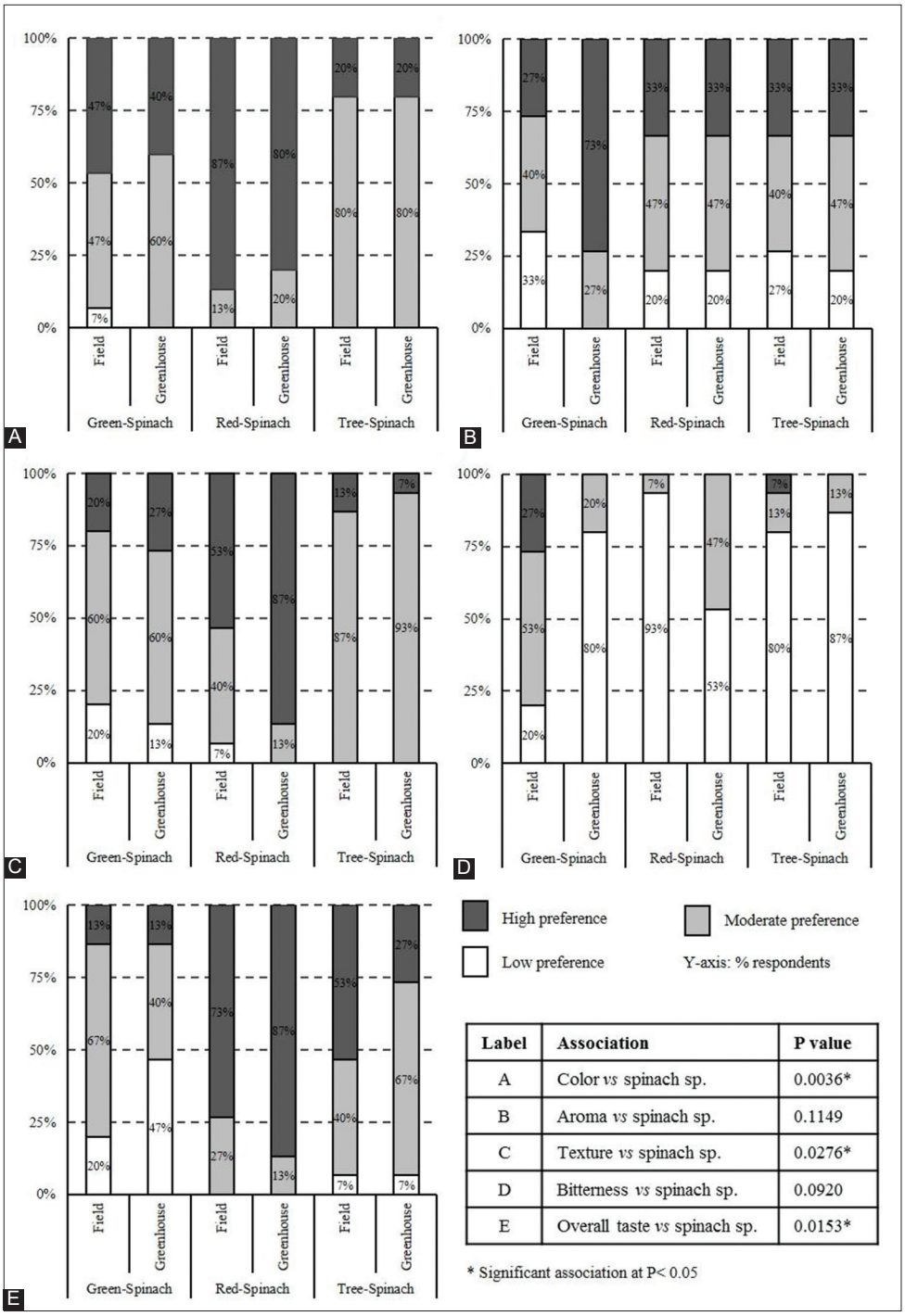


Fig 6. The cooked samples of the spinach shoot-tops. 1: Green spinach (*Basella alba*); 2: Red spinach (*Basella rubra*); 3: Tree spinach (*Talinum fruticosum*); A: From the Leaf samples collected from the field; B: From the Leaf samples collected from the greenhouse.



Label	Association	P value
A	Color *vs* spinach sp.	0.0036*
B	Aroma *vs* spinach sp.	0.1149
C	Texture *vs* spinach sp.	0.0276*
D	Bitterness *vs* spinach sp.	0.0920
E	Overall taste *vs* spinach sp.	0.0153*

* Significant association at P< 0.05

Fig 7. The association analysis between each organoleptic property and the three spinach species; Green spinach (*Basella alba*); 2: Red spinach (*Basella rubra*); 3: Tree spinach (*Talinum fruticosum*). The detailed legend is shown in the left-bottom cage.

GS and RS. However, the locus *rbcL* comprised of four non-synonymous and four synonymous mutations along with three mutations in *atpB-rbcL* and one mutation in *matK-trnT* (Fig. 5). Remarkably GS and RS got variations in the *rbcL* deduced amino acid sequences. There were P20Y, Q21K, and N26T AA substitutions found RS and GS respectively (Fig. 5). Therefore, *rbcL* sequence polymorphism can be successfully employed to discriminate the two species as *B. alba* and B. *rubra*. Since, some of the existing classification attempts to refer *B. rubra* also as *B. alba* (Deshmukh and Gaikwad, 2014; Roy et al., 2010), based on the *rbcL* polymorphism it can be suggested that *B. rubra* is a separate species as previously classified by Dassanayake and Fosberg, (1995). When determining the taxonomic position of TS, it got clustered with another member (*Portulaca pilosa*) which is also coming under the family Portulacaceae

(Fig. 3). Moreover, the clade consisting of *T. fruticosum* and *P. pilosa* was the most recently diverged clade from the *Basella* clade within the family Portulacaceae (Fig. 3). The close phylogenetic relationship between *Talinum*/*Portulaca* clade and the *Basella* clade must have been the reason for having the similar culinary and sensory attributes in TS to those of GS and RS. The phylogenetic closeness between GS and RS to TS would have contributed to the naming of TS as a spinach species in Sri Lanka.

Assessment of the consumer preference

The latest studies have indicated that consumers tend to prefer greenhouse-grown GLVs than the field grown counterparts (Gruda, 2005; GPN, 2019; The Food Journal and Food, Nutrition and Science, 2015). In order, to check whether greenhouse-grown GLVs are preferred more than the field grown plants, a taste panel was performed with cooked dishes of GS, RS and TS grown under field and greenhouse conditions. The images of the prepared dishes are given in Fig. 6. The dishes of *B. alba* (Fig. 6.1A and 6.1B) and *B. rubra* (Fig. 6.2A and 6.2B) were more intense green in color where as a dull green appearance was observed in the dish of *T. fruticosum* (Fig. 6.3A and 6.3B). However, within the species, the appearance of the dishes made out of greenhouse and field grown material were not significantly different except in *B. rubra*, where the dish prepared from greenhouse-grown material had less intensity in its color (Fig. 6).

The significant associations were observed between the preferred levels of color, texture and overall taste with the species ($P<0.05$) (Fig. 7). In all sensory parameters, *B. rubra* was the most preferred spinach species compared with the other two species. There was no effect of the growth condition (field grown, or greenhouse grown) for the association between color and the species ($P<0.05$) (Fig. 7A). Similarly, there was no significant difference in the preferred level of aroma and the species for *B. rubra* and *T. fruticosum*. However, the aroma of greenhouse grown *B. alba* was significantly more preferred by the panelists ($P<0.05$) (Fig. 7B). Moreover, based on the association between preferred level of texture and the species, the greenhouse-grown plants were more preferred than the field grown stuff of all three species ($P<0.05$) (Fig. 7C). The species level variations were observed for the preferred levels of bitterness according the growth condition where greenhouse-grown *B. alba* and *T. fruticosum* and the field grown were least bitter for the tasters ($P<0.05$) (Fig. 7D). There was an observable difference in the preferred levels of overall taste based on the growth condition only for *T. fruticosum* ($P<0.05$) (Fig. 7E). The overall taste of *B. alba* was the least preferred while that of *B. rubra* was the highest preferred ($P<0.05$) (Fig. 7E).

CONCLUSIONS

The phylogenetic analysis based on *rbcL* and *ITS* markers separated GS and RS into two well -supported clades, implying that they should be considered under *B. alba* and *B. rubra* respectively. These two species also have sequence polymorphisms for *atpB-rbcL* and *matK-trnT* markers supporting the delimitation into two species. The TS, *T. fruticosum* of family Portulacaceae, is in a closely related clade of the family Bacillaceae. This evolutionary relatedness between *B. alba*/*B. rubra* with *T. fruticosum* must be the reason for the similar taste in prepared dishes and basis to designate under same vernacular name "spinach" in Sri Lanka. The organoleptic analysis based on the dishes prepared using greenhouse and field grown GS, RS, and TS showed that there is no differential preference for the two types of material (greenhouse and field). Therefore, the greenhouse-grown spinach shoot-tops can be popularized for the safety concerns.

Authors' contributions

Thenuwara Hannadige Ishara Gayathree maintained the plants, prepared dishes, led the laboratory experiments and contributed in conceptualization, data analysis and writing. Sachinthani Isurika Karunarathne, Lahiru Thilanka Ranaweera, Hashan Sri Madhubhashana Jayarathne, Sachithrani Kanchana Kannangara, and Agas Pathirannahalage Disnie Tharindi Ranathunga contributed in conceptualization, maintained plants, assisted in preparing dishes, conducted laboratory experiments, assisted in data analysis and drafting the manuscript. Cholani Weebadde and Suneth Sithumini Sooriyapathirana conceptualized the research questions and designs, secured funding, and lead the experiments, data analysis and preparation of the manuscript. Suneth Sithumini Sooriyapathirana administered the project and approved the manuscript for publication.

REFERENCES

Acikgoz, F. E. and S. Adiloglu. 2018. A review on a new exotic vegetable for Turkey: Malabar Spinach (*Basella alba* L. or *Basella rubra* L.). J. Hortic. 5: 239.

Afolabi, O. B. and O. I. Oloyede. 2014. Antioxidant properties of the extracts of *Talinum triangulare* and its effect on antioxidant enzymes in tissue homogenate of swiss albino rat. Int. J. Toxicol. 21: 307-313.

Alexandre, E. C. F., Pereira, L. S., Andrade, J. W. S., Filho, S. C. V and A. Jakelaitis. 2017. Plant biometric characterization and leaf micromorphometry of *Talinum* triangulare (Jacq.) Willd cultivated under shade. Rev. Ceres. 65: 44-55.

Anderson, C. R., Ponce, A. M and R. J. Price. 2004. Immunohistochemical identification of an extracellular matrix scaffold that microguides capillary sprouting *in vivo*. J. Histochem. Cytochem. 52: 1063-1072.

Bamidele, O., Akinnuga, A. M., Olorunfemi, J. O., Odetola, O. A., Oparaji, C. K and N. Ezeigbo. 2010. Effects of aqueous extract of *Basella alba* leaves on haematological and biochemical parameters in albino rats. Afr. J. Biotechnol. 9: 6952-6955.

Cavanaugh, J. E. 1997. Unifying the derivations for the Akaike and corrected Akaike information criteria. Stat. Prob. Lett. 33: 201-208.

Dassanayake, M. D. and F. R. Fosberg 1995. A Revised Handbook to the Flora of Ceylon. 7th Ed. Smithsonian Institution and the National Science Foundation, Sri Lanka.

de Boer, H. J., A. Kool, A. Broberg, W. R. Mziray, I. Hedberg and J. J. Levenfors. 2005. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol. 96: 461-469.

DOA. 2018. Department of Agriculture, Sri Lanka: Ministry of Agriculture, Government of Sri Lanka. Available from: https://www.doa.gov.lk. [Last accessed on 2018 Aug 04].

Deshmukh, S. A. and D. K. Gaikwad. 2014. A review of the taxonomy, ethnobotany, phytochemistry and pharmacology of *Basella alba* (*Basellaceae*). J. Appl. Pharm. Sci. 4: 153-165.

Ezekwe, M. O., Besong, S. A and P. E. Igbokwe. 2001. Beneficial influence of purslane and water leaf supplement to humans. FASEB J. 16: 639-645.

Flora and Fauna Web. 2019. Available from: https://www.florafaunaweb.nparks.gov.sg. [Last accessed on 2019 Jan 05].

Folarin, O. M., Bamiro, F. O and K. O. Esuoso. 2001. Distribution of nutritive elements in water leaf. Glob. J. Pure Appl. Sci. 7: 285-290.

Frandsen, P. B., B. Calcott, C. Mayer and R. Lanfear. 2015. Automatic selection of partitioning schemes for phylogenetic analyses using iterative k-means clustering of site rates. BMC Evol. Biol. 15: 13.

Glassgen, W. E., J. W. Metzger, S. Heuer and D. Strack. 1993. Betacyanins from fruits of Basella rubra. Phytochemistry. 33: 1525-1527.

GPN. 2019. Greenhouse Product News. Available from: https://www.gpnmag.com/article/greenhouse-vegetables-your-worst-enemies. [Last accessed on 2019 Jan 02].

Gruda, N. 2005. Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. Crit. Rev. Plant Sci. 24: 227-247.

Hoot, S. B. and W. C. Taylor. 2001. The utility of nuclear ITS, a LEAFY homolog intron, and chloroplast atpB-rbcL spacer region data in phylogenetic analyses and species delimitation in Isoetes. Am. Fern J. 91: 166-177.

Huelsenbeck, J. P. and F. Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17: 754-755.

Joshua, J. S., Mazumder, P. M., Rathinavelusamy, P and D. Sasmal. 2012. Role of antioxidants in phytomedicine with special reference to antidiabetic herbs. Asian Pac. J. Trop. Dis. 1: 969-979.

Kress, W. J. and D. L. Erickson. 2007. A two-locus global DNA barcode for land plants: The coding rbcL gene complements the non-coding trnH-psbA spacer region. PLoS One. 2: e508.

Kristine, B., S. Chien-chang and R. Consolacion. 2015. Chemical constituents of *Talinum* triangulare. Res. J. Pharm. Biol. Chem. Sci. 6: 167-171.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870-1874.

Lanfear, R., B. Calcott, D. Kainer, C. Mayer and A. Stamatakis. 2014. Selecting optimal partitioning schemes for phylogenomic datasets. BMC Evol. Biol. 14: 82.

Lanfear, R., P. B. Frandsen, A. M. Wright, T. Senfeld and B. Calcott. 2016. Partition finder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34: 772-773.

Levin, R. A., Wagner, W. L., Hoch, P. C., Nepokroeff, M., Pires, J. C., Zimmer, E. A and K. J. Sytsma. 2003. Family-level relationships of *Onagraceae* based on chloroplast rbcL and ndhF data. Am. J. Bot. 90: 107-115.

Liang, D., Q. Zhou, W. Gong, Y. Wang, Z. Nie, H. He, J. Li, J. Wu, C. Wu and J. Zhang. 2011. Studies on the antioxidant and hepatoprotective activities of polysaccharides from Tallinum triangulare. J. Ethnopharmacol. 136: 316- 321.

Liao, D. Y., Y. C. Chai, S. H. Wang, C. W. Chen and M. S. Tsai. 2015. Antioxidant activities and contents of flavonoids and phenolic acids of *Talinum triangulare* extracts and their immunomodulatory effects. J. Food Drug Anal. 23: 294-302.

Maisuthisakul, P., S. Pasuk and P. Ritthiruangdej. 2008. Relationship between antioxidant properties and chemical composition of some Thai plants. J. Food Compost. Anal. 21: 229-240.

McDonald, B. A. and E. H. Stukenbrock. 2016. Rapid emergence of pathogens in agro-ecosystems: Global threats to agricultural sustainability and food security. Philos. T. R. Soc. B. 371: 20160026.

Miller, M. A., W. Pfeiffer and T. Schwartz. 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. Gateway Computing Environments Workshop (GCE). Available from: http://www.ieeexplore.ieee.org/abstractdocument/5676129. [Last accessed on 2018 Dec 20].

Njoroge, G. N., R. W. Bussmann, B. Gemmill, L. E. Newton and V. W. Ngumi. 2004. Utilisation of weed species as sources of traditional medicines in central Kenya. Lyonia. 7: 71-87.

Norton-Brown Herbarium. 2018. Plant Identification Guides. Available from: http://www.nbh.psla.umd.edu/guides/guides.html. [Last accessed on 2018 Dec 30].

Palanuvej, C., S. Hokputsa, T. Tunsaringkarn and N. Ruangrungsi. 2009. *In vitro* glucose entrapment and alpha-glucosidase inhibition of mucilaginous substances from selected Thai medicinal plants. Sci. Pharm. 77: 837-850.

Planet, P. J. 2006. Tree disagreement: Measuring and testing incongruence in phylogenies. J. Biomed. Inform. 39: 86-102.

Premakumari, K. B., A. Siddiqua, S. Banu, J. Josephine, L. Jenita and B. Raj. 2010. Comparative antimicrobial studies of methanolic extract of muntingiacalabura, *Basella alba* and *Basella rubra* leaves. J. Pharmacogn. Phytochem. 2: 246-248.

Rahamatullah, M., M. R. Islam, M. Z. Kabir, M. H. Rashid, R. Jahan, R. Begum, S. Seraj, M. A. Khatun and A. Chowdhury. 2010. Folk medicinal practices in Vasu Bihar village, Bogra district, Bangladesh. Am. Eur. J. Agric. Environ. Sci. 4: 86-93.

Rambaut, A. 2014. FigTree, a Graphical Viewer of a Phylogenetic Trees. Available from: http://www.tree.bio.ed.ac.uk/software/figtree. [Last accessed on 2018 Dec 20].

Rambaut, A. and A. Drummond. 2007. Tracer v1. Available from: http://www.beast.bio.ed.ac.uk/tracer. [Last accessed on 2018 Dec 15].

Roy, S. K., G. Gangopadhyay and K. K. Mukherjee. 2010. Is stem twining form of Basella alba L. a naturally occurring variant? Curr. Sci. 98: 1370-1375.

Sen, K., A. Goel, S. Rawal, N. Mahajan, S. Baboo and S. Alak. 2010. Antimicrobial activity of Basella rubra leaves. Int. J. Pharm. Sci. 1: 88-91.

Shruthi, S. D., H. N. N. Kumar and R. Adhikari. 2012. A review on medicinal importance of Basella alba L. Int. J. Pharm. Pharm. Res. 4: 110-114.

Simmen, B., P. Pasquet and C. H. Hladik. 2004. Methods for assessing taste abilities and hedonic responses in human and non-human primates. Researching Food Habits: Methods and Problems. Berghahn Books, Oxford, pp. 87-99.

Singh, M., R. Singh and M. Kotecha. 2016. *Basella rubra* Linn. a review.Int. J. Basic Med. Sci. Pharm. 5: 206-223.

Slavin, J. L. and B. Lloyd. 2012. Health benefits of fruits and vegetables. Adv. Nutr. 3: 506-516.

Smith, M. A. K. 2004. Pendimethalin phytotoxicity and seedling weed control in Indian spinach (*Basella alba* L.). Crop Prot. 23: 201-204.

Sonkar, D. S., R. Gupta and S. A. Saraf. 2012. Effect of Basella rubra L. leaf extract on heamatological parameters and amylase activity. Pharmacogn. Commun. 2: 10-13.

Sperling, C. R. and V. Bittrich. 1993. Flowering plants dicotyledons. In: K. Kubitzki, J. G. Rohwer, V. Bittrich (Eds.), The Families and Genera of Vascular Plants, Basellaceae. Vol. 2. Springer, Berlin, Heidelberg.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 22: 2688-2690.

Stamatakis, A., P. Hoover and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57: 758-771.

The Food Journal and Food, Nutrition and Science. 2015. An Alliance between the Lempert Report and the Center for Food Integrity. Available from: https://www.foodnutritionscience.com/articles/greenhouse-growing. [Last accessed on 2019 Jan 02].

Toshiyuki, M., H. Kazuhiro and Y. Masayuki. 2001. Medicinal foodstuffs XXIII structures of new oleanane type Triterpene-oligoglycosides, *Basella* saponins A, B, C and D from the fresh aerial parts of *Basella rubra* L. Chem. Pharm. Bull. 49: 776-779.

White, T. J., T. Bruns, S. J. Lee and J. L. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. Vol. 18. Academic Press, Cambridge, Massachusetts, pp. 315-322.

Wicke, S. and D. Quandt. 2009. Universal primers for the amplification of the plastid trnK/matK region in land plants. Anales. Jard. Bot. Madrid. 66: 285-288.

Wood, J. R. 2013. Taxonomic revision of *Talinum* (*Talinaceae*) in Bolivia with a note on the occurrence of *Phemeranthus* (*Montiaceae*). Kew Bull. 68: 233-247.

Xu, S. Z., Z. Y. Li and X. H. Jin. 2018. DNA barcoding of invasive plants in China: A resource for identifying invasive plants. Mol. Ecol. Resour. 18: 128-136.

Yanadaiah, J. P., S. M. Lakshmi, K. N. Jayveera and Y. Sudhakar. 2011. Hepatoprotective activity of aqueous ethanolic extract of aerial parts of *Basella rubra* L. against carbon tetrachloride and paracetamol induced hepatotoxicity in rats. Int. J. Pharm. Pharm. Res. 3: 502-506.