Title

Systematics of the miracle berry genus, Synsepalum, sensu lato, and relatives (Sapotaceae)

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Systematics of the Miracle Berry Genus, *Synsepalum*, sensu lato, and Relatives (Sapotaceae)

By

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DISSERTATION

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Overview

This dissertation comprises three distinct but related research. The first chapter deals with the phylogenetic relationship of the miracle plants and relatives, the second is on the evolution of miraculin, and the third chapter is on the ethnobotanical survey of African immigrants in the Sacramento region of California. In the phylogeny chapter, species in the genera *Synsepalum* and *Englerophytum* were studied using molecular techniques to investigate if the two genera are closely related. *Synsepalum* is a genus of trees and shrubs native to tropical lowland areas of Africa (Ayensu, 1978). It has glabrous staminodes and imbricate to valvate corolla lobes (Anderberg & Swenson, 2003). A very common feature in the genus is their fused sepals, a character that gave the name to the genus *Synsepalum*. The genus has a very wide range of distribution and is very prominent in Africa. There are no published reports on phylogenetic relationships within the genus. (Borg et al., 2019) their studies of the *Englerophytum*-*Synsepalum* complex reported six lineages of which four of them were composed solely of species in *Synsepalum*. There are, however, some concerns with the lineages reported. Some of the lineages had just a single species which is not the type species. More species are needed to better understand the clades in *Synsepalum* and to confirm the taxonomic relationships among the species in the genus. *Synsepalum* is confirmed to constitute more than one lineage suggesting it is not monophyletic in its current definition. Overall, the study suggests the need for a comprehensive evaluation of all species currently recognized in the two genera.

On the evolution of miraculin, *S. dulcificum* (miracle berry) is one of the most prominent species in the genus due to its presence in the plant glycoprotein miraculin, a sweetening agent that helps in modifying taste. There are no reports as to whether miraculin is also produced in other species

of the genus or not. In this study, using a PCR protocol, the presence of the gene responsible for miraculin was investigated in other species in the genera *Synsepalum* and *Englerophytum*. Two species of *Synsepalum* produced PCR amplification products for the gene for miraculin (Chr10G0299340) while none of the species of *Englerophytum* implicated in this study showed any sign of miraculin. The inferred amino acid sequences of miraculin from the species that produced PCR amplification products for the gene for miraculin were combined with those from species containing miraculin-like proteins to investigate the evolution of miraculin. It is revealed that miraculin is distinct from miraculin-like proteins due to the presence of histidine 30 and histidine 60, the residue responsible for the taste-modifying property of miracle berries. Miraculin-like protein contains only histidine 60.

In chapter three, using a semi-structured questionnaire, African immigrants residing in different locations in and around Sacramento were interviewed on their use of medicinal plant knowledge after prior informed consent. Immigrants play a significant role in the transfer of medicinal plant knowledge, traditions, and cultures from their home country to their new area of primary residence. A total of 105 participants from 15 different African countries responded to the questionnaire. Participants were, between the ages of 20 and 60 comprising 65% female and 34% male. The result from this survey shows that many immigrants, though living in the global north where Western medicine is accessible, have rich knowledge of medicinal plants and still prioritize herbal medicines as an effective way to treat a wide variety of ailments.

Table of Contents

Chapter 1: Systematics of the miracle berry genus, *Synsepalum*, sensu lato, and relatives (Sapotaceae)

Abstract

Synsepalum and *Englerophytum* are two closely related genera of the tribe Chrysophylleae in the family Sapotaceae. It has been reported that the two genera are a monophyletic group, and their generic limitations are uncertain. *Synsepalum* is an economically important genus that includes the medicinally and culinarily important plant, -miracle berry, *S. dulcificum*. The phylogenetic relationships among the species are poorly understood. This study has been conducted to refine the phylogenetic relationships between and within the two genera utilizing nuclear and chloroplast DNA data. Bayesian analyses and Maximum likelihood of nuclear internal transcribed spacer (ITS) and plastid (trnH-psbA and rps16-trnK) sequences were used to reconstruct the phylogeny of the two genera. Phylogenetic trees generated for both regions resulted in the resolution of five major clades. Three of the clades correspond to species in the genus *Synsepalum* and two clades include species of *Englerophytum*. The two clades of *Englerophytum* are nested within *Synsepalum* suggesting that the two genera are closely related and may not merit their current circumscription as distinct genera. Also, *Synsepalum* is confirmed to constitute more than one lineage suggesting it is not monophyletic in its current definition. Overall, the study suggests the need for a comprehensive evaluation of all species currently recognized in the two genera.

Introduction

Sapotaceae is an angiosperm family having 53 genera and comprising about 1,200 species (Bartish et al., 2005). Diagnostic characters used to recognize Sapotaceae include the production of latex when cut at the bark- a common feature in trees growing in wet conditions, alternate, simple, and entire leaves except for a few genera like *Sarcoperma*. Flowers are either simple or entire, except for genera with staminodes in the corolla lobes. Stamens in Sapotaceace are generally opposite the corolla lobes. They also possess Malpighian hairs on different organs. Although they are known to be more prominent in the tropics, they are also found in many other parts of the world. Several studies of the family have shown that the classification of the family is notoriously complicated due to varied morphological differences and homoplasy (Bartish *et al*., 2005). Some authors (Aubréville, 1964), (Baehni, 1965), and (Pennington, 1991) have proposed different classifications for the family. (Aubréville, 1964) recognized 122 genera. This was reduced to 63 by (Baehni, 1965). Pennington (1991) further reduced the genera to 53. All the taxonomic work reported thus far for the different authors was based purely on morphological characters. The most utilized is the one by Pennington, 1991. Pennington recognized five tribes. Namely, Chrysophylleae, Isonandreae, Omphalocarpeae, Mimusopeae, and Sideroxyleae. In a more recent classification combining morphological character and molecular evidence, (Anderberg & Swenson 2003) in their evolutionary studies of the family reported three evolutionary lineages for Sapotaceae. Tribes Isonandreae, Mimusopeae, and Sideroxyleae formed a clade; Chrysophylleae and Omphalocarpeae formed a clade; Sarcosperma formed a clade relating to the other clades. *Synsepalum* and *Englerophytum* are part of the tribe Chrysophylleae, the largest of the five tribes recognized by Pennington. Species in this tribe are uniquely identified by the 4-5 merous flowers with a calyx in a single whorl.

Synsepalum (A.DC.) Daniell and *Englerophytum* K. Krause are two closely related genera of the tribe Chrysophylleae in the family Sapotaceae. These two genera comprise 35 and 19 internationally recognized species respectively and are predominantly distributed across West-Central tropical Africa (Borg *et al.*, 2019; Tchokponhoué *et al*., 2021). Both genera share the frequent presence of stipules, usually 5-merous flower structures with the irregular presence of small staminodes, similar seeds, and embryos. They are however considered to be different genera due to the consistent striate brochidodromous venation and strong fusion of the filaments into a staminal tube found in species of *Englerophytum*, whereas in species of *Synsepalum* leaf venation tends to be eucamptodromous and the filaments are free.

Figure 1a: Flower of Englerophytum

Source: (Kew, 2023)

Figure 1b: Fruits of Synsepalum

Source: (Britannica, 2023)

History of the Classification of *Synsepalum*

Synsepalum has undergone several taxonomic changes throughout history as new species have been discovered. It is comprised of trees and shrubs native to tropical lowland areas of Africa (Ayensu, 2008). It was described in 1852 and currently consists of about 35 species (Borg *et al.*, 2019), including the very popular miracle berry plant, *S. dulcificum* (Schumach. & Thonn.) Daniell which is the type species on which the genus is based. Like the genus *Diploon* Cronguist, *Synsepalum* has glabrous staminodes and imbricate to valvate corolla lobes (Anderberg & Swenson, 2003). A very common feature in the genus is their fused sepals, a character that gave the name to the genus. *Synsepalum* can also be characterized by its long spreading corolla lobes and large stipules (Anderberg & Swenson, 2003). Many of the species are very different from each other, making previous experts segregate them into smaller genera where the species are reasonably uniform. The current 35 recognized species in the genus are a combination of species from these previously recognized smaller genera, including *Afrosersalisia* A.Chev., *Pachystela* Radlk, *Vincentella* Pierre, *Synsepalum*, and *Tulestea*.

Pennington, (1991) considered previous generic classifications unsatisfactory and united all these genera under *Synsepalum*. He merged the small genera using overlapping characters highlighted in Table 1 to form the currently recognized genus. The combination of the following characters was used to describe *Synsepalum*: frequent occurrence of large stipules, eucamptodromous venation, 5-merous flowers, corolla nearly always rotate, cyathiform or shortly tubular with wide-spreading lobes, corolla lobe aestivation imbricate or induplicate valvate, stamens fixed at or near the top of the corolla tube, exerted with well-developed filaments. The seed is broad and not laterally compressed, with a broad adaxial scar that sometimes extends to cover most of the surface. The embryo has plano-convex cotyledons and endosperm is known to be generally absent in the genus. Due to the inconsistency in the characters of the small genera that were merged, species in the genus are often individually very distinct. This has complicated the taxonomic revision of the genus and caused many synonyms to have emerged. Also, the lumping of these genera to form the genus *Synsepalum* has been disputed by many authors as the conclusion was purely based on morphological characters without any consideration given to molecular evidence.

History of the Classification of *Englerophytum*

Englerophytum K. Krause was described as a genus (Krause, 1914), with *Englerophytum stelechanthum as* the type species. Five species were added to the genus (Aubréville, 1960, 1961, 1964a), two of which were newly described while the other three were products of new combinations of species previously classified in different genera. As opposed to the views of (Liben, 1989), who advocated for the distinct status of the genera *Englerophytum*, *Wildemaniodoxa* Aubrév. & Pellegr. and Zeyherella (Pierre ex Baill.) Aubrév. & Pellegr, (Pennington, 1991) united the genera based on the fusion of their filaments and the number of floral parts. Although (Pennington, 1991) considered *Synsepalum* to be closely related to *Englerophytum* because of the frequent presence of stipules, usually 5-merous flowers, irregular presence of small staminodes, and similar structure of seeds and embryo, he considered *Synsepalum* distinct from *Englerohpytum* due to the brochidodromous venation and strong tendency of fusion of the filaments in the staminal tube. While *Englerophytum* has a striate brochidodromous leaf venation and a tendency for fused filaments, *Synsepalum* exhibits eucamptodromous leaf venation patterns and free filaments.

Morphological Variation

When comparing *Synsepalum* and *Englerophytum*, it is seen from Figure 1 in the appendix that they are morphologically similar except for their leaf venation and staminal fusion. *Synsepalum* is eucamptodromous while *Englerophytum* is brochidodromous, and there is a strong tendency towards fusion of the filaments in the staminal tube in *Englerophytum*. These major variations have led some taxonomists to hold the view that the two genera should be merged. In contrast, (Borge *et al.*, 2019) reported that combined data from nuclear DNA, chloroplast DNA and morphology analyzed using parsimony suggested that *Synsepalum* and *Englerophytum* are distinct genera. The views of (Borg et al., 2019) are however completely different from the previously obtained results from (Swensen et al., 2005 & 2008) in which the two genera formed a single heterogeneous clade where species of *Synsepalum* genus were grouped within species of *Englerophytum*. (Swensen et al., 2005, 2008) viewed that the two genera form a clade but that neither is monophyletic by itself and the two should therefore be considered as single genus. The very slim differences between the two genera are a call for concern as the decision to either merge the two genera or separate them is yet to be resolved.

Distribution of species in the *Synsepalum* and *Englerophytum* complex

Species of *Synsepalum* and *Englerophytum* are distributed across tropical Africa. While some are endemic to just one country like *S. aubrevillei* in Cote d'Ivoire, others have a wide range of distribution in different countries in tropical Africa. Also, many species in the genus *Englerophytum* are endemic to Gabon. Species in both genera are mostly trees and are mostly found in the wet tropic biomes, with few species found in the seasonally dry region. Table 2 shows species in the genera, their distribution, habitat, and habits.

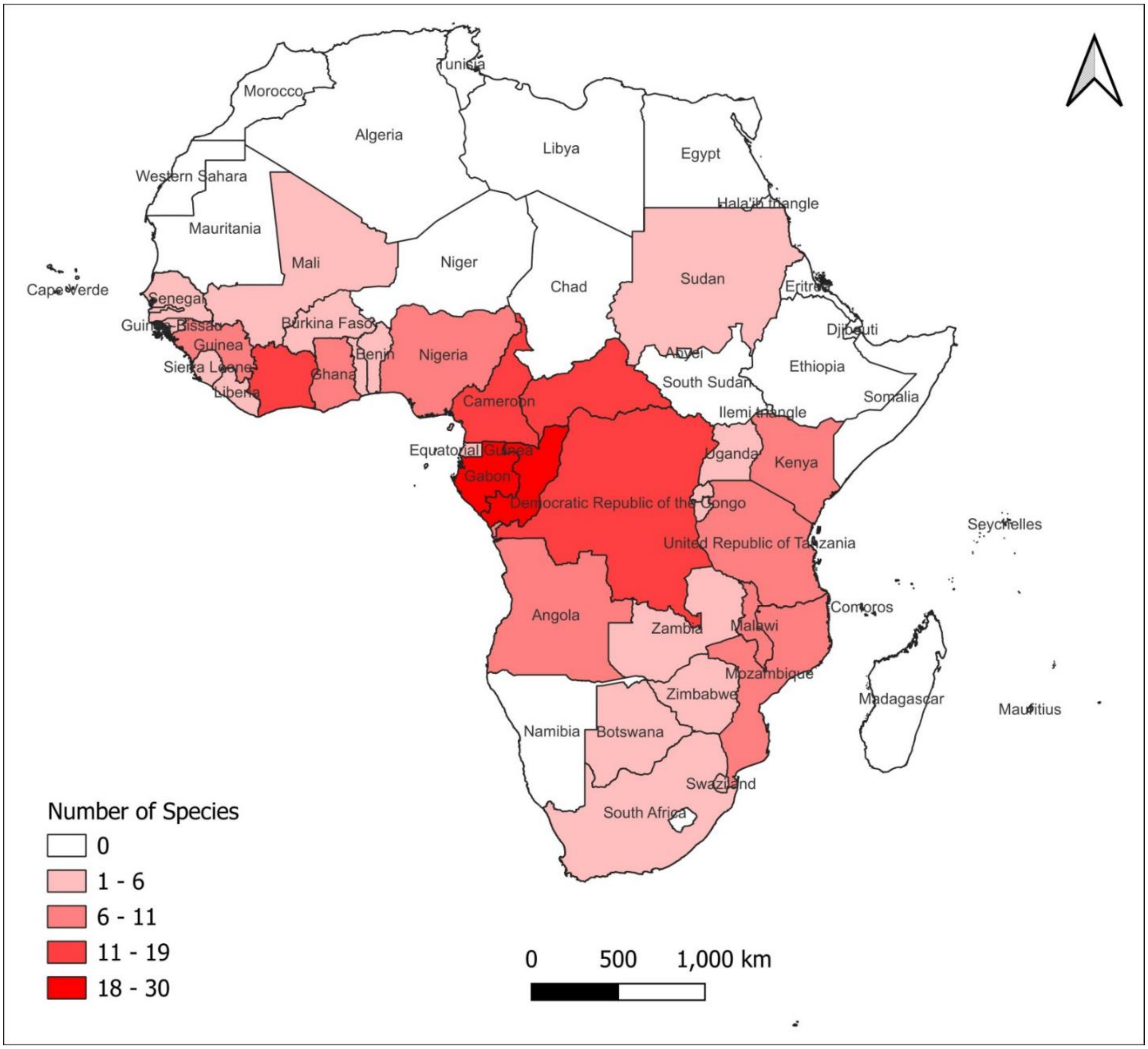


Figure 2. Map of Africa showing species distribution in *Synsepalum* and *Englerophytum*

Ethnomedical importance of *Synsepalum* and *Englerophytum*.

Numerous uses of species in both genera have been reported including the very famous miracle berry (*S. dulficicum* (Schum. et Thonn.) Daniell) in *Synsepalum* and stamvrug (*Englerophytum magalismontanum* (Sond.) T.D. Penn.) in *Englerophytum*. Seventy-six different uses have been reported for *S. dulcificum* alone (Tchokponhoué et al., 2021). One of the most prominent applications is in the treatment of socioeconomically burdensome ailments such as cancer and diabetes (Tchokponhoué et al., 2021). The fruits of miracle berry when consumed, mask certain taste receptors on the tongue and temporarily make sour foods taste sweet. This property is due to the presence of the glycoprotein, miraculin. This property in the berry has made it very useful for cancer patients undergoing chemotherapy as it helps to restore their sense of taste that may be affected while undergoing chemotherapy (Hudson et al., 2018). Some other species are used for charcoal production, timber *(S. msolo* and *S. passargei*), and the making of poles (S. passargei and (*Synsepalum Afzelii - Useful Tropical Plants*, n.d.). Just like many tropical plants, some species in the two genera have been implicated in traditional herbal medicine. *S. brevipes* is widespread from Senegal east to Kenya and south to Angola, Zimbabwe, and Mozambique. The sweetly sour fruit pulp is edible and sometimes used with sugar to prepare fruit juice. It is also used to treat jaundice and nausea, and the latex from the fruit is applied as a galactagogue. The root decoction is used in the treatment of malaria. Sap from the roots and bark is drunk to treat coughs, colds, hernias, and stomach complaints. It is also used in treating snake bites, stomach upset, and fever. The leaves are used against hookworm infection of the small intestine. A bark decoction is drunk to treat swellings. In Benin, it is reported that all parts of *S. dulcificum* are used (E. G. Achigan-Dako et al., 2015; Fandohan et al., 2017) mostly for medicine (Fandohan et al., 2017). *E. magalismontanum* is used in traditional medicine to treat ailments such as infertility, coughs, colds, bronchial problems, fever, asthma, abdominal pains, chronic coughs, pleurisy, and skin disorders (Olaokun et al., 2022).

Previous Phylogenetic Analysis

Classification of the angiosperm family Sapotaceae (Pennington, 1991) was purely morphological. Borg *et al*. (2019) attempted the use of nuclear DNA and plastid *trn*H-*psb*A to estimate phylogeny within the clade. Their results do not support the classification by Pennington, and the species of the two focal genera of this study that they analyzed were resolved in a polytomy of six clades: two comprising the species of *Englerophytum* and four of *Synsepalum*. However, their result cannot be considered final due to incomplete sampling, as only 11 out of the 35 accepted species of *Synsepalum* and 8 out of the 19 species of *Englerophytum* were used for the study. They also recommended that more work is required before a comprehensive taxonomic conclusion about the clade can be reached.

Aside from the work of (Borg *et al*. 2019), there are no published reports on phylogenetic relationships within the *Synsepalum*-*Englerophytum* clade. (Borg *et al*., 2019), their studies of the *Synsepalum*-*Englerophytum* complex reported that four of the six lineages comprised *Synsepalum* species, and three out of the four lineages of *Synsepalum* corresponded to the smaller genera of the earlier generic classification (Aubréville, 1964). There are, however, some concerns with the lineages reported. Some of the lineages had just a single species, which does not correspond with the type species of the small, segregated genus (e.g. the *Vincentella* clade). More species need to be investigated to better understand the phylogenetic relationships among species currently classified in *Synsepalum* and *Englerophytum* and to determine the number, names, and circumscriptions of genera that should be recognized in a phylogenetically-based classification.

Significance of the study

In general, plant phylogenetic studies provide a framework for understanding the fundamental processes of evolution and help in organizing the diverse plants of the earth in a way that will make sense to all. In the genus *Synsepalum,* although the presence of stipules and 5-merous flowers has been suggested as diagnostic characters for the genus, the presence of stipules is not consistent. They are missing in some species, (e.g. *S. dulcificum*); these may represent secondary losses, however. Phylogenetic analyses based on molecular data should make it possible to evaluate relationships among species in the group and compare them with the ancient generic concept. Moreover, not much has been done in resolving the divergent views of researchers on the merging of the small genera by Pennington to form *Synsepalum sensu lato* this research proffers a solution to taxonomic problems in the *Synsepalum-Eglerophytum* complex.

The objective of the Research

The primary goal of this project is to investigate the monophyly of the genera *Synsepalum* and *Englerophytum* and the internal relationships of the species in the genera using molecular data and a broader sampling than the ones used by (Borg *et al*., 2019).

Materials and Methods

Taxon sampling

It is generally believed that fresh materials from the field are more reliable for DNA extraction but due to the outbreak of the Covid-19 pandemic, getting to the field to sample materials was not an option to be explored for this study. Thus, materials for both *Synsepalum* and *Engleropytum* were mostly accessed from herbarium material. Materials were obtained as loans through the University of California Davis Herbarium (DAV). Samples were collected from Missouri Botanical Gardens (MO), New York Botanical Gardens (NY), Harvard University Herbarium (HUH) and The Conservatory and Botanical Garden of the city of Geneva (**Conservatoire et Jardin botaniques de la Ville de Genève**) (G). A few other samples (species No. 44 – 47 in Table 1) were collected in silica gel from people who grow them in their gardens. Leaf material sufficient for use in extracting DNA was removed from the herbarium samples. To avoid the destructive removal of leaf samples, leaves already placed in the fragmented packet in the herbarium sheets were first used. Where there were no leaves in the fragment packet, a single leaf was removed and used for the experiment. A total of 103 leaf samples were used for this study, comprising 43 from different herbaria in the United States (MO, NY, and Harvard herbarium), 56 from Switzerland, and four were fresh samples.

Genomic Regions Selection

In this study, for the nuclear region, ITS4 & ITS5 primers were used while for the chloroplast regions, trnH-psbA, and rps16-trnK primers were used. The choices of the regions and primers were based on previous studies of the family *Sapotaceae*. Several researched articles on phylogenetic studies in *Sapotaceae* have shown that both ITS and trnH-psbA are excellent primers in the study of species relationships in the genus and family in general (Richardson et al., 2014; Swenson et al., 2008).

DNA Extraction

DNA was extracted from all the 103 samples. Two methods were used for grinding. Liquid nitrogen was applied to 20 mg of leaf tissue in a mortar and pestle and the leaf was ground to produce a fine paste. In some cases, about 20 mg of leaf tissue mixed with 20 mg of PVP was ground in two 30-s cycles in a BeadBug Mini Homogenizer Model D1030. DNA was extracted from ground leaves using the DNAeasy plant DNA extraction kit (Qiagen, Valencia, California, USA). The extraction of DNA was according to the manufacturer's instructions with slight modifications for some samples. Where DNA extracted using Qiagen did not provide good bands during PCR, CTAB was used to extract DNA.

Amplification and sequencing

For nuclear DNA, 10 µM of the primers ITS4 (TCCTCCGCTTATTGATATGC), ITS5 (GGAAGTAAAAGTCGTAACAAGG) (Abbas et al., 2020) was used. The reactions were carried out in 50 uL comprising of the master mix in the table below:

Reaction	Volume (uL)
1. DD water	41
2. Coral load buffer	5
3. DNA template	2
4. DNTP	1
5. Taq	0.50
6. Forward primer	0.25
7. Reverse primer	0.25
8. Total	50

Applied Biosystems 2720 thermal cycler made in Singapore was used. The thermal cycling profile was generally that suggested by the manufacturer: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 90 s, and additional cycle at 72°C for 8 min.

For the chloroplast genome, two separate regions were amplified, each having a distinct pair of primers. 10 µM of the following primers were used. trnH (ACTGCCTTGATCCACTTGGC), psbA (CGAAGCTCCATCTACAAATGG) (Zhou et al., 2010), rps16-trnK (AAAGTGGGTTTTTATGATCC) rps16-trnK (TTAAAAGCCGAGTACTCTACC) (Armstrong, 2011). The reactions were carried out in 25 uL comprising the master mix in the table below:

Reaction	Volume (uL)
1. DD water	17.25
2. Ammonium (NH4)	2.5
3. MGCL2	1.25
4. DNA template	1
5. Forward primer	0.75
6. Reverse primer	0.75
7. DNTP	0.5
8. BSA	0.8
9. Taq	0.2
10. Total	25

The thermal cycling profile setting for the chloroplast region was: 80°C for 5 min, followed by 35 cycles of 95°C for 1 min, 50°C for 1 min, 62°C for 5 mins, and an additional cycle at 65°C for 5 mins at a ramp rate of 0.3°C/s.

Gel Electrophoresis

The amplified fragments for both regions were controlled for their quality by electrophoresis. 1.8g of powdered agarose gel was added to 100 mL of 1X TAE buffer. The mixture was shaken vigorously to ensure the agarose gel was completely immersed in the 1X TAE buffer. The mixture is heated in the microwave for 1 minute or 90 seconds to ensure the agarose gel has completely melted. After heating, 1 µL of Sybrsafe DNA gel stain is added to the beaker containing the agarose gel, which is placed in a bath containing water for a few seconds until the beaker is cool enough to be handled with the hand using hand gloves. The gel solution was poured into a tray fitted with combs and allowed to stay for 20 minutes until it solidified. After solidification, the comb is removed, and the wells are loaded with PCR. The chamber containing the loaded DNA is connected to power at 76 KVA and allowed to run for 1 hour. The gel is then visualized under UV light. Wells that produce bands are considered successful. The bands are excised using a razor blade. DNA was extracted from the bands and purified by application of a QIA quick PCR purification kit from Qiagen (Qiagen, Valencia, California, USA).

Sequence editing and alignment.

To obtain DNA sequences, extracted purified DNA from the gel was sent to the UC Davis sequencing center. For each direction of the primer, six micro-liters were used. Raw data from the facility were opened on Sequencher 5.4.6 (Gene Codes Corporation, Ann Arbor MI, USA) which was used to assemble contigs and edit the sequences. The first nucleotides of each end of the sequences were trimmed until readable bases were obtained. After trimming up the sequences, BLAST searches were performed to ensure the results obtained were that of Sapotaceae. In cases of contamination, blast results give different plant families and in some cases insects. Whenever contamination was observed the experiment was repeated to be sure the right species was used for the research. Alignment was done using muscle in MEGA X.

Phylogenetic analysis

The evolutionary history was inferred using the Bayesian Inference and Maximum Likelihood methods.

Bayesian analysis.- The dataset was analyzed with Bayesian inference using the program MrBayes version 3.2.7a (Ronquist et al., 2012). Sequence data was subjected to a general time reversal model including the estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G). The relative fit of various models of nucleotide substitution for the ITS region, chloroplast regions, and combined data set to identify the best model was examined. The best model was selected based on the Akaike Information Criterion (AIC). The Markov Chain Monte Carlo (MCMC) sampling, starting from random trees and priors, was run for 1,000,000 generations and every 100th tree was sampled. Four MCMC chains comprising three heated chains and a single cold chain were used in the analyses. Majority rule consensus trees and posterior probabilities for nodes were assembled from all post-burn-in sampled trees. Phylogenetic reconstructions were estimated after a couple of independent runs to confirm that they converged on similar stationary parameter estimates. For the combined data set, the data for each region were merged and aligned using muscle in MEGA before running on MrBayes.

Maximum Likelihood. - For Maximum Likelihood estimation, different regions used different models. For the nuclear region, ITS 4 and ITS 5, sequence alignment was performed using the muscle tool included in the MEGA 10 suite, and the Kimura-2 parameter model was applied. For the chloroplast region involving trnHpsbA and rps16-trnK primers, alignments were also performed using muscle, but the best model was the Hasegawa-Kishino model. For the combined data, alignments were performed using muscle while the best model was the Tamura 3-parameter model. The bootstrap consensus tree inferred from 1,000 replicates in Maximum Likelihood, was taken to represent the evolutionary history of the taxa analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches in the trees generated.

Results

After extraction of DNA, poor bands were obtained during PCR for many of the species studied. This could be due to the old nature of the herbarium specimens used. A higher number of successful PCR reactions were obtained for the nuclear ITS region than for the chloroplast regions. Among the chloroplast regions, the highest number of successful reactions was obtained for the trnH-psbA and the lowest for the rps16-trnk region. Although rps16-trnK primers were not used at the start of the study, it was later introduced when other primers failed. Considering the success recorded for the primers even though introduced later, it is safe to say rps16-trnK is a good primer for study in the *Sapotaceae* family.

Nuclear region

Unlike the result reported by (Borg *et al*., 2019) amplification was much easier with the ITS region compared to the chloroplast regions. Most of the sequences obtained for the nuclear region were between 500 to 550bp.

Chloroplast region

Several primers were tried for the chloroplast region but for many of them, only very few specimens were amplified during PCR. The two primers that gave better results among the different primers used for the chloroplast regions were trnHpsbA and rps16-trnK. When samples were sent for sequencing, for the trnHpsbA region, most of the results obtained were good for one direction, and rarely did we get good results for both directions. To protect the integrity of the result, only directions with clean DNA sequences were used for alignment and analysis. This problem with trnH-psbA that is reported here was also encountered by (Borg *et al.*, 2019). Fewer samples were used for rps16-trnK as the primers were introduced very late during the laboratory work.

Combined datasets

Clean reads that are suitable for analysis were obtained for all three primer sets used for this study, but more samples were amplified for the nuclear region. To combine the data, only samples that we got sequences for both regions were used. This is to ensure that none of the regions will influence the topology of the tree more than the other. Also, for ease of comparison of clades obtained from the combined dataset to clades from the separate regions.

Bayesian Inference

Trees from Bayesian analysis for ITS, trnHpsbA, rps16-trnK, and combined dataset are shown below in figures 3.1 – 3.4 respectively. The values of the posterior probabilities of the branches obtained are used as measures of branch support. Values below 0.95 are considered to have very low support. All the trees obtained from Bayesian inference and Maximum likelihood show a close relationship between *Englerophytum* and *Synsepalum*.

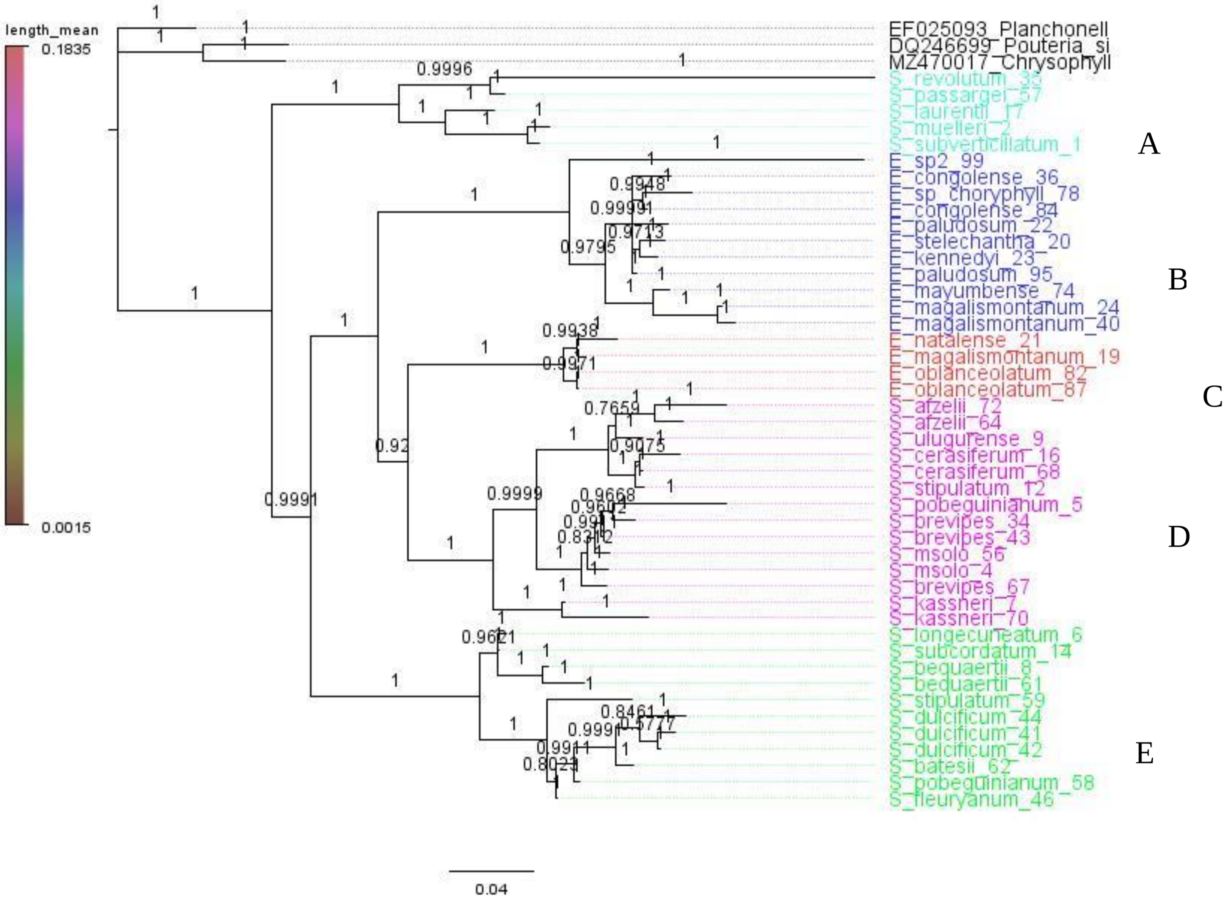


Figure 3.1 – A 50% majority rule Bayesian consensus tree of the ITS dataset revealing the different clades and their posterior probability.

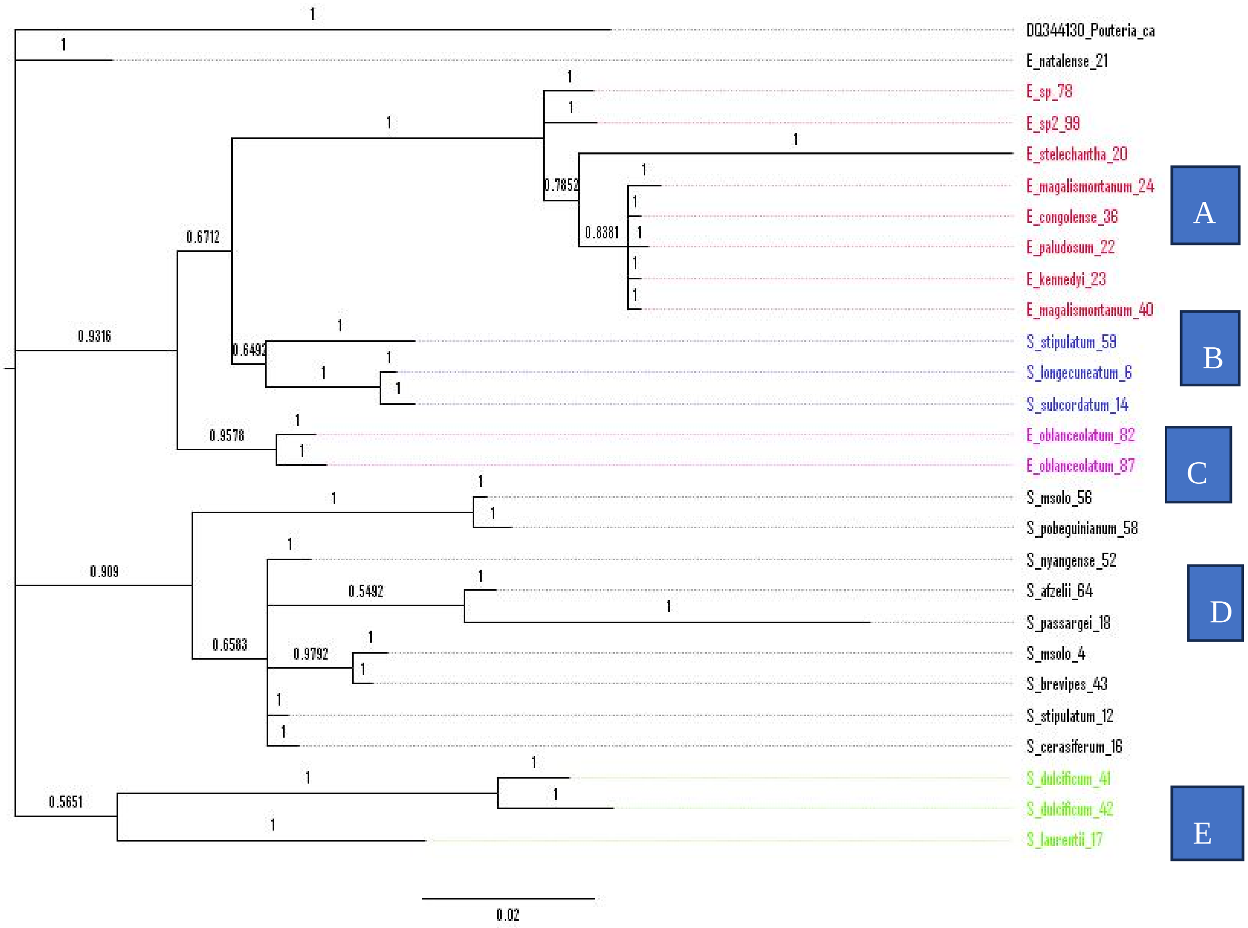


Figure 3:2 – A 50% majority rule Bayesian consensus tree of the chloroplast region (trnHpsbA) dataset revealing the different clades and their posterior probability.

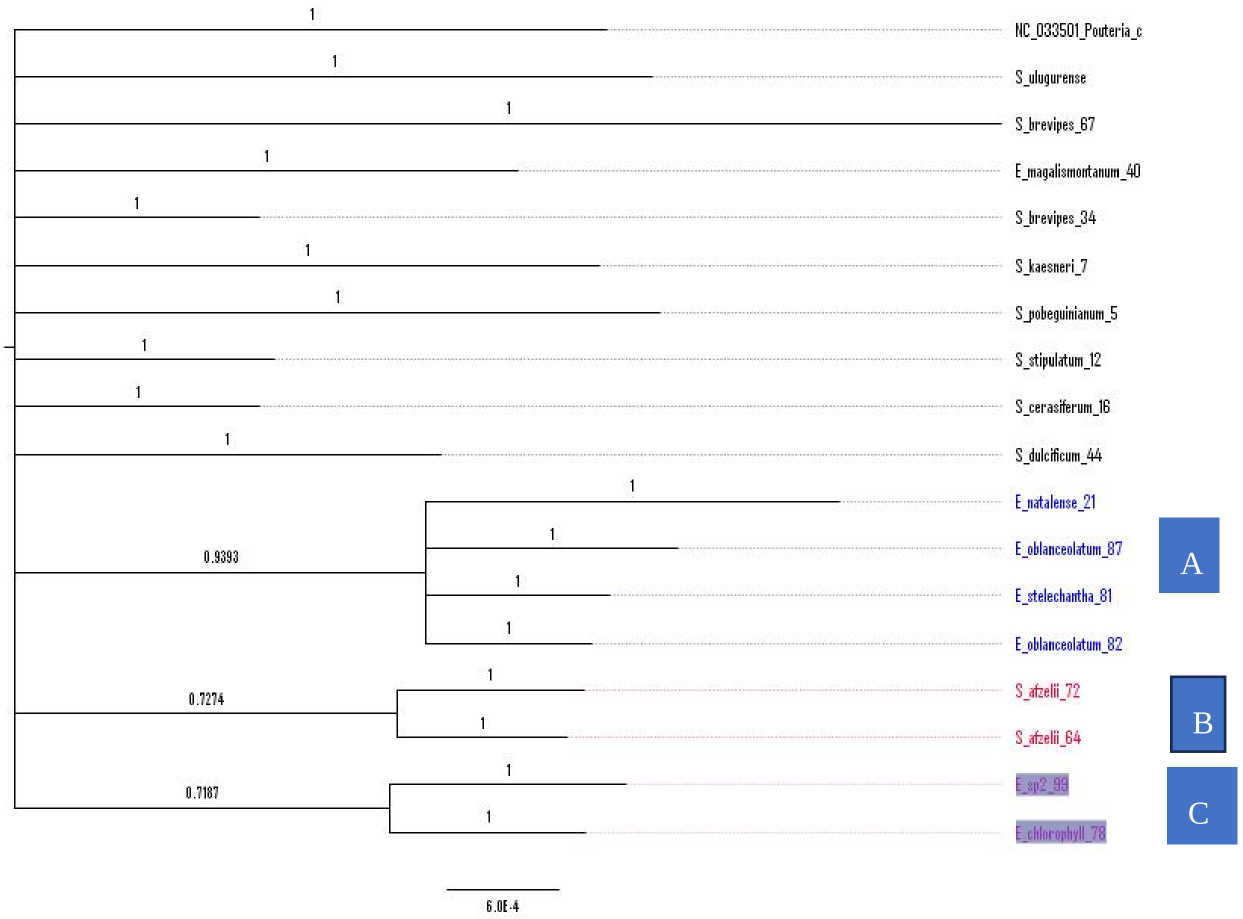


Figure 3.3 – A 50% majority rule Bayesian consensus tree of the chloroplast region (rps16-trnK) dataset revealing the different clades and their posterior probability.

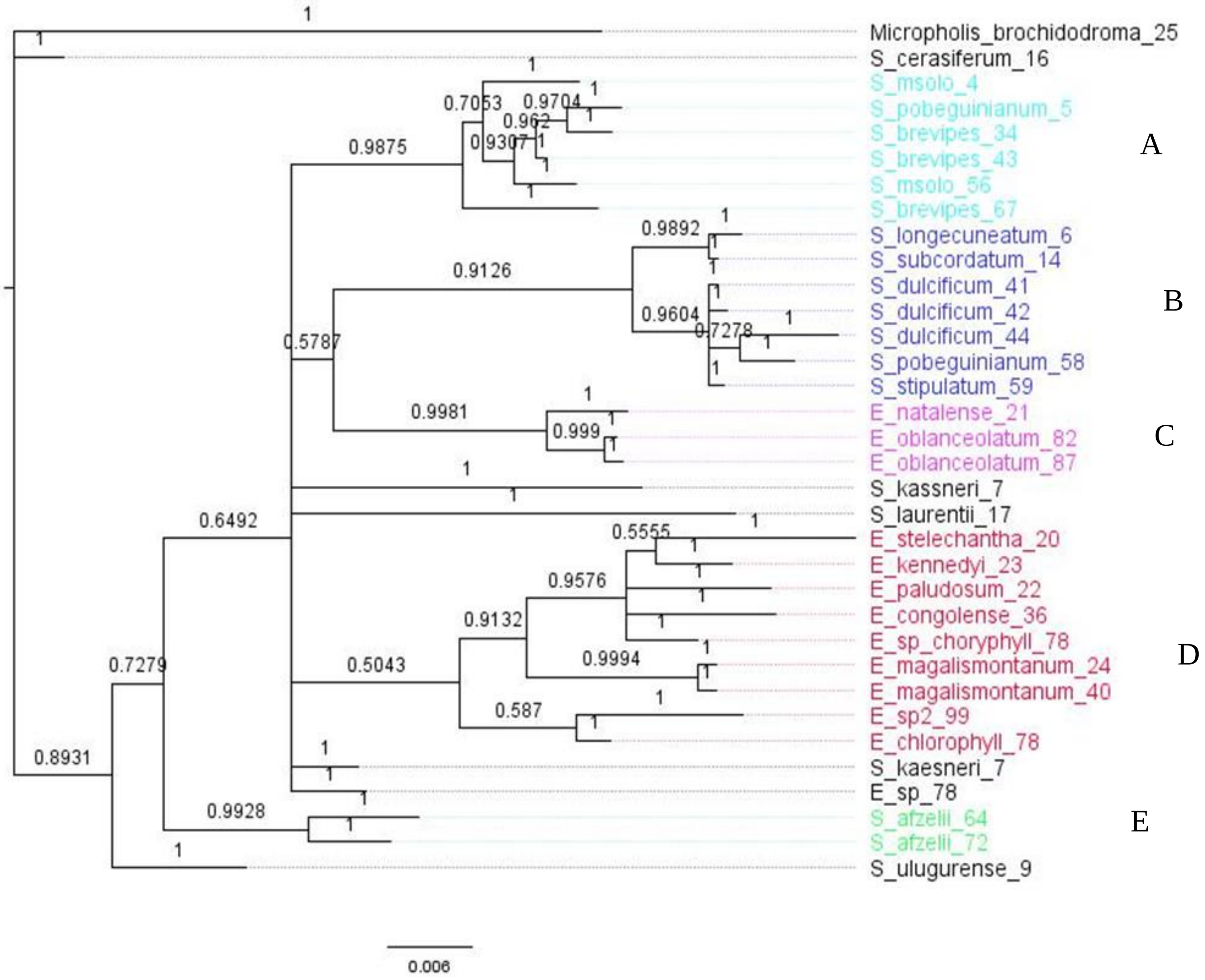


Figure 3.4 – A 50% majority rule Bayesian consensus tree of the combined dataset revealing the different clades and their posterior probability.

3.3 Maximum Likelihood Statistics

About 37% of the ITS region is considered variable and 66% of the variable site (277) is parsimony informative. Table 3.1 below shows the statistics for maximum Likelihood carried out for the three datasets. For the chloroplast region, trnH-psbA had only 16% variability and 31% of the variable sites (80) being parsimony-informative, while rps16-trnK had 5% variability and 16% of the variable sites (18) being parsimony informative. Trees for maximum likelihood for the different regions and combined data sets are shown in Figures 3.5 – 3.8.

Table 3.1: Parsimony Statistics

	ITS	trnH-psbA	rps16-trnK
Total aligned length	753	486	357
Parsimony informative characters	184	25	3
Variable sites	277	80	18
Percentage variability	37%	16%	5%
Conserved sites	422	362	338

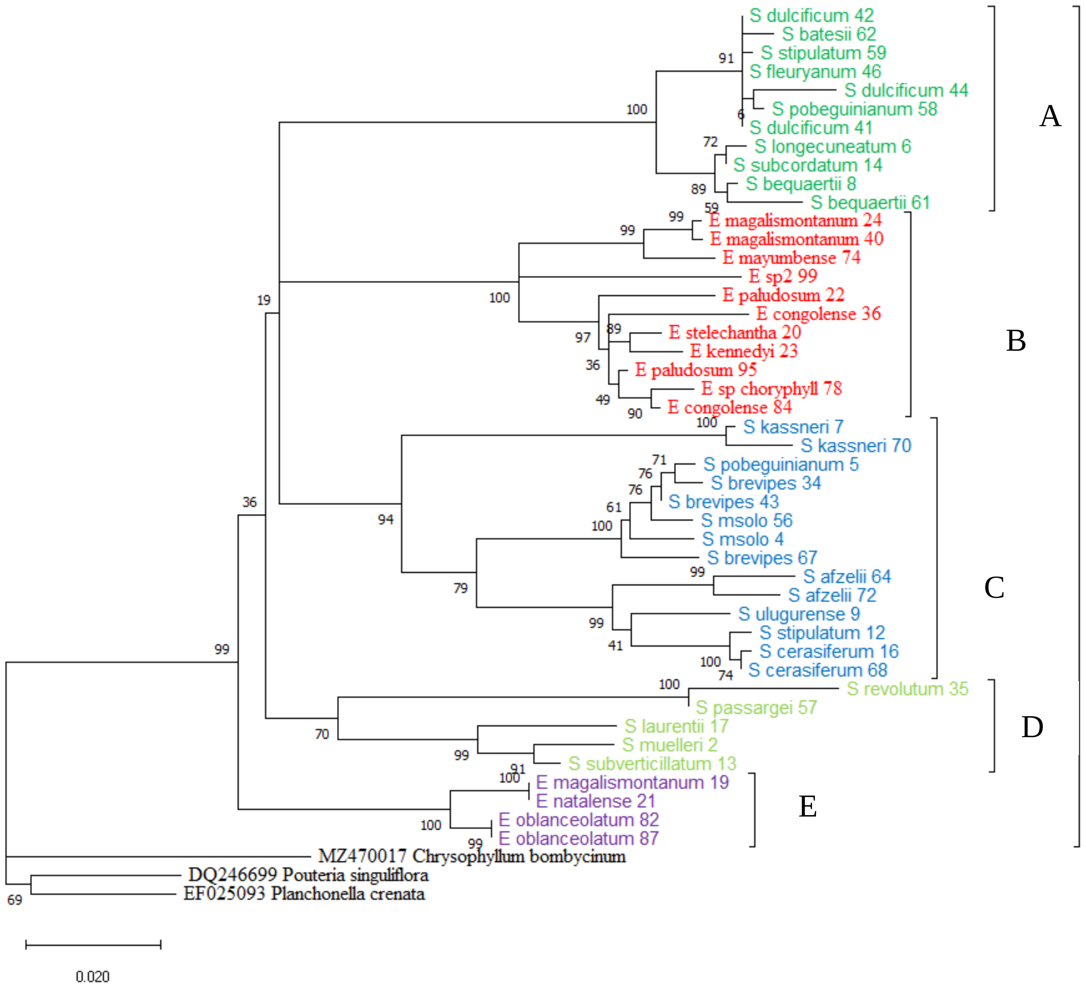


Figure 3.5 – ITS most parsimonious tree using Maximum likelihood

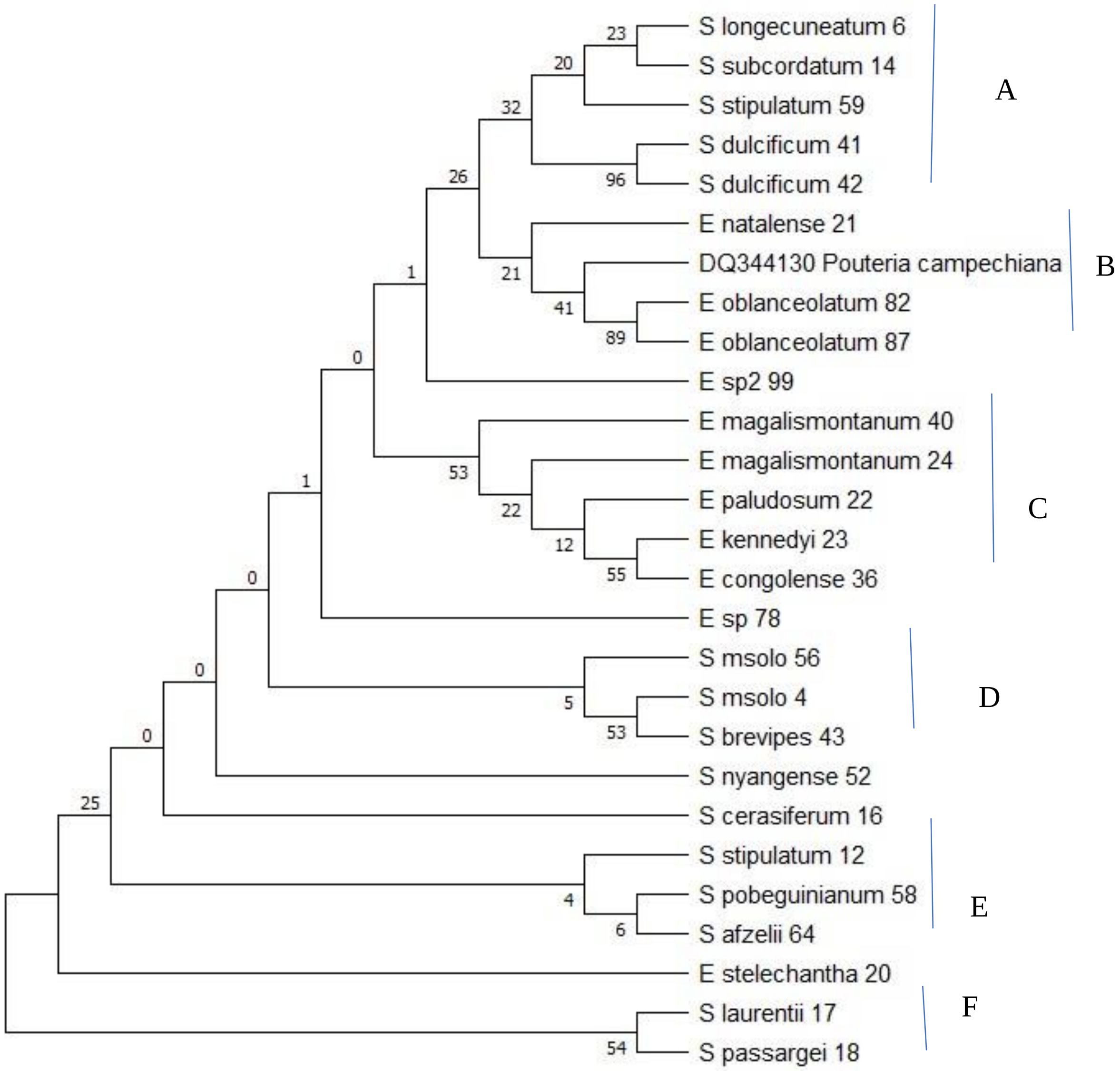


Figure 3.6 trnHpsbA most parsimonious tree using the maximum likelihood method.

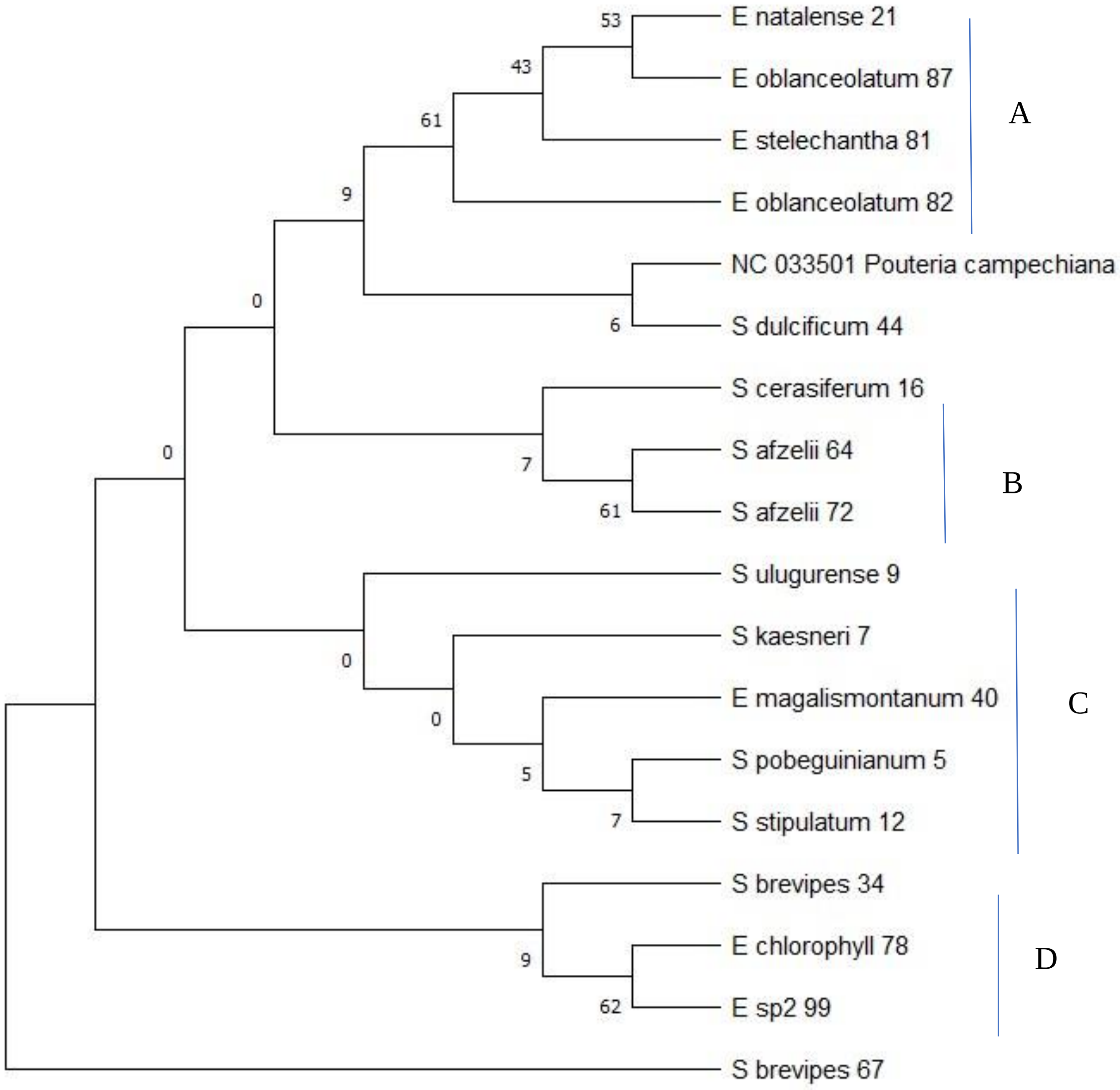


Figure 3.7 – rps16-trnK most parsimonious tree using maximum likelihood.

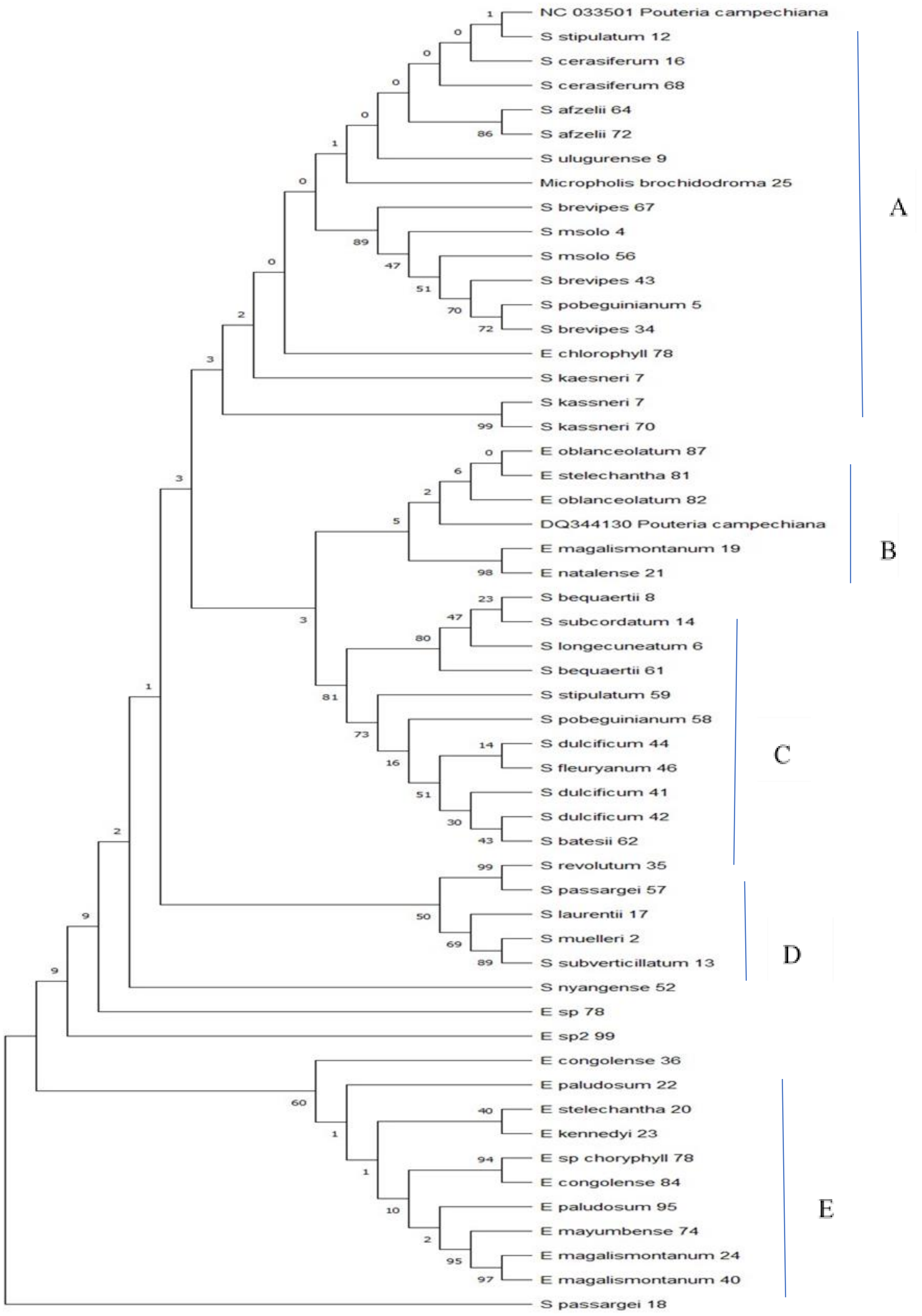


Figure 3.8 – Most parsimonious tree for the combined dataset (ITS. trnH-psbA, and rps16-trnK) using the maximum likelihood method.

Monophyly of Synsepalum -Englerophytum

Tree topology from our Bayesian and Maximum likelihood analysis support the monophyly of a group including species of *Synsepalum* and *Englerophytum*. *Englerophytum* is nested within *Synsepalum* in all the phylogenetic trees obtained. The ITS data analyzed using Bayesian inference resulted in five different clades. Two clades comprise the species in the genus *Englerophytum* – clade B and C while the other three clades include species in the genus *Synsepalum*. For trnH-psbA, five clades also were identified. Two of the clades were species exclusively belonging to *Englerophytum* – clades A and C, while three clades were species in *Synsepalum*. These include clades B, D, and E. Neither *Englerophytum* nor *Synsepalum* resolved as monophyletic in all the trees generated for both chloroplast and nuclear regions. Due to the few samples used for rps16-trnK, the tree obtained did not provide a robust contribution like the trees from ITS and trnH-psbA. It is, however, important to state that the three clades generated had species that were exclusive of *Engelophytum* and *Synsepalum*. One of the clades had a good bootstrap value of 0.93 while the other two clades have 0.7 as their bootstrap values. The tree generated for the combined data set is still resolved in a polytomy of 5 clades. Two out of the five clades generated were exclusively for species in the genus *Englerophytum*, while three clades were species in the genus *Synsepalum*.

Monophyly of *Synsepalum*

The genus *Synsepalum* was not resolved as monophyletic in the trees generated but rather polyphyletic because some of the clades in the polytomy corresponded to the previously recognized genera by Aubréville. Three of the species previously grouped under the genus *Afrosersalisia* were used in this study namely *S. afzelli, S. cerasifera,* and *S. kassneri*. As seen in Figure 3.1, they belong to the same clade, - clade D. The same thing is observed in Figure 3.5. For the *Vincentella* genus, out of the four species, data were successfully obtained for three species. These include *S. muelleri*, *S. passargei*, and *S. revoluta* (now revolutum). None of the herbaria we loaned specimens from had *S. brenanii*. Just like the report for *Afrosersalisia*, the species are in the same clade, -clade D. They were united with another species that was described after the merging of Pennington-*S. larentii*. The case is slightly different for the genus *Pachystella* as seen in both Figures 3.1 and 3.5. Out of the three species in *Pachystella*, two species (*S. msolo* and *S. brevipes*) formed a clade, clade D, separating them from *S. subverticillatum*, which was found in clade A in Figure 3.1. For the genus *Synsepalum* sensu stricto, four species were previously recognized. These include *S. dulcificum*- the type species for the genus, *S. glycydorum*, *S. stipulatum*, and *S. subcordatum*. Here again, three out of the four species were used in this study. We could not get samples for *S. glycydorum*. In this group, all the species also corresponded to the previous classification in *Synsepalum*. The species were in one clade, clade E. It is important to note, however, that these three species were not the only species found in the clade, other species formed the same clade with them.

Discussion

Unlike the results of (Bartish et al., 2011; Swenson, Richardson, et al., 2008; Swenson & Anderberg, 2005; Borg et al., 2019) who reported difficulty in getting good results for ITS regions, ITS 4 and ITS 5 gave the best results for all the primers used in this experiment. Many of the herbarium specimens did not provide good bands in PCR for some chloroplast primers. For the ITS primers, PCR did not only provide good bands for many of the species but also had good sequence data when the extracted DNA from the gel was sent for sequencing. The trees obtained from the different regions show that the ITS region had a better success as it amplified more species compared to the chloroplast regions. The chloroplast region, trnH-psbA gave more reliable results than rps16-trnK primers as it amplified more species. Although almost all of the samples used for this research were herbarium samples, it would be appropriate to say if fresh samples from the field were used for this study, more sequence data would have been generated for all primers used in this study.

4.1 **Clades**

4.1.1 Clades from ITS

Clade A

As shown in Table 6 below, clade A for the ITS tree consists of *S. revolutum*, *S. passargei, S. laurentii*, S. *muelleri*, and *S. subverticillatum*. Aside from *S. passargei*, they are mostly trees. Although there is no single morphological character that unites all the species, stipules are present for most of them. Also, they all have alternate leaves except for *S. passargei* its leaves are crowded at the branch end. They must have widely spread secondary venation, a character that is quite consistent in the clade. This clade is very similar to the genus *Vincentella*, one of the previously recognized genera merged by Pennington. The only species in the genus that is missing in this clade is *S. brenani.*

Clade B and **C** comprise of species in the genus *Englerophytum*. The genus did not resolve as a monophyletic. This was also observed by (Borg et al., 2019).

Clade D

The clade comprises *S. afzelii*, *S. ulugurense, S. cerasiferum, S. stipulatum, S. pobeguinianum, S. brevipes*, *S. msolo*, and *S. kassneri*. This clade does not correspond to any of the previously recognized genera. There are species from the different genera found in this clade. While *S. afzelii* and *S. cerasiferum* are from the previous genus *Afrosersalisa*, *S. msolo* and *S. brevipes* are from the genus *Pachystella*. The two species present in this clade (*S. brevipes, S. msolo)* both have stipules. They both have seeds that are ellipsoid in shape.

Clade E

In the previous genera, *Synsepalum* in the strict sense had four species. This includes *S. dulcificum*, *S. stipulatum*, *S. subcordatum* and *S. glycodorum*. We could not get leaf materials for *Synsepalum glycodurum*. Aside from *S. glycodorum*, all the species in *Synsepalum* in the strict sense are in clade E. The clade also contained other species that were described after the merging of the small genera by Pennington.

4.1.2 Clades from Chloroplast (trnH-psbA) tree.

There are five clades in the chloroplast tree. Two of the clades (A and C) are species in the genus *Englerophytum*. The C clade had only one species otherwise the genus would have been a single clade.

Clade B. All of the species in clade B for the trnH-psbA tree are also found in clade E of the ITS tree.

Clade D None of the species of *Englerophytum* used for this study were found in this clade and the clade is not similar to any of the previously merged small genera. Species from all the previously merged genera, except for the genus *Tulesta*, are contained in this clade.

4.1.3 Clades for rps16-trnK

As previously mentioned, the tree generated for this region does not provide robust information. This is because the primer was used when we were concluding laboratory work and had exhausted leaf materials for many species used for the research. Despite the few samples of species used for this region, the tree for this region has three clades and it is important to note also that the clades did not have species from the two different genera combined. The species of *Englerophytum* formed a clade with a 0.93 bootstrap value.

4.1.4 Combined data tree

The combined data tree is not very different from the trees from ITS and chloroplast regions, the species are separated into five clades comprising two *Englerophytum* and three *Synsepalum*. We see here again that the generic limit within *Synsepalum* and *Englerophytum* remains unclear even when the data were combined. It is good to emphasize here again that the current delimitation of *Synsepalum* and *Englerophytum* as circumscribed by Pennington cannot be substantiated using molecular evidence.

Implications of result for *Synsepalum*.

Two principal issues are addressed in this work. One is the merging of the small genera (Pennington, 1991), while two is the divergent view of some authors on the *Synsepalum-Englerophytum* complex. The previously recognized small genera and their species before the revision (Pennington, 1991) include (***Afrosersalisia***: afzelli, cerasifera, and kassneri. ***Vincentella***: brenanii, muelleri, passargei, revoluta. ***Pachystela***: brevipes, msolo, subverticillata. ***Synsepalum***: dulcificum, glycydorum, stipulatum, subcordatum. ***Tulesta***: garbonensis, sereti). After the revision of *Sapotaceae* by Pennington in 1991, 19 more species have been added to the genus *Synsepalum* bringing the total to 35 species currently recognized. Some authors including (Anderberg & Swenson, 2003; Swenson & Anderberg, 2005, Borg et al 2019) have called for the separation of *Synsepalum* from the previous small genera that were combined (Pennington, 1991). The results obtained in this research do support the suggestion to merge the two genera with additional suggestions.

As seen in all of the different trees generated, some of the clades corresponded to the previous *Synsepalum* that was recognized by Aubréville. Data analysis using Bayesian inference and Maximum likelihood for ITS grouped all three species of *Synsepalum* sensu stricto (*S. dulcificum*, *S. subcordatum,* and *S. stipulatum*) used in this study into one clade. A dichotomous tree having species of *Synsepalum* in one clade and species of *Englerophytum* in another clade would have been an excellent reflection of the monophyly of *Synsepalum,* however, the tree obtained from the nuclear, chloroplast region and combined dataset resulted in a polytomy. In all the trees the clades of *Englerophytum* are nested within *Synsepalum*. This consistent nesting of *Englerophytum* within *Synsepalum* further suggests that the genera are closely related. Leaf venation, presence or absence of stipules, inflorescence, fusion of sepals, stamens insertion, anther position, and number of ovaries tend to overlap in the previously merged genera. The overlapping morphological character in Table 1 and the molecular evidence seen in Figures 3.1 and 3.5 suggest very strongly that *Synsepalum* sensu lato should be reversed into small genera.

Implications of results for the *Synsepalum* and *Englerophytum* complex

Molecular evidence obtained from this study and all other phylogenetic studies involving the species merged by Pennington and the newly described ones for both *Synsepalum* and

Englerophytum shows that the two genera are not very distinct from each other. As a rule, phylogenetic analyses are used to test if morphological characters are reliable indicators of phylogenetic relationships. The trees obtained in this research do not agree with the circumscription of the genera as defined by Pennington. All the trees obtained for ITS, trnHpsbA, rps16-trnK, and combined data using MrBayes for Bayesian inference and MEGA X for Maximum likelihood show that *Englerophytum* is nested within *Synsepalum*.

It is important to state here that only a few morphological characters were used by (Pennington, 1991) to separate *Englerophytum* from *Synsepalum*. This includes leaf venation and fusion of the filaments. *Synsepalum* (Figure 2); has an eucamptodromous venation and *Englerophytum* (Figure 3) has a brochidodromous venation and a strong fusion of the filament into a staminal tube. Although it is taxonomically correct, in some cases, to use few morphological characters to separate genera, this is not just the case with the *Englerophytum-Synsepalum* complex. The nesting of *Englerophytum* within *Synsepalum* in trees generated for both nuclear chloroplast regions indicates very strongly that the genera do not merit distinct generic status.

Conclusion and Recommendation

In this study of the phylogenetic relationships of the *Synsepalum-Englerophytum* complex, neither of the two genera resolved as monophyletic but rather resulted in polytomy, with clades of *Englerophytum* nested within *Synsepalum*. This shows that the morphological circumscription of the two genera does not align with the molecular evidence. We think that morphological character states that distinguish *Synsepalum* might be ancestral (symplesiomorphies) while the character states that distinguish *Englerophytum* may be independently derived (homoplasious) synapomorphies for each subclade.

The polytomy observed in the genus *Synsepalum* shows that the genus is not a single lineage. Some of the clades in the phylogenetic tree correspond to some of the small genera merged by Pennington. The *Synsepalum-Englerophytum* complex certainly requires some formal taxonomic changes to align with molecular evidence but such changes cannot be made until a broader sampling of all species currently recognized in the genera is collected for comprehensive morphological as well as molecular studies. The complete genome of *Synsepalum* has been published. If the complete genome of *Englerophytum* is published, it would serve as additional information in making a comprehensive conclusion on the *Englerophytum-Synsepalum* complex.

References

Abbas, H. S., Falih, M. A., Hamad, B. S., & Eesa, S. H. (2020). Detection of local isolates of the entomopathogenic fungus Metarhizium anisopliae using specific primers. *Acta Entomology and Zoology*, *1*(1), 01–04. https://doi.org/10.33545/27080013.2020.v1.i1a.1

Achigan-Dako, E. (2015). Current knowledge and breeding perspectives for the miracle plant Synsepalum dulcificum (Schum. Et Thonn.) Daniell. *Genetic Resources and Crop Evolution*, *62*. https://doi.org/10.1007/s10722-015-0225-7

Achigan-Dako, E. G., Tchokponhoué, D. A., N'Danikou, S., Gebauer, J., & Vodouhè, R. S. (2015). Current knowledge and breeding perspectives for the miracle plant Synsepalum dulcificum (Schum. Et Thonn.) Daniell. *Genetic Resources and Crop Evolution*, *62*(3), 465–476. https://doi.org/10.1007/s10722-015-0225-7

Africa—Climate, Regions, Variations | Britannica. (n.d.). Retrieved February 23, 2024, from https://www.britannica.com/place/Africa/Climate

Akinmoladun, A. C., Adetuyi, A. R., Komolafe, K., & Oguntibeju, O. O. (2020). Nutritional benefits, phytochemical constituents, ethnomedicinal uses and biological properties of Miracle fruit plant (Synsepalum dulcificum Shumach. & Thonn. Daniell). *Heliyon*, *6*(12), e05837. https://doi.org/10.1016/j.heliyon.2020.e05837

Alizadeh-Navaei, R., Roozbeh, F., Saravi, M., Pouramir, M., Jalali, F., & Moghadamnia, A. A. (2008). Investigation of the effect of ginger on the lipid levels. A double blind controlled clinical trial. *Saudi Medical Journal*, *29*(9), 1280–1284.

Ameh, G. I., & Nwammoh, A. O. (2010). Phytochemical and Ethnobotanical Evaluation of Garlic Bulb (Allium sativum L.). *Bio-Research*, *8*. https://doi.org/10.4314/br.v8i1.62545

Amir, H. M., Grace, O. M., Wabuyele, E., & Manoko, M. L. K. (2019). Ethnobotany of Aloe L. (Asphodelaceae) in Tanzania. *South African Journal of Botany*, *122*, 330–335. https://doi.org/10.1016/j.sajb.2019.01.038

Anderberg, A. A., & Swenson, U. (2003). Evolutionary Lineages in Sapotaceae (Ericales): A Cladistic Analysis Based on ndhF Sequence Data. *International Journal of Plant Sciences*, *164*(5), 763–773. https://doi.org/10.1086/376818

Ang, L., Song, E., Hu, X.-Y., Lee, H. W., Chen, Y., & Lee, M. S. (2022). Herbal Medicine Intervention for the Treatment of COVID-19: A Living Systematic Review and Cumulative Meta-Analysis. *Frontiers in Pharmacology, 13,* 906764. https://doi.org/10.3389/fphar.2022.906764

Ang, L., Song, E., Lee, H. W., & Lee, M. S. (2020). Herbal Medicine for the Treatment of Coronavirus Disease 2019 (COVID-19): A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Journal of Clinical Medicine*, *9*(5), 1583. https://doi.org/10.3390/jcm9051583

Armstrong, K. (2011). *Systematics and biogeography of the pantropical genus Manilkara Adans. (Sapotaceae)*. https://era.ed.ac.uk/handle/1842/4871

Asiimwe, S., Namukobe, J., Byamukama, R., & Imalingat, B. (2021). Ethnobotanical survey of medicinal plant species used by communities around Mabira and Mpanga Central Forest Reserves, Uganda. *Tropical Medicine and Health*, *49*, 52. https://doi.org/10.1186/s41182-021-00341-z

Aubréville, A. (1961). Notes sur les Sapotacées de l'Afrique équatoriale. Notul. Syst. (Paris) 16: 223–279.

Aubréville, A. (1964). Flore du Caméroun. Monograph 2. Sapotaceés. Paris: Muséum national d'Histoire naturelle.

Baehni, C. (1965). Mémoires sur les Sapotacées 3. Inventaire des genres. Boissiera 11: 1–262.

Bartish, I. V., Swenson, U., Munzinger, J., & Anderberg, A. A. (2005). Phylogenetic relationships among New Caledonian Sapotaceae (Ericales): Molecular evidence for generic polyphyly and repeated dispersal. *American Journal of Botany*, *92*(4), 667–673. https://doi.org/10.3732/ajb.92.4.667

Bartish, I. V., Antonelli, A., Richardson, J. E.& Swenson, U. (2011). Vicariance or long-distance dispersal: historical biogeography of the pantropical subfamily Chrysophylloideae (Sapotaceae). J. Biogeogr. 38(1): 177–190.

Borg, D., Richardson, J.-E., Harris, D., Gautier, L., Hughes, M., & Mackinder, B. (2019). PHYLOGENY of TWO AFRICAN GENERA of SAPOTACEAE - ENGLEROPHYTUM and SYNSEPALUM. *Edinburgh Journal of Botany, 76,* 1–37. https://doi.org/10.1017/S0960428619000040

Caballero-Serrano, V., McLaren, B., Carrasco, J. C., Alday, J. G., Fiallos, L., Amigo, J., & Onaindia, M. (2019). Traditional ecological knowledge and medicinal plant diversity in Ecuadorian Amazon home gardens. *Global Ecology and Conservation*, *17*, e00524. https://doi.org/10.1016/j.gecco.2019.e00524

Ceuterick, M., Vandebroek, I., & Pieroni, A. (2011). Resilience of Andean urban ethnobotanies: A comparison of medicinal plant use among Bolivian and Peruvian migrants in the United Kingdom and in their countries of origin. *Journal of Ethnopharmacology*, *136*(1), 27–54. https://doi.org/10.1016/j.jep.2011.03.038

Chaudhari, S., Ruknuddin, G., & Prajapati, P. (2016). Ethno medicinal values of Citrus genus: A review. *Medical Journal of Dr. D.Y. Patil University*, *9*, 560. https://doi.org/10.4103/0975-2870.192146

Choi, S. E., & Garza, J. (2021). Consumer Likings of Different Miracle Fruit Products on Different Sour Foods. *Foods*, *10*(2), 406. https://doi.org/10.3390/foods10020406

Coleman, M. (2016, March 18). Discovering the Sapotaceae family. *Botanics Stories*. https://stories.rbge.org.uk/archives/19291

Contributors, W. E. (n.d.). *Health Benefits of Bitter Kola*. WebMD. Retrieved August 16, 2023, from https://www.webmd.com/diet/health-benefits-bitter-kola

Crichton, M., Davidson, A. R., Innerarity, C., Marx, W., Lohning, A., Isenring, E., & Marshall, S. (2022). Orally consumed ginger and human health: An umbrella review. *The American Journal of Clinical Nutrition*, *115*(6), 1511–1527. https://doi.org/10.1093/ajcn/nqac035

Cupp, M. J. (1999). Herbal Remedies: Adverse Effects and Drug Interactions. *American Family Physician*, *59*(5), 1239–1244.

Daimei, P., & Kumar, Y. (2014). Ethnobotanical uses of gingers in Tamenglong district, Manipur, Northeast India. *Genetic Resources and Crop Evolution*, *61*(1), 273–285. https://doi.org/10.1007/s10722-013-0054-5

de Medeiros, P. M., Soldati, G. T., Alencar, N. L., Vandebroek, I., Pieroni, A., Hanazaki, N., & de Albuquerque, U. P. (2012a). The Use of Medicinal Plants by Migrant People: Adaptation, Maintenance, and Replacement. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2012*, 807452. https://doi.org/10.1155/2012/807452 de Medeiros, P. M., Soldati, G. T., Alencar, N. L., Vandebroek, I., Pieroni, A., Hanazaki, N., & de Albuquerque, U. P. (2012b). The Use of Medicinal Plants by Migrant People: Adaptation, Maintenance, and Replacement. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2012*, 807452. https://doi.org/10.1155/2012/807452

Del Campo, R., Zhang, Y., & Wakeford, C. (2017). Effect of Miracle Fruit (Synsepalum dulcificum) Seed Oil (MFSO®) on the Measurable Improvement of Hair Breakage in Women with Damaged Hair. *The Journal of Clinical and Aesthetic Dermatology*, *10*(11), 39–48.

Doddawad, V. G., Shivananda, S., Vidya, C. S., Madhu, B., & Gurupadayya, B. M. (2022). Insight on Synsepalum dulcificum: A Bioactive Compound on Taste Modification and Its Biological Properties. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, *12*(3), 93. https://doi.org/10.4103/ijnpnd.ijnpnd_19_22

Douglas Kinghorn, A., Chin, Y.-W., Pan, L., & Jia, Z. (2010). 3.10—Natural Products as Sweeteners and Sweetness Modifiers. In H.-W. (Ben) Liu & L. Mander (Eds.), *Comprehensive Natural Products II* (pp. 269–315). Elsevier. https://doi.org/10.1016/B978-008045382-8.00077-0

Du, L., Shen, Y., Zhang, X., Prinyawiwatkul, W., & Xu, Z. (2014). Antioxidant-rich phytochemicals in miracle berry (*Synsepalum dulcificum*) and antioxidant activity of its extracts. *Food Chemistry*, *153*, 279–284. https://doi.org/10.1016/j.foodchem.2013.12.072

DV. (2022, April 25). *Flavor-Tripping With the Miracle Fruit*. The Somm Journal. https://www.sommjournal.com/flavor-tripping-with-the-miracle-fruit/

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, *4*, 177. https://doi.org/10.3389/fphar.2013.00177

Eltis, D., Morgan, P., & Richardson, D. (2007). Agency and Diaspora in Atlantic History: Reassessing the African Contribution to Rice Cultivation in the Americas. *The American Historical Review*, *112*(5), 1329–1358. https://doi.org/10.1086/ahr.112.5.1329

Fandohan, A., Chadare, F. J., Gouwakinnou, G., Tovissodé, C. F., Bonou, A., Djonlonkou, S., Houndelo, L. F. H., Sinsin, C., & Assogbadjo, A. (2017). Traditional uses and economic value of Synsepalum dulcificum in South Benin. *Bois et Forets Des Tropiques*, 17–30.

Fitzgerald, M., Heinrich, M., & Booker, A. (2020). Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques. *Frontiers in Pharmacology*, *10*. https://www.frontiersin.org/articles/10.3389/fphar.2019.01480

Gonfa, N., Robi, D., & Hundera, K. (2020). Ethnobotanical study of medicinal plants, its utilization, and conservation by indigenous people of Gera district, Ethiopia Ethnobotanical study of medicinal plants, its utilization, and conservation by indigenous people of Gera district, Ethiopia PUBLIC INTEREST STATEMENT. *Cogent Food & Agriculture*, *6*. https://doi.org/10.1080/23311932.2020.1852716

Gonfa, N., Tulu, D., Hundera, K., & Raga, D. (2020). Ethnobotanical study of medicinal plants, its utilization, and conservation by indigenous people of Gera district, Ethiopia. *Cogent Food & Agriculture*, *6*(1), 1852716. https://doi.org/10.1080/23311932.2020.1852716

Hasani, H., Arab, A., Hadi, A., Pourmasoumi, M., Ghavami, A., & Miraghajani, M. (2019). Does ginger supplementation lower blood pressure? A systematic review and meta-analysis of clinical trials. *Phytotherapy Research: PTR*, *33*(6), 1639–1647. https://doi.org/10.1002/ptr.6362

Hirai, T., Shohael, A. M., Kim, Y.-W., Yano, M., & Ezura, H. (2011). Ubiquitin promoter–terminator cassette promotes genetically stable expression of the taste-modifying protein miraculin in transgenic lettuce. *Plant Cell Reports*, *30*(12), 2255–2265. https://doi.org/10.1007/s00299-011-1131-x

Howard, P. (2006). *Gender Bias in Ethnobotany: Propositions and Evidence of a Distorted Science and Promises of a Brighter Future*.

Hudson, S. D., Sims, C. A., Odabasi, A. Z., Colquhoun, T. A., Snyder, D. J., Stamps, J. J., Dotson, S. C., Puentes, L., & Bartoshuk, L. M. (2018). Flavor Alterations Associated with Miracle Fruit and Gymnema sylvestre. *Chemical Senses*, *43*(7), 481–488. https://doi.org/10.1093/chemse/bjy032

Izawa, K., Amino, Y., Kohmura, M., Ueda, Y., & Kuroda, M. (2010). 4.16—Human–Environment Interactions – Taste. In H.-W. (Ben) Liu & L. Mander (Eds.), *Comprehensive Natural Products II* (pp. 631–671). Elsevier. https://doi.org/10.1016/B978-008045382-8.00108-8

Kiyama, R. (2020). Nutritional implications of ginger: Chemistry, biological activities and signaling pathways. *The Journal of Nutritional Biochemistry*, *86*, 108486. https://doi.org/10.1016/j.jnutbio.2020.108486

Klimek-Szczykutowicz, M., Szopa, A., & Ekiert, H. (2020). Citrus limon (Lemon) Phenomenon—A Review of the Chemistry, Pharmacological Properties, Applications in the Modern Pharmaceutical, Food, and Cosmetics Industries, and Biotechnological Studies. *Plants*, *9*, 119. https://doi.org/10.3390/plants9010119

Koizumi, A., Tsuchiya, A., Nakajima, K., Ito, K., Terada, T., Shimizu-Ibuka, A., Briand, L., Asakura, T., Misaka, T., & Abe, K. (2011). Human sweet taste receptor mediates acid-induced sweetness of miraculin. *Proceedings of the National Academy of Sciences*, *108*(40), 16819–16824. https://doi.org/10.1073/pnas.1016644108

Krause, K. (1914). Englerophytum, eine neue afrikanische gattung der Sapotaceen. Bot. Jahrb. Syst. 50(Suppl.): 343–348.

Kurihara, K., & Beidler, L. M. (1969). Mechanism of the Action of Taste-modifying Protein. *Nature*, *222*(5199), Article 5199. https://doi.org/10.1038/2221176a0

Lipatova, O., & Campolattaro, M. M. (2016). The Miracle Fruit: An Undergraduate Laboratory Exercise in Taste Sensation and Perception. *Journal of Undergraduate Neuroscience Education*, *15*(1), A56–A60.

Liben, L. (1989). La véritable identité des genres et espèces confondus sous le nom de “Bequaertiodendron magalismontanum” (Sond.) Heine & Hemsley (Sapotaceae) en Afrique centrale et occidentale. Bull. Jard. Bot. Natl. Belg. 59(1/2): 151–169.

Liu, Y.-X., Zhou, Y.-H., Jiang, C.-H., Liu, J., & Chen, D.-Q. (n.d.). Prevention, treatment and potential mechanism of herbal medicine for Corona viruses: A review. *Bioengineered*, *13*(3), 5480–5508. https://doi.org/10.1080/21655979.2022.2036521

Luo, L., Jiang, J., Wang, C., Fitzgerald, M., Hu, W., Zhou, Y., Zhang, H., & Chen, S. (2020). Analysis on herbal medicines utilized for treatment of COVID-19. *Acta Pharmaceutica Sinica. B*, *10*(7), 1192–1204. https://doi.org/10.1016/j.apsb.2020.05.007

Luo, X., Zhang, Y., Li, H., Ren, M., Liu, Y., Liu, Y., Zhang, Y., Kuang, Z., Cai, Y., Chen, Y., & Ni, X. (2022). Clinical Evidence on the Use of Chinese Herbal Medicine for Acute

Infectious Diseases: An Overview of Systematic Reviews. *Frontiers in Pharmacology, 13*. https://www.frontiersin.org/articles/10.3389/fphar.2022.752978

Mahomoodally, M. F., Protab, K., & Aumeeruddy, M. Z. (2019). Medicinal plants brought by Indian indentured immigrants: A comparative review of ethnopharmacological uses between Mauritius and India. *Journal of Ethnopharmacology, 234*, 245–289. https://doi.org/10.1016/j.jep.2019.01.012

McHugh, P. (2018, September 15). *Record 44.5 Million Immigrants in 2017*. CIS.Org. https://cis.org/Report/Record-445-Million-Immigrants-2017

Medicinal plants. (2023). In *Wikipedia*. https://en.wikipedia.org/w/index.php?title=Medicinal_plants&oldid=1171382764

Miracle fruit | Description, Effects, & Uses | Britannica. (2024, January 30). https://www.britannica.com/plant/miracle-fruit

MIRACLE FRUIT OIL COMPANY DONATES MIRACLE FRUIT TREE TO BAPTIST HEALTH – Miracle Fruit Oil. (n.d.). Retrieved February 23, 2024, from https://www.miraclefruitoil.com/blogs/news/miracle-fruit-oil-company-donates-miracle-fruit-tree-to-baptist-health-hospitals-to-improve-the-lives-of-cancer-patients

Misaka, T. (2013). Molecular mechanisms of the action of miraculin, a taste-modifying protein. *Seminars in Cell & Developmental Biology*, *24*(3), 222–225. https://doi.org/10.1016/j.semcdb.2013.02.008

Momodu, S. (2022, February 9). *African Americans in the California Gold Rush (1848-1860) •*. https://www.blackpast.org/african-american-history/events-african-american-history/african-americans-in-the-california-gold-rush-1848-1860/

Moneim, A., & Sulieman, E. (2019). Garcinia Kola (Bitter Kola): Chemical Composition. In A. A. Mariod (Ed.), *Wild Fruits: Composition, Nutritional Value and Products* (pp. 285–299). Springer International Publishing. https://doi.org/10.1007/978-3-030-31885-7_23

Morgan, A. (2020, August 18). Roots of African American Herbalism: Herbal Use by Enslaved Africans. *Herbal Academy*. https://theherbalacademy.com/african-american-herbalism-history/

Nguyen, A., Kim, S. J., Phuoc Long, N., Jung Eun Min, Yoon, Y. C., Eun Goo Lee, Mina Kim, Tae Joon Kim, Yoon Young Yang, Eui Young Son, Yoon, S. J., Nguyen Co, D., Kim, H. M., & Sung Won Kwon. (2020). Ginger on Human Health: A Comprehensive Systematic Review of 109 Randomized Controlled Trials. *Nutrients*, *12*, 157. https://doi.org/10.3390/nu12010157

Nicholson, S. E. (2001). Climatic and environmental change in Africa during the last two centuries. *Climate Research*, *17*(2), 123–144. https://doi.org/10.3354/cr017123

Niu, Y., Ni, S., & Liu, J. (n.d.). Complete chloroplast genome of Synsepalum dulcificum D.: A magical plant that modifies sour flavors to sweet. *Mitochondrial DNA. Part B, Resources*, *5*(3), 3052–3053. https://doi.org/10.1080/23802359.2020.1798299

Okoro, B. C., Dokunmu, T. M., Okafor, E., Sokoya, I. A., Israel, E. N., Olusegun, D. O., Bella-Omunagbe, M., Ebubechi, U. M., Ugbogu, E. A., & Iweala, E. E. J. (2023). The ethnobotanical, bioactive compounds, pharmacological activities and toxicological evaluation of garlic (Allium sativum): A review. *Pharmacological Research - Modern Chinese Medicine*, *8*, 100273. https://doi.org/10.1016/j.prmcm.2023.100273

Olaokun, O. O., Manonga, S. A., Zubair, M. S., Maulana, S., & Mkolo, N. M. (2022). Molecular Docking and Molecular Dynamics Studies of Antidiabetic Phenolic Compound Isolated from Leaf Extract of Englerophytum magalismontanum (Sond.) T.D.Penn. *Molecules*, *27*(10), Article 10. https://doi.org/10.3390/molecules27103175

Onder, G., & Liperoti, R. (2016). Herbal Medications. *JAMA*, *315*(10), 1068. https://doi.org/10.1001/jama.2015.19388

Oyebade, A. (2017). African immigrants and their churches. In *The New African Diaspora in the United States*. Routledge.

Oyetunji, O. (n.d.). Ethno-botanical survey, review and informatics of medicinal plants used by indigenous people of Lekki, Ibeju-Lekki Local Government Area of Lagos State, Nigeria. *JOURNAL OF RESEARCH AND REVIEW IN SCIENCE*. Retrieved February 23, 2024, from https://www.academia.edu/63065076/Ethno_botanical_survey_review_and_informatics_of_medicinal_plants_used_by_indigenous_people_of_Lekki_Ibeju_Lekki_Local_Government_Area_of_Lagos_State_Nigeria

Pagnocca, T. S., Zank, S., & Hanazaki, N. (2020). "The plants have axé": Investigating the use of plants in Afro-Brazilian religions of Santa Catarina Island. *Journal of Ethnobiology and Ethnomedicine*, *16*(1), 20. https://doi.org/10.1186/s13002-020-00372-6

Paladino, A., Costantini, S., Colonna, G., & Facchiano, A. (2008). Molecular modelling of miraculin: Structural analyses and functional hypotheses. *Biochemical and Biophysical Research Communications*, *367*, 26–32. https://doi.org/10.1016/j.bbrc.2007.12.102

Parreño, R., Rodríguez-Alcocer, E., Martínez-Guardiola, C., Carrasco, L., Castillo, P., Arbona, V., Jover-Gil, S., & Candela, H. (2023). Turning Garlic into a Modern Crop: State of the Art and Perspectives. *Plants*, *12*(6), Article 6. https://doi.org/10.3390/plants12061212

Pennington, T. D. (1991). The Genera of the Sapotaceae. Richmond: Royal Botanic Gardens, Kew.

Posadzki, P., Watson, L. K., & Ernst, E. (2013). Adverse effects of herbal medicines: An overview of systematic reviews. *Clinical Medicine (London, England)*, *13*(1), 7–12. https://doi.org/10.7861/clinmedicine.13-1-7

Prasad, S., & Tyagi, A. K. (2015). Ginger and Its Constituents: Role in Prevention and Treatment of Gastrointestinal Cancer. *Gastroenterology Research and Practice*, *2015*, e142979. https://doi.org/10.1155/2015/142979

Richardson, J. E., Bakar, A. M., Tosh, J., Armstrong, K., Smedmark, J., Anderberg, A. A., Slik, F., & Wilkie, P. (2014). The influence of tectonics, sea-level changes and dispersal on migration and diversification of Isonandreae (Sapotaceae). *Botanical Journal of the Linnean Society*, *174*(1), 130–140. https://doi.org/10.1111/boj.12108

Romanus, P. C., Mendes, F. R., & Carlini, E. de A. (2018). Factors affecting the use of medicinal plants by migrants from rural areas of Brazilian Northeast after moving to a metropolitan region in Southeast of Brazil. *Journal of Ethnobiology and Ethnomedicine*, *14*(1), 72. https://doi.org/10.1186/s13002-018-0270-3

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian

Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Sarić-Kundalić, B., Dobeš, C., Klatte-Asselmeyer, V., & Saukel, J. (2011). Ethnobotanical survey of traditionally used plants in human therapy of east, north and north-east Bosnia and Herzegovina. *Journal of Ethnopharmacology*, *133*(3), 1051–1076. https://doi.org/10.1016/j.jep.2010.11.033

Selvakumar, P., Gahloth, D., Tomar, P. P. S., Sharma, N., & Sharma, A. K. (2011). Molecular Evolution of Miraculin-Like Proteins in Soybean Kunitz Super-Family. *Journal of Molecular Evolution*, *73*(5), 369–379. https://doi.org/10.1007/s00239-012-9484-5

Shah, A. A., Badshah, L., Khalid, N., Shah, M. A., Manduzai, A. K., Faiz, A., De Chiara, M., Mattalia, G., Sõukand, R., & Pieroni, A. (2023). Disadvantaged Economic Conditions and Stricter Border Rules Shape Afghan Refugees' Ethnobotany: Insights from Kohat District, NW Pakistan. *Plants*, *12*(3), Article 3. https://doi.org/10.3390/plants12030574

Shahrajabian, M. H., Sun, W., & Cheng, Q. (2019). Clinical aspects and health benefits of ginger (Zingiber officinale) in both traditional Chinese medicine and modern industry. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, *69*(6), 546–556. https://doi.org/10.1080/09064710.2019.1606930

Silveira, D., Prieto-Garcia, J. M., Boylan, F., Estrada, O., Fonseca-Bazzo, Y. M., Jamal, C. M., Magalhães, P. O., Pereira, E. O., Tomczyk, M., & Heinrich, M. (2020). COVID-19: Is There Evidence for the Use of Herbal Medicines as Adjuvant Symptomatic Therapy? *Frontiers in Pharmacology*, *11*. https://www.frontiersin.org/articles/10.3389/fphar.2020.581840

Singh, D. R. K. (2019). Uses of Medicinal Plants In Traditional and Modern Medicine. *International Journal of Engineering Research & Technology*, *8*(8). https://doi.org/10.17577/IJERTV8IS080241

Singh, P., Mishra, G., Pottoo, F. H., Singh, B., & Zeleke, M. M. (2022). Zingiber officinale: Its Ethanobotanical Uses, Phytochemistry, and Pharmacology. In M. H. Masoodi & M. U. Rehman (Eds.), *Edible Plants in Health and Diseases: Volume II : Phytochemical and Pharmacological Properties* (pp. 1–42). Springer. https://doi.org/10.1007/978-981-16-4959-2_1

Sintayehu, D. W. (2018). Impact of climate change on biodiversity and associated key ecosystem services in Africa: A systematic review. *Ecosystem Health and Sustainability*, *4*(9), 225–239. https://doi.org/10.1080/20964129.2018.1530054

Snider, P., & Zeff, J. (2019). Unifying Principles of Naturopathic Medicine Origins and Definitions. *Integrative Medicine: A Clinician's Journal*, *18*(4), 36–39.

Sohail, A., Barry, A., Auburn, S., Cheng, Q., Lau, C. L., Lee, R., Price, R. N., Furuya-Kanamori, L., Bareng, P., McGuinness, S. L., & Leder, K. (2024). Imported malaria into Australia: Surveillance insights and opportunities. *Journal of Travel Medicine*, *31*(3), taad164. https://doi.org/10.1093/jtm/taad164

Sulaiman, A. N., Arzai, A. H., & Taura, D. W. (2022). Ethnobotanical survey: A comprehensive review of medicinal plants used in treatment of gastro intestinal diseases in Kano state, Nigeria. *Phytomedicine Plus*, *2*(1), 100180. https://doi.org/10.1016/j.phyplu.2021.100180

Swenson, U. & anderberg, A. A. (2005). Phylogeny, character evolution, and classification of Sapotaceae (Ericales). Cladistics 21(2): 101–130.

Swenson, U., Lowry, P. P., Munzinger, J., Rydin, C., & Bartish, I. V. (2008). Phylogeny and generic limits in the Niemeyera complex of New Caledonian Sapotaceae: Evidence of multiple origins of the anisomerous flower. *Molecular Phylogenetics and Evolution*, *49*(3), 909–929. https://doi.org/10.1016/j.ympev.2008.09.022

Synsepalum afzelii—Useful Tropical Plants. (n.d.). Retrieved August 8, 2023, from https://tropical.theferns.info/viewtropical.php?id=Synsepalum+afzelii

Tareau, M.-A., Greene, A., Palisse, M., & Odonne, G. (2022). Migrant Pharmacopoeias: An Ethnobotanical Survey of Four Caribbean Communities in Amazonia (French Guiana). *Economic Botany*, *76*(2), 176–188. https://doi.org/10.1007/s12231-021-09529-0

Tchokponhoué, D. A., N'Danikou, S., Fassinou Hotegni, N. V., Nyadanu, D., Kahane, R., Odindo, A. O., Achigan-Dako, E. G., & Sibiya, J. (2021). Use Patterns, Knowledge Diversity and Drivers for the Cultivation of the Miracle Plant [Synsepalum dulcificum (Schumach & Thonn.) Daniell] in Benin and Ghana. *Plants*, *10*(11), 2253. https://doi.org/10.3390/plants10112253

Thakur, K. S., Kumar, M., Bawa, R., & Bussmann, R. W. (2014). Ethnobotanical Study of Herbaceous Flora along an Altitudinal Gradient in Bharmour Forest Division, District Chamba of Himachal Pradesh, India. *Evidence-Based Complementary and Alternative Medicine*, *2014*, e946870. https://doi.org/10.1155/2014/946870

The Cardiovascular Benefits and Harms of Ginger. (n.d.). LIVESTRONG.COM. Retrieved August 15, 2023, from https://www.livestrong.com/article/557445-the-cardiovascular-benefits-and-harms-of-ginger/

Torres-Avilez, W., Medeiros, P. M. de, & Albuquerque, U. P. (2016). Effect of Gender on the Knowledge of Medicinal Plants: Systematic Review and Meta-Analysis. *Evidence-Based Complementary and Alternative Medicine*, *2016*, e6592363. https://doi.org/10.1155/2016/6592363

Tugume, P., Kakudidi, E. K., Buyinza, M., Namaalwa, J., Kamatenesi, M., Mucunguzi, P., & Kalema, J. (2016). Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda. *Journal of Ethnobiology and Ethnomedicine*, *12*(1), 5. https://doi.org/10.1186/s13002-015-0077-4

Uchejeso, O., & Chinaza, I. (2020). *Bitter kola assisted the Igbo Speaking People in Nigeria against COVID-19*.

Volpato, G., Godínez, D., Beyra, A., & Barreto, A. (2009). Uses of medicinal plants by Haitian immigrants and their descendants in the Province of Camagüey, Cuba. *Journal of Ethnobiology and Ethnomedicine*, *5*, 16. https://doi.org/10.1186/1746-4269-5-16

Wang, Z., & Yang, L. (2021). Chinese herbal medicine: Fighting SARS-CoV-2 infection on all fronts. *Journal of Ethnopharmacology*, *270*, 113869. https://doi.org/10.1016/j.jep.2021.113869

Wink, M., Botschen, F., Gosmann, C., Schäfer, H., & Waterman, P. G. (2010). Chemotaxonomy Seen from a Phylogenetic Perspective and Evolution of Secondary Metabolism. In *Annual*

Plant Reviews Volume 40: Biochemistry of Plant Secondary Metabolism (pp. 364–433). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781444320503.ch7

Xiang, L., Chen, Z., Wei, S., & Zhou, H. (2022). Global Trade Pattern of Traditional Chinese Medicines and China's Trade Position. *Frontiers in Public Health*, *10*. https://www.frontiersin.org/articles/10.3389/fpubh.2022.865887

Yang, Z., Liu, Z., Xu, H., Chen, Y., Du, P., Li, P., Lai, W., Hu, H., Luo, J., & Ding, Y. (2022). The Chromosome-Level Genome of Miracle Fruit (Synsepalum dulcificum) Provides New Insights Into the Evolution and Function of Miraculin. *Frontiers in Plant Science*, *12*. https://www.frontiersin.org/articles/10.3389/fpls.2021.804662

Yeshi, K., Turpin, G., Jamtsho, T., & Wangchuk, P. (2022). Indigenous Uses, Phytochemical Analysis, and Anti-Inflammatory Properties of Australian Tropical Medicinal Plants. *Molecules*, *27*(12), 3849. https://doi.org/10.3390/molecules27123849

Yogom, B. T., Avana-Tientcheu, M.-L., Mboujda, M. F. M., Momo, S. T., Fonkou, T., Tsobeng, A., Barnaud, A., & Duminil, J. (2020). Ethnicity Differences in Uses and Management Practices of Bitter Kola Trees (Garcinia kola) in Cameroon. *Economic Botany*, *74*(4), 429–444. https://doi.org/10.1007/s12231-020-09508-x

Zahn, R., Perry, N., Perry, E., & Mukaetova-Ladinska, E. B. (2019). Use of herbal medicines: Pilot survey of UK users' views. *Complementary Therapies in Medicine*, *44*, 83–90. https://doi.org/10.1016/j.ctim.2019.02.007

Zhou, G., Yang, L., Li, C., Xu, W., & Chen, G. (2010). Genetic diversity in endangered *Notopterygium forbesii* Boissieu based on intraspecies sequence variation of chloroplast

DNA and implications for conservation. *Biochemical Systematics and Ecology*, *38*(5), 911–916. https://doi.org/10.1016/j.bse.2010.09.012

Appendix

Table 1: Morphological characters of small genera merged by Pennington 1991

Species	Current name	Encampt/Brochido	Tertiaries	Stipules	Inflorescence	Free/fused sepals	Corolla tube length	Corolla aestivation	Stamen insertion
Afrosersalisia afzellii	*Synsepalum afzelii*	+ -	reticulate	-	ax	+ --	1	2.5	?
A. ceracifera	*S. ceraciferum*	+ -	?	-	ramif	--+	1.5	2	Imb
A. kassneri	*S. kassneri*	+ -	areolate	-	ax	-+-	1.25	1.75	?
Vincentella brenanii	*S. brenanii*	+ -	Oblique and retic	+	ax cauli	+--	1	2	valv
V. muelleri	*S. muelleri*	+ -	Oblique and areol	+	ax	+--	8	2	valv
V. passargei	*S. passargei*	+ -	Retic	+	ax	+--	0.25	2.75	
V. revoluta	*S. revolutum*	+ -	Oblique	+	ax	+--	0.5	2	Imb
Pachystella brevipes	*S. brevipes*	+ -	Horiz	+	ax	+--	1	4	Imb
P. msolo	*S. msolo*	+ -	Horiz	+	Cauli/rami	+--	2	5.5	?
P. subverticillata	*S. suverticilatum*	+ -	Oblique	+	ax	+--	3.5	4.5	Valv

S. dulcificum	*S. dulcificum*	+ -	Retic	-	ax	--+	2.5	2.5	imb
S. glycodorum	*S. glycodorum*	+ -	Oblique/horizontal	+	ax	--+	4	3	?imb
S. stipulatum	*S. stipulatum*	+ -	Retic	+	ax	-+-	2	2	?
S. subcordatum	*S. subcordatum*	+ -	Oblique	-	?	-+-	2.5	3.5	imb
Tulestea gabonensis	*S. gabonensis*	-+	Oblique	-	ax	-+-	2	3.5	Imb
T. seretii	*S. seretii*	+ -	Oblique	-	ax	+--	2	3	quin

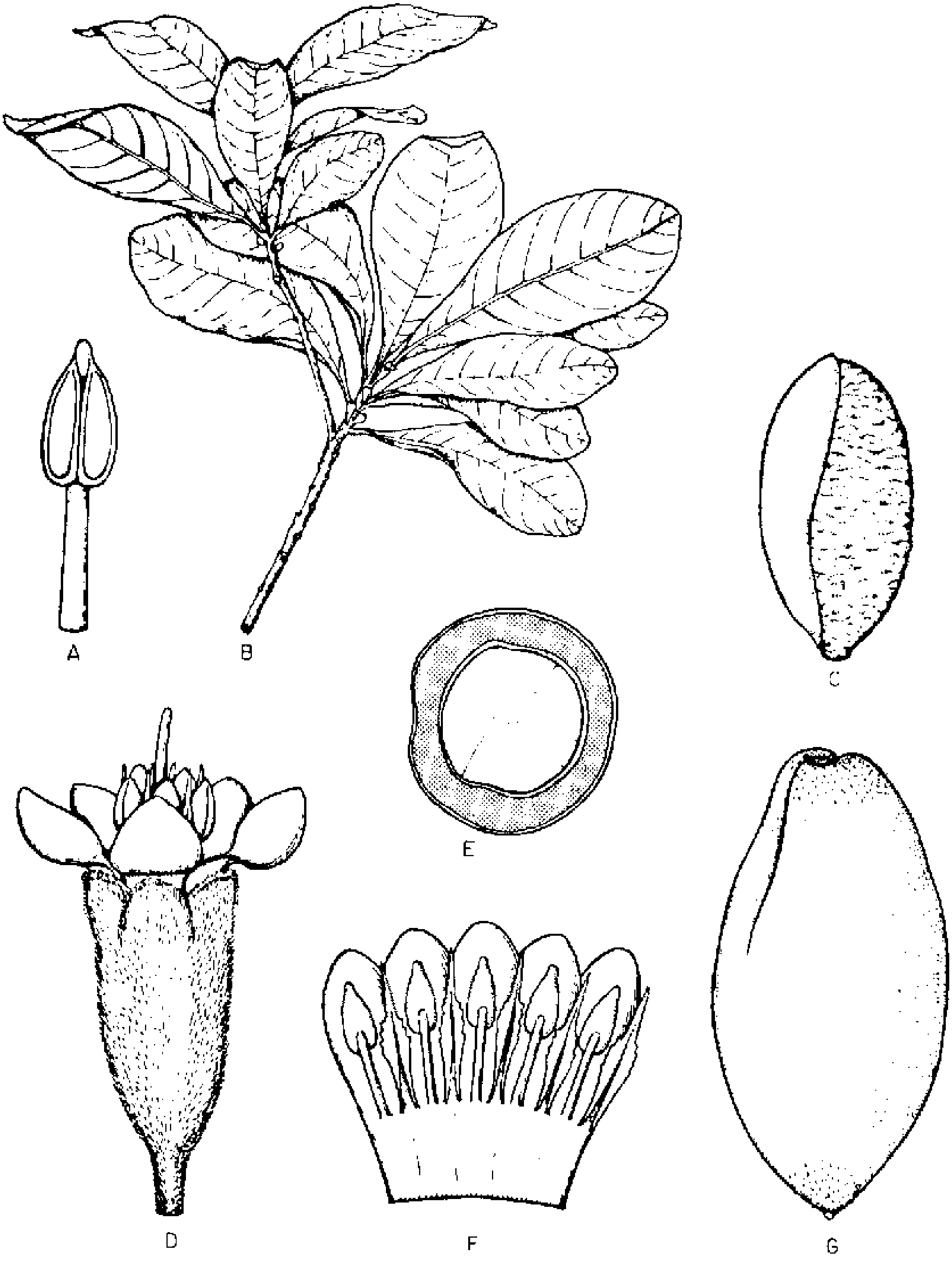
species	Stamens insertion	Stamens exerted	Stamens included	Anther position	Anther hairy or glabrous	Staminode length (fraction of corolla length)	Staminode number	Style exerted or included	Seed scar width	Position of corolla lobes (erect or spreading)	Number of ovary loculi
Afrosersalisia afzellii	t	+	-	ex	g		0	ex	¾	Sp	5
A. ceracifera	T	+	-	ex	h	v	5	ex	2/3	Sp	5

A. kassneri	T	+	-	ex	g	1/6	5	ex	½	Sp	5
Vincentella brenanii	T	+	-	ex	g	2	5	incl	?	Sp	5
V. muelleri	T	-	+	incl	g		0	ex	?	Sp	7
V. passargei	T	+	-	ex	g	1	5	ex	¼-1/3	sp	4
V. revoluta	T	+	-		g	7/8	5	ex	1/2	sp	5
Pachystella brevipes	T	+	-	?ex	g	2/3	1-3	ex	2/3	sp	5-6
P. msolo	T	+	-	ex	g		0	ex	½	Erect	?
P. subverticillata	T	+	-	ex	g		0	ex	?	Sp	5
S. dulcificum	T	+	-	ex	g	4/5	5	ex	2/3	sp	5
S. glycodorum	T	+	-	ex	g	½-1	5	ex	7/8	Sp	5-6
S. stipulatum	T	+	-	ex	g	3/4	5	ex	?	Sp	5

S. subcordatum	T	+	-	ex	g	1/2	5	ex	?	Sp	5
Tulestea gabonensis	T	+	-	ex	h	v	5	ex	?	±sp	5
T. sereti	3/4	-	+	ex	g	v	5	ex	1/2	erect	5

Key

Areol – areolate, AX – axillary, Cauli – cauliflorous, ex – exserted, g – glabrous, h – hairy, horiz – horizontal, imb – imbricate, incl – included, quin – quincuncial, ramif – ramiflorous, retic – reticulate, sp – spreading, valv – valvate, v – vestige.


A
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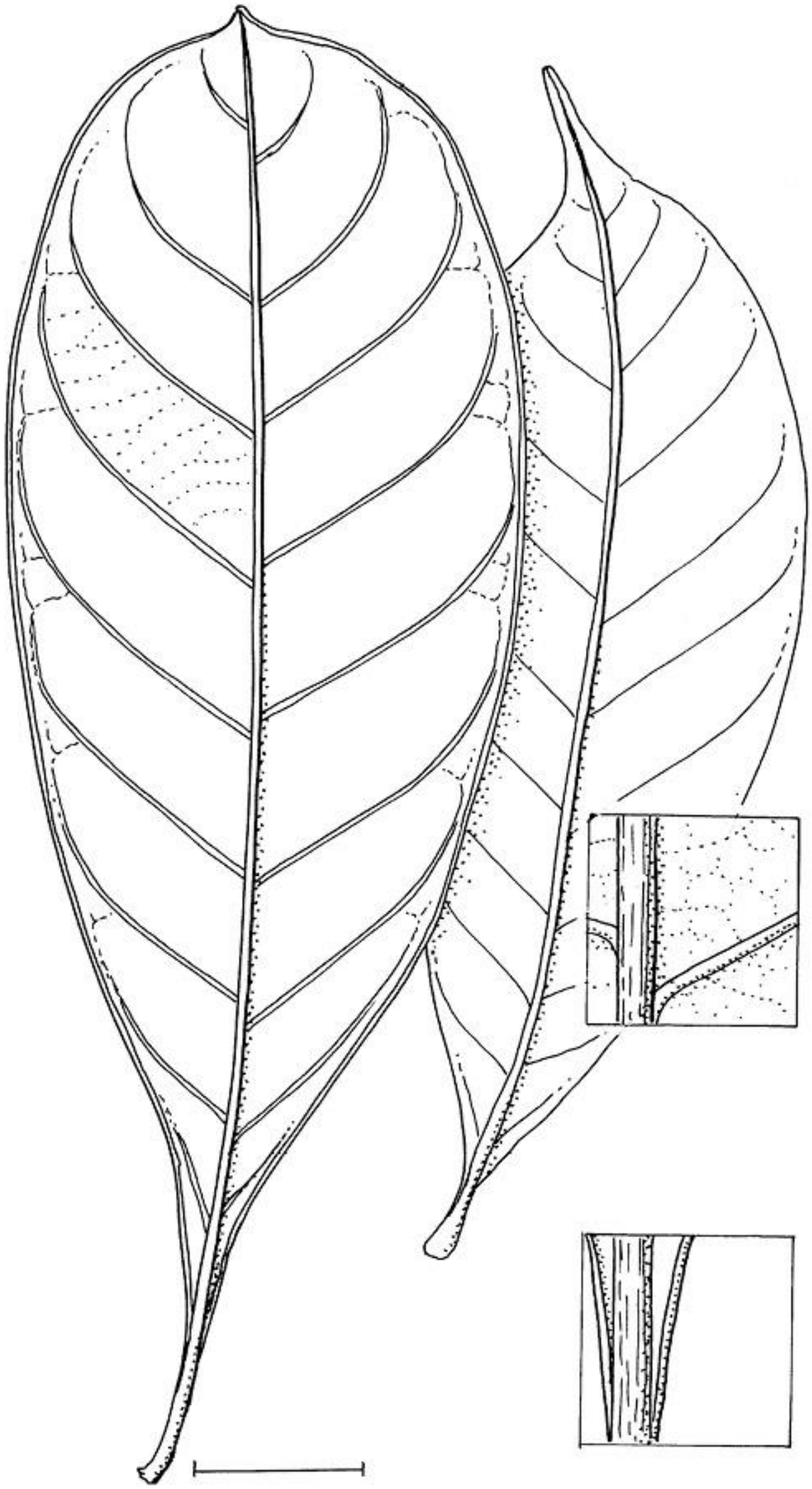


Figure 1: ***S. dulcificum*** **– type species for** Synsepalum

Figure 2: Leaf venation for Synsepalum

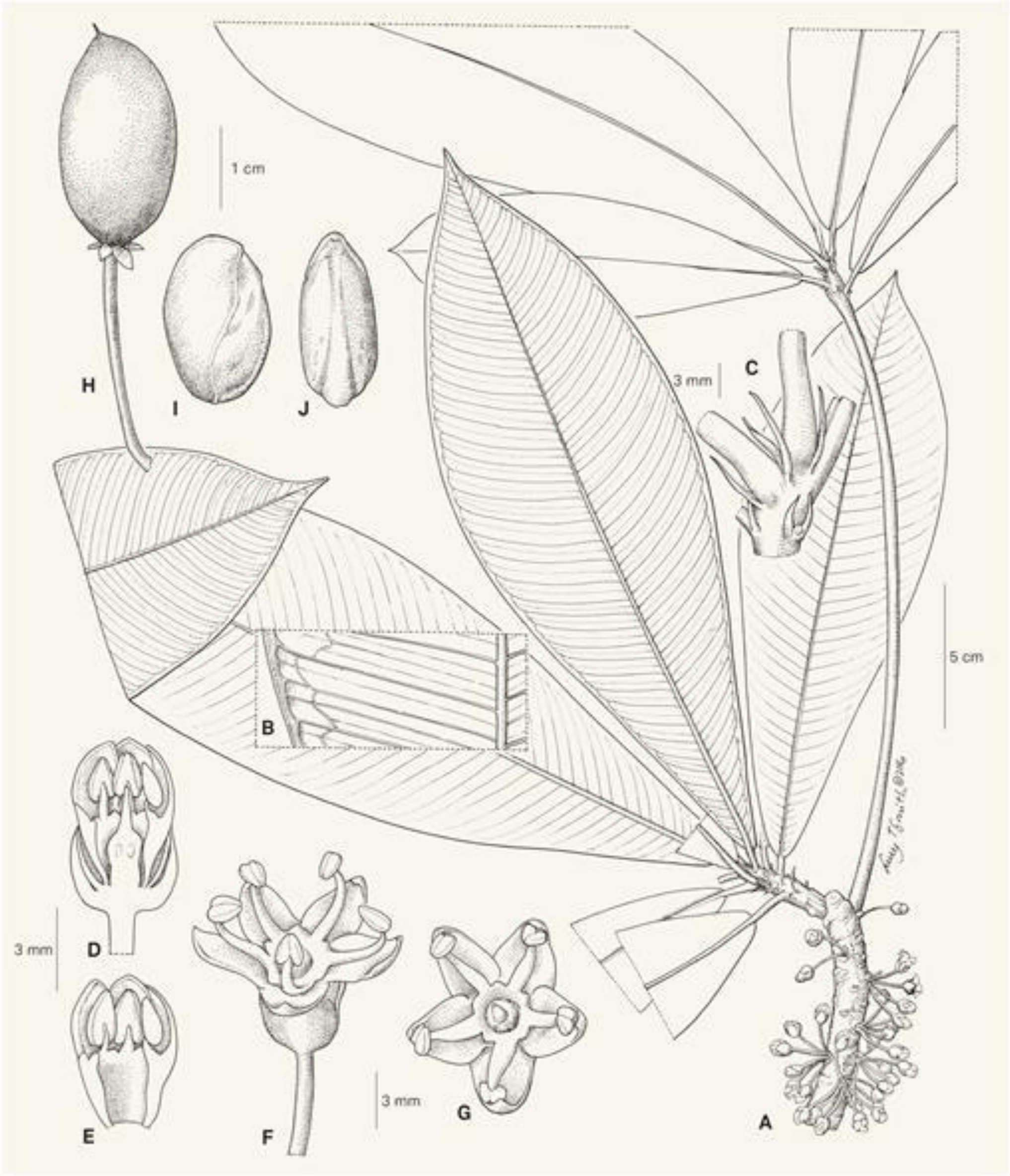


Figure 3: *Englerophytum paludosum* L. Gaut., Burgt & O. Lachenaud. A. Aerial roots at the base of the trunk; B. Leaves; C. Inflorescence; D. Apex of twig showing stipules; E. Flowers, Gabon; F. Flowers.

Table 2: Geographical distribution of species of *Synsepalum* and *Englerophytum*

S/NO.	Species	Distribution	Habitat	Habit
1.	*S. dulcificum* (Schumach. & Thonn.) Daniell	Benin, Cameroon, Central African Republic, Congo, Gabon, Ghana, Ivory Coast, Nigeria, and Zaïre.	Wet tropical biome.	Trees or shrubs
2.	*S. afzelii* (Engl.) T.D.Penn.	Cameroon, Gabon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria, Sierra Leone.	Grows primarily in the wet tropical biome.	Trees
3.	*S. aubrevillei* (Pellegr.) Aubrév. & Pellegr.	Ivory Coast	Wet tropical biome.	Trees
4.	*S. batesii* (A.Chev.) Aubrév. & Pellegr.	Cameroon	Wet tropical biome.	Trees
5.	*S. bequaertii* De Wild.	DR Congo	Wet tropical biome.	Trees

6.	*S. brenanii* (Heine) T.D.Penn.	Cameroon	Wet tropical biome.	Trees
7.	*S. brevipes* (Baker) T.D.Penn.	Angola, Benin, Burkina, Cameroon, Central African Republic, Congo, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Kenya, Liberia, Malawi, Mali, Mozambique, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zambia, Zaïre, Zimbabwe	Wet tropical biome.	Trees
8.	*S. buluensis* (Greves) ined.	Gabon & Cabinda	Wet tropical biome	Trees
9.	*S. carrieanum* (Dubard) Pierre ex ined.	Congo	Wet tropical biome	Trees
10.	*S. cerasiferum* (Welw.) T.D.Penn.	Angola, Burundi, Cameroon, Central African Republic, Gabon, Guinea, Ivory Coast, Kenya, Malawi, Mozambique, Sierra Leone, Sudan, Tanzania, Uganda, Zambia	Wet tropical biome	Trees

11.	*S. Chimanimani* Rokni & I.Darbysh.	Mozambique, Zimbabwe	Wet tropical biome	Shrub or tree
12.	*S. congolense* Lecomte	Gabon & Congo	Wet tropical biome	Shrub or tree
13.	*S. fleuryanum* A.Chev.	Gabon	Wet tropical biome	Shrub
14.	*S. gabonense* (Aubrév. & Pellegr.) T.D.Penn.	Gabon	Wet tropical biome	Tree
15.	*S. kaessneri* (Engl.) T.D.Penn.	SE. Kenya to NE. & E. Central Tanzania	Grows primarily in the seasonally dry tropical biome.	Shrub or tree
16.	*S. laurentii* (De Wild.) D.J.Harris	Central African Republic, Zaïre	Grows primarily in the seasonally dry tropical biome.	Tree
17.	*S. lastoursvillense* (Aubrév. & Pellegr.) ined.	Gabon	Wet tropical biome.	Shrub
18.	*S. letestui* Aubrév. & Pellegr.	Central African Republic, Congo, and Gabon	Wet tropical biome	Shrub
19.	*S. letouzeyi* Aubrév.	Cameroon to Central African Republic	Wet tropical biome	Shrub or tree
20.	*S. msolo* (Engl.) T.D.Penn.	Benin, Cameroon, Congo, Gabon, Ghana, Ivory Coast, Kenya,	Wet tropical biome	Tree

		Tanzania, Uganda, Zaïre.		
21.	*S. muelleri* (Kupicha) T.D.Penn.	S. Malawi to N. Central Mozambique	Grows primarily in the seasonally dry tropical biome.	Tree
22.	*S. ntimii* W.D.Hawth.	Liberia & Ghana	Grows primarily in the seasonally dry tropical biome.	Tree
23.	*S. nyangense* (Pellegr.) McPhersen & J.T.White	Gabon	Wet tropical biome	Shrub or tree
24.	*S. ogouense* (Aubrév. & Pellegr.) ined.	Gabon	Wet tropical biome	Shrub or tree
25.	*S. passargei* (Engl.) T.D.Penn.	Angola, Benin, Burundi, Cameroon, Central African Republic, Ghana, Guinea, Ivory Coast, Malawi, Mozambique, Nigeria, Sierra Leone, Tanzania, Zambia, Zaïre, Zimbabwe	Wet tropical biome	Shrub or tree
26.	*S. pobeguinianum* (Dubard) Aké Assi & L.Gaut.	Burkina, Guinea, Guinea-Bissau, Ivory Coast, Mali, Senegal	Wet tropical biome	Tree
27.	*S. revolutum* (Baker) T.D.Penn.	Cameroon, Central African Republic, Ghana, Gulf of	Wet tropical biome	Tree

		Guinea Is., Ivory Coast, Nigeria, Zaïre		
28.	*S. seretii* (De Wild.) T.D.Penn.	Congo, Gabon, Zaïre	Wet tropical biome	Tree
29.	*S. stipulatum* (Radlk.) Engl.	Cabinda, Cameroon, Central African Republic, Congo, Gabon, Nigeria, Zaïre	Wet tropical biome	Tree
30.	*S. subcordatum* De Wild.	Central African Republic, Congo, Gabon, Zaïre	Wet tropical biome	Tree
31.	*S. subverticillatum* (E.A.Bruce) T.D.Penn.	Kenya	Grows primarily in the seasonally dry tropical biome.	Shrub or tree
32.	*S. tomentosum* (Aubrév. & Pellegr.) ined.	Gabon	Wet tropical biome	Shrub
33.	*S. tsounkpe* Aubrév. & Pellegr.	Ivory Coast	Wet tropical biome	Tree
34.	*S. ulugurense* (Engl.) Engl.	Tanzania (Uluguru Mountains)	Grows primarily in the seasonally dry tropical biome.	Tree
35.	*S. zenkeri* Aubrév. & Pellegr.	Cameroon & Congo	Wet tropical biome	Tree
36.	Englerophytum congolense (De Wild.) Aubrév. & Pellegr.	Gabon to DR Congo	Wet tropical biome	Tree

37.	*E. ferrugineum* L.Gaut. & O.Lachenaud	Gabon	Wet tropical biome	Tree
38.	*E. gigantifolium* O.Lachenaud & L.Gaut.	Gabon	Wet tropical biome	Tree
39.	*E. iturense* (Engl.) L.Gaut.	Gabon	Wet tropical biome	Tree
40.	*E. koulamoutouense* (Aubrév. & Pellegr.) ined.	Gabon to Congo	Wet tropical biome	Shrub
41.	*E. laurentii* (De Wild.) L.Gaut.	Angola, Cameroon, Central African Republic, Gabon, Zaïre	Wet tropical biome	Tree
42.	*E. letestui* (Aubrév. & Pellegr.) L.Gaut.	Gabon to Congo	Wet tropical biome	Tree
43.	*E. libenii* O.Lachenaud & L.Gaut.	Cameroon to Gabon	Wet tropical biome	Tree
44.	*E. longepedicellatum* (De Wild.) L.Gaut.	Gabon to DR Congo	Wet tropical biome	Shrub or tree
45.	*E. magalismontanum* (Sond.) T.D.Penn.	Angola, Botswana, KwaZulu-Natal, Malawi, Mozambique, Northern Provinces, Swaziland, Tanzania, Zambia, Zaïre, Zimbabwe	Grows primarily in the seasonally dry tropical biome.	Tree
46.	*E. mayumbense* (Greves) L.Gaut.	Cabinda, Congo, Gabon	Wet tropical biome	Shrub

47.	*E. natalense* (Sond.) T.D.Penn.	Cape Provinces, Kenya, KwaZulu-Natal, Malawi, Mozambique, Northern Provinces, Swaziland, Tanzania, Uganda, Zimbabwe	Grows primarily in the seasonally dry tropical biome.	Tree
48.	*E. oblanceolatum* (S.Moore) T.D.Penn.	Benin, Cameroon, Central African Republic, Congo, Equatorial Guinea, Ghana, Guinea, Ivory Coast, Kenya, Liberia, Nigeria, Sierra Leone, Togo, Uganda.	Wet tropical biome	Tree
49.	*E. oubanguiense* (Aubrév. & Pellegr.) Aubrév. & Pellegr.	Cameroon, Central African Republic, Ghana, Guinea, Ivory Coast, Liberia, Nigeria.	Wet tropical biome	Tree
50.	*E. paludosum* L.Gaut., Burgt & O.Lachenaud	Cameroon, Congo, Gabon	Wet tropical biome	Tree
51.	*E. rwandense* (Troupin) ined.	Burundi & Rwanda	Wet tropical biome	Tree
52.	*E. somiferanum* Aubrév.	Gabon	Wet tropical biome	Tree

53.	*E. stelechantha* K.Krause	Cameroon, Congo, Gabon	Wet tropical biome	Tree
54.	*E. sylverianum* Kenfack & L.Gaut.	Cameroon, Equatorial Guinea	Wet tropical biome	Tree

Table 3: Species used for the study collected within the United States.

S/NO	Species name	Collector(s) Name	Collector number	Herbarium
1	*E. magalismontanum*	D.A McCallum	1074	MO
2.	*S. longecuneatum*	Gordon McPherson	16736	MO
3.	*S. bequaertii*	Lee White	1115	MO
4.	*S. congolense*	G. Dauby	1078	MO
5.	*S. cerasiferum*	Gordon McPherson	21391	MO
6.	*E. iturense*	J. Bokdam	4529	MO
7.	*E. congolense*	Terese Butler Hart	1336	MO
8.	*S. subverticillatum*	Lenin Festo	2760	MO
9.	*S. stipulatum*	Roy E. Gereau	7596	MO
10.	*S. subcordatum*	Terese Butler Hart	1092	MO
11.	*S. revolutum*	Thomas D.W	7998	MO
12.	*S. nyangense*	Gordon McPherson	1527	MO

13.	*S. muelleri*	J.D & E.G Chapman	9339	MO
14.	*S. afzelii*	H.J. Beentje	633	MO
15.	*S. letouzei*	J.J.F.E. de Wilde	8441	MO
16.	*S. laurentii*	David J. Harris	2770	MO
17.	*S. dulcificum*	B.O. Daramola	395	MO
18.	*S. ulugurense*	Moses A. Mwangoka	6515	MO
19.	*S. brevipes*	M.Merello	1603	MO
20.	*S. kaessneri*	C. J. Kayombo	2962	MO
21.	*S. aubrevillei*	J. de Koning	5334	MO
22.	*E. paludosum*	Ehoarn Bidault	1728	MO
23.	*Chrysophyllum acreanum*	Jose Campos	3216	MO
24.	*E. stelechantha*	J.J. Wieringa	2321	MO
25.	*S. pobeguniana*	Chris S. Duvall	424	MO
26.	*S. passargei*	N. Sokpon	247	MO
27.	*Pradosia brevipes*	L.R. Landrum	8676	MO
28.	*E. kennedyi*	J. Nemba	571	MO
29.	*Pycnandra balansae*	P.P Lowry II et al.	5959	MO

30.	*Micropholis brochidodroma*	Juan Ruiz	813	MO
31.	*Pouteria aff. congestifolia*	Walter Palacios	4034	MO
32.	*E. magalismontanum*	E. Kaaya	2559	MO
33.	*E. natalense*	O.A. Kibure	1453	MO
34.	*S. msolo*	M.A. Mwangoka	216	MO
35.	*S. nyangense*	L.J.T White	1597	NY
36.	*S. cerasiferum*	J. de Koning	5935	NY
37.	*S. dulcificum*		2458	NY
38.	*E. magalismontanum*	R. Brand	49	NY
39.	*S. subcordatum*	J.M. Reitsma	2008	NY
40.	*S. seretii*	J.Louis	5658	NY
41.	*S. attenuatum*	J.D. Kennedy	1664	Harvard
42.	*S. seretii*	J. Louis	7932	Harvard
43.	*S. congolense*	M. L.E. Testu	1769	Harvard
44.	*S. dulcificum*			Silica gel
45.	*S. dulcificum*			Silica gel
46.	*S. brevipes*			Silica gel
47.	*S. dulcificum*			Silica gel

Table 4: Species collected outside the United States for this study

S/NO.	Species name	Species number	Herbarium
1.	*E. laurentii*-98	600412486	G
2.	*E. oubanguinense*-97	600412485	G
3.	*E. laurentii*-96	P00099736	G
4.	*E. parludosum*-95	600418157	G
5.	*E. letestui*-94	600412484	G
6.	*E. mayubense*-93	600412476	G
7.	*E. stelechatum*-92	600412483	G
8.	*E. loongepedicellatum*-91	MO2220167	G
9.	*E. sylverianum*-90	MO3340932	G
10.	*E. vermosenii*-89	600412490	G
11.	*E. congolense*-88	MO4314351	G
12.	*E. oblanceolatum*-87	600412472	G
13.	*E. congolense*-86	P00099763	G
14.	*E. vermosenii*-85	P00099652	G
15	*E. congolense*-84	MO3701061	G
16.	*E. sp chrysophyllum*-83	600418335	G
17.	*E. oblanceolatum*-82	600412473	G
18.	*E. stelechantum*-81	600412482	G
19.	*E. natalense*-80	600412475	G
20.	*E. natalense*-79	600412477	G

21.	*E. chrysophyll sp.*-78	600412478	G
22.	*E. longipedicillatum*-77	MO2211311	G
23.	*E. longipedicillatum*-76	MO2211315	G
24.	*E. sylverianum*-75	600412489	G
25.	*E. mayubense*-74	600412480	G
26.	*E. magalismontanum*-73	600412479	G
27.	*S. afzelii*-72	600412443	G
28.	*S. glycodorum*-71	600412421	G
29.	*S. kaessneri*-70	600412422	G
30.	*S. cerasiferum*-69	600412435	G
31.	*S. cerasiferum*-68	600412437	G
32.	*S. brevipes*-67	600412439	G
33.	*S. brevipes*-66	600412438	G
34.	*S. passargei*-65	600412427	G
35.	*S. afzelii*-64	600412419	G
36.	*S. aubrevillei*-63	600412442	G
37.	*S. batesii*-62	600412441	G
38.	*S. bequaertii*-61	600412440	G
39.	*S. stipulatum*-60	600412465	G
40.	*S. stipulatum*-59	600412464	G
41.	*S. pobeguinianum*-58	600412428	G
42.	*S. passargai*-57	600412426	G

43.	*S. msolo*-56	600412424	G
44.	*S. longecunatum*-55	600412423	G
45.	*Tulesta tomentosa*-54	600412467	G
46.	*S. subcordatum*-53	600412466	G
47.	*S. nyangense*-52	600412431	G
48.	*Afrosersalisa maudensis*-51	600412430	G
49.	*S. revolutum*-50	600412429	G
50.	*S. seretii*-49	600412463	G
51.	*S. laurentii*-48	600412468	G
52.	*S. fleuryanum*-47	600412432	G
53.	*S. fleuryanum*-46	600412433	G
54.	*S. dulcificum*-45	600412434	G
55.	*Pseudopachystela lastrovillense*-100	600390399	G
56.	*E. sp.*2-99	600418336	G

Table 5: Clades from ITS for Englerophytum- Synsepalum complex

S/NO.	**CLADES**	**TAXA**
1.	A	*S. revolutum, S. passargei, S. laurentii, S. muelleri, S. subverticillatum, S. longecuneatum, S. subcordatum, S. bequaertii, S. stipulatum, S. dulcificum, S. batesii, S. pobeguinianum, S. fleuryanum*
2.	B	*E species, E. stelechantha, E. Kennedy, E. congolense, E. paludosum, E. mayumbese, E. magalismontanum, E. natalense, E. oblanceolatum*
3.	C	*S. kassneri*

4.	D	*S. pobeguinianum, S. brevipes, S. msolo*
5.	E	*S. afzelli, S. ulugurense*
6.	F	*S. cerasiferum*

Taxonomic description of species of *Synsepalum* used in the study.

The morphological descriptions as described by Pennington 1991, of the different species of *Synsepalum* used in the study are highlighted below.

***S revolutum* (Baker) T.D. Penn.:** It is a tree that grows up to 20m. Like some other species in the genus, it does not have stipules. The leaves are up to 65cm long and alternate. The leaves are also described as simple and strongly clustered at the twig tip. They are large and obovate. The apex of the leaf is obtuse with large sub-triangular acumen. The leaf base is rounded/ cordate-decurrent. The secondary venation is widely spaced more than 10mm, a character that is found in many species in the genus. The secondary venation is also parallel forming continuous sub-marginal loops, while the tertiary venation is reticulate and sometimes scalariform. The abaxial lamina has long silvery hairs. The midrib and petiole feel like pubescent. It is reported in the protologue that the inflorescence and fruit are unknown.

***S. passargei* (Engl.) T.D. Penn:** It is a shrub or much–branched evergreen tree that grows up to 8 m. It has a spreading crown and drooping branches. The trunk slightly fluted at the base. The bark is smooth, light brown or grey. Young branchlets are densely ferrugineous–pubescent. Stipules are present and are about 5–9 mm. long, they are subulate and persistent. Leaves crowded at the branch end. Lamina 5-12 x 2·5–5 cm. The leaves are narrowly obovate with obtuse or rounded apex and acute base. The margin is slightly revolute and undulated with lateral nerves that are about 7–11 on each side. The leaf petiole is 6–10 mm. The upper leaf surface is smooth, glossy, glabrous, drying brownish– or greyish-green, with impressed nerves. The lower surface mat is glabrous, greyish green to pinkish brown with prominent hirsute to glabrescent pale brown to orange nerves. Flowers are fascicled in axils of current and recently fallen leaves, on slender pedicels 3–6 mm long. Calyx is about 1·6 mm. long, pubescent, divided almost to the base into narrowly oblong segments. The corolla is about 3·5 mm. long and greenish white. The petals are almost free, narrowly obovate, becoming reflexed at anthesis. The filaments are slender, and about 3 mm. long with anthers 0–6 mm. long and extrorse. The staminodes are a little shorter than to equaling petals, lanceolate in outline, with ± lacerate margin. The gynoecium is about 2·5 mm. long with a hemispherical ovary, densely and conspicuously hirsute, crowned with a slender style. Fruit up to 1·5 cm. long, ellipsoid, yellow or orange, puberulous to ± glabrous, with withered flower parts persisting at the base. The flesh is edible. Seed up to 12 x 8 mm. having testa shiny pale greyish brown with paler ± elliptic lateral scar.

***S. laurentii* (De Wild.) D.J. Harris:** It is a tree that is about 20m in height. Stipules are present. It has simple leaves that are alternate and have an obovate shape. The apex of the leaves is obtuse with no acumen, while the base is acute. The secondary venation is parallel, forming continuous sub-marginal loops and widely spaced that extend beyond 10 mm. The tertiary venation is reticulate. The abaxial lamina is glabrous. The midrib and petiole feel like pubescent. Just like *Synsepalum revolutum*, the protologue of *Synsepalum laurentii* indicated that the inflorescence and fruit characters are unknown.

***S. muelleri* (Kupicha) T.D. Penn:** it is an understory tree that grows up to 7 m high. The slash is pinkish with white latex. It has a smooth bark. The flowers are white and sessile on the trunk and branches. The fruit is pink with white pulp. The seeds are shiny and brown in color.

S. subverticillatum **(E.A. Bruce) T.D Penn:** It is described as a shrub or small tree that grows up to 8 m high. The growth increases by repeated subapical branching. The young shoots and petioles are densely pubescent with ± stiffly erect hairs. Leaves ± whorled at ends of long and short shoots. Petioles up to 8 mm. long; stipules setose, up to 1 cm. long. Leaf-lamina obovate to oblanceolate and about 5–13 cm. long and 2–6 cm. wide. The apex of the leaf is described as obtuse to emarginate, narrowly cuneate; with glabrous upper surface. The lower surface is pubescent on the midrib and nerves of young leaves, later becoming practically glabrous; lateral nerves 7–12 on each side, venation reticulate. Flowers sessile. Sepals closely clasping corolla-tube, ± free to base, broadly ovate, up to 3 mm. long, 2.5 mm. wide, rusty pilose outside. Corolla-tube urceolate, up to 4 mm. long; lobes spreading, ± ovate, up to 5 mm. long and 3 mm. wide. The free part of the filament is up to 7 mm. long; anthers narrowly obcordate. Staminodes absent. Ovary subglobose, up to 2 mm. long, densely pilose; style long and slender, up to 1.2 cm. long, tapering to simple stigma. Fruits unknown.

S. longecuneatum **De. Wild.:** It is a tree that grows up to 20m tall. The bole and slash character shows that the slash is pinkish with white latex. Stipules absent. Leaves alternate, simple, oblanceolate; apex acute with elongate sub-triangular acumen (length 2-3x base width); base long decurrent. Secondary venation is widely spaced (>10mm) and parallel, forming continuous sub-marginal loops; tertiary venation is scalariform, barely visible. Abaxial surfaces glabrous. Inflorescences in short axillary fascicles. Calyx lobes are short. The corolla tube is up to 3mm long, lobes to 2mm long. Stamens slightly exceeding lobes. The ovary is unknown. Fruit ellipsoid, shiny, green, and cherry red.

***S. subcordatum* De Wild.:** It is a tree that grows up to 2-3m tall, slash deep pink, fibrous with white latex. Stipules present. Leaves are alternate, simple, obovate. The apex is obtuse with large sub-triangular acumen, sometimes absent; base acute. The secondary venation is widely spaced (>10mm) parallel, forming continuous sub-marginal loops. Tertiary venation is scalariform that is obscured below and impressed above. Abaxial lamina glabrous; midrib and petiole with long hairy pubescence, pubescent when young. Inflorescences in fascicles, 8-20 flowers per fascicle borne on 1–2-year-old branches. Calyx is almost free, short, pubescent. Corolla tube is very short, with lobes to 2.2mm long. Stamens and staminodes are nearly free up to 2mm long. Ovary 5-locular, densely hairy. Fruit unknown.

***S. bequaertii* De Wild.:** The taxonomic description in the protologue reveals that it is a tree that grows up to 35m and produces pinkish exudate when slashed. It has a persistent stipule and alternate leaves that are simple and obovate-oblanceolate in shape. The apex of the leaves is described as rounded but sometimes obtuse-blunt with elongated sub-triangular acumen and decurrent base. The venation of the leaves is widely spaced (> 10mm). The venation is also parallel and barely visible, curved but not forming submarginal loops. The tertiary venation is reticulate and obscured below. The abaxial surface of the leaf is glabrous but pubescent when young. It has a fascicle type of inflorescence. The calyx of the flower is about 4mm long while the corolla is up to 2mm long. The corolla lobe is about 4.5 mm long. With stamen growing up to 6mm. the ovary is 5-locular. The fruit is edible and has an ellipsoid shape.

***S. stipulatum* Engl.:** It is a tree that grows up to 15 m high. It is found in the lowland rainforest in southern Nigeria, and into the Congo basin. The wood is very hard, grey, and perhaps flexible. It is cut into planks and is fibrous. When fresh it has a pleasant smell. Stems of small trees serve as axe-handle.

***S. dulcificum* (Schumach. & Thonn.) Baill.:** This is the type species for the genus popularly known as miracle berry. A shrub or small tree, up to 4.5 m tall. Leaves alternate, simple, entire, clustered near ends of branchlets. The petiole is very short; blade obovate-oblanceolate, 5-10 cm x 1.5-4 cm, glabrous below, with about 8 pairs of lateral veins. Flowers solitary, in small, axillary, subsessile clusters; calyx tubular, 4-5-lobed, ribbed; corolla tubular, tube as long as calyx, lobes 4-5, brown; stamens 4-5; pistil with simple style and inconspicuous stigma. Fruit a one-seeded, ellipsoidal berry,1.5-1.8 cm long, dark red; pulp whitish-pink. The seed is large with a hard, shiny testa. *S. dulcificum* prefers damp localities, like along rivers. It is often grown around dwellings. Propagation is by seed but it is slow-growing. The active principle is a basic glycoprotein (miraculin) with a high molecular weight (44 000) which is difficult to stabilize. The purified protein is potentially an interesting sweetening agent. It may potentially be of interest to Southeast Asia as a source of a natural sweetening agent.

***S. batesii* (A.Chev.) Aubrév. & Pellegr.**

A tree with lanceolate leaves that has an obtuse apex and above is glabrous. Veins are inconspicuous. The petiole is 1 cm long. Flowers are in fascicules with very short pedicels. Sepals are 5 and ovate in shape while the corolla lobes are 5 and oblong. Leaves are dull green and are united at the end of the branches. Flowers grouped in clusters on the branches, below the leaves, and sometimes in their axils, subsessile; calyx ovoid, urceolate, divided into 5 lobes. Corolla is a little longer than the calyx, tube is almost as long as the lobes. Five stamens are opposite with long filaments. The ovary is divided into five lobes and covered with stiff hairs at the base.

***S. pobeguinianum* (Pierre ex Lecomte) Aké Assi & L.Gaut**. A tree of up to 45 ft. high, leaves with whitish tomentum when young, but never silvery-silky and shining, nerves very prominent and distinct beneath, ascending at an angle of about 45°; flowers like those of *Synsepalum brevipes*.

***S. fleuryanum* A. Chev.**

It is a 2-meter-high shrub. The leaves are gathered in a false whorl. It has stipules that are filiform and about 1.5 – 3.5 cm in width. The secondary veins are about 6 – 8 pairs. Short petiole that is pubescent then glabrous. Sessile flower buds, pubescent ferruginous appresses.

***S. brevipes* (Baker) T.D.Penn**. Evergreen medium-sized tree up to 25 m tall, but usually smaller; bole straight, strongly fluted, up to 100 cm in diameter, slightly buttressed at base; bark surface brown to grey, scaly inner bark pink-red, exuding latex; crown with spreading branches, terminal parts drooping. The young branches are shortly brown and hairy. The leaves are arranged spirally, clustered at ends of branches, simple and entire. The stipules are filiform and grow up to 0.5–2 cm long. It is persistent. The petiole is up to 1 cm long. The blade is obovate to oblanceolate, 8–25 cm × 4–9 cm, cuneate at base, rounded to shortly acuminate at apex, leathery, initially silvery hairy below but glabrescent, pinnately veined with 5–11 pairs of distinct lateral veins. Flowers in fascicles on branches below the leaves. Flowers bisexual, regular, 5-merous, greenish to creamy white, fragrant, with short pedicels; sepals free, ovate, appressed hairy; corolla 5–6 mm long, with short tube and longer, oblong lobes; stamens inserted at apex of corolla tube opposite the corolla lobes, staminodes minute or absent; ovary superior, globose, hairy, 5-celled, gradually narrowing into the long cylindrical style. Fruit an ellipsoid or ovoid berry 2–2.5 cm long, yellow to orange when ripe, with persistent style on top, 1-seeded. Seed ellipsoid, c. 2 cm long, brown, with a very large scar. Seedling with epigeal germination; hypocotyl 0.5–2.5 cm long, epicotyl 7–11 cm long; cotyledons elliptical, plano-convex, 1–1.5 cm long, fleshy; first two leaves opposite, with linear stipules up to 3 mm long.

***S. msolo* (Engl.) T.D. Penn.:** Medium to tall tree with much-branched and spreading canopy; height up to 50 m.; bole deeply fluted and pillared near the base. Young shoots with dense appressed hairs, later becoming glabrous. Petioles short and stout, 4–8 mm. long; stipules subulate, up to 1.5 cm. long. Leaf-lamina oblanceolate to obovate-oblong, 10–35(–55) cm. long, 4–14(–16) cm. wide, apex rounded or shortly acuminate, tapering to an abruptly obtuse or sub auriculate base; upper surface glabrous, lower surface with small greyish or silvery ± appressed or spreading hairs; lateral nerves 10–20 on each side, veins oblique. Flowers are fragrant, usually clustered on warty projections on older branches. Pedicels 4–6 mm. long. Sepals connate at base, broadly ovate to suborbicular, up to 5 mm. long and 5 mm. wide, cinereous pubescent externally and pilose internally. Corolla greenish-white; tube up to 2 mm. long; lobes ± elliptic, up to 4 mm. long, 3 mm. wide. The free part of the filament is up to 5.5 mm. long; anthers ovate, dehiscence extrorse. Ovary conical, ± 2 mm. long; style up to 5 mm. long. Fruit dull yellow, subglobose, up to 2.5 cm. in diameter; skin thin; flesh juicy. Seed ellipsoid, slightly flattened, up to 1.8 cm. long; scar prominent, lateral, and occupying over half of the surface.

S. afzelii* (Engl.) T.D.Penn.:* Evergreen medium-sized tree up to 30 m tall; bole often fluted at base and sinuous, up to 170(–200) cm in diameter, often with broad buttresses at base; bark surface shallowly fissured and finely scaly, inner bark orange-brown to pinkish, soft-fibrous, exuding latex; young branches initially shortly hairy, but soon glabrous. Leaves arranged spirally, clustered at ends of branches, simple and entire; stipules small, early caducous; petiole c. 1 cm long; blade elliptical to obovate, 6–13 cm × 2.5–4 cm, cuneate at base, rounded to shortly acuminate at apex, glabrous, pinnately veined with c. 10 pairs of indistinct lateral veins. Flowers in fascicles on branches below the leaves. Flowers bisexual, regular, 5-merous, small, with short pedicels; sepals fused at base, broadly ovate, shortly reddish hairy; corolla c. 3.5 mm long, with short tube and longer, oblong lobes; stamens inserted at apex of corolla tube opposite the corolla lobes, alternating with short staminodes toothed at margins; ovary superior, globose, hairy, 5-celled, gradually narrowing into the cylindrical style. Fruit an ellipsoid berry c. 2.5 cm long, red when ripe, 1-seeded. Seed ellipsoid, c. 2 cm long, brown, with a very large scar. Seedling with epigeal germination; hypocotyl 2–3 cm long, epicotyl 5–6 cm long; cotyledons fleshy, c. 2 cm × 1 cm.

***S. ulugurense* Engl.:**

A tree with thin branches that is very densely leafy at the tip. Young with stalks, the petioles and ribs, and densely rusty-hair Stipules are present and are narrowly lanceolate and deciduous with very short petioles. Glabrous except for the rib, narrowly lanceolate-oblong or lanceolate. Narrowed from the upper third towards the base. Apex far acuminates with lateral veins.

***S. cerasiferum* (Welw.) T.D.Penn**.: Evergreen tree 740 m. tall; trunk straight, often unbranched for a considerable distance, with rough, pale to dark grey, scaly or longitudinally fissured bark; slash pale brown or pink, turning orange; lower part of trunk fluted and buttressed. Young shoots are glabrous. Leaf lamina 321 x 1255 cm., elliptic to narrowly elliptic obovate, the apex rounded, the base acuminate; petiole 14 cm. long. Upper leaf surface smooth, glabrous, somewhat glossy, with faintly impressed midrib and inconspicuous lateral nerves 714 on each side; lower surface glabrous, mat, with fairly prominent main nerves and a faintly visible reticulation; leaves often drying brownish. Flowers densely fasciculate in axils of current and fallen leaves; pedicels up to

7 mm. long. Calyx c. 25 mm. long, comprising 5 hard ovate sepals fused to half their length and forming a firm cylinder constricted at the apex. Corolla yellowish cream to greenish, the lower half forming a narrow tube within the calyx, expanding above into 5 patent to reflexed free lobes c. 25 mm. long. Anthers c. 15 mm. long, apiculate. Staminodes c. 05 mm. long, inconspicuous. Ovary c. 4 mm. long, narrowly conical. Fruit solitary, up to 25 x 2 cm., a red ovoid to subglobose berry with persistent style, persistent calyx forming a woody cupule at the base, and thick woody stalk 310 mm. long. Seed up to 2 cm. long; scar occupying j or more of surface area.

Chapter 2. Molecular evolution of miraculin in the genus *Synsepalum*

Abstract

Since the discovery of the glycoprotein, miraculin, in miracle berry (*S. dulcificum*), diverse kinds of research have been done on the protein including investigations of the taste-modifying activity, structure, and subcellular localization. There is, however, no published report about the investigation of the presence of miraculin in other species in the genus. In this study, using a PCR protocol, the presence of the gene responsible for miraculin (Chr10G0299340) was investigated in other species in the genera *Synsepalum* and *Englerophytum*. Two species of *Synsepalum* produced PCR amplification products for the gene for miraculin while none of the species of *Englerophytum* implicated in this study showed any sign of miraculin. The inferred amino acid sequences of miraculin from the species that produced PCR amplification products for the gene for miraculin were combined with those from species containing miraculin-like proteins to investigate the evolution of miraculin. It is revealed that miraculin is distinct from miraculin-like proteins due to the presence of histidine 30 and histidine 60, the residues responsible for the taste-modifying property of miracle berry. Miraculin-like protein contains only histidine 60.

Introduction

Miraculin is a taste-modifying glycoprotein (Lipatova & Campolattaro, 2016; Paladino et al., 2008) that was first extracted from the berry of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell, commercially known as miracle berry (Akinmoladun et al., 2020). It was purified in 1968 (Kurihara & Beidler, 1969) and sequenced in 1989 (Izawa et al., 2010). Miraculin is confirmed to contain 191 amino acids and some carbohydrates (Akinmoladun et al., 2020; Douglas Kinghorn et al., 2010). Due to the flavor-changing properties of the homodimeric protein, it is used both in the beverage industry and in the treatment of insulin-resistant diabetic patients. Miraculin is reported to be 400,000 times sweeter than sucrose on a molecular basis hence it is used as a replacement for sugar in juice lemonade and some other non-alcoholic beverages. It contains 31% glucosamine, 30% mannose, 22% fucose, 10% xylose and 7% galactose (DV, 2022; Izawa et al., 2010).

The presence of miraculin-like protein is reported in many angiosperm families (Selvakumar et al., 2011). The sequences obtained from miraculin-like proteins are very similar to sequences from native miraculin. From the amino terminus to the 60th position, miraculin sequences are 36.3% identical with miraculin-like sequences while from the 143rd to the carboxy terminus miraculin is 51.1% similar to miraculin-like protein. However, unlike the miraculin found in miracle berry, the miraculin-like proteins found in other plant families do not alter the taste bud. Histidine 30 and 60, found only in miracle berry, are responsible for the taste-altering ability of miraculin (Selvakumar et al., 2011). For the other plants containing miraculin-like protein, only histidine 60 is present and there are no reports of taste-altering ability in miraculin-like proteins. It has also been reported that not only do miraculin and miraculin-like proteins have nearly identical homology, but their molecular weights are also similar. The molecular weights of miraculin-like proteins are estimated to be 20,000, while sequence analysis of purified miraculin shows that the protein has a molecular weight of about 28,000 as estimated by SDS-PAGE and is composed of a single polypeptide. This includes the amount of carbohydrate in the protein which is estimated to be 13.9% of the total weight. Miraculin helps the plant resist germ infection, regulate its growth, and defend the plant against environmental pressure (Yang et al., 2022). Similar functions are also reported for miraculin-like proteins where they show trypsin inhibitory activity, and antifungal properties and help in plant defense (Selvakumar et al., 2011).

S. dulcificum is an evergreen shrub of West African origin (E. Achigan-Dako, 2015; Du et al., 2014; Izawa et al., 2010; Misaka, 2013). There are numerous reports of the plant being used for different purposes (E. Achigan-Dako, 2015; Akinmoladun et al., 2020; Tchokponhoué et al., 2021). In tropical Africa where the plant is found in the natural forest, the berry is used to improve the taste of foods that are sour because of fermentation or fruits that are acidic in nature (Akinmoladun et al., 2020; DV, 2022). The ability of the berry to give a sweet sensation to acidic food led to the name miracle berry (Choi & Garza, 2021).

Taxonomic description of miracle berry

S. dulcificum is an evergreen shrub or small tree in the family Sapotaceae (Niu et al., 2020). It grows up to 4 meters in its natural habitat (E. Achigan-Dako, 2015). The leaves are alternate, simple, entire, and clustered near the ends of branchlets. The petiole is very short; the blade is obovate-oblanceolate and about 5-10 cm x 1.5-4 cm long. The leaf is glabrous below, with about 8 pairs of lateral veins. Flowers solitary, in small, axillary, subsessile clusters; calyx is tubular, 4-5-lobed, ribbed; corolla tubular, tube as long as calyx, lobes 4-5, brown; stamens 4-5; pistil with simple style and inconspicuous stigma. Fruit is a one-seeded, ellipsoidal berry that is 1.5-1.8 cm long. It is dark red; pulp whitish pink. The seed is large with a hard, shiny testa. *S. dulcificum* prefers damp localities, like along rivers. It is often grown around dwellings. Propagation is by seed, but it is slow growing. Its back-slash is fibrous and exudes a little white latex.

Ethnobotanical uses of miracle berry

There is a rich literature on the ethnobotanical application of miracle berry, especially in tropical Africa where it grows naturally. Different herbal preparations are made from the plant as all parts of the plants are reported to be effective in managing different human diseases(Akinmoladun et al., 2020). The leaf is the most used part of the plant in traditional medicine. In Ghana, the twigs are used locally as chewing sticks while the wood serves as firewood. A root decoction is used in Congo to treat kidney and stomach upsets. The berry is used to disguise other foods that are spoiling. In Nigeria, the pulp is used to treat parasitic skin infections. People in the rural communities of the Akwa Ibom State in Nigeria, use the macerated root in soda water to treat gonorrhea. Also, in Lagos, Nigeria, the leaves are employed in the treatment of asthma, male infertility, diabetes, weight loss and cancer (Akinmoladun et al., 2020). People in the southeast and other states in southwest Nigeria use the leaf juice to treat diabetes. In Benin, the root is used to treat sexual weakness, cough, and tuberculosis. Also, leaves are used in the treatment of diabetes, malaria, hyperthermia, and enuresis while the bark is employed in the treatment of prostate ailments. The branches are used as a vegetable toothbrush for good dental and oral health. In Japan, the berry is used by diabetic and obese patients (Akinmoladun et al., 2020).

Application of miracle berry in the food and beverage industry

Miracle berry is a highly valued plant that has both traditional and modern applications. It is used in various industries, including food and beverages, cosmetic and pharmaceutical sectors (Tchokponhoué et al., 2021). In the food industry, it is used as a non-calorie natural sweetener and beverage colorant due to the presence of anthocyanins. It is considered a suitable replacement for synthetic sugar in non-alcoholic beverages and yogurt preparation. Humans are biologically predisposed to crave sugar, so finding a way to satisfy that craving that is not harmful to well-being will immensely benefit human health. The idea of replacing sugar with miraculin is increasingly becoming attractive to small and large organizations. This is important considering the high number of people living with diabetes and other sugar-related ailments in the world. Companies in the United States, such as Joywell Foods in California, MiraBurst in New York, and Miracle Fruit Farm LCC in Miami, are already using miracle berries for producing drinks and nutritional supplements. Significantly, Joywell Foods uses a proprietary microbial fermentation process to produce sweet proteins nearly identical to those found in exotic fruits and berries. Though these proteins taste like sugar — and are around 2,000 times sweeter than sugar — they do not impact blood sugar levels or gut microbiomes. Joywell Foods is on a mission to utilize exotic proteins from miracle berries to improve mainstream food formulations by reducing the level of unhealthy sugars in foods.

Application of miracle berry in the cosmetic industry

In the cosmetic industry, oils extracted from the seeds of miracle berry are used to prevent hair breakage. Miracle fruit seed oil contains high amounts of beneficial phytonutrients: Palmitic acid and squalene. These are essential lipid components at levels most closely like those produced by our glands that naturally lubricate, moisturize, strengthen, and protect hair and skin from damage and aging. Vitamin K1, linoleic acid, and elemental silicon are essential phytonutrients found in the fruit of miracle berry and are important in maintaining the structural integrity of human skin, hair, nails, and joints. Also, two randomized, double-blind, placebo-controlled clinical studies performed on the oil from the seeds of miracle berry by certified medical doctors, dermatologists, and orthopedists further revealed the numerous benefits of the oil (Del Campo et al., 2017). For hair, the oil outperformed users of leading hair oil brands, and those who used no oils felt the difference in the healthier performance of their hair. Miracle seed oil hair treatment is the only hair oil product clinically proven to be safe and effective in strengthening hair, reducing breakage, decreasing hair loss due to breakage, and restoring hair from damage (Del Campo et al., 2017). The research on the Vitabrace wristband showed improvement in hand and finger mobility. Improvements in people's performance during daily living tasks were also recorded. The cosmetic and beauty products manufacturing industry in the United States is a multi-billion-dollar industry. In 2022, it was reported that the market size, measured by revenue was $49bn. The miracle berry plant has market value in the United States and in other countries growing miracle berry commercially.

Application of miracle berry in the pharmaceutical industry

There is also published evidence revealing the pharmacological properties of miracle berry. These include antidiabetic, blood cholesterol-lowering, anti-hyperuricemia, antioxidant, anticonvulsant, and anticancer properties. Interestingly, the Baptist Health System, a faith-based, non-profit organization spread across many states in the United States, has been using miracle fruit to help cancer patients who lose their sense of taste due to chemotherapy treatments. Miracle fruit cubes are now available through the pharmacy of the Miami Cancer Institute. They are the first and only dietary supplement ever approved by Baptist Health System for sale and distribution to their patients within their entire network of hospitals. The berry improves insulin resistance hence it is used in the prevention and treatment of heavily economic burdensome non-communicable diseases like cancer and diabetes. Cancer patients undergoing chemotherapy use miracle berries to restore their taste and can enjoy their meals during chemotherapy. It is generally believed that patients undergoing chemotherapy are not able to eat thereby affecting their response to treatment. In a study on the effect of miraculin on the ability of cancer patients to eat, it was reported that all the participants confirmed a positive response to eating after taking the berry.

Geographical distribution of miracle berry

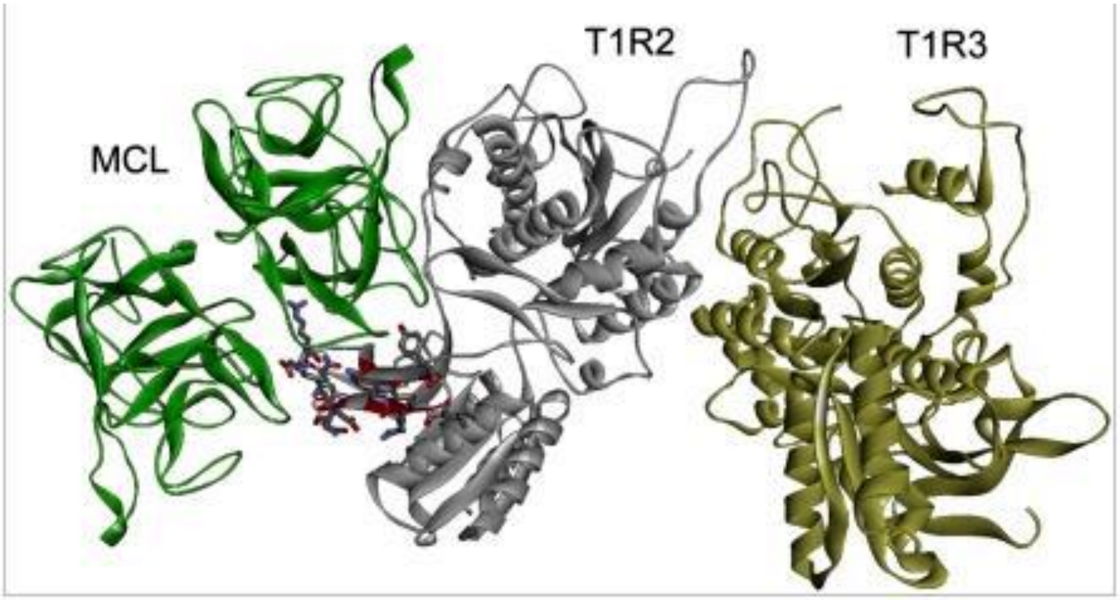
Although the plant is increasingly grown globally because of its enormous commercial potential, it originated in tropical West Africa, grown in Nigeria, Ghana, and Congo (Akinmoladun et al., 2020). The plant requires tropical conditions to thrive (E. Achigan-Dako, 2015) as it does not tolerate frost. The plant has been introduced to many locations where the weather is close to its natural habitat. These include Southeast Asian countries, Australia, and the United States. In the 1960s the people of Ghana gifted one of the prominent leaders of China, Enlai Zhou miracle berry fruit as a national gift (Yang et al., 2022). This may have contributed to the widespread use of the crop in some parts of Asia. In the United States, miracle berry is commercially grown in some states especially Florida due to the subtropical conditions in the state. Most of the countries in tropical Africa experience high temperatures that sometimes exceed 35 °C (Nicholson, 2001) due to the close position of the African continent to the equator. Miracle berries do well in acid soils ranging from pH 4.5–5.8 and climates that vary between 30-40 °C, hence their distribution in tropical Africa (Akinmoladun et al., 2020). Its multipurpose application which is now of high commercial value is reported in many parts of the world including the United States, Asia, and some countries in Europe.

Constraints growing miracle berry

The fact that the miracle berry is a tropical plant that takes about four years before it starts fruiting(Akinmoladun et al., 2020) and the difficulty experienced growing it from seeds, has hampered the interest of many who would have loved to engage in the large-scale establishment of the plantation. Additionally, it has been reported that the taste-modifying property of the berry is lost within 2-3 hours after harvesting at room temperature, minimizing the shelf life of the natural glycoprotein. However, as reported by Hiwasa-Tanase et al. (2012) several attempts have been made by different scientists to overcome these obstacles including the use of *E. coli*, as a delivery vector to express transgenic miraculin in *Aspergillus. oryzae*, *Lactusa sativa* (lettuce), *Fragaria* x *ananassa* (strawberry), and *Solanum lycopersicum* (tomatoes) to produce transgenic miraculin. Recombinant miraculin obtained from *E. coli* showed less potency compared to miraculin obtained from miracle berries, however. The activity of recombinant miraculin in both *A. oryzae* and *Saccharomyces cerevisiae* was evaluated at one-fifth the concentration of native miraculin. In transgenic lettuce, miraculin expression gave a very positive result as the taste-modifying activity of the miraculin was equal in strength to the one obtained from miracle berry. Unfortunately, progeny studies revealed gene silencing in subsequent generations. In transgenic strawberries, recombinant miraculin obtained was at very low levels. In transgenic tomatoes, stable gene expression even up to the fifth generation with a very high level of miraculin that is as stable as the miraculin in the natural source was obtained.

Mechanism of miraculin

Miraculin is regarded as a glycoprotein because of the presence of a carbohydrate group attached to the polypeptide chain. When the fleshy part of the fruit of *S. dulcificum* is eaten, the miraculin molecule binds to the tongue's taste buds, causing sour foods to taste sweet and leading to its generally known name as ‘miracle fruit’(Lipatova & Campolattaro, 2016). Miraculin binds to the human taste receptor (hT1R2–hT1R3) as an antagonist at neutral pH and changes into an agonist at acid pH. In other words, miraculin binds to the sweet taste receptor and activates the receptor as the pH decreases (Doddawad et al., 2022; Misaka, 2013). The effect lasts for up to 2 hours or until the miraculin-sweet receptor complex is deactivated by the alpha-amylase enzyme released from the saliva. It is believed that the taste modification happens in the T1R2-T1R3 sweetness receptor, as shown in the diagram below (Fig. 1).



Source: (Koizumi et al., 2011)

Figure 1: Ribbon representation of a docking model of miraculin taste-modifying protein to the T1T2-T1T3 sweet-taste receptor.

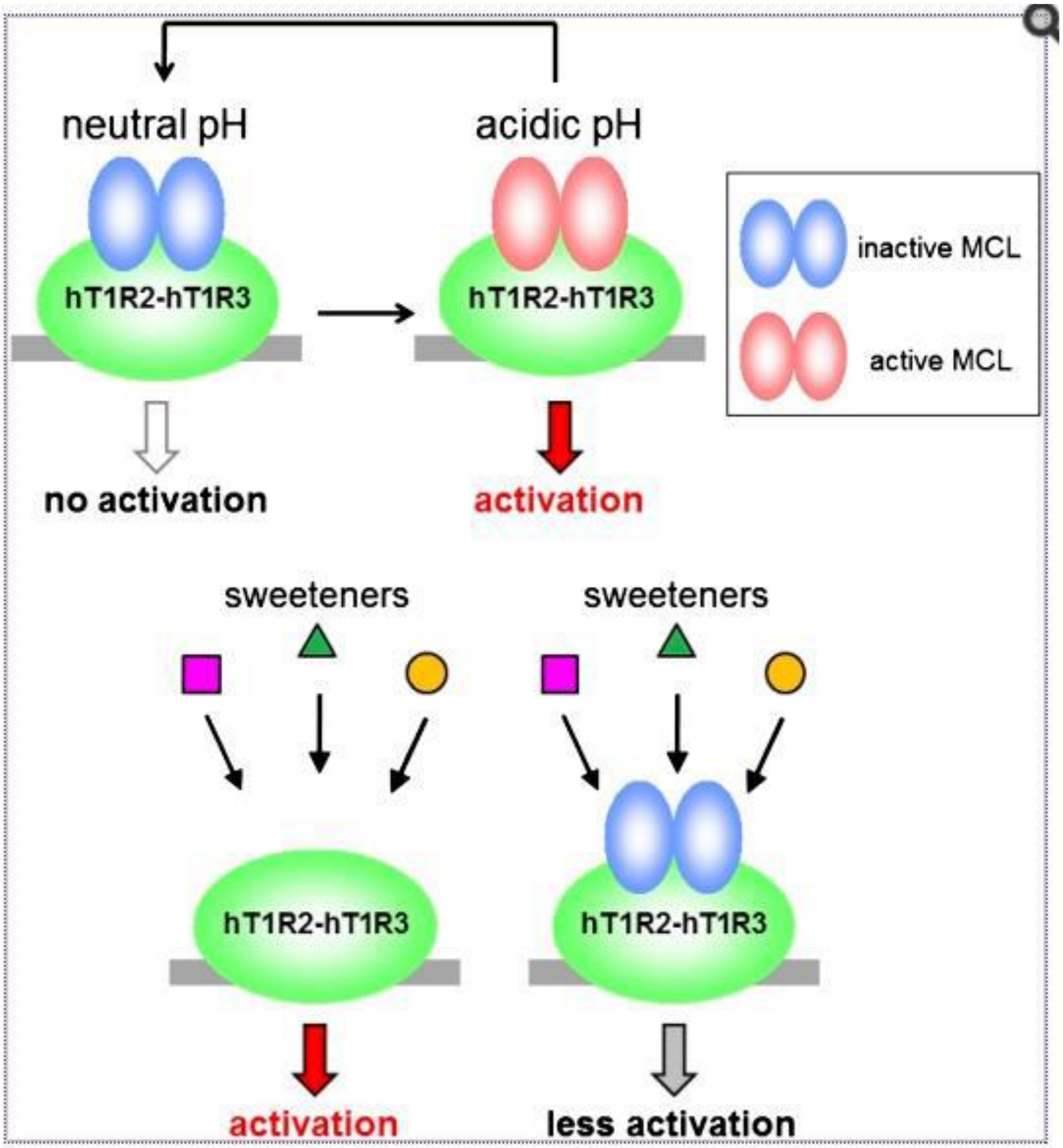
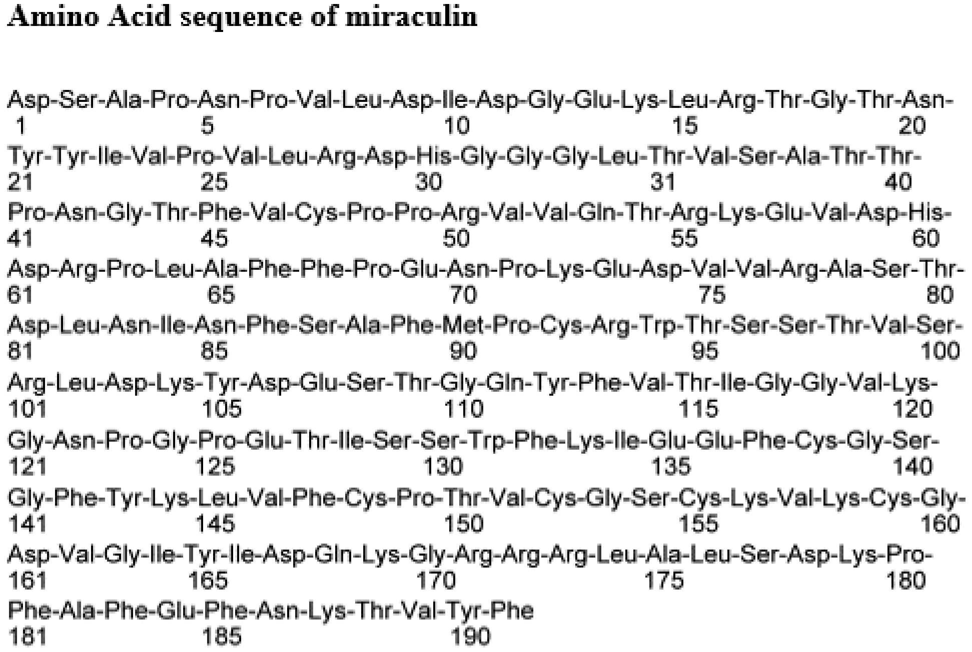


Figure 2: Steps in the miraculin taste-modifying protein to the T1T2-T1T3 sweet-taste receptor

Source: (Misaka, 2013)



Amino Acid sequence of miraculin

Asp-Ser-Ala-Pro-Asn-Pro-Val-Leu-Asp-Ile-Asp-Gly-Glu-Lys-Leu-Arg-Thr-Gly-Thr-Asn-
1 5 10 15 20
Tyr-Tyr-Ile-Val-Pro-Val-Leu-Arg-Asp-His-Gly-Gly-Gly-Leu-Thr-Val-Ser-Ala-Thr-Thr-
21 25 30 31 40
Pro-Asn-Gly-Thr-Phe-Val-Cys-Pro-Pro-Arg-Val-Val-Gln-Thr-Arg-Lys-Glu-Val-Asp-His-
41 45 50 55 60
Asp-Arg-Pro-Leu-Ala-Phe-Phe-Pro-Glu-Asn-Pro-Lys-Glu-Asp-Val-Val-Arg-Ala-Ser-Thr-
61 65 70 75 80
Asp-Leu-Asn-Ile-Asn-Phe-Ser-Ala-Phe-Met-Pro-Cys-Arg-Trp-Thr-Ser-Ser-Thr-Val-Ser-
81 85 90 95 100
Arg-Leu-Asp-Lys-Tyr-Asp-Glu-Ser-Thr-Gly-Gln-Tyr-Phe-Val-Thr-Ile-Gly-Gly-Val-Lys-
101 105 110 115 120
Gly-Asn-Pro-Gly-Pro-Glu-Thr-Ile-Ser-Ser-Trp-Phe-Lys-Ile-Glu-Glu-Phe-Cys-Gly-Ser-
121 125 130 135 140
Gly-Phe-Tyr-Lys-Leu-Val-Phe-Cys-Pro-Thr-Val-Cys-Gly-Ser-Cys-Lys-Val-Lys-Cys-Gly-
141 145 150 155 160
Asp-Val-Gly-Ile-Tyr-Ile-Asp-Gln-Lys-Gly-Arg-Arg-Arg-Leu-Ala-Leu-Ser-Asp-Lys-Pro-
161 165 170 175 180
Phe-Ala-Phe-Glu-Phe-Asn-Lys-Thr-Val-Tyr-Phe
181 185 190

Figure 3: 191 amino acids contained in miraculin highlighting A 30 and 60

Source: Douglas Kinghorn et al., 2010

There is no published report on the presence of miraculin in other species in the genus. In this research, other species of the genera *Synsepalum* and *Englerophytum* were investigated for the presence of a functional miraculin gene. The identification of the gene for miraculin in other species will create an avenue for the possibility of large-scale in-vitro synthesis by pharmaceutical companies and other industries involved in the use of miracle berry. Interestingly, miraculin was found in two different species in the genus *Synsepalum* (*S. longecuneatum* and *S. bequaertii*). This study was carried out at the peak of the COVID-19 pandemic hence going to gather fresh materials from the field was not an option. Hence, herbarium specimens were used for the study. Since fresh material was not available, we could only test for the presence of the miraculin gene using a PCR approach. Although we could not test for the actual presence of the protein, we reasoned that if we could amplify a gene whose sequence encoded a potentially functional protein, it is likely that the plant expresses the gene and produces the protein, at least in some tissues. Phylogenetic and secondary structure analyses were conducted to understand the evolutionary history of miraculin.

Geographical distribution and taxonomic description of species that amplified for miraculin- *S. longecuneatum* and *S. bequaertii.*

According to the International Union for Conservation of Nature (IUCN), *S. bequaertii* is considered endangered. It is native to Congo DR and grows primarily in the wet tropical biome. *S. longecuneatum* is a tree that grows primarily in the seasonally dry tropical biome. It is native to Cabinda, Cameroon, Central African Republic, Congo, Gabon, Nigeria, and Zaïre.

***S. bequaertii* De Wild.:** The taxonomic description in the protologue reveals that it is a tree that grows up to 35m and produces pinkish exudate when slashed. It has a persistent stipule and alternate leaves that are simple and obovate-oblanceolate in shape. The apex of the leaves is described as rounded but sometimes obtuse-blunt with elongated sub-triangular acumen and decurrent base. The venation of the leaves is widely spaced (> 10mm). The venation is also parallel and barely visible, curved but not forming submarginal loops. The tertiary venation is reticulate and obscured below. The abaxial surface of the leaf is glabrous but pubescent when young. It has a fascicle type of inflorescence. The calyx of the flower is about 4mm long while the corolla is up to 2mm long. The corolla lobe is about 4.5 mm long. With stamen growing up to 6mm. the ovary is 5-locular. The fruit is edible and has an ellipsoid shape.

***S. longecuneatum* De. Wild.:** It is a tree that grows up to 20m tall. The bole and slash character shows that the slash is pinkish with white latex. Stipules absent. Leaves alternate, simple, oblanceolate; apex acute with elongate sub-triangular acumen (length 2-3x base width); base long decurrent. Secondary venation is widely spaced (>10mm) and parallel, forming continuous sub-marginal loops; tertiary venation is scalariform, barely visible. Abaxial surfaces glabrous. Inflorescences in short axillary fascicles. Calyx lobes are short. The corolla tube is up to 3mm long, lobes to 2mm long. Stamens slightly exceeding lobes. The ovary is unknown. Fruit ellipsoid, shiny, green, and cherry red.

Materials and methods

Taxon sampling

102 species of *Synsepalum* and *Englerophytum* were sampled for this study. Herbarium specimen samples were obtained from Missouri Botanical Garden (MO), New York Botanical Garden (NY), Harvard University Herbarium (HUH), and Herbarium in Geneva (G) and fresh samples were collected from the Botanical Conservatory at the University of California, Davis.

DNA extraction

Genomic DNA was isolated from leaves taken from herbarium specimens or leaves collected from living plants and dried in silica gel. About 20 mg of leaf tissue mixed with 20 mg of PVP was ground in two 30-s cycles in a BeadBug Mini Homogenizer Model D1030. DNA was extracted from ground leaves using the DNAeasy plant DNA extraction kit (Qiagen, Valencia, California, USA). The extraction of DNA was according to the manufacturer's instructions with slight modifications for some samples.

Amplification and sequencing

PCR was performed with 10 µM of the primers mirR (TTT GAG CTC TTA GAA GTA TAC GGT TTT GT) and mirF (TTT TCT AGA ATG AAG GAA TTA ACA ATGCT) (Hirai et al., 2011). The reactions were carried out in 25 µL of reaction mixture using prepared beads from Pharmacia Biotech (Uppsala, Sweden). The thermal cycling profile was generally that suggested by the manufacturer: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 90 s, and an additional cycle at 72°C for 8 min. The amplified fragments were controlled for their quality by electrophoresis in 0.8% agarose gel and purified by application of QIAquick PCR purification kit from Qiagen (VWR International AB, Stockholm, Sweden). After purification, samples were sent to the UC Davis Genomic Center for sequencing reaction.

Assembly—Sequences of amplified DNA Fragments were assembled and cross-checked against sequence chromatograms using Sequencher 5.4.6. Blasting of cleaned sequences was done on NCBI to confirm the obtained sequences were from the Sapotaceae family and related to the famous miracle berry.

Analysis:

Identification of sequences. Amino acid sequences from *Synsepalum* were used to blast search against the NCBI database. Sequences with significant homology were used for the analysis.

Alignment: Multiple sequence alignment of protein sequences was made using Muscle taking default parameters. The alignment shows a remarkable degree of similarities between the various sequences.

Phylogenetic analysis: Maximum likelihood analysis on aligned sequences was performed using MEGA X. The model of protein evolution that best fit the protein data was applied. The best scoring model for the alignment was the Jones-Taylor-Thornton (JTT) probability model (Jones et al., 19912), with rate variation among sites calculated as a gamma distribution (+G). The reliability of the branching was tested by bootstrap statistical analysis (1,000 replications).

Jal view and Bioinformatic tool kits were used for secondary structure analysis and principal component analysis. The amino acid sequences were also used in Rosetta to generate the tertiary structure of the protein.

Results

Out of the 102 samples of *Synsepalum* and *Englerophytum* that were used for the experiment, only three species of *Synsepalum* produced PCR amplification products for the gene for miraculin. These include *S. dulcificum*, the plant already known to contain miraculin, *S. longecuneatum*, and *S. bequaertii*. *S. dulcificum* was included in the research to confirm the reliability of the protocol that was adopted. Around 588 to 630 base pair fragments were obtained after sequencing. None of the species in the genus *Englerophytum* showed signs of the presence of miraculin.

NCBI Blast

After assembling the contigs, the nucleotides were run through the NCBI database by blast to check for similar plant species. Amino acid sequences deduced were used to perform the BLAST search against the NCBI database, 87 homologs were returned belonging to different angiosperm families. A total of 19 accessions from different angiosperm families were carefully selected from the result of the blast search and combined with amino acid sequences deduced from their DNA.

The data set of twenty-three samples was used for phylogenetic analysis and secondary structure prediction. The table below shows the different species used.

Table 1: Species used for phylogenetic analysis and secondary structure prediction.

S/NO.	FAMILY	PLANT	GENBANK NUMBER
1.	Sapotaceae	*S. dulcificum*	AB512278
2.	Sapotaceae	*S. longecuneatum*	Fresh material
3.	Sapotaceae	*S. bequaertii*	Fresh material
4.	Sapotaceae	*S. dulcificum*	Fresh material
5.	Sapotaceae	*S. dulcificum*	Fresh material
6.	Sapindaceae	*Acer yangbiense*	TXG47971.1
7.	Malvaceae	*Corchorus olitorius*	OMP05594.1
8.	Meliaceae	*Melia azedarach*	KAJ4713686.1
9.	Salicaceae	*Populus alba*	XP_034912243.1
10.	Celastraceae	*Tripterygium wilfordii*	XP_038678858.1
11.	Olacaceae	*Malania oleifera*	XP_057968178.1
12.	Fagaceae	*Quercus rubra*	KAK4567357.1
13.	Cannabaceae	*Trema orientale*	PON53619.1
14.	Rosaceae	*Prunus persica*	XP_020419805.1
15.	Vitaceae	*Vitis vinifera*	CAN81015.1
16.	Cornaceae	*Cornus florida*	XP_059647223.1
17.	Solanaceae	*Lycium barbarum*	XP_060204345.1
18.	Rhamnaceae	*Ziziphus jujuba*	XP_048330366.2

19.	Theaceae	*Camellia sinensis*	XP_028083598.1
20.	Ericaceae	*Vaccinium darrowii*	KAH7852499.1
21.	Paulowniaceae	*Paulownia fortunei*	KAI3454283.1
22.	Bignoniaceae	*Handroanthus impetiginosus*	PIN11664.1
23.	Ebenaceae	*Diospyros lotus*	XP_052182837.1

Phylogenetic tree.

To investigate the phylogenetic relationship of the miraculin gene to other species containing miraculin, a phylogenetic tree was constructed using sequences from miracle berry, two additional species in *Synsepalum* that produced PCR amplification products for the gene for miraculin, and eighteen other species containing miraculin-like protein from different angiosperm families. Amino acid sequences were aligned using Muscle and a maximum likelihood tree was constructed from the complete dataset using MEGA-X. Three clades were recovered from the tree. All four species of *Synsepalum* united in clade C at 100% similarity confirming they share a common ancestor (Fig. 4). The other species having miraculin-like protein divided into two clades- A and B. The figures on the branch are the percentage of how close they are in each clade.

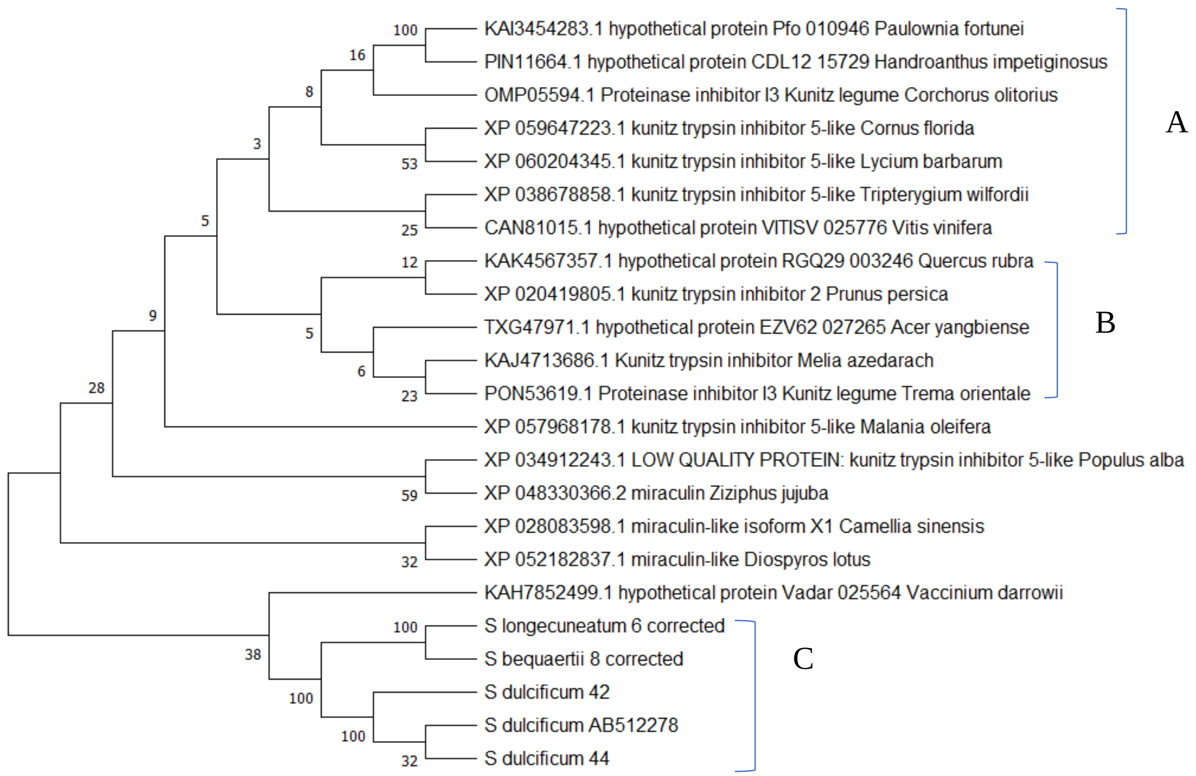
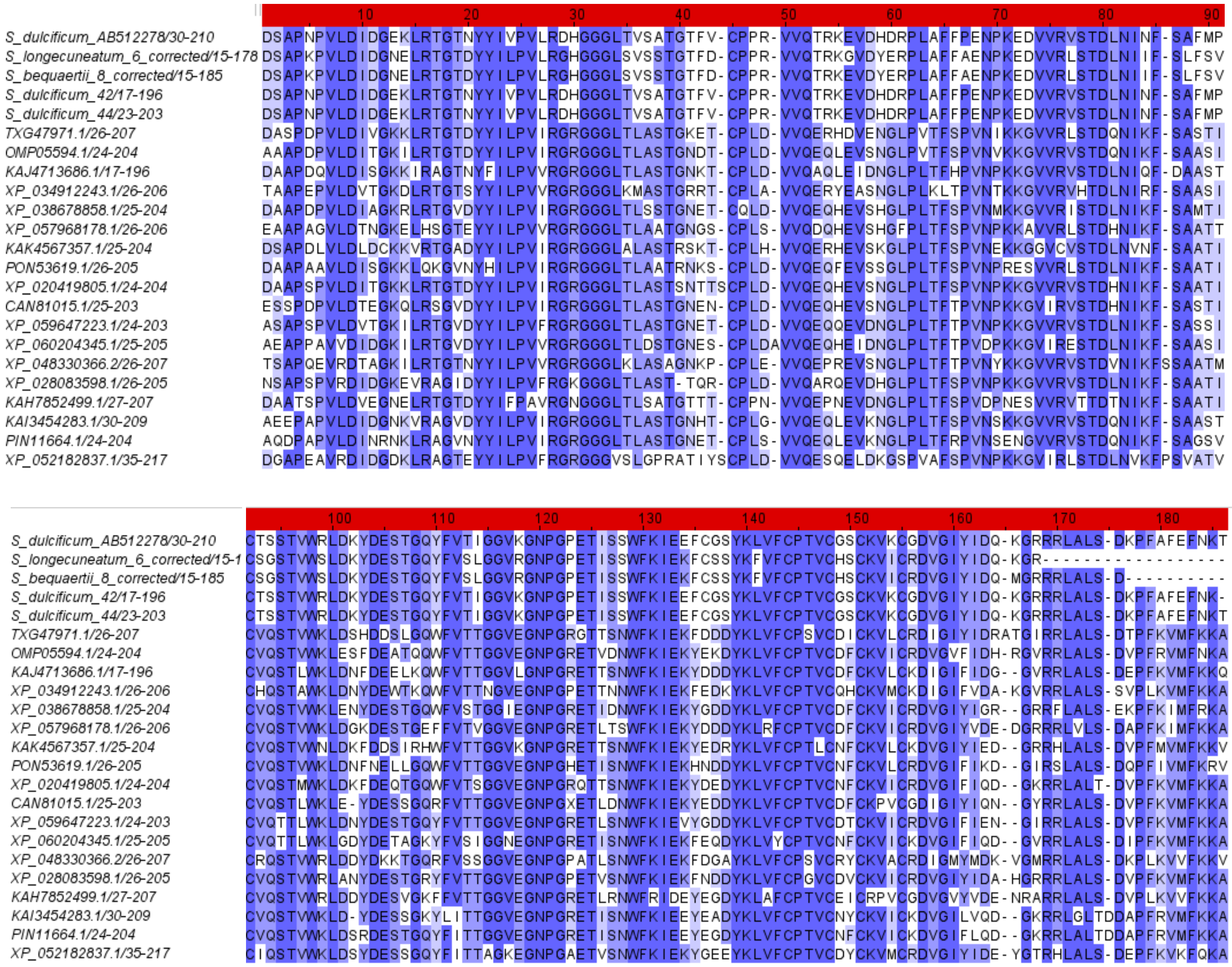


Figure 4: the phylogenetic tree was constructed by the maximum likelihood method. The numbers by the branch points indicate the confidence levels for the relationship of the paired sequences as determined by bootstrap statistical analysis.

Alignment for secondary structure analysis

Amino acid sequence analysis of all the new and old sequences (23 sequences) was used in the secondary structure analysis. Alignment was done using the Jal view algorithm to show the level of similarity among sequences. The multiple sequence alignment shows very interesting results, indicating very close similarity among the species used for the study (Fig. 5). The blue color shows the percentage of similarity among the different species included in the study.



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S_longecuneatum_6_corrected/15-178      DSAPKPVLDIDGNELRTGTDYYILPVLRGHGGGLSVSSTGTFD-CPPR-VVQTRKGVDYERPLAFFAENPKEDVVRLSTDLNIIF-SLFSV
S_bequaertii_8_corrected/15-185         DSAPKPVLDIDGNELRTGTDYYILPVLRGHGGGLSVSSTGTFD-CPPR-VVQTRKEVDYERPLAFFAENPKEDVVRLSTDLNIIF-SLFSV
S_dulcificum_42/17-196                  DSAPNPVLDIDGEKLRTGTNYYIVPVLRDHGGGLTVSATGTFV-CPPR-VVQTRKEVDHDRPLAFFPENPKEDVVRVSTDLNINF-SAFMP
S_dulcificum_44/23-203                  DSAPNPVLDIDGEKLRTGTNYYIVPVLRDHGGGLTVSATGTFV-CPPR-VVQTRKEVDHDRPLAFFPENPKEDVVRVSTDLNINF-SAFMP
TXG47971.1/26-207                       DASPDPVLDIVGKKLRTGTDYYILPVIRGRGGGLTLASTGKET-CPLD-VVQERHDVENGLPVTFSPVNIKKGVVRLSTDQNIKF-SASTI
OMP05594.1/24-204                       AAAPDPVLDITGKILRTGTDYYILPVIRGRGGGLTLASTGNDT-CPLD-VVQEQLEVSNGLPVTFSPVNVKKGVVRVSTDQNIKF-SAASI
KAJ4713686.1/17-196                     DAAPDQVLDISGKKIRAGTNYFILPVVRGRGGGLTLASTGNKT-CPLD-VVQAQLEIDNGLPLTFHPVNPKKGVVRVSTDLNIQF-DAAST
XP_034912243.1/26-206                   TAAPEPVLDVTGKDLRTGTSYYILPVVRGRGGGLKMASTGRRT-CPLA-VVQERYEASNGLPLKLTPVNTKKGVVRVHTDLNIRF-SAASI
XP_038678858.1/25-204                   DAAPDPVLDIAGKRLRTGVDYYILPVIRGRGGGLTLSSTGNET-CQLD-VVQEQHEVSHGLPLTFSPVNMKKGVVRISTDLNIKF-SAMTI
XP_057968178.1/26-206                   EAAPAGVLDTNGKELHSGTEYYILPVVRGRGGGLTLAATGNGS-CPLS-VVQDQHEVSHGFPLTFSPVNPKKAVVRLSTDHNIKF-SAATT
KAK4567357.1/25-204                     DSAPDLVLDLDCKKVRTGADYYILPVIRGRGGGLALASTRSKT-CPLH-VVQERHEVSKGLPLTFSPVNEKKGGVCVSTDLNVNF-SAATI
PON53619.1/26-205                       DAAPAAVLDISGKKLQKGVNYHILPVIRGRGGGLTLAATRNKS-CPLD-VVQEQFEVSSGLPLTFSPVNPRESVVRLSTDLNIKF-SAATI
XP_020419805.1/24-204                   DAAPSPVLDITGKKLRTGTDYYILPVIRGRGGGLTLASTSNTTSCPLD-VVQEQHEVSNGLPLTFSPVNPKKGVVRVSTDHNIKF-SAATI
CAN81015.1/25-203                       ESSPDPVLDTEGKQLRSGVDYYILPVIRGRGGGLTLASTGNEN-CPLD-VVQEQHEVSNGLPLTFTPVNPKKGVIRVSTDHNIKF-SASTI
XP_059647223.1/24-203                   ASAPSPVLDVTGKILRTGVDYYILPVFRGRGGGLTLASTGNET-CPLD-VVQEQQEVDNGLPLTFTPVNPKKGVVRVSTDLNIKF-SASSI
XP_060204345.1/25-205                   AEAPPAVVDIDGKILRTGVDYYILPVVRGRGGGLTLDSTGNES-CPLDAVVQEQHEIDNGLPLTFTPVDPKKGVIRESTDLNIKF-SAASI
XP_048330366.2/26-207                   TSAPQEVRDTAGKILRTGTNYYILPVVRGRGGGLKLASAGNKP-CPLE-VVQEPREVSNGLPLTFTPVNYKKGVVRVSTDVNIKFSSAATM
XP_028083598.1/26-205                   NSAPSPVRDIDGKEVRAGIDYYILPVFRGKGGGLTLAST-TQR-CPLD-VVQARQEVDHGLPLTFSPVNPKKGVVRVSTDLNIKF-SAATI
KAH7852499.1/27-207                     DAATSPVLDVEGNELRTGTDYYIFPAVRGNGGGLTLSATGTTT-CPPN-VVQEPNEVDNGLPLTFSPVDPNESVVRVTTDTNIKF-SAATI
KAI3454283.1/30-209                     AEEPAPVLDIDGNKVRAGVDYYILPVIRGRGGGLTLASTGNHT-CPLG-VVQEQLEVKNGLPLTFSPVNSKKGVVRVSTDQNIKF-SAAST
PIN11664.1/24-204                       AQDPAPVLDINRNKLRAGVNYYILPVIRGRGGGLTLASTGNET-CPLS-VVQEQLEVKNGLPLTFRPVNSENGVVRVSTDQNIKF-SAGSV
XP_052182837.1/35-217                   DGAPEAVRDIDGDKLRAGTEYYILPVFRGRGGGVSLGPRATIYSCPLD-VVQESQELDKGSPVAFSPVNPKKGVIRLSTDLNVKFPSVATV

S_dulcificum_AB512278/30-210            CTSSTVWRLDKYDESTGQYFVTIGGVKGNPGPETISSWFKIEEFCGSYKLVFCPTVCGSCKVKCGDVGIYIDQ-KGRRRLALS-DKPFAFEFNKT
S_longecuneatum_6_corrected/15-1        CSGSTVWSLDKYDESTGQYFVSLGGVRGNPGPETISSWFKIEKFCSSYKFVFCPTVCHSCKVICRDVGIYIDQ-KGR------------------
S_bequaertii_8_corrected/15-185         CSGSTVWSLDKYDESTGQYFVSLGGVRGNPGPETISSWFKIEKFCSSYKFVFCPTVCHSCKVICRDVGIYIDQ-MGRRRLALS-D----------
S_dulcificum_42/17-196                  CTSSTVWRLDKYDESTGQYFVTIGGVKGNPGPETISSWFKIEEFCGSYKLVFCPTVCGSCKVKCGDVGIYIDQ-KGRRRLALS-DKPFAFEFNK-
S_dulcificum_44/23-203                  CTSSTVWRLDKYDESTGQYFVTIGGVKGNPGPETISSWFKIEEFCGSYKLVFCPTVCGSCKVKCGDVGIYIDQ-KGRRRLALS-DKPFAFEFNKT
TXG47971.1/26-207                       CVQSTVWKLDSHDDSLGQWFVTTGGVEGNPGRGTTSNWFKIEKFDDDYKLVFCPSVCDICKVLCRDIGIYIDRATGIRRLALS-DTPFKVMFKKA
OMP05594.1/24-204                       CVQSTVWKLESFDEATQQWFVTTGGVEGNPGRETVDNWFKIEKYEKDYKLVFCPTVCDFCKVICRDVGVFIDH-RGVRRLALS-DVPFRVMFNKA
KAJ4713686.1/17-196                     CVQSTLWKLDNFDEELKQWFVTTGGVLGNPGRETTSNWFKIEKYDDDYKLVFCPTVCDFCKVLCKDIGIFIDG--GVRRLALS-DEPFKVMFKKQ
XP_034912243.1/26-206                   CHQSTAWKLDNYDEWTKQWFVTTNGVEGNPGPETTNNWFKIEKFEDKYKLVFCPTVCQHCKVMCKDIGIFVDA-KGVRRLALS-SVPLKVMFKKA
XP_038678858.1/25-204                   CVQSTVWKLENYDESTGQWFVSTGGIEGNPGRETIDNWFKIEKYGDDYKLVFCPTVCDFCKVICRDVGIYIGR--GRRFLALS-EKPFKIMFRKA
XP_057968178.1/26-206                   CVQSTVWKLDGKDESTGEFFVTVGGVEGNPGRETLTSWFKIEKYDDDYKLRFCPTVCDFCKVICRDVGIYVDE-DGRRRLVLS-DAPFKIMFKKA
KAK4567357.1/25-204                     CVQSTVWNLDKFDDSIRHWFVTTGGVKGNPGRETTSNWFKIEKYEDRYKLVFCPTLCNFCKVLCKDVGIYIED--GRRHLALS-DVPFMVMFKKV
PON53619.1/26-205                       CVQSTVWKLDNFNELLGQWFVTTGGVEGNPGHETISNWFKIEKHNDDYKLVFCPTVCNFCKVLCRDVGIFIKD--GIRSLALS-DQPFIVMFKRV
XP_020419805.1/24-204                   CVQSTMWKLDKFDEQTGQWFVTSGGVEGNPGRQTTSNWFKIEKYDEDYKLVFCPTVCNFCKVICRDVGIFIQD--GKRRLALT-DVPFKVMFKKA
CAN81015.1/25-203                       CVQSTLWKLE-YDESSGQRFVTTGGVEGNPGXETLDNWFKIEKYEDDYKLVFCPTVCDFCKPVCGDIGIYIQN--GYRRLALS-DVPFKVMFKKA
XP_059647223.1/24-203                   CVQTTLWKLDNYDESTGQYFVTTGGVEGNPGRETLSNWFKIEVYGDDYKLVFCPTVCDTCKVICRDVGIFIEN--GIRRLALS-DVPFKVMFKKA
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XP_048330366.2/26-207                   CRQSTVWRLDDYDKKTGQRFVSSGGVEGNPGPATLSNWFKIEKFDGAYKLVFCPSVCRYCKVACRDIGMYMDK-VGMRRLALS-DKPLKVVFKKV
XP_028083598.1/26-205                   CVQSTVWRLANYDESTGRYFVTTGGVEGNPGPETVSNWFKIEKFNDDYKLVFCPGVCDVCKVICRDVGIYIDA-HGRRRLALS-DVPFKVMFKKA
KAH7852499.1/27-207                     CVQSTVWRLDDYDESVGKFFVTTGGVEGNPGRETLRNWFRIDEYEGDYKLAFCPTVCEICRPVCGDVGVYVDE-NRARRLALS-DVPLKVVFKKA
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PIN11664.1/24-204                       CVQSTVWKLDSRDESTGQYFITTGGVEGNPGRETISNWFKIEEYEGDYKLVFCPTVCNFCKVICKDVGIFLQD--GKRRLALTDDAPFRVMFKKA
XP_052182837.1/35-217                   CIQSTVWKLDSYDESSGQYFITTAGKEGNPGAETVSNWFKIEKYGEEYKLVFCPTVCDYCKVMCRDVGIYIDE-YGTRHLALS-DEPFKVKFQKA
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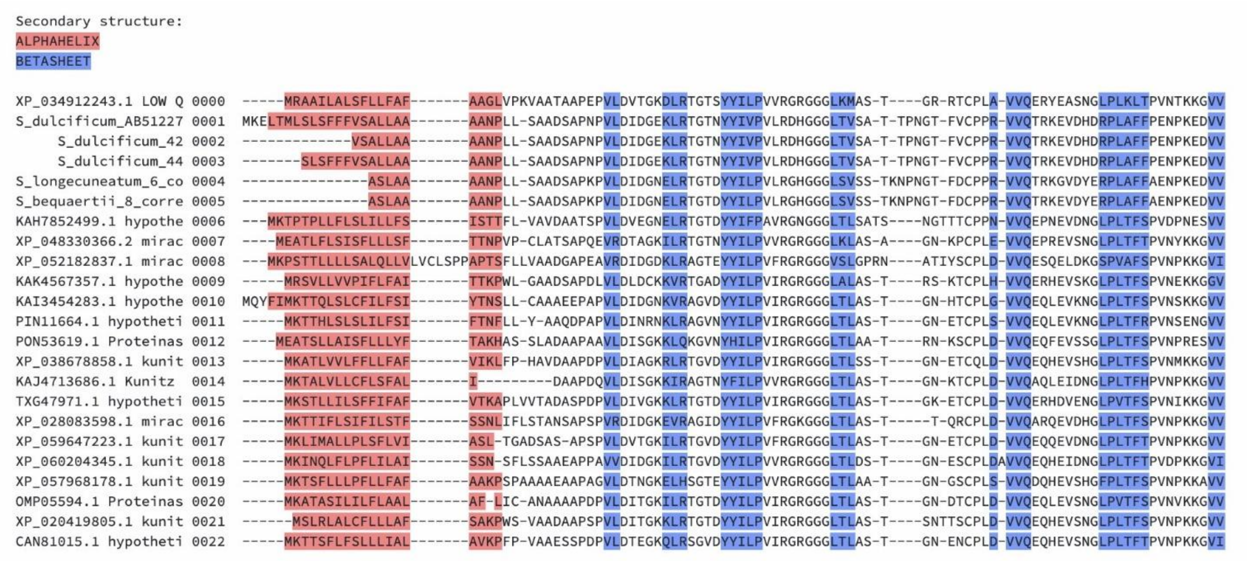
Figure 5: Alignment of species using Jal view to show the percentage of similarity among species.

Secondary structure analysis

Secondary structure analysis was done using the Bioinformatics toolkit. The red column shows the sections of the sequences that are alpha helices while the blue part shows the beta-sheet (Table 2).

Table 2: secondary structure analysis showing alpha helices and beta-sheet.



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Secondary structure:
ALPHAHELIX
BETASHEET

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S_dulcificum_AB51227 0001  MKELTMLSLSFFFVSALLAA-------AANPLL-SAADSAPNPVLDIDGEKLRTGTNYYIVPVLRDHGGGLTVSA-T-TPNGT-FVCPPR-VVQTRKEVDHDRPLAFFPENPKEDVV
     S_dulcificum_42 0002  -------------VSALLAA-------AANPLL-SAADSAPNPVLDIDGEKLRTGTNYYIVPVLRDHGGGLTVSA-T-TPNGT-FVCPPR-VVQTRKEVDHDRPLAFFPENPKEDVV
     S_dulcificum_44 0003  -------SLSFFFVSALLAA-------AANPLL-SAADSAPNPVLDIDGEKLRTGTNYYIVPVLRDHGGGLTVSA-T-TPNGT-FVCPPR-VVQTRKEVDHDRPLAFFPENPKEDVV
S_longecuneatum_6_co 0004  ---------------ASLAA-------AANPLL-SAADSAPKPVLDIDGNELRTGTDYYILPVLRGHGGGLSVSS-TKNPNGT-FDCPPR-VVQTRKGVDYERPLAFFAENPKEDVV
S_bequaertii_8_corre 0005  ---------------ASLAA-------AANPLL-SAADSAPKPVLDIDGNELRTGTDYYILPVLRGHGGGLSVSS-TKNPNGT-FDCPPR-VVQTRKEVDYERPLAFFAENPKEDVV
KAH7852499.1 hypothe 0006  ---MKTPTPLLFLSLILLFS-------ISTTFL-VAVDAATSPVLDVEGNELRTGTDYYIFPAVRGNGGGLTLSATS----NGTTTCPPN-VVQEPNEVDNGLPLTFSPVDPNESVV
XP_048330366.2 mirac 0007  ----MEATLFLSISFLLLSF-------TTNPVP-CLATSAPQEVRDTAGKILRTGTNYYILPVVRGRGGGLKLAS-A----GN-KPCPLE-VVQEPREVSNGLPLTFTPVNYKKGVV
XP_052182837.1 mirac 0008  ---MKPSTTLLLLSALQLLVLVCLSPPAPTSFLLVAADGAPEAVRDIDGDKLRAGTEYYILPVFRGRGGGVSLGPRN----ATIYSCPLD-VVQESQELDKGSPVAFSPVNPKKGVI
KAK4567357.1 hypothe 0009  -----MRSVLLVVPIFLFAI-------TTKPWL-GAADSAPDLVLDLDCKKVRTGADYYILPVIRGRGGGLALAS-T----RS-KTCPLH-VVQERHEVSKGLPLTFSPVNEKKGGV
KAI3454283.1 hypothe 0010  MQYFIMKTTQLSLCFILFSI-------YTNSLL-CAAAEEPAPVLDIDGNKVRAGVDYYILPVIRGRGGGLTLAS-T----GN-HTCPLG-VVQEQLEVKNGLPLTFSPVNSKKGVV
PIN11664.1 hypotheti 0011  -----MKTTHLSLSLILFSI-------FTNFLL-Y-AAQDPAPVLDINRNKLRAGVNYYILPVIRGRGGGLTLAS-T----GN-ETCPLS-VVQEQLEVKNGLPLTFRPVNSENGVV
PON53619.1 Proteinas 0012  ----MEATSLLAISFLLLYF-------TAKHAS-SLADAAPAAVLDISGKKLQKGVNYHILPVIRGRGGGLTLAA-T----RN-KSCPLD-VVQEQFEVSSGLPLTFSPVNPRESVV
XP_038678858.1 kunit 0013  -----MKATLVVLFFLLFAF-------VIKLFP-HAVDAAPDPVLDIAGKRLRTGVDYYILPVIRGRGGGLTLSS-T----GN-ETCQLD-VVQEQHEVSHGLPLTFSPVNMKKGVV
KAJ4713686.1 Kunitz  0014  -----MKTALVLLCFLSFAL-------I---------DAAPDQVLDISGKKIRAGTNYFILPVVRGRGGGLTLAS-T----GN-KTCPLD-VVQAQLEIDNGLPLTFHPVNPKKGVV
TXG47971.1 hypotheti 0015  -----MKSTLLILSFFIFAF-------VTKAPLVVTADASPDPVLDIVGKKLRTGTDYYILPVIRGRGGGLTLAS-T----GK-ETCPLD-VVQERHDVENGLPVTFSPVNIKKGVV
XP_028083598.1 mirac 0016  -----MKTTIFLSIFILSTF-------SSNLIFLSTANSAPSPVRDIDGKEVRAGIDYYILPVFRGKGGGLTLAS-T-----T-QRCPLD-VVQARQEVDHGLPLTFSPVNPKKGVV
XP_059647223.1 kunit 0017  -----MKLIMALLPLSFLVI-------ASL-TGADSAS-APSPVLDVTGKILRTGVDYYILPVFRGRGGGLTLAS-T----GN-ETCPLD-VVQEQQEVDNGLPLTFTPVNPKKGVV
XP_060204345.1 kunit 0018  -----MKINQLFLPFLILAI-------SSN-SFLSSAAEAPPAVVDIDGKILRTGVDYYILPVVRGRGGGLTLDS-T----GN-ESCPLDAVVQEQHEIDNGLPLTFTPVDPKKGVI
XP_057968178.1 kunit 0019  -----MKTSFLLLPFLLFAF-------AAKPSPAAAAEAAPAGVLDTNGKELHSGTEYYILPVVRGRGGGLTLAA-T----GN-GSCPLS-VVQDQHEVSHGFPLTFSPVNPKKAVV
OMP05594.1 Proteinas 0020  -----MKATASILILFLAAL-------AF-LIC-ANAAAAPDPVLDITGKILRTGTDYYILPVIRGRGGGLTLAS-T----GN-DTCPLD-VVQEQLEVSNGLPVTFSPVNVKKGVV
XP_020419805.1 kunit 0021  ------MSLRLALCFLLLAF-------SAKPWS-VAADAAPSPVLDITGKKLRTGTDYYILPVIRGRGGGLTLAS-T----SNTTSCPLD-VVQEQHEVSNGLPLTFSPVNPKKGVV
CAN81015.1 hypotheti 0022  -----MKTTSFLFSLLLIAL-------AVKPFP-VAAESSPDPVLDTEGKQLRSGVDYYILPVIRGRGGGLTLAS-T----GN-ENCPLD-VVQEQHEVSNGLPLTFTPVNPKKGVI
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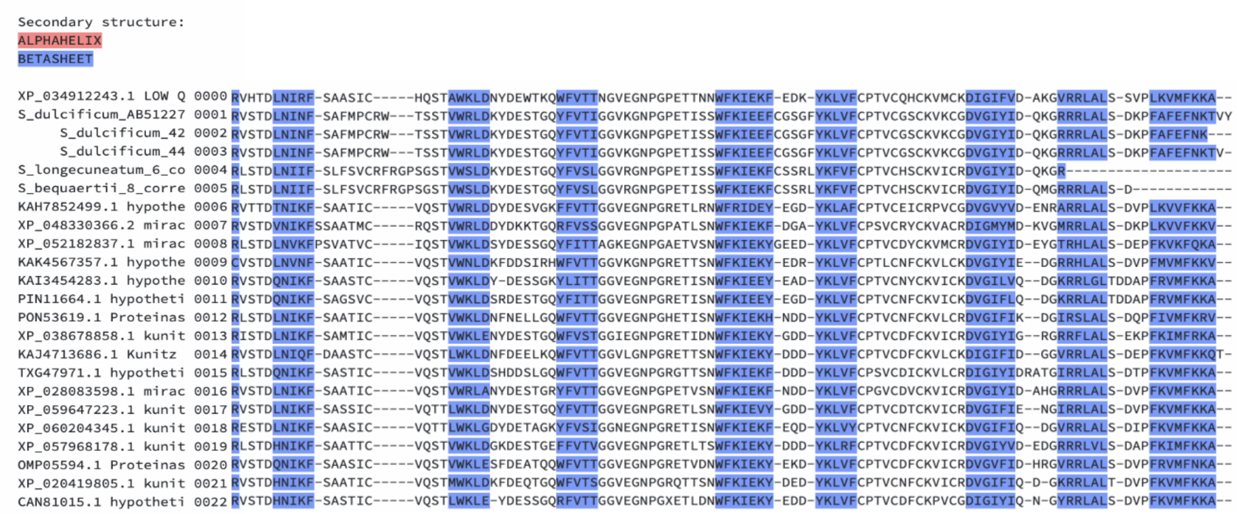

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Secondary structure:
ALPHAHELIX
BETASHEET

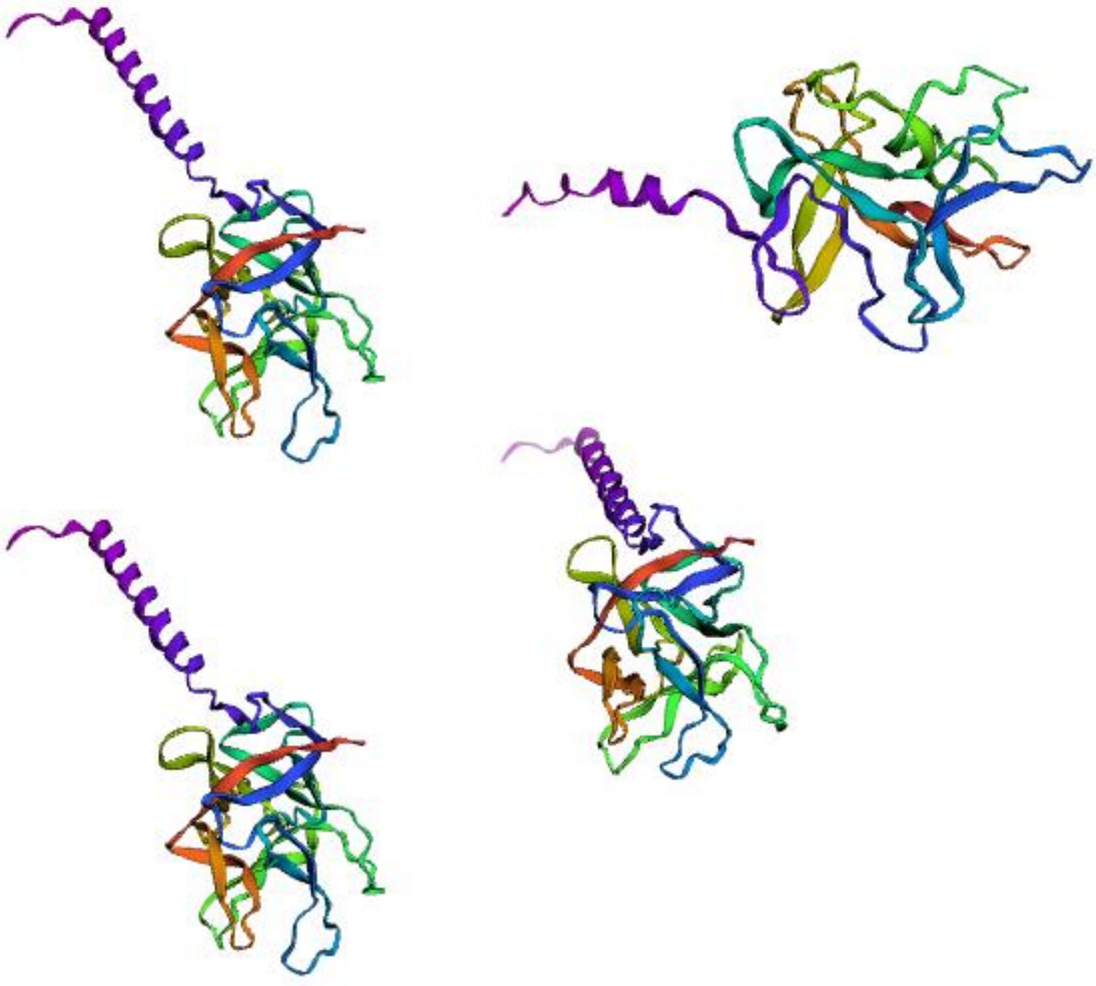
XP_034912243.1 LOW Q 0000 RVHTDLNIRF-SAASIC-----HQSTAWKLDNYDEWTKQWFVTTNGVEGNPGPETTNNWFKIEKF-EDK-YKLVFCPTVCQHCKVMCKDIGIFVD-AKGVRRLALS-SVPLKVMFKKA--
S_dulcificum_AB51227 0001 RVSTDLNINF-SAFMPCRW---TSSTVWRLDKYDESTGQYFVTIGGVKGNPGPETISSWFKIEEFCGSGFYKLVFCPTVCGSCKVKCGDVGIYID-QKGRRRLALS-DKPFAFEFNKTVY
     S_dulcificum_42 0002 RVSTDLNINF-SAFMPCRW---TSSTVWRLDKYDESTGQYFVTIGGVKGNPGPETISSWFKIEEFCGSGFYKLVFCPTVCGSCKVKCGDVGIYID-QKGRRRLALS-DKPFAFEFNK---
     S_dulcificum_44 0003 RVSTDLNINF-SAFMPCRW---TSSTVWRLDKYDESTGQYFVTIGGVKGNPGPETISSWFKIEEFCGSGFYKLVFCPTVCGSCKVKCGDVGIYID-QKGRRRLALS-DKPFAFEFNKTV-
S_longecuneatum_6_co 0004 RLSTDLNIIF-SLFSVCRFRGPSGSTVWSLDKYDESTGQYFVSLGGVRGNPGPETISSWFKIEKFCSSRLYKFVFCPTVCHSCKVICRDVGIYID-QKGR--------------------
S_bequaertii_8_corre 0005 RLSTDLNIIF-SLFSVCRFRGPSGSTVWSLDKYDESTGQYFVSLGGVRGNPGPETISSWFKIEKFCSSRLYKFVFCPTVCHSCKVICRDVGIYID-QMGRRRLALS-D------------
KAH7852499.1 hypothe 0006 RVTTDTNIKF-SAATIC-----VQSTVWRLDDYDESVGKFFVTTGGVEGNPGRETLRNWFRIDEY-EGD-YKLAFCPTVCEICRPVCGDVGVYVD-ENRARRLALS-DVPLKVVFKKA--
XP_048330366.2 mirac 0007 RVSTDVNIKFSSAATMC-----RQSTVWRLDDYDKKTGQRFVSSGGVEGNPGPATLSNWFKIEKF-DGA-YKLVFCPSVCRYCKVACRDIGMYMD-KVGMRRLALS-DKPLKVVFKKV--
XP_052182837.1 mirac 0008 RLSTDLNVKFPSVATVC-----IQSTVWKLDSYDESSGQYFITTAGKEGNPGAETVSNWFKIEKYGEED-YKLVFCPTVCDYCKVMCRDVGIYID-EYGTRHLALS-DEPFKVKFQKA--
KAK4567357.1 hypothe 0009 CVSTDLNVNF-SAATIC-----VQSTVWNLDKFDDSIRHWFVTTGGVKGNPGRETTSNWFKIEKY-EDR-YKLVFCPTLCNFCKVLCKDVGIYIE--DGRRHLALS-DVPFMVMFKKV--
KAI3454283.1 hypothe 0010 RVSTDQNIKF-SAASTC-----VQSTVWKLDY-DESSGKYLITTGGVEGNPGRETISNWFKIEEY-EAD-YKLVFCPTVCNYCKVICKDVGILVQ--DGKRRLGLTDDAPFRVMFKKA--
PIN11664.1 hypotheti 0011 RVSTDQNIKF-SAGSVC-----VQSTVWKLDSRDESTGQYFITTGGVEGNPGRETISNWFKIEEY-EGD-YKLVFCPTVCNFCKVICKDVGIFLQ--DGKRRLALTDDAPFRVMFKKA--
PON53619.1 Proteinas 0012 RLSTDLNIKF-SAATIC-----VQSTVWKLDNFNELLGQWFVTTGGVEGNPGHETISNWFKIEKH-NDD-YKLVFCPTVCNFCKVLCRDVGIFIK--DGIRSLALS-DQPFIVMFKRV--
XP_038678858.1 kunit 0013 RISTDLNIKF-SAMTIC-----VQSTVWKLENYDESTGQWFVSTGGIEGNPGRETIDNWFKIEKY-GDD-YKLVFCPTVCDFCKVICRDVGIYIG--RGRRFLALS-EKPFKIMFRKA--
KAJ4713686.1 Kunitz  0014 RVSTDLNIQF-DAASTC-----VQSTLWKLDNFDEELKQWFVTTGGVLGNPGRETTSNWFKIEKY-DDD-YKLVFCPTVCDFCKVLCKDIGIFID--GGVRRLALS-DEPFKVMFKKQT-
TXG47971.1 hypotheti 0015 RLSTDQNIKF-SASTIC-----VQSTVWKLDSHDDSLGQWFVTTGGVEGNPGRGTTSNWFKIEKF-DDD-YKLVFCPSVCDICKVLCRDIGIYIDRATGIRRLALS-DTPFKVMFKKA--
XP_028083598.1 mirac 0016 RVSTDLNIKF-SAATIC-----VQSTVWRLANYDESTGRYFVTTGGVEGNPGPETVSNWFKIEKF-NDD-YKLVFCPGVCDVCKVICRDVGIYID-AHGRRRLALS-DVPFKVMFKKA--
XP_059647223.1 kunit 0017 RVSTDLNIKF-SASSIC-----VQTTLWKLDNYDESTGQYFVTTGGVEGNPGRETLSNWFKIEVY-GDD-YKLVFCPTVCDTCKVICRDVGIFIE--NGIRRLALS-DVPFKVMFKKA--
XP_060204345.1 kunit 0018 RESTDLNIKF-SAASIC-----VQTTLWKLGDYDETAGKYFVSIGGNEGNPGRETISNWFKIEKF-EQD-YKLVYCPTVCNFCKVICKDVGIFIQ--DGVRRLALS-DIPFKVMFKKA--
XP_057968178.1 kunit 0019 RLSTDHNIKF-SAATTC-----VQSTVWKLDGKDESTGEFFVTVGGVEGNPGRETLTSWFKIEKY-DDD-YKLRFCPTVCDFCKVICRDVGIYVD-EDGRRRLVLS-DAPFKIMFKKA--
OMP05594.1 Proteinas 0020 RVSTDQNIKF-SAASIC-----VQSTVWKLESFDEATQQWFVTTGGVEGNPGRETVDNWFKIEKY-EKD-YKLVFCPTVCDFCKVICRDVGVFID-HRGVRRLALS-DVPFRVMFNKA--
XP_020419805.1 kunit 0021 RVSTDHNIKF-SAATIC-----VQSTMWKLDKFDEQTGQWFVTSGGVEGNPGRQTTSNWFKIEKY-DED-YKLVFCPTVCNFCKVICRDVGIFIQ-D-GKRRLALT-DVPFKVMFKKA--
CAN81015.1 hypotheti 0022 RVSTDHNIKF-SASTIC-----VQSTLWKLE-YDESSGQRFVTTGGVEGNPGXETLDNWFKIEKY-EDD-YKLVFCPTVCDFCKPVCGDIGIYIQ-N-GYRRLALS-DVPFKVMFKKA--
```

The tertiary structure of the miracle berry protein and the two other species that produced PCR amplification products for the gene for miraculin were generated.

Tertiary structure comparison

trRosetta algorithm was used to generate the tertiary structure of *Synseplaum dulcificum* and the two species of Synsepalum (*S. longecuneatum* and *S. bequaertii*) that produced PCR amplification products for the gene for miraculin.

a. *S.dulcificum*　　*b. S. longecuneatum*



c. S. dulcificum　　*d. S. bequaertii*

Figure six a-d: Tertiary structure of different species of Synsepalum containing miraculin

Sequence Comparison

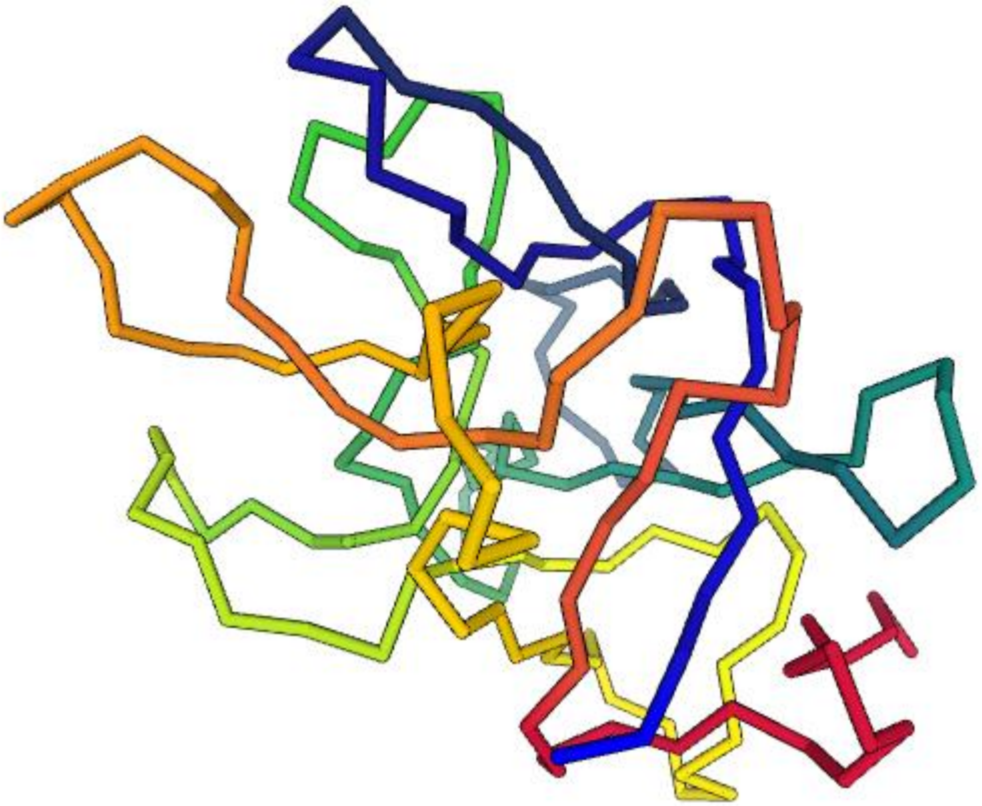
The DAL algorithm for protein comparison was used to compare the tertiary structure of *S. dulcificum* with the structures of *S. beaquetii* and *S. longecuneatum* to evaluate the level of similarity of their protein. The structure below is a comparison between *S. dulcificum* and *S. beaquetii*. Structurally equivalent residues are shown in uppercase while structurally non-equivalent residues are in lowercase. The vertical bars between sequences are used to indicate amino acid identities. *S. dulcificum* and *S. beaquetii* are 80% identical with a Z score of 31.4 while *S. dulcificum* and *S. longecuneatum* are 78% similar with a similar Z-score of 29.1. It is generally accepted that a Z-score above 20 means the two structures are homologous.

```
DSSP  LLLLLLLLLLLLLLLLLLLEEEELLLLLLLLEEEEELlLLLL--LLLLLLEEEEELLLLLL
Query APNPVLDIDGEKLRTGTNYYIVPVLRDHGGGLTVSAtTPNG--TFVCPPRVVQTRKEVDH   58
ident || |||||||  ||||| ||| |||| ||||| ||  | |   || |||||||||||||
Sbjct APKPVLDIDGNELRTGTDYYILPVLRGHGGGLSVSS-TKNPngTFDCPPRVVQTRKEVDY   59
DSSP  LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLEEEEEL-LLLLllLLLLLLEEEEELLLLLL


DSSP  LLLEEEELLLLLLLLLLLLLLLLEEEELLLLLLL---LLLLLLLEEELLLLLLLLLLEEELL
Query DRPLAFFPENPKEDVVRVSTDLNINFSAFMPCR---WTSSTVWRLDKYDESTGQYFVTIG  115
ident  |||||| ||||||||| |||||| || |  ||      |||| |||||||||||||  |
Sbjct ERPLAFFAENPKEDVVRLSTDLNIIFSLFSVCRfrgPSGSTVWSLDKYDESTGQYFVSLG  119
DSSP  LLLEEEEEELLLLLLLLLLLLLEEEEEELLLLLLlllLLLLLLLLEEELLLLLLLLLLEEELL

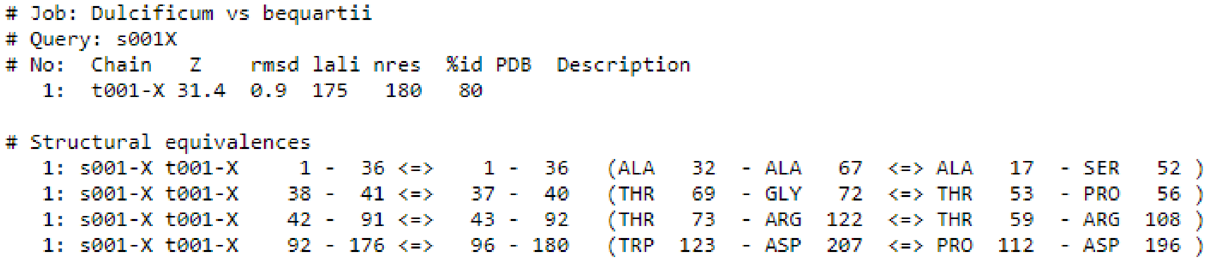

DSSP  LLLLLLLHHHHHHLLEEEEELLLLLLEEEEEELLLLLLLLLLLLEEEEEEEELLLLLEEEEEE
Query GVKGNPGPETISSWFKIEEFCGSGFYKLVFCPTVCGSCKVKCGDVGIYIDQKGRRRLALS  175
ident || ||||||||||||||| || |  || ||||||| |||| | |||||||| ||||||||
Sbjct GVRGNPGPETISSWFKIEKFCSSRLYKFVFCPTVCHSCKVICRDVGIYIDQMGRRRLALS  179
DSSP  LLLLLLLHHHHHHLLEEEEELLLLLLEEEEEELLLLLLLLLLLLEEEEEEEELLLLLEEEEEE


DSSP  Lllllleeeel
Query Dkpfafefnkt  186
ident |
Sbjct D----------  180
DSSP  L----------
```



Combined structure of *S. dulcificum* and *S. beaquetii*.

Table 3: showing structural equivalence of amino acid of *S. dulcificum* and *S. beaquetii*



```
# Job: Dulcificum vs bequartii
# Query: s001X
# No:  Chain   Z    rmsd lali nres  %id PDB  Description
   1:  t001-X 31.4  0.9  175   180   80

# Structural equivalences
   1: s001-X t001-X     1 -  36 <=>    1 -  36   (ALA   32  - ALA   67  <=> ALA   17  - SER   52 )
   1: s001-X t001-X    38 -  41 <=>   37 -  40   (THR   69  - GLY   72  <=> THR   53  - PRO   56 )
   1: s001-X t001-X    42 -  91 <=>   43 -  92   (THR   73  - ARG  122  <=> THR   59  - ARG  108 )
   1: s001-X t001-X    92 - 176 <=>   96 - 180   (TRP  123  - ASP  207  <=> PRO  112  - ASP  196 )
```

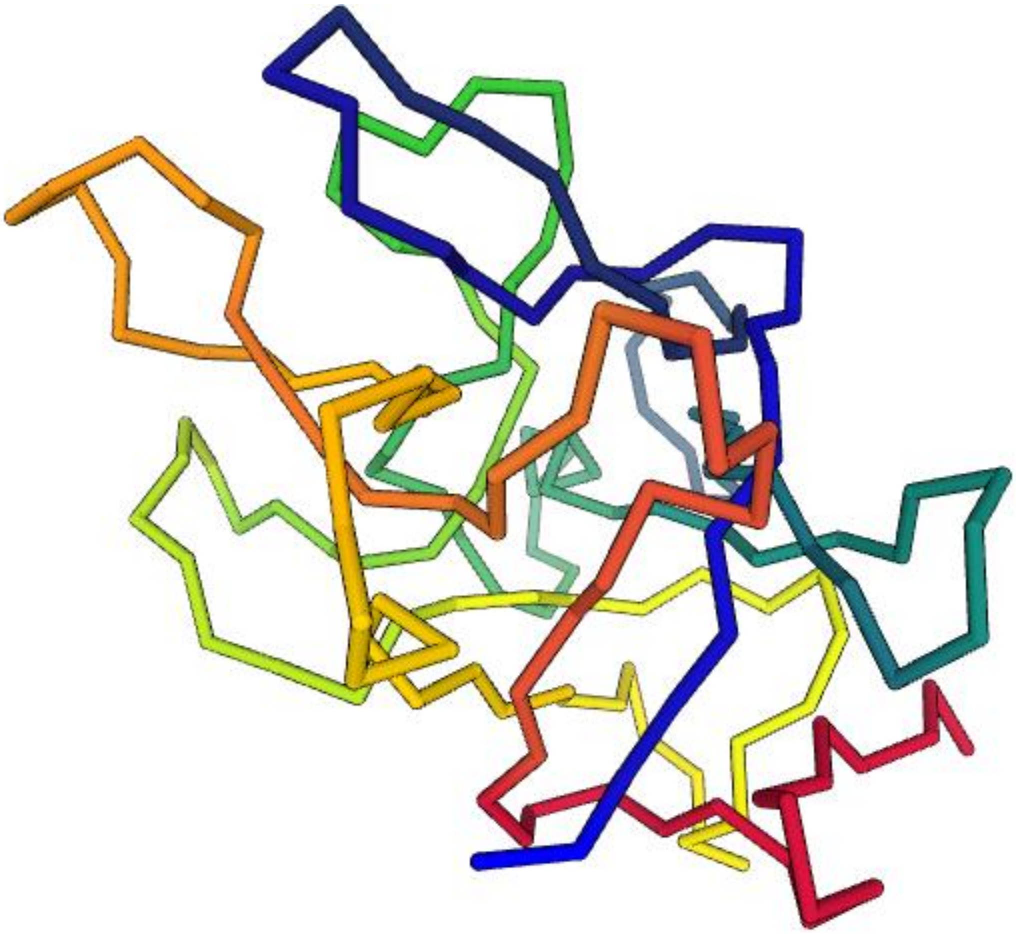
Structural comparison between *S. dulcificum* and *S. longecuneatum*

```
DSSP  LLLLLLLLLLLLLLLLLLEEEELLLLLLLLEEEEELLLLLL-LLLLLLEEEEELLLLLLL
Query APNPVLDIDGEKLRTGTNYYIVPVLRDHGGGLTVSATTPNG-TFVCPPRVVQTRKEVDHD   59
ident || |||||||  ||||| ||| |||| ||||| || |     || |||||||||| ||
Sbjct APKPVLDIDGNELRTGTDYYILPVLRGHGGGLSVSSTKNPNgTFDCPPRVVQTRKGVDYE   60
DSSP  LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLEEEELLLLLLlHHHLLLEEEELLLLLLL

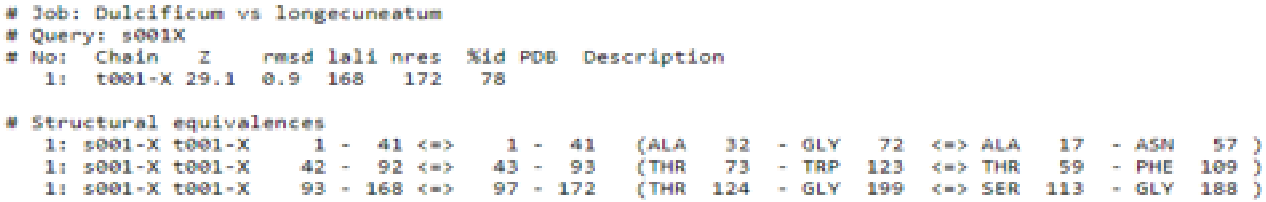

DSSP  LLEEEELLLLLLLLLLLLLLLLEEEELLLLLLLL---LLLLLLEEELLLLLLLLLEEELLL
Query RPLAFFPENPKEDVVRVSTDLNINFSAFMPCRW---TSSTVWRLDKYDESTGQYFVTIGG  116
ident |||||| ||||||||| |||||| || |  ||      |||| |||||||||||||  ||
Sbjct RPLAFFAENPKEDVVRLSTDLNIIFSLFSVCRFrgpSGSTVWSLDKYDESTGQYFVSLGG  120
DSSP  LLEEEEELLLLLLLLLLLLLLEEEEELLLLLLLLlllLLLLLLEEELLLLLLLLLEEELLL


DSSP  LLLLLLHHHHHHLLEEEELLLLLLEEEEELLLLLLLLLLLLLLEEEEEEEELLLLleeeeeel
Query VKGNPGPETISSWFKIEEFCGSGFYKLVFCPTVCGSCKVKCGDVGIYIDQKGrrrlalsd  176
ident | ||||||||||||||| || |  || ||||||| |||| | ||||||||||
Sbjct VRGNPGPETISSWFKIEKFCSSRLYKFVFCPTVCHSCKVICRDVGIYIDQKG--------  172
DSSP  LLLLLLHHHHHHLLEEEELLLLLLEEEEELLLLLLLLLLLLLLEELLLLLLLLL--------


DSSP  llllleeeel
Query kpfafefnkt  186
ident
Sbjct ----------  172
DSSP  ----------
```



Combined structure of *S. dulcificum* and *S. longecuneatum*.



```
# Job: Dulcificum vs longecuneatum
# Query: s001X
# No:  Chain   Z    rmsd lali nres  %id PDB  Description
   1:  t001-X 29.1  0.9  168   172   78

# Structural equivalences
   1: s001-X t001-X     1 -  41 <=>    1 -  41   (ALA   32  - GLY   72  <=> ALA   17  - ASN   57 )
   1: s001-X t001-X    42 -  92 <=>   43 -  93   (THR   73  - TRP  123  <=> THR   59  - PHE  109 )
   1: s001-X t001-X    93 - 168 <=>   97 - 172   (THR  124  - GLY  199  <=> SER  113  - GLY  188 )
```

Discussion

This study focuses on the presence and evolution of miraculin within the *Synsepalum-Englerophytum* clade in the family Sapotaceae. Only three species of *Synsepalum* and none of the species of *Englerophytum* used for this study produced PCR amplification products for the gene for miraculin. This shows that the presence of miraculin in some species of *Synsepalum* and none in *Englerophytum* can contribute to studying the phylogenetic relationship of the two genera. Multiple sequence alignment of amino acid sequences from species containing miraculin and related species from GenBank shows great similarity between native miraculin and other species containing miraculin-like protein. Among the 18 families combined with Sapotaceae, phylogenetic analysis shows that *Vaccinium darrowii* from the family Ericaceae is closely related to the Sapotaceae family. This result contradicts the findings of Yang *et al*., 2022 where they reported that Sapotaceae is closer to Theaceae and Ebenaceae. It is, however, important to note that none of the species of Ericaceae was implicated in their study as is the case in this study. Species from *Synsepalum* clustered separately from other species from GenBank implying they have diverged long back from common ancestor. The other species having miraculin-like protein divided into two groups similar to the result of (Selvakumar *et al*., 2011) where they reported miraculin-like sequences forming distinct groups from typical Kunitz family inhibitors.

As stated earlier, the taste-modifying property in miraculin is due to the presence of HIS 30 and HIS 60 which is only found in *S. dulcificum* (Selvakumar *et al*., 2011). The nucleotide sequences obtained for the two species (*S. longecuneatum* and *S. bequaertii*) are nearly identical to sequences from *S. dulcificum.* However, His 60, one of the residues responsible for the taste altering ability of miracle berry, is absent in the amino acid sequences of *S. longecuneatum* and *S. bequaertii*.

The principal components of the secondary structure are the alpha helix, beta sheet, and random coil. Secondary structure analysis of the species that produced PCR amplification products for the gene for miraculin shows close similarities between them and miraculin found in miracle berry. When combined with species having miraculin-like proteins, it is seen that miraculin-like proteins are very similar to native miraculin.

Conclusion

Here we report that miraculin is not only found in miracle berry but also *S. bequartii* and *S. longecuneatum*. This implies that taste-modifying properties may not only be present in miracle berry but may possibly be found in *S. bequartii* and *S. longecuneatum*. It is however, important to state that actual fresh materials of the two species will be needed to acertain the taste altering ability in the new species. The importance of this discovery cannot be over-emphasized considering the wide use of miraculin. The applications of miraculin are rapidly expanding from its use in dramatically reducing sugar contents in popular soft drinks, to allowing cancer patients undergoing chemotherapy to overcome food aversions and so improve both physical and emotional health. Humans are biologically predisposed to crave sugar, so finding a way to satisfy that craving that is not harmful to our well-being will be of immense benefit to human health considering the high number of people living with diabetes and other sugar-related ailments. There is strong evidence indicating that sugar-sweetened soft drinks contribute to the development of diabetes, which is increasing at an alarming rate in many parts of the world especially in wealthy nations like the United States. According to the Centers for Disease Control and Prevention's National Diabetes Statistics Report for 2022 cases of diabetes have risen to an estimated 37.3 million, or 11.3% of the US population. Aside from the many health consequences of diabetes, the estimated total economic cost of diagnosed diabetes cases in the U.S. was $327 billion in 2017.

The discovery of the functional gene of miraculin in other species provides additional information for people interested in the commercial production of miraculin. The result obtained from transgenic tomatoes is a pointer to the possibility of obtaining commercial quantities of miraculin within a short period and the need for further research in fast-growing plants that may give similar results to tomatoes or even a superior outcome.

References

Achigan-Dako, E. (2015). Current knowledge and breeding perspectives for the miracle plant Synsepalum dulcificum (Schum. Et Thonn.) Daniell. Genetic Resources and Crop Evolution, 62. https://doi.org/10.1007/s10722-015-0225-7

Akinmoladun, A. C., Adetuyi, A. R., Komolafe, K., & Oguntibeju, O. O. (2020). Nutritional benefits, phytochemical constituents, ethnomedicinal uses and biological properties of Miracle fruit plant (Synsepalum dulcificum Shumach. & Thonn. Daniell). Heliyon, 6(12), e05837. https://doi.org/10.1016/j.heliyon.2020.e05837

Choi, S. E., & Garza, J. (2021). Consumer Likings of Different Miracle Fruit Products on Different Sour Foods. Foods, 10(2), 406. https://doi.org/10.3390/foods10020406

Del Campo R, Zhang Y, Wakeford C. (2017). Effect of Miracle Fruit (Synsepalum dulcificum) Seed Oil (MFSO®) on the Measurable Improvement of Hair Breakage in Women with Damaged Hair: A Randomized, Double-blind, Placebo-controlled, Eight-month Trial. J Clin Aesthet Dermatol.10(11):39-48.

Doddawad, V. G., Shivananda, S., Vidya, C. S., Madhu, B., & Gurupadayya, B. M. (2022). Insight on Synsepalum dulcificum: A Bioactive Compound on Taste Modification and Its Biological Properties. International Journal of Nutrition, Pharmacology, Neurological Diseases, 12(3), 93. https://doi.org/10.4103/ijnpnd.ijnpnd_19_22.

Douglas Kinghorn, A., Chin, Y.-W., Pan, L., & Jia, Z. (2010). 3.10—Natural Products as Sweeteners and Sweetness Modifiers. In H.-W. (Ben) Liu & L. Mander (Eds.), Comprehensive Natural Products II (pp. 269–315). Elsevier. https://doi.org/10.1016/B978-008045382-8.00077-0

Du, L., Shen, Y., Zhang, X., Prinyawiwatkul, W., & Xu, Z. (2014). Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts. Food Chemistry, 153, 279–284. https://doi.org/10.1016/j.foodchem.2013.12.072

DV. (2022, April 25). Flavor-Tripping with the Miracle Fruit. The Somm Journal. https://www.sommjournal.com/flavor-tripping-with-the-miracle-fruit/

Gómez de Cedrón, M.; Wagner, S.; Reguero, M.; Menéndez. (2020) Rey, A.; Ramírez de Molina, A. Miracle Berry as a Potential Supplement in the Control of Metabolic Risk Factors in Cancer. Antioxidants 9, 1282 https://doi.org/10.3390/antiox9121282.

Hiwasa-Tanase K, Hirai T, Kato K, Duhita N, Ezura H. (2012) From miracle fruit to transgenic tomato: mass production of the taste-modifying protein miraculin in transgenic plants. Plant Cell Rep. Vol. 31(3):513-25.

Izawa, K., Amino, Y., Kohmura, M., Ueda, Y., & Kuroda, M. (2010). 4.16—Human–Environment Interactions – Taste. In H.-W. (Ben) Liu & L. Mander (Eds.), Comprehensive Natural Products II (pp. 631–671). Elsevier. https://doi.org/10.1016/B978-008045382-8.00108-8.

Koizumi, A., Tsuchiya, A., Nakajima, K., Ito, K., Terada, T., Shimizu-Ibuka, A., Briand, L., Asakura, T., Misaka, T., & Abe, K. (2011). Human sweet taste receptor mediates the acid-induced sweetness of miraculin. Proceedings of the National Academy of Sciences, 108(40), 16819–16824. https://doi.org/10.1073/pnas.1016644108.

Kurihara, K., & Beidler, L. M. (1969). Mechanism of the Action of Taste-modifying Protein. Nature, 222(5199), Article 5199. https://doi.org/10.1038/2221176a0.

Lipatova, O., & Campolattaro, M. M. (2016). The Miracle Fruit: An Undergraduate Laboratory Exercise in Taste Sensation and Perception. Journal of Undergraduate Neuroscience Education, 15(1), A56–A60.

Miracle Fruit | Description, Effects, & Uses | Britannica. (2024, January 30). https://www.britannica.com/plant/miracle-fruit.

Misaka, T. (2013). Molecular mechanisms of the action of miraculin, a taste-modifying protein. Seminars in Cell & Developmental Biology, 24(3), 222–225. https://doi.org/10.1016/j.semcdb.2013.02.008.

Niu YF, Ni SB, Liu J. (2020). The complete chloroplast genome of Synsepalum dulcificum D.: a magical plant that modifies sour flavors to sweet. Mitochondrial DNA B Resour. 5(3):3052-3053.

Paladino, A., Costantini, S., Colonna, G., & Facchiano, A. (2008). Molecular modeling of miraculin: Structural analyses and functional hypotheses. Biochemical and Biophysical Research Communications, 367, 26–32. https://doi.org/10.1016/j.bbrc.2007.12.102.

Selvakumar, P., Gahloth, D., Tomar, P. P. S., Sharma, N., & Sharma, A. K. (2011). Molecular Evolution of Miraculin-Like Proteins in Soybean Kunitz Super-Family. Journal of Molecular Evolution, 73(5), 369–379. https://doi.org/10.1007/s00239-012-9484-5.

Tchokponhoué, D. A., N'Danikou, S., Fassinou Hotegni, N. V., Nyadanu, D., Kahane, R., Odindo, A. O., Achigan-Dako, E. G., & Sibiya, J. (2021). Use Patterns, Knowledge Diversity, and Drivers for the Cultivation of the Miracle Plant [Synsepalum dulcificum (Schumach & Thonn.) Daniell] in Benin and Ghana. Plants, 10(11), 2253. https://doi.org/10.3390/plants10112253.

Yang, Z., Liu, Z., Xu, H., Chen, Y., Du, P., Li, P., Lai, W., Hu, H., Luo, J., & Ding, Y. (2022). The Chromosome-Level Genome of Miracle Fruit (Synsepalum dulcificum) Provides New Insights Into the Evolution and Function of Miraculin. Frontiers in Plant Science, 12. https://www.frontiersin.org/articles/10.3389/fpls.2021.804662.

Chapter 3. Ethnomedical survey of plants used in the treatment of ailments by African immigrants in the Sacramento region of California.

Abstract

Background: Immigrants play a significant role in the transfer of medicinal plant knowledge, traditions, and cultures from their home country to their new area of primary residence. Ethnomedical studies on the use of plants are of great relevance in understanding and documenting their health-seeking behavior. It is erroneously assumed that many immigrants have little knowledge about herbal medicine and therefore stop using it when they migrate to developed nations. This study was therefore conducted to investigate and document ethnobotanical knowledge of African immigrants in the Sacramento region of California.

Methodology: A semi-structured questionnaire was administered to willing African immigrants, after prior informed consent, in different locations in Sacramento. These include churches, African food stores, and Christian students' fellowship. A total of 105 participants responded to the questionnaire. Participants were from 15 different African countries, between the ages of 20 and 60 comprising 65% female and 34% male.

Results and conclusion: Among the respondents, the most common occupation (50) was health professionals. In addition, 90% of respondents confirmed being aware of medicinal plants, having ginger as the most cited medicinal plant. The results from this survey show that many immigrants, though living in the global north where Western medicine is accessible, have rich knowledge of medicinal plants and still prioritize herbal medicines as an effective way to treat a wide variety of ailments.

Introduction

The world is in dire need of solutions to the many health challenges confronting humanity and there is a need to explore all possible avenues. Plants have been used for centuries for the management of health but there has been a decline in the use of plants due to the increasing spread and adoption of Western medicine (Ogunkunle and Ladejobi, 2006) and the absence of scientific information on what constitutes the required dosage of herbs in the treatment of ailments (Ekor, 2014). Nonetheless, the need to revisit the potency of plants in treating ailments is now gaining ground the world over. It has been reported that about 85% of all synthetic drugs are derived from plants (Fitzgerald et al., 2020). The United States Department of Agriculture (USDA) reported that about 40% of drugs in the pharmacies of developed nations are derived from plants, and this percentage includes the top 20 best-selling prescription drugs in the United States.

Immigrants are a good source of documenting ancestral knowledge on the use of medicinal plants as some of them have rich knowledge of how plants are used medicinally in their home countries (de Medeiros et al., 2012a; Mahomoodally et al., 2019; Romanus et al., 2018). The Sacramento region of California is home to many African immigrants but there is no documented information about medicinal plants used by these immigrants. This research, therefore, seeks to document plants used as medicine by African immigrants in the Sacramento region of California. This will serve as baseline information for research on various aspects of medicinal plants, including drug discovery, genetic diversity, and nutrition, and will help to emphasize the importance of biodiversity conservation, the sustainable use of plant resources, and the role of immigrant communities in understanding the value and importance of herbal medicine.

History and status of African immigrants in the Sacramento region

It has been reported that over 200 million people live in different locations outside their country of origin (Medeiros *et al.*, 2012). The United States is a country with a rich presence of immigrants (McHugh, 2018), with more immigrants than any other country in the world. In California, it is reported that the earliest black residents were brought in by the Spanish. There were 952 African Americans residing in California as of 1850. This population had doubled by 1952 when the total number of African Americans in California was estimated to be 2,000 (Momodu, 2022). The passage of the Immigration and Nationality Act of 1965, which abolished the national origin quota system and opened immigration from non-European countries, marked a turning point for African immigration to California. The population of African immigrants in California has grown rapidly since 1965. According to the 2019 United States Census Bureau estimates, 5.8% or 2,282,144 of the people in California are African Americans or black. This is also evident in the diverse communities with their own cultural and religious traditions. Some of the African immigrants in California have established churches, social networks, and ethnic associations (Oyebade, 2017).

History of traditional uses of Plants for Medicine in Africa

About 80% of primary health care in developing nations is based on herbal treatment (Caballero-Serrano *et al.*, 2019; D. R. K. Singh, 2019; Yeshi *et al.*, 2022). This high percentage is due to cultural traditions and insufficient access to Western medicine in some cases. There are however people in some parts of the world such as Australia, the UK, other parts of Europe, and North America who still rely heavily on medicinal plants for the treatment of ailments despite the availability of Western medicine (Ekor, 2014; Yeshi et al., 2022).

Ethnomedical system and source of medicinal plants used by African immigrants

Africa is known to have a very rich biodiversity due to the varied climate on the continent (Sintayehu, 2018). In discussing immigrants in this context, there are two categories of immigrants; those who were brought to the United States as slaves and those who migrated willfully or through the US lottery system. These two groups tend to have slightly different ethnomedical systems and sources of medicinal plants. African immigrants who were brought to the United States as slaves relied heavily on herbalism for their healing as there were few other options available to them (Morgan, 2020). They used plants extensively to treat ailments such as colds, fevers, typhoid, and pneumonia while working in plantations in South Carolina (Morgan, 2020). They used the plants to prepare remedies the same way they had in their ancestral homes. In situations where the combination of plants they would have used in their home country became difficult to obtain, African immigrants in Brazil replaced the missing combination with plants from their new environment that may be similar to plants from their place of origin (Pagnocca et al., 2020). This method of replacement is also reported for the Yoruba tribe from western Nigeria who migrated to Brazil and adopted plants with morphological similarity to plants from their ancestral home (Volpato et al., 2009). It can be inferred that this replacement could be possible when both species are closely related as species with phylogenetic proximity may contain similar compounds (Wink et al., 2010). Some adopted the ethno-medical system in their new environment. Some of the immigrants who were brought in as slaves arrived with seeds and stems of crops, they were using for herbal preparation in their home country (Eltis et al., 2007). These include among others, herbs, spices, leafy greens, legumes, fruits, and vegetables.

In contrast, voluntary immigrants generally rely on the ethnomedical systems with which they were familiar before emigration. Unlike the slaves who had limited options, this category of immigrants could prepare herbs based on the knowledge they had or call family or friends in their home country to guide them on how to prepare herbs. There are also many videos on how to prepare different types of herbs on the internet, an opportunity that was not available for immigrants who arrived as slaves. Presently, there are several options for immigrants to now get medicinal plants. These include among others, African shops where immigrants purchase dried and fresh leaves from their home countries and online sources. In addition, some immigrants maintain social ties with people in their home countries. This helps them to get medicinal plants that are not available in African stores in California from their home countries, either when they visit their home country or when they see people traveling to or from their home country. It is worth noting that these plants are usually inspected at the point of entry to minimize the illegal importation of plant products. Some plants are acquired with relative ease when there is a continuous flow of people between the immigrant’s ancestral country and the United States. If the plants are not grown in the United States and cannot be imported due to customs regulations, immigrants get advice from family and friends in their home countries on possible substitutes they can use. Some immigrants experiment with the growing of frequently used plants in their home gardens. From the gardens, they collect plants and use them in combination with other plants they are familiar with in the local flora to prepare herbs to treat their ailments (de Medeiros et al., 2012b). There are cases where some plants grow successfully while others do not succeed due to different climatic conditions between their ancestral homes and their place of primary residence. Some medicinal plants like *Vernonia amygdalina* (bitter leaf) are grown in the United States. It is reported that juice from the leaf is used to treat stomach and abdominal pains, cure insomnia, lower high blood pressure, and enhance fertility.

The relevance of documenting immigrants' ethnomedical knowledge.

Ethnomedical knowledge is fading away at a fast pace due to the use of Western medicine so documenting available information will be of great help to the present and future generations. Immigrants serve as a good source of medicinal knowledge. This is because many of them rely on medicinal plants because of the high cost of western medicine and for some of them, their immigration status may serve as an impediment to their ability to access medical health insurance. Despite the rich knowledge of medicinal plants by immigrants, there is no published study on how African immigrants in Sacramento use plants for their ailments. Therefore, this study seeks to document plants used in the treatment of ailments by African immigrants in the Sacramento region of California. This documented knowledge about medicinal plants will serve as baseline information for plant genetic resources, nutritionists, and people in the pharmaceutical industry to explore other possible applications.

Objectives of the Study

The specific objectives of this study are to:

1. Assess the demographic characteristics and original ancestry of respondents in Sacramento.
2. Document ethnobotanical knowledge of African immigrants in Sacramento.
3. Investigate the variation in the use of plants among the different African countries represented in California.

Materials and Methods

Study area

This survey and distribution of questionnaire was carried out in Sacramento, the part of California in the central west of the United States. As the capital city of California, Sacramento is regarded as the principal political and economic center of the state. It is on **Lat:** 38° 34' 32.7504" N and **Long:** 121° 28' 43.8636" W. Respondents, however, included people who live elsewhere but have some regular connections in Sacramento.

The map below shows the primary residence of respondents.

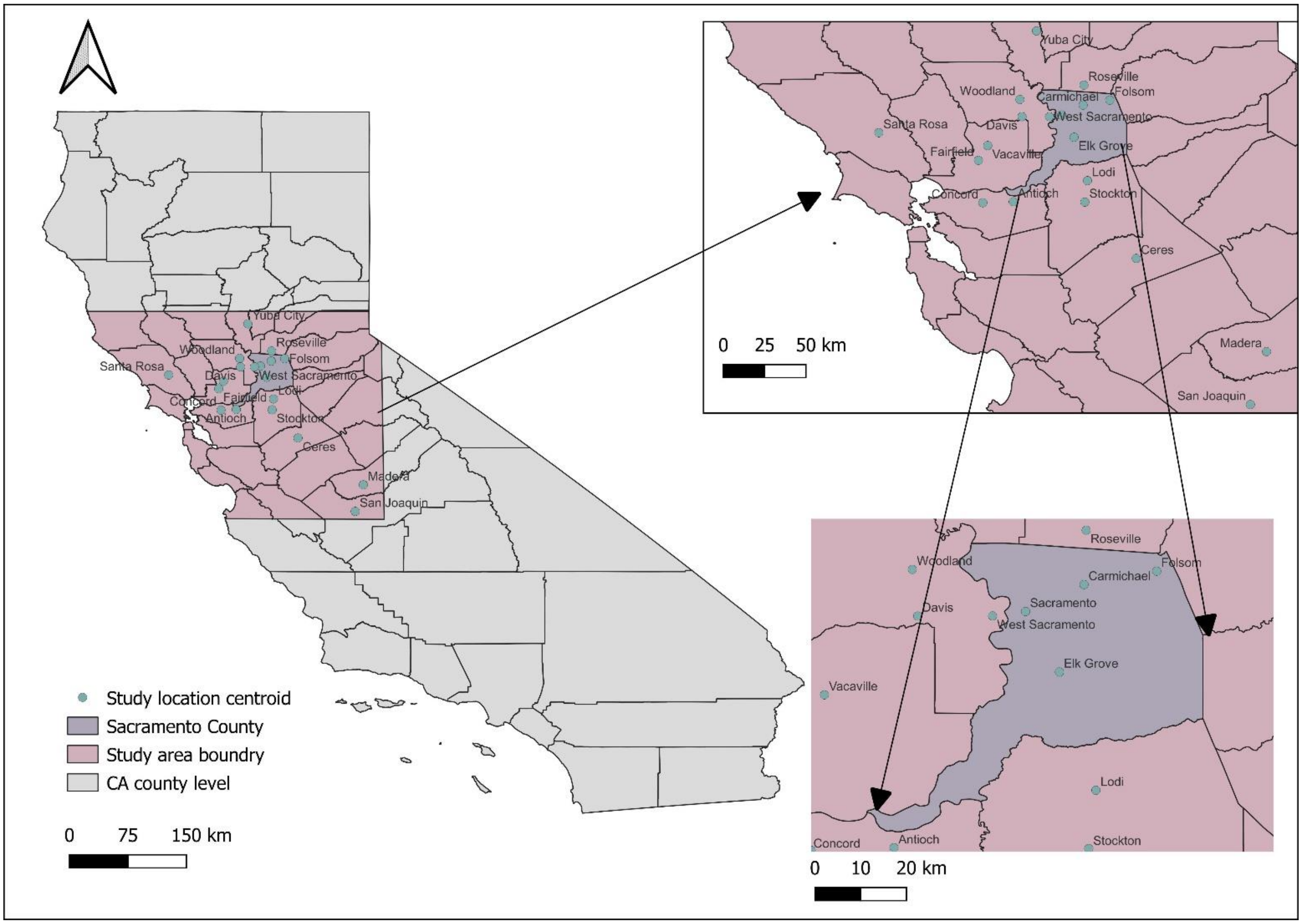


Figure 1:0

Experimental Design and Data Collection Technique

Questionnaire were administered to African immigrants who reside in or carry out transactions in Sacramento. Places with high presence of African immigrants were the focus of the study. These include African churches, African markets, and Christian students’ fellowship. These places were visited after prior informed consent. Before the administration of the questionnaire to respondents, training at the Institutional Review Board at the University of California, Davis was completed, and the permit was granted. The traditional botanical knowledge of respondents was recorded using in-person interviews and administration of semi-structured questionnaires to willing African immigrants, in which they were requested to freely list plants they use for medicine. The questionnaire was structured as follows: Section A: Personal information of respondents (respondent’s name, gender, religion, age bracket, country of ancestry, region of primary residence); Section B: Ethnobotanical information of plants: (marking names of plants used, part of plants mostly used, listing of local names of plants used for medicine that do not appear in the questionnaire, local preparation, and medicinal administration). A total of 105 copies of the questionnaire were completed and returned.

Table 1. Residence of people interviewed.

Location	**Frequency**	**Percentage**
Others	12	11.5
Sacramento	39	37.1
Davis	18	17.1
Vacaville	5	4.8
Fairfield	5	4.8
Elk Groove	6	5.7
Folsom	3	2.9
West Sac	3	2.9
Ceres	1	1.0
San Joaquin	1	1.0
Concord	1	1.0
Yuba	1	1.0
Car michael	1	1.0
Lodi	1	1.0
Roseville	1	1.0
Stockton	1	1.0
Antioch	1	1.0
Santa Rosa	2	1.9
Madera	2	1.9
Woodland	1	1.0
Total	105	100.0

Data Analysis

Responses from the returned questionnaires were entered into an Excel spreadsheet to have the data in an analyzable format, after which they were analyzed (bar chart for frequency distribution) using IBM Statistical Package for Social Sciences (SPSS) version 26 Armonk, NY.

Results

Demographic information about respondents is presented in Table 2. In this study, a total of 105 African immigrants were surveyed, comprising 68 women, 36 men, and 1 not declared. The proportion of women (65%) involved in this study was higher than that of men (34%) (Table 1). People within the age range of 41-50 were the highest respondents (50), followed by ages 31-40 (21), 51-60(17) and 21-30(12). Out of the 105 people interviewed, health professionals were the highest number of respondents (46) while lawyers, accountants, engineers, and researchers were the least number of respondents (one each). The highest number of respondents (50) was recorded for people with bachelor's degrees while the least (5) was recorded for people with a high school diploma.

Table 2: Demographic characteristics of respondents

Variable	**Frequency**	**Percentage**	**Standard error**
Gender			
Undeclared	1	1.0	
Male	36	34.3	0.49
Female	68	64.8	
Total	105	100	
Age Range			
Others	4	3.8	
< or equal to 20 years	1	1.0	
21-30	12	11.4	
31-40	21	20.0	0.11
41-50	50	47.6	
51-60	17	16.2	
Total	105	100.0	
Occupation			
Others	17	16.2	
Professor	5	4.8	
Students	11	10.5	0.7
Researcher	1	1.0	
Scientist	4	3.8	
Health professional	46	43.8	
Clergy	2	1.9	
Administration	8	7.6	
IT	4	3.8	
Engineer	4	3.8	
Accountant	1	1.0	
Lawyer	1	1.0	
Pharmacist	1	1.0	
Total	105	100.0	

Level of Education			
Others	6	5.7	
PhD	9	8.6	
MSc	29	27.6	0.12
Bachelor's	50	47.6	
High School	6	5.7	
Diploma	5	4.8	
Total	105	100.0	

Residence of participants: The 105 people interviewed were scattered across 19 locations in the Sacramento region. Most of the locations had just one participant while the highest number (39) in a single location was recorded for Sacramento City.

Original ancestry of immigrants: Immigrants from 15 African countries were interviewed. The highest number (59) of people interviewed are Nigerians. This was followed by Kenya. Five other African countries had one person per country.

Table 3: Original ancestry of immigrants interviewed.

Original Ancestry	Frequency	Percentage	Standard error
Others	7	5.8	
Nigeria	59	62.5	0.42
Gambia	1	63.5	
Ivory Coast	2	65.4	
Ghana	3	68.3	
Eritrea	2	70.2	
Part of East Africa	1	71.2	
Kenya	6	76.9	

Cameroon	3	79.8	
South Africa	7	86.5	
Sierra Leone	2	88.5	
Uganda	4	92.3	
Malawi	1	93.3	
D R Congo	1	94.2	
Burkina Faso	5	99.0	
Rwanda	1	100.0	
Total	**105**		

Medicinal plants awareness: Out of the 105 people interviewed, **89**% of the people confirmed their awareness of medicinal plants while 3.8% said they are not aware. Although the percentage of people who are not aware may not be high 3.8% is notable.

Table 4: Medicinal plants awareness among immigrants interviewed.

Awareness	Frequency	Percent	Standard error
Undecided	2	1.9	
Yes	94	89.5	0.05
No	4	3.8	
Not Sure	5	4.8	
Total	105	100.0	

Table 5: Source of medicinal plant knowledge:

Grandparents/elders recorded the highest (39) while the least (1) were recorded for friends and the combination option of friends and grandparents.

Source	Frequency	Percent	Standard error
NA	29	26.7	0.24
Grandparents/Elders	39	37.1	
Parents	15	14.3	
Friends	1	1.0	
Online/Literature	5	4.8	
Grandparents/Elders/Parents	5	4.8	
Grandparents/Elders/Parents/Friends	3	2.9	
Grandparents/Parents	2	1.9	
Grandparents/Friends	1	1.0	
Grandparents/Internet/Books	3	2.9	
Others	2	1.9	
Total	105	100.0	

Table 6: List of medicinal plants and families

S/No.	Scientific name	Common name	Family	Frequency	Percentage (%)	Standard error
1.	*Allium sativum*	Garlic	Amaryllidaceae	89	8.9	0.04
2.	*Aloe vera*	Aloe vera	Asphodelaceae	81	8.1	0.04
3.	*Anacardium occidentale*	Cashew	Anacardiaceae	23	2.3	0.04
4.	*Azadirachta indica*	Neem/Dogoyaro	Malvaceae	41	4.1	0.02
5.	*Carica papaya*	Pawpaw	Caricaceae	54	5.4	0.05
6.	*Ceiba pentandra*	Kapok tree	Malvaceae	4	0.4	0.02
7.	*Citrus jambhiri*	Rough lemon	Rutaceae	32	3.2	0.05
8.	*Cymbopogon citratus*	Lemon grass	Poaceae	63	6.3	0.05
9.	*Elaeis guineensis*	Palm tree	Arecaceae	26	2.6	0.04
10.	*Garcinia kola*	Bitter kola	Clusiaceae	56	5.6	0.05

11.	*Gmelina arborea*	Gmelina	Lamiaceae	4	0.4	0.02
12.	*Mangifera indica*	Mango	Anacardiaceae	47	4.7	0.05
13.	*Moringa oleifera*	Moringa	Moringaceae	55	5.5	0.05
14.	*Ocimum basilicun*	Basil	Lamiaceae	29	2.9	0.04
15.	*Ocimum gratissimum*	Scent leaf	Lamiaceae	44	4.4	0.03
16.	*Parkia biglobosa*	Locus bean	Fabaceae	20	2.0	0.04
17.	*Prosopis africana*	Iron tree	Fabaceae	32	3.2	0.05
18.	*Psidium guajava*	Guava	Myrtaceae	41	4.1	0.05
19.	*Synsepalum dulcificum*	Miracle berry	Sapotaceae	44	4.4	0.02
20.	*Tectona grandis*	Teak tree	Lamiaceae	4	0.4	0.02
21.	*Vernonia amygdalina*	Bitter leaf	Asteraceae	68	6.8	0.05

22.	*Vitellaria paradoxa*	Shea butter	Sapotaceae	52	5.2	0.05
23.	*Zingiber officinale*	Ginger	Zingiberaceae	92	9.2	0.03
Total	23		16	1,001	100	

List of plants used: The 105 African immigrants reported being aware of 23 medicinal plants. The highest number (92) was recorded for Ginger, followed by Garlic 89 and the least for Teak and Gmelina having 4 each.

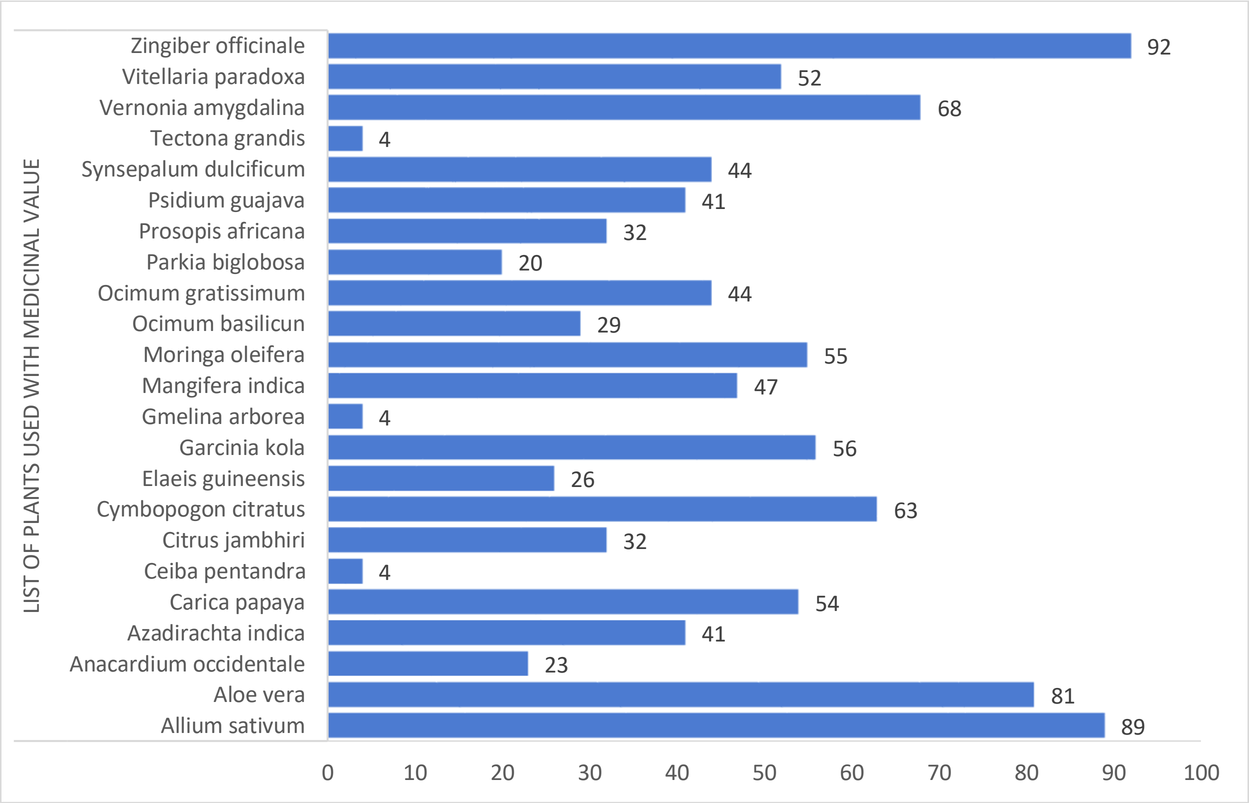


Figure 2: Frequency distribution of medicinal plants used among African immigrants

The most used part of the plant was recorded for a combination of leaves, roots, and seeds (46.7%), followed by leaves only (28.6%). Many parts of the plants fall into the category of least used.

Table 7: List of parts of plants mostly used.

Parts of plant	Frequency (F)	Percentage (%)
Others	49	46.7
Leaves	30	28.6
Roots	2	1.9
Seeds	2	1.9
Stems	2	1.9
Leaves & Roots	5	4.8
Leaves and Seeds	3	2.9
Leaves and stems	3	2.9
Roots, stems	1	1.0
Leaves, roots, seeds and	4	3.8
Stem	2	1.9
Leaves, roots, and stem	1	1.0
Leaves, seeds, and stem	1	1.0
Leaves, roots, and seeds	49	46.7

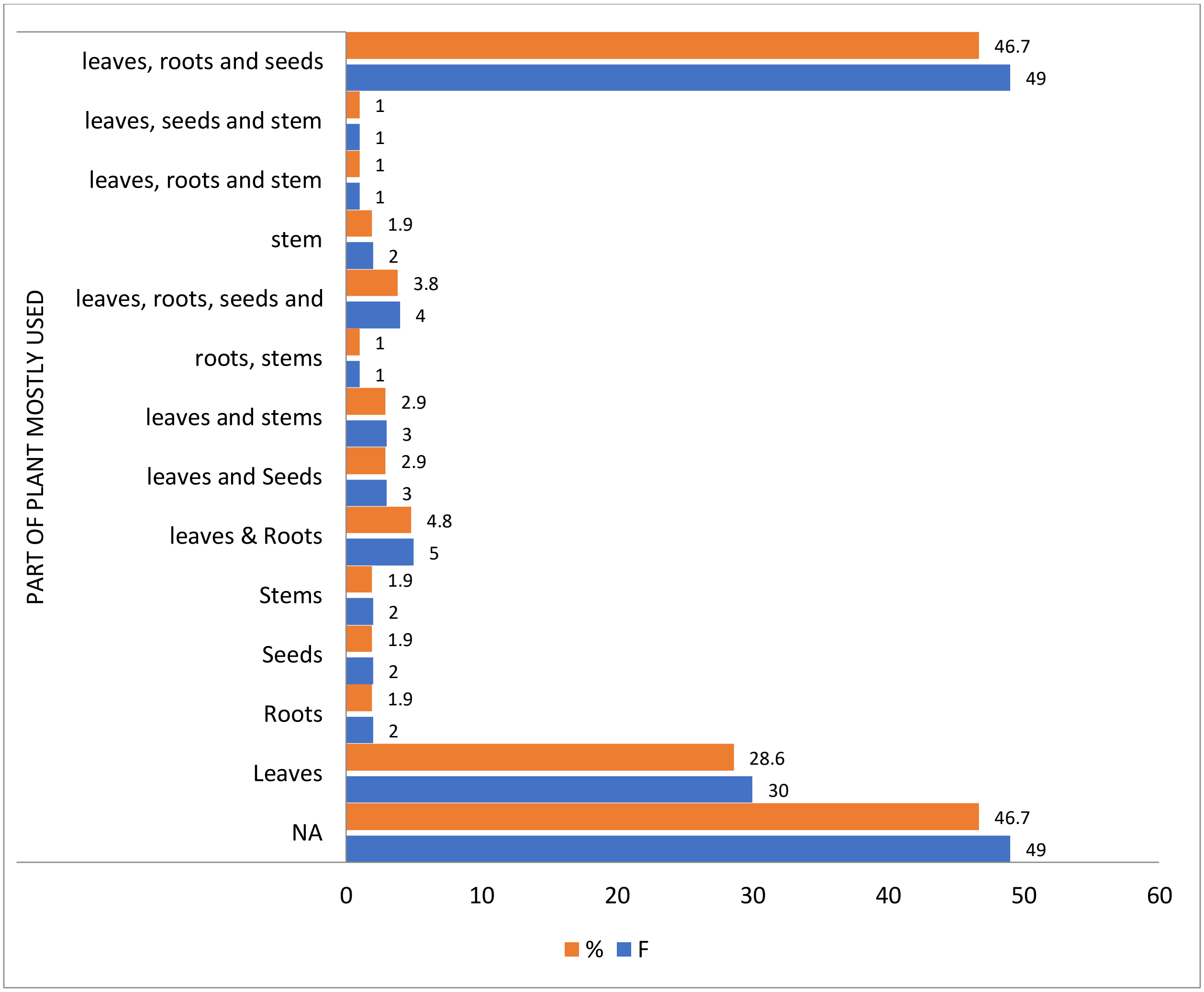


Figure 3: Frequency of most used part of the plant.

Discussion

Medicinal Plants and their use by African immigrants in the Sacramento region of California

On the list of medicinal plants used as recorded in Table 2, ginger (*Z. officinale*) recorded the highest number of usages. The highest usage attributed to the aromatic herb, ginger, is not strange as ginger has been used as traditional medicine and spice for a long period. Ginger has both medicinal and nutritional value, containing over 400 compounds (Prasad & Tyagi, 2015) and has at least 60 active constituents (Shahrajabian et al., 2019). In Chinese traditional medicine, it is reported that ginger has been used as a spice and medicine for well over 200 years (Kiyama, 2020; Shahrajabian et al., 2019). The extended period stated here suggests the aromatic herb is of immense benefit to man. Ginger has been used in the treatment of different types of ailments including arthritis, colds, headaches, viral infections, and digestive problems (Kiyama, 2020; Shahrajabian et al., 2019). The powder obtained from the dried rhizome of ginger is used in lowering both high blood pressure (Hasani et al., 2019) and high cholesterol (Alizadeh-Navaei et al., 2008; Shahrajabian et al., 2019). In a study involving 70 diabetic patients, it was confirmed that patients who were administered ginger for three months had lower triglyceride cholesterol scores when compared with patients who used a placebo. It was also revealed in a study of over 4,000 subjects that people who use ginger had a lower chance of having chronic heart disease or hypertension (Alizadeh-Navaei et al., 2008). Not only is ginger used as medicine and spices but also as flavoring agents by many people in different parts of the world. Pharmacologically, it is reported that ginger is antidiabetic, analgesic, antiarthritic, anticancer, antioxidant, and antiulcer. There are many publications on the invaluable uses of ginger (Crichton et al., 2022; Daimei & Kumar, 2014; Nguyen et al., 2020; P. Singh et al., 2022). Ginger is grown both in tropical and temperate regions. North and West Africa are among the areas with the top ginger farmers in the world, hence African immigrants were very familiar with ginger before migrating to the United States.

Other useful medicinal plants gathered from the survey include garlic, aloe vera, lemon, and bitter kola among others. Just like ginger, garlic has been extensively used in many parts of Africa. Hence its use by African immigrants in Sacramento is not unexpected. It is used in the treatment of high blood cholesterol levels, cholera, and dysentery (Okoro et al., 2023). Although 80% of the global production of garlic comes from China and India (Parreño et al., 2023), many Africans use garlic extensively. This is seen in the survey reported here for African immigrants in Sacramento. The type of diseases reported here is a pointer to why garlic is extensively used by Africans. The ailments are quite common in developing nations and are harmful. In a publication on the phytochemical and ethnobotanical evaluation of garlic in Nigeria, the authors also acknowledged the therapeutic and nutritional uses of garlic (Ameh & Nwammoh, 2010). Their findings agree with the result that is reported in this paper where garlic was rated as one of the most used medicinal plants.

Aloe vera is one of the most popular plants in the genus Aloe. Its popularity can be traced to the numerous extensive uses of the plant. In an ethnobotanical survey of the genus Aloe in Tanzania, the authors reported thirty-seven uses of only 11 species (Amir et al., 2019). Most of the respondents reported using aloe for malaria treatment. This again strongly suggests why most of the respondents in this study reported using *Aloe vera*. Malaria is a very common ailment in Africa and has claimed many lives on the continent. According to WHO, at least 619, 000 malaria deaths out of 247 million cases were reported in 2021(Sohail et al., 2024). This staggering number of malaria cases, the cosmetic uses, and available evidence of *Aloe vera*'s ability to combat malaria and other forms of ailments like blood pressure, blood sugar, and soothing sunburn among others, is most likely responsible for the high number of African immigrants using the plants.

Among the many plants listed in the questionnaire, lemon is one of the topmost plants used by African immigrants in Sacramento. Lemon is considered one of the dietary supplements that has been used for ages (Chaudhari et al., 2016). It is essential not only for the pharmaceutical properties it contains but also for its use in cosmetic and culinary purposes (Klimek-Szczykutowicz et al., 2020). Lemon juice is mixed with coconut oil and used in the treatment of fever, common cold, and high blood pressure (Chaudhari et al., 2016). This essential use could be responsible for why many African immigrants reported using it. In general, the citrus family is known to possess analgesic, anti-inflammatory, antioxidant, anthelmintic, antibacterial, antifungal, and hypolipidemic properties (Chaudhari et al., 2016). Lemon is used in the preparation of many herbal medicines because of its potency. Its use is not only among Africans but in many parts of the world. In an ethnobotanical study in Bosnia and Herzegovia, lemon was reported as one of the main constituents used in the preparation of herbs (Sarić-Kundalić et al., 2011).

Bitter kola, a plant from which the popular Coca-Cola got its brand name, is a common plant in many African countries especially in Central and Western Africa because of its medicinal value (Contributors, n.d.; Moneim & Sulieman, 2019; Yogom et al., 2020). It is used in the treatment of cough, snake bite, bacterial infection, and viral infection (Moneim & Sulieman, 2019). In this survey, it is one of the plants used by many of the respondents. Some people in southeastern Nigeria use it to entertain guests(Uchejeso & Chinaza, 2020). The diverse usage of the plants could be responsible for their use among many of the African immigrants who were interviewed.

The plants mostly used as noted here, are responses of the people from the different countries that were interviewed. This implies the usage of these plants is universal and not restricted to any single country. Although the African immigrants interviewed are not from the same country, there seems to be consistency in plant usage across the continent. This shows that many countries in Africa have adopted methods of herbal preparation from neighboring countries. This should not be so much of a surprise due to the proximity some of these countries have to one another. People in Nigeria travel by car to Ghana and the same thing happens for Ghanaians who reside in Nigeria. In East Africa, there is also this interconnection between the different East African countries.

Parts of plants are mostly used.

For the parts of plants mostly used, the combination of leaves, roots, and seeds was mostly used by people for the preparation of herbs. The use of leaves for most of the methods of the combination used in preparing herbs agrees with that of (Gonfa, Tulu, et al., 2020). They reported in their ethnobotanical study of medicinal plants used by the people of Gera district in Ethiopia that leaves were the most used part of plants by the residents of Gera district. Also, (Gonfa, Robi, et al., 2020) reported that leaves are the most used part of plants in the preparation of herbal medicine. The use of leaves by most respondents could be because of the ease of harvesting.

Characteristics of respondents

Gender

The results of this survey buttress the views of other published ethnobotanical surveys that the use of the medicinal plant is not limited to one gender but practiced by both men and women. This agrees with the findings of (Asiimwe et al., 2021; Howard, 2006; Torres-Avilez et al., 2016) where they reported that men and women are involved in the use of traditional medicine. In this survey most of the respondents were women. These findings are contrary to the report of Tugume et al. (2016) where they had more men as respondents than women in their survey of communities around Mabira forest. Although more women were reported as respondents in this research, this does not negate the African belief that traditional healers are mostly males. The high percentage recorded here could be due to the people who were administered the questionnaire. More women were given the questionnaire than men. This is because most of the places visited for the survey were areas that women tend to frequent than men.

Age of respondents

The most frequent age range recorded for respondents was people between the ages of 41 and 50. This is generally true for many ethnobotanical studies (Sulaiman et al., 2022). People between the ages of forty and above tend to have richer knowledge about herbal plants than the younger folks who are less than thirty years old. Not very many younger generation folks are interested in medicinal plants (Thakur et al., 2014). This is not a comforting one as the rapid disappearance of medicinal plant knowledge among the younger generation either due to education or civilization, suggests very strongly that unrecorded ethnomedical knowledge may be lost forever.

Level of education of respondents

Among the number of people interviewed, some had PhD, Master, Bachelor, Diploma, and High School qualifications. The highest number was recorded for people with bachelor’s degrees. The different classes of degrees of the respondents further help to strengthen that medicinal plant knowledge does not lie solely with the unschooled. Some people irrespective of their level of education, still have high regard for the importance of medicinal plant knowledge.

Residence of respondents

Respondents reside in about nineteen different parts of Sacramento with Sacramento city having the highest number of respondents. The highest number of respondents recorded for Sacramento city does not in any way suggest that African immigrants in another part of Sacramento region do not use plants. The major areas questionnaires were administered to were areas in Sacramento city hence the high number of respondents from the city. If the administration of the questionnaire had tilted to other cities, the result would have been different.

Source of medicinal plant knowledge

On the source of medicinal plant knowledge, grandparents recorded the highest. This is a call for concern as these set of people are aged and may not have a good education. If there were no documented evidence of how these plants are used, then traditional knowledge, especially in developing nations, would be threatened when these people are no more.

Medicinal plant awareness

On the level of awareness, 90% of the total number of people interviewed confirmed that they are very much aware of medicinal plants. This report includes both the well-educated and people with little education. With the level of awareness recorded for people of different levels of education, it is safe to say ethnobotanical knowledge is very important. It is however important to note that being aware of medicinal plants does not suggest that all people are knowledgeable about the therapeutic and nutritional benefits of herbal plants and how it is applied.

Occupation of respondents

The 105 respondents in this survey were people from various fields of endeavor. The highest number of respondents (50) was recorded for health professionals. This points to the potency of medicinal plants. One would think that people working in the healthcare industry would be more glued to Western medicine than people in other fields of endeavor but that is not the case here. The high numbers of healthcare workers who patronize traditional medicine suggest very strongly that traditional medicine is very potent and should be given more attention. This finding is similar to that of (Zahn et al., 2019) in their survey of the use of medicinal plants in the United Kingdom. They reported that nearly half of the medical professionals interviewed accepted the use of medicinal plants. Also, the findings of this research are in many respects similar to those of (Tareau et al., 2022) who reported how the people of French Guiana who are residents in South America depend very strongly on medicinal plants for the treatment of their ailments. Just like the African immigrants in Sacramento, the people of French Guiana do not only rely on plants from their home country but also replace them with plants in South America when they have difficulty getting some types of plants. Also in an article published in the Journal of Ethnopharmacology about a comparative study on the use of medicinal plants between Peruvian and Bolivian migrants that are resident in London, it was reported that both communities still depend strongly on the use of medicinal plants especially for treating ailments that they are familiar with from their ancestral homes (Ceuterick *et al*., 2011). Not only do they rely on medicinal plants, just like African immigrants in the Sacramento region of California, the Peruvians, and the Bolivians rely on primary food species for treating ailments. They also expressed difficulty in getting fresh plants in London. This is not completely strange as some of the species they used back in their home countries are not native to the flora in their new location and getting such plants fresh from their home countries can be very complicated due to customs and immigration issues and the difficulty involved in getting the needed permits. Just as expressed here, Afghan refugees in Pakistan also expressed difficulty in getting medicinal plants from their home country due to the strict border policy (Shah *et al*., 2023). The fear of introducing invasive species has also made transporting plants from one location to another difficult.

Conclusions

The results obtained from this survey conducted in Northern California show that medicinal plants are still actively used by immigrants despite living in the global North where the use of orthodox medicine is pronounced. The result also suggests very strongly that many immigrants depend heavily on medicinal plants irrespective of their level of education and place of primary residence. If immigrants could take the pain of getting medicinal plants from their home country despite all the logistics involved, it, therefore, points to how invaluable medicinal plants are to man. Neglecting them will be to the disadvantage of the present and future generations.

Recommendation

From personal experience, except for one walk to the emergency room, it is possible to be in the queue for about five months to see a consultant. When a sick patient is referred to see a consultant, it is because the situation demands superior attention so waiting further may lead to deterioration of one’s health hence the search for a suitable alternative. As a suitable alternative to orthodox medicine in the treatment of many ailments, different herbal products are now used all over the world and the resultant benefits have helped to establish the efficacy of medicinal plants (Ekor, 2014). Also, in the recent outbreak of covid-19, many reports showed that some herbal medicines are very effective in treating covid-19, some even reported that some herbal medicines have superior safety margins when compared to reference drugs (Ang et al., 2020, 2022; Y.-X. Liu et al., n.d.; L. Luo et al., 2020; X. Luo et al., 2022; Silveira et al., 2020; Wang & Yang, 2021). There are however many views that do not support the use of medicinal plants. Several papers (Cupp, 1999; Ekor, 2014; Onder & Liperoti, 2016; Posadzki et al., 2013) have listed concerns about the use of medicinal plants. These include poor regulation of the herbal market, side effects from prolonged use or overdose, and threats to the environment due to the destruction of habitat leading to climate change because of over-collection to meet the market demand ("Medicinal Plants," 2023). While these concerns may be true, almost all the concerns raised about herbal medicine tend to apply to orthodox medicine. If the use of orthodox medicines is not stopped because of the concerns with it, then discouraging the use of herbal medicine does not look like the most appropriate approach. There is a need to consider the many benefits of herbal medicine that have been evident for centuries. In ancient times, medicinal plants were used even by the elites in the society. The Greek physician, Dioscorides documented over 1,000 recipes for medicines using medicinal plants ("Medicinal Plants," 2023). It is believed that his work formed the basis for pharmacopeias. Due to the efficacy and cost-effectiveness of medicinal plants, the following recommendations are made:

- Health institutions globally should incorporate the safe use of medicinal plants by creating a unit that helps in carrying out the correct identification of medicinal plants and controlled dose-dependent trials before any plant with medicinal properties can be made public. If this is standardized, people are more likely to embrace herbal medicine in the management of their health. Also, biodiversity loss due to excessive harvesting of medicinal plants will be minimized as only licensed people can harvest medicinal plants for herb preparation. In the United States, naturopathic doctors, who are health care professionals who emphasize the use of natural medicine, are considered legitimate in twenty-two (22) states and are licensed (Snider & Zeff, 2019). This is a very encouraging approach. It is hoped that more countries will embrace what is being done in these 22 states in the United States not only for the health benefits but also as an avenue to generate income and create jobs, especially in developing nations where there is a high rate of unemployment. Already China is taking the lead. In 2019, it was reported that traditional Chinese medicine (TCM) export was valued at $4.019 billion (Xiang et al., 2022). This is achievable even in African countries if the right approach is used. Also, care should be taken to conserve biodiversity so that we do not lose these important resources. One of the ways this can be achieved is by encouraging commercial production of medicinal plants.

References

Abbas, H. S., Falih, M. A., Hamad, B. S., & Eesa, S. H. (2020). Detection of local isolates of the entomopathogenic fungus Metarhizium anisopliae using specific primers. *Acta Entomology and Zoology*, *1*(1), 01–04. https://doi.org/10.33545/27080013.2020.v1.i1a.1

Achigan-Dako, E. (2015). Current knowledge and breeding perspectives for the miracle plant Synsepalum dulcificum (Schum. Et Thonn.) Daniell. *Genetic Resources and Crop Evolution*, *62*. https://doi.org/10.1007/s10722-015-0225-7

Achigan-Dako, E. G., Tchokponhoué, D. A., N’Danikou, S., Gebauer, J., & Vodouhè, R. S. (2015). Current knowledge and breeding perspectives for the miracle plant Synsepalum dulcificum (Schum. Et Thonn.) Daniell. *Genetic Resources and Crop Evolution*, *62*(3), 465–476. https://doi.org/10.1007/s10722-015-0225-7

Africa—Climate, Regions, Variations | Britannica. (n.d.). Retrieved February 23, 2024, from https://www.britannica.com/place/Africa/Climate

Akinmoladun, A. C., Adetuyi, A. R., Komolafe, K., & Oguntibeju, O. O. (2020). Nutritional benefits, phytochemical constituents, ethnomedicinal uses and biological properties of Miracle fruit plant (Synsepalum dulcificum Shumach. & Thonn. Daniell). *Heliyon*, *6*(12), e05837. https://doi.org/10.1016/j.heliyon.2020.e05837

Alizadeh-Navaei, R., Roozbeh, F., Saravi, M., Pouramir, M., Jalali, F., & Moghadamnia, A. A. (2008). Investigation of the effect of ginger on the lipid levels. A double blind controlled clinical trial. *Saudi Medical Journal*, *29*(9), 1280–1284.

Ameh, G. I., & Nwammoh, A. O. (2010). Phytochemical and Ethnobotanical Evaluation of Garlic Bulb (Allium sativum L.). *Bio-Research*, *8*. https://doi.org/10.4314/br.v8i1.62545

Amir, H. M., Grace, O. M., Wabuyele, E., & Manoko, M. L. K. (2019). Ethnobotany of Aloe L. (Asphodelaceae) in Tanzania. *South African Journal of Botany*, *122*, 330–335. https://doi.org/10.1016/j.sajb.2019.01.038

Anderberg, A. A., & Swenson, U. (2003). Evolutionary Lineages in Sapotaceae (Ericales): A Cladistic Analysis Based on ndhF Sequence Data. *International Journal of Plant Sciences*, *164*(5), 763–773. https://doi.org/10.1086/376818

Ang, L., Song, E., Hu, X.-Y., Lee, H. W., Chen, Y., & Lee, M. S. (2022). Herbal Medicine Intervention for the Treatment of COVID-19: A Living Systematic Review and Cumulative Meta-Analysis. *Frontiers in Pharmacology, 13,* 906764. https://doi.org/10.3389/fphar.2022.906764

Ang, L., Song, E., Lee, H. W., & Lee, M. S. (2020). Herbal Medicine for the Treatment of Coronavirus Disease 2019 (COVID-19): A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Journal of Clinical Medicine*, *9*(5), 1583. https://doi.org/10.3390/jcm9051583

Armstrong, K. (2011). *Systematics and biogeography of the pantropical genus Manilkara Adans. (Sapotaceae)*. https://era.ed.ac.uk/handle/1842/4871

Asiimwe, S., Namukobe, J., Byamukama, R., & Imalingat, B. (2021). Ethnobotanical survey of medicinal plant species used by communities around Mabira and Mpanga Central Forest Reserves, Uganda. *Tropical Medicine and Health*, *49*, 52. https://doi.org/10.1186/s41182-021-00341-z

Bartish, I. V., Swenson, U., Munzinger, J., & Anderberg, A. A. (2005). Phylogenetic relationships among New Caledonian Sapotaceae (Ericales): Molecular evidence for generic polyphyly and repeated dispersal. *American Journal of Botany*, *92*(4), 667–673. https://doi.org/10.3732/ajb.92.4.667

Borg, D., Richardson, J.-E., Harris, D., Gautier, L., Hughes, M., & Mackinder, B. (2019). PHYLOGENY of TWO AFRICAN GENERA of SAPOTACEAE - ENGLEROPHYTUM and SYNSEPALUM. *Edinburgh Journal of Botany*, *76,* 1–37. https://doi.org/10.1017/S0960428619000040

Caballero-Serrano, V., McLaren, B., Carrasco, J. C., Alday, J. G., Fiallos, L., Amigo, J., & Onaindia, M. (2019). Traditional ecological knowledge and medicinal plant diversity in Ecuadorian Amazon home gardens. *Global Ecology and Conservation*, *17*, e00524. https://doi.org/10.1016/j.gecco.2019.e00524

Ceuterick, M., Vandebroek, I., & Pieroni, A. (2011). Resilience of Andean urban ethnobotanies: A comparison of medicinal plant use among Bolivian and Peruvian migrants in the United Kingdom and in their countries of origin. *Journal of Ethnopharmacology*, *136*(1), 27–54. https://doi.org/10.1016/j.jep.2011.03.038

Chaudhari, S., Ruknuddin, G., & Prajapati, P. (2016). Ethno medicinal values of Citrus genus: A review. *Medical Journal of Dr. D.Y. Patil University*, *9*, 560. https://doi.org/10.4103/0975-2870.192146

Choi, S. E., & Garza, J. (2021). Consumer Likings of Different Miracle Fruit Products on Different Sour Foods. *Foods, 10*(2), 406. https://doi.org/10.3390/foods10020406

Coleman, M. (2016, March 18). Discovering the Sapotaceae family. *Botanics Stories*. https://stories.rbge.org.uk/archives/19291

Contributors, W. E. (n.d.). *Health Benefits of Bitter Kola*. WebMD. Retrieved August 16, 2023, from https://www.webmd.com/diet/health-benefits-bitter-kola

Crichton, M., Davidson, A. R., Innerarity, C., Marx, W., Lohning, A., Isenring, E., & Marshall, S. (2022). Orally consumed ginger and human health: An umbrella review. *The American Journal of Clinical Nutrition*, *115*(6), 1511–1527. https://doi.org/10.1093/ajcn/nqac035

Cupp, M. J. (1999). Herbal Remedies: Adverse Effects and Drug Interactions. *American Family Physician*, *59*(5), 1239–1244.

Daimei, P., & Kumar, Y. (2014). Ethnobotanical uses of gingers in Tamenglong district, Manipur, Northeast India. *Genetic Resources and Crop Evolution*, *61*(1), 273–285. https://doi.org/10.1007/s10722-013-0054-5

de Medeiros, P. M., Soldati, G. T., Alencar, N. L., Vandebroek, I., Pieroni, A., Hanazaki, N., & de Albuquerque, U. P. (2012a). The Use of Medicinal Plants by Migrant People: Adaptation, Maintenance, and Replacement. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2012*, 807452. https://doi.org/10.1155/2012/807452

de Medeiros, P. M., Soldati, G. T., Alencar, N. L., Vandebroek, I., Pieroni, A., Hanazaki, N., & de Albuquerque, U. P. (2012b). The Use of Medicinal Plants by Migrant People: Adaptation, Maintenance, and Replacement. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2012*, 807452. https://doi.org/10.1155/2012/807452

Del Campo, R., Zhang, Y., & Wakeford, C. (2017). Effect of Miracle Fruit (Synsepalum dulcificum) Seed Oil (MFSO®) on the Measurable Improvement of Hair Breakage in Women with Damaged Hair. *The Journal of Clinical and Aesthetic Dermatology*, *10*(11), 39–48.

Doddawad, V. G., Shivananda, S., Vidya, C. S., Madhu, B., & Gurupadayya, B. M. (2022). Insight on Synsepalum dulcificum: A Bioactive Compound on Taste Modification and Its Biological Properties. *International Journal of Nutrition, Pharmacology, Neurological Diseases, 12*(3), 93. https://doi.org/10.4103/ijnpnd.ijnpnd_19_22

Douglas Kinghorn, A., Chin, Y.-W., Pan, L., & Jia, Z. (2010). 3.10—Natural Products as Sweeteners and Sweetness Modifiers. In H.-W. (Ben) Liu & L. Mander (Eds.), *Comprehensive Natural Products II* (pp. 269–315). Elsevier. https://doi.org/10.1016/B978-008045382-8.00077-0

Du, L., Shen, Y., Zhang, X., Prinyawiwatkul, W., & Xu, Z. (2014). Antioxidant-rich phytochemicals in miracle berry (*Synsepalum dulcificum*) and antioxidant activity of its extracts. *Food Chemistry, 153*, 279–284. https://doi.org/10.1016/j.foodchem.2013.12.072

DV. (2022, April 25). *Flavor-Tripping With the Miracle Fruit*. The Somm Journal. https://www.sommjournal.com/flavor-tripping-with-the-miracle-fruit/

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4*, 177. https://doi.org/10.3389/fphar.2013.00177

Eltis, D., Morgan, P., & Richardson, D. (2007). Agency and Diaspora in Atlantic History: Reassessing the African Contribution to Rice Cultivation in the Americas. *The American Historical Review, 112*(5), 1329–1358. https://doi.org/10.1086/ahr.112.5.1329

Fandohan, A., Chadare, F. J., Gouwakinnou, G., Tovissodé, C. F., Bonou, A., Djonlonkou, S., Houndelo, L. F. H., Sinsin, C., & Assogbadjo, A. (2017). Traditional uses and economic value of Synsepalum dulcificum in South Benin. *Bois et Forets Des Tropiques*, 17–30.

Fitzgerald, M., Heinrich, M., & Booker, A. (2020). Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques. *Frontiers in Pharmacology*, *10*. https://www.frontiersin.org/articles/10.3389/fphar.2019.01480

Gonfa, N., Robi, D., & Hundera, K. (2020). Ethnobotanical study of medicinal plants, its utilization, and conservation by indigenous people of Gera district, Ethiopia Ethnobotanical study of medicinal plants, its utilization, and conservation by indigenous people of Gera district, Ethiopia PUBLIC INTEREST STATEMENT. *Cogent Food & Agriculture*, *6*. https://doi.org/10.1080/23311932.2020.1852716

Gonfa, N., Tulu, D., Hundera, K., & Raga, D. (2020). Ethnobotanical study of medicinal plants, its utilization, and conservation by indigenous people of Gera district, Ethiopia. *Cogent Food & Agriculture*, *6*(1), 1852716. https://doi.org/10.1080/23311932.2020.1852716

Hasani, H., Arab, A., Hadi, A., Pourmasoumi, M., Ghavami, A., & Miraghajani, M. (2019). Does ginger supplementation lower blood pressure? A systematic review and meta-analysis of clinical trials. *Phytotherapy Research: PTR*, *33*(6), 1639–1647. https://doi.org/10.1002/ptr.6362

Hirai, T., Shohael, A. M., Kim, Y.-W., Yano, M., & Ezura, H. (2011). Ubiquitin promoter–terminator cassette promotes genetically stable expression of the taste-modifying protein miraculin in transgenic lettuce. *Plant Cell Reports*, *30*(12), 2255–2265. https://doi.org/10.1007/s00299-011-1131-x

Howard, P. (2006). *Gender Bias in Ethnobotany: Propositions and Evidence of a Distorted Science and Promises of a Brighter Future*.

Hudson, S. D., Sims, C. A., Odabasi, A. Z., Colquhoun, T. A., Snyder, D. J., Stamps, J. J., Dotson, S. C., Puentes, L., & Bartoshuk, L. M. (2018). Flavor Alterations Associated with Miracle Fruit and Gymnema sylvestre. *Chemical Senses*, *43*(7), 481–488. https://doi.org/10.1093/chemse/bjy032

Izawa, K., Amino, Y., Kohmura, M., Ueda, Y., & Kuroda, M. (2010). 4.16—Human–Environment Interactions – Taste. In H.-W. (Ben) Liu & L. Mander (Eds.), *Comprehensive Natural Products II* (pp. 631–671). Elsevier. https://doi.org/10.1016/B978-008045382-8.00108-8

Kiyama, R. (2020). Nutritional implications of ginger: Chemistry, biological activities and signaling pathways. *The Journal of Nutritional Biochemistry*, *86*, 108486. https://doi.org/10.1016/j.jnutbio.2020.108486

Klimek-Szczykutowicz, M., Szopa, A., & Ekiert, H. (2020). Citrus limon (Lemon) Phenomenon—A Review of the Chemistry, Pharmacological Properties, Applications in the Modern Pharmaceutical, Food, and Cosmetics Industries, and Biotechnological Studies. *Plants*, *9*, 119. https://doi.org/10.3390/plants9010119

Koizumi, A., Tsuchiya, A., Nakajima, K., Ito, K., Terada, T., Shimizu-Ibuka, A., Briand, L., Asakura, T., Misaka, T., & Abe, K. (2011). Human sweet taste receptor mediates acid-induced sweetness of miraculin. *Proceedings of the National Academy of Sciences*, *108*(40), 16819–16824. https://doi.org/10.1073/pnas.1016644108

Kurihara, K., & Beidler, L. M. (1969). Mechanism of the Action of Taste-modifying Protein. *Nature*, *222*(5199), Article 5199. https://doi.org/10.1038/2221176a0

Lipatova, O., & Campolattaro, M. M. (2016). The Miracle Fruit: An Undergraduate Laboratory Exercise in Taste Sensation and Perception. *Journal of Undergraduate Neuroscience Education*, *15*(1), A56–A60.

Liu, Y.-X., Zhou, Y.-H., Jiang, C.-H., Liu, J., & Chen, D.-Q. (n.d.). Prevention, treatment and potential mechanism of herbal medicine for Corona viruses: A review. *Bioengineered*, *13*(3), 5480–5508. https://doi.org/10.1080/21655979.2022.2036521

Luo, L., Jiang, J., Wang, C., Fitzgerald, M., Hu, W., Zhou, Y., Zhang, H., & Chen, S. (2020). Analysis on herbal medicines utilized for treatment of COVID-19. *Acta Pharmaceutica Sinica. B*, *10*(7), 1192–1204. https://doi.org/10.1016/j.apsb.2020.05.007

Luo, X., Zhang, Y., Li, H., Ren, M., Liu, Y., Liu, Y., Zhang, Y., Kuang, Z., Cai, Y., Chen, Y., & Ni, X. (2022). Clinical Evidence on the Use of Chinese Herbal Medicine for Acute Infectious Diseases: An Overview of Systematic Reviews. *Frontiers in Pharmacology*, *13*. https://www.frontiersin.org/articles/10.3389/fphar.2022.752978

Mahomoodally, M. F., Protab, K., & Aumeeruddy, M. Z. (2019). Medicinal plants brought by Indian indentured immigrants: A comparative review of ethnopharmacological uses between Mauritius and India. *Journal of Ethnopharmacology*, *234*, 245–289. https://doi.org/10.1016/j.jep.2019.01.012

McHugh, P. (2018, September 15). *Record 44.5 Million Immigrants in 2017*. CIS.Org. https://cis.org/Report/Record-445-Million-Immigrants-2017

Medicinal plants. (2023). In *Wikipedia*. https://en.wikipedia.org/w/index.php?title=Medicinal_plants&oldid=1171382764

Miracle fruit | Description, Effects, & Uses | Britannica. (2024, January 30). https://www.britannica.com/plant/miracle-fruit

MIRACLE FRUIT OIL COMPANY DONATES MIRACLE FRUIT TREE TO BAPTIST HEALTH – Miracle Fruit Oil. (n.d.). Retrieved February 23, 2024, from https://www.miraclefruitoil.com/blogs/news/miracle-fruit-oil-company-donates-miracle-fruit-tree-to-baptist-health-hospitals-to-improve-the-lives-of-cancer-patients

Misaka, T. (2013). Molecular mechanisms of the action of miraculin, a taste-modifying protein. *Seminars in Cell & Developmental Biology*, *24*(3), 222–225. https://doi.org/10.1016/j.semcdb.2013.02.008

Momodu, S. (2022, February 9). *African Americans in the California Gold Rush (1848-1860) •*. https://www.blackpast.org/african-american-history/events-african-american-history/african-americans-in-the-california-gold-rush-1848-1860/

Moneim, A., & Sulieman, E. (2019). Garcinia Kola (Bitter Kola): Chemical Composition. In A. A. Mariod (Ed.), *Wild Fruits: Composition, Nutritional Value and Products* (pp. 285–299). Springer International Publishing. https://doi.org/10.1007/978-3-030-31885-7_23

Morgan, A. (2020, August 18). Roots of African American Herbalism: Herbal Use by Enslaved Africans. *Herbal Academy*. https://theherbalacademy.com/african-american-herbalism-history/

Nguyen, A., Kim, S. J., Phuoc Long, N., Jung Eun Min, Yoon, Y. C., Eun Goo Lee, Mina Kim, Tae Joon Kim, Yoon Young Yang, Eui Young Son, Yoon, S. J., Nguyen Co, D., Kim, H. M., & Sung Won Kwon. (2020). Ginger on Human Health: A Comprehensive Systematic

Review of 109 Randomized Controlled Trials. *Nutrients*, *12*, 157. https://doi.org/10.3390/nu12010157

Nicholson, S. E. (2001). Climatic and environmental change in Africa during the last two centuries. *Climate Research*, *17*(2), 123–144. https://doi.org/10.3354/cr017123

Niu, Y., Ni, S., & Liu, J. (n.d.). Complete chloroplast genome of Synsepalum dulcificum D.: A magical plant that modifies sour flavors to sweet. *Mitochondrial DNA. Part B, Resources*, *5*(3), 3052–3053. https://doi.org/10.1080/23802359.2020.1798299

Okoro, B. C., Dokunmu, T. M., Okafor, E., Sokoya, I. A., Israel, E. N., Olusegun, D. O., Bella-Omunagbe, M., Ebubechi, U. M., Ugbogu, E. A., & Iweala, E. E. J. (2023). The ethnobotanical, bioactive compounds, pharmacological activities and toxicological evaluation of garlic (Allium sativum): A review. *Pharmacological Research - Modern Chinese Medicine*, *8*, 100273. https://doi.org/10.1016/j.prmcm.2023.100273

Olaokun, O. O., Manonga, S. A., Zubair, M. S., Maulana, S., & Mkolo, N. M. (2022). Molecular Docking and Molecular Dynamics Studies of Antidiabetic Phenolic Compound Isolated from Leaf Extract of Englerophytum magalismontanum (Sond.) T.D.Penn. *Molecules*, *27*(10), Article 10. https://doi.org/10.3390/molecules27103175

Onder, G., & Liperoti, R. (2016). Herbal Medications. *JAMA*, *315*(10), 1068. https://doi.org/10.1001/jama.2015.19388

Oyebade, A. (2017). African immigrants and their churches. In *The New African Diaspora in the United States*. Routledge.

Oyetunji, O. (n.d.). Ethno-botanical survey, review and informatics of medicinal plants used by indigenous people of Lekki, Ibeju-Lekki Local Government Area of Lagos State, Nigeria.

JOURNAL OF RESEARCH AND REVIEW IN SCIENCE. Retrieved February 23, 2024, from https://www.academia.edu/63065076/Ethno_botanical_survey_review_and_informatics_of_medicinal_plants_used_by_indigenous_people_of_Lekki_Ibeju_Lekki_Local_Government_Area_of_Lagos_State_Nigeria

Pagnocca, T. S., Zank, S., & Hanazaki, N. (2020). “The plants have axé”: Investigating the use of plants in Afro-Brazilian religions of Santa Catarina Island. *Journal of Ethnobiology and Ethnomedicine*, *16*(1), 20. https://doi.org/10.1186/s13002-020-00372-6

Paladino, A., Costantini, S., Colonna, G., & Facchiano, A. (2008). Molecular modelling of miraculin: Structural analyses and functional hypotheses. *Biochemical and Biophysical Research Communications*, *367*, 26–32. https://doi.org/10.1016/j.bbrc.2007.12.102

Parreño, R., Rodríguez-Alcocer, E., Martínez-Guardiola, C., Carrasco, L., Castillo, P., Arbona, V., Jover-Gil, S., & Candela, H. (2023). Turning Garlic into a Modern Crop: State of the Art and Perspectives. *Plants*, *12*(6), Article 6. https://doi.org/10.3390/plants12061212

Posadzki, P., Watson, L. K., & Ernst, E. (2013). Adverse effects of herbal medicines: An overview of systematic reviews. *Clinical Medicine (London, England)*, *13*(1), 7–12. https://doi.org/10.7861/clinmedicine.13-1-7

Prasad, S., & Tyagi, A. K. (2015). Ginger and Its Constituents: Role in Prevention and Treatment of Gastrointestinal Cancer. *Gastroenterology Research and Practice*, *2015*, e142979. https://doi.org/10.1155/2015/142979

Richardson, J. E., Bakar, A. M., Tosh, J., Armstrong, K., Smedmark, J., Anderberg, A. A., Slik, F., & Wilkie, P. (2014). The influence of tectonics, sea-level changes and dispersal on migration and diversification of Isonandreae (Sapotaceae). *Botanical Journal of the Linnean Society*, *174*(1), 130–140. https://doi.org/10.1111/boj.12108

Romanus, P. C., Mendes, F. R., & Carlini, E. de A. (2018). Factors affecting the use of medicinal plants by migrants from rural areas of Brazilian Northeast after moving to a metropolitan region in Southeast of Brazil. *Journal of Ethnobiology and Ethnomedicine*, *14*(1), 72. https://doi.org/10.1186/s13002-018-0270-3

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Sarić-Kundalić, B., Dobeš, C., Klatte-Asselmeyer, V., & Saukel, J. (2011). Ethnobotanical survey of traditionally used plants in human therapy of east, north and north-east Bosnia and Herzegovina. *Journal of Ethnopharmacology*, *133*(3), 1051–1076. https://doi.org/10.1016/j.jep.2010.11.033

Selvakumar, P., Gahloth, D., Tomar, P. P. S., Sharma, N., & Sharma, A. K. (2011). Molecular Evolution of Miraculin-Like Proteins in Soybean Kunitz Super-Family. *Journal of Molecular Evolution*, *73*(5), 369–379. https://doi.org/10.1007/s00239-012-9484-5

Shah, A. A., Badshah, L., Khalid, N., Shah, M. A., Manduzai, A. K., Faiz, A., De Chiara, M., Mattalia, G., Sõukand, R., & Pieroni, A. (2023). Disadvantaged Economic Conditions and Stricter Border Rules Shape Afghan Refugees' Ethnobotany: Insights from Kohat District, NW Pakistan. *Plants*, *12*(3), Article 3. https://doi.org/10.3390/plants12030574

Shahrajabian, M. H., Sun, W., & Cheng, Q. (2019). Clinical aspects and health benefits of ginger (Zingiber officinale) in both traditional Chinese medicine and modern industry. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, *69*(6), 546–556. https://doi.org/10.1080/09064710.2019.1606930

Silveira, D., Prieto-Garcia, J. M., Boylan, F., Estrada, O., Fonseca-Bazzo, Y. M., Jamal, C. M., Magalhães, P. O., Pereira, E. O., Tomczyk, M., & Heinrich, M. (2020). COVID-19: Is There Evidence for the Use of Herbal Medicines as Adjuvant Symptomatic Therapy? *Frontiers in Pharmacology*, *11*. https://www.frontiersin.org/articles/10.3389/fphar.2020.581840

Singh, D. R. K. (2019). Uses of Medicinal Plants In Traditional and Modern Medicine. *International Journal of Engineering Research & Technology*, *8*(8). https://doi.org/10.17577/IJERTV8IS080241

Singh, P., Mishra, G., Pottoo, F. H., Singh, B., & Zeleke, M. M. (2022). Zingiber officinale: Its Ethanobotanical Uses, Phytochemistry, and Pharmacology. In M. H. Masoodi & M. U. Rehman (Eds.), *Edible Plants in Health and Diseases: Volume II : Phytochemical and Pharmacological Properties* (pp. 1–42). Springer. https://doi.org/10.1007/978-981-16-4959-2_1

Sintayehu, D. W. (2018). Impact of climate change on biodiversity and associated key ecosystem services in Africa: A systematic review. *Ecosystem Health and Sustainability*, *4*(9), 225–239. https://doi.org/10.1080/20964129.2018.1530054

Snider, P., & Zeff, J. (2019). Unifying Principles of Naturopathic Medicine Origins and Definitions. *Integrative Medicine: A Clinician's Journal*, *18*(4), 36–39.

Sohail, A., Barry, A., Auburn, S., Cheng, Q., Lau, C. L., Lee, R., Price, R. N., Furuya-Kanamori, L., Bareng, P., McGuinness, S. L., & Leder, K. (2024). Imported malaria into Australia: Surveillance insights and opportunities. *Journal of Travel Medicine*, *31*(3), taad164. https://doi.org/10.1093/jtm/taad164

Sulaiman, A. N., Arzai, A. H., & Taura, D. W. (2022). Ethnobotanical survey: A comprehensive review of medicinal plants used in treatment of gastro intestinal diseases in Kano state, Nigeria. *Phytomedicine Plus*, *2*(1), 100180. https://doi.org/10.1016/j.phyplu.2021.100180

Swenson, U., Lowry, P. P., Munzinger, J., Rydin, C., & Bartish, I. V. (2008). Phylogeny and generic limits in the Niemeyera complex of New Caledonian Sapotaceae: Evidence of multiple origins of the anisomerous flower. *Molecular Phylogenetics and Evolution*, *49*(3), 909–929. https://doi.org/10.1016/j.ympev.2008.09.022

Synsepalum afzelii—Useful Tropical Plants. (n.d.). Retrieved August 8, 2023, from https://tropical.theferns.info/viewtropical.php?id=Synsepalum+afzelii

Tareau, M.-A., Greene, A., Palisse, M., & Odonne, G. (2022). Migrant Pharmacopoeias: An Ethnobotanical Survey of Four Caribbean Communities in Amazonia (French Guiana). *Economic Botany*, *76*(2), 176–188. https://doi.org/10.1007/s12231-021-09529-0

Tchokponhoué, D. A., N'Danikou, S., Fassinou Hotegni, N. V., Nyadanu, D., Kahane, R., Odindo, A. O., Achigan-Dako, E. G., & Sibiya, J. (2021). Use Patterns, Knowledge Diversity and Drivers for the Cultivation of the Miracle Plant [Synsepalum dulcificum (Schumach & Thonn.) Daniell] in Benin and Ghana. *Plants*, *10*(11), 2253. https://doi.org/10.3390/plants10112253

Thakur, K. S., Kumar, M., Bawa, R., & Bussmann, R. W. (2014). Ethnobotanical Study of Herbaceous Flora along an Altitudinal Gradient in Bharmour Forest Division, District Chamba of Himachal Pradesh, India. *Evidence-Based Complementary and Alternative Medicine*, *2014*, e946870. https://doi.org/10.1155/2014/946870

The Cardiovascular Benefits and Harms of Ginger. (n.d.). LIVESTRONG.COM. Retrieved August 15, 2023, from https://www.livestrong.com/article/557445-the-cardiovascular-benefits-and-harms-of-ginger/

Torres-Avilez, W., Medeiros, P. M. de, & Albuquerque, U. P. (2016). Effect of Gender on the Knowledge of Medicinal Plants: Systematic Review and Meta-Analysis. *Evidence-Based Complementary and Alternative Medicine*, *2016*, e6592363. https://doi.org/10.1155/2016/6592363

Tugume, P., Kakudidi, E. K., Buyinza, M., Namaalwa, J., Kamatenesi, M., Mucunguzi, P., & Kalema, J. (2016). Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda. *Journal of Ethnobiology and Ethnomedicine*, *12*(1), 5. https://doi.org/10.1186/s13002-015-0077-4

Uchejeso, O., & Chinaza, I. (2020). *Bitter kola assisted the Igbo Speaking People in Nigeria against COVID-19*.

Volpato, G., Godínez, D., Beyra, A., & Barreto, A. (2009). Uses of medicinal plants by Haitian immigrants and their descendants in the Province of Camagüey, Cuba. *Journal of Ethnobiology and Ethnomedicine*, *5*, 16. https://doi.org/10.1186/1746-4269-5-16

Wang, Z., & Yang, L. (2021). Chinese herbal medicine: Fighting SARS-CoV-2 infection on all fronts. *Journal of Ethnopharmacology*, *270*, 113869. https://doi.org/10.1016/j.jep.2021.113869

Wink, M., Botschen, F., Gosmann, C., Schäfer, H., & Waterman, P. G. (2010). Chemotaxonomy Seen from a Phylogenetic Perspective and Evolution of Secondary Metabolism. In *Annual Plant Reviews Volume 40: Biochemistry of Plant Secondary Metabolism* (pp. 364–433). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781444320503.ch7

Xiang, L., Chen, Z., Wei, S., & Zhou, H. (2022). Global Trade Pattern of Traditional Chinese Medicines and China's Trade Position. *Frontiers in Public Health*, *10*. https://www.frontiersin.org/articles/10.3389/fpubh.2022.865887

Yang, Z., Liu, Z., Xu, H., Chen, Y., Du, P., Li, P., Lai, W., Hu, H., Luo, J., & Ding, Y. (2022). The Chromosome-Level Genome of Miracle Fruit (Synsepalum dulcificum) Provides New Insights Into the Evolution and Function of Miraculin. *Frontiers in Plant Science*, *12*. https://www.frontiersin.org/articles/10.3389/fpls.2021.804662

Yeshi, K., Turpin, G., Jamtsho, T., & Wangchuk, P. (2022). Indigenous Uses, Phytochemical Analysis, and Anti-Inflammatory Properties of Australian Tropical Medicinal Plants. *Molecules*, *27*(12), 3849. https://doi.org/10.3390/molecules27123849

Yogom, B. T., Avana-Tientcheu, M.-L., Mboujda, M. F. M., Momo, S. T., Fonkou, T., Tsobeng, A., Barnaud, A., & Duminil, J. (2020). Ethnicity Differences in Uses and Management Practices of Bitter Kola Trees (Garcinia kola) in Cameroon. *Economic Botany*, *74*(4), 429–444. https://doi.org/10.1007/s12231-020-09508-x

Zahn, R., Perry, N., Perry, E., & Mukaetova-Ladinska, E. B. (2019). Use of herbal medicines: Pilot survey of UK users' views. *Complementary Therapies in Medicine*, *44*, 83–90. https://doi.org/10.1016/j.ctim.2019.02.007

Zhou, G., Yang, L., Li, C., Xu, W., & Chen, G. (2010). Genetic diversity in endangered *Notopterygium forbesii* Boissieu based on intraspecies sequence variation of chloroplast DNA and implications for conservation. *Biochemical Systematics and Ecology*, *38*(5), 911–916. https://doi.org/10.1016/j.bse.2010.09.012