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Faculty of Tropical AgriSciences

Nutritional content of Zambian underutilized species: The case of mahwahwa (*Strychnos pungens* Soler) and mahuluhulu (*Strychnos cocculoides* Baker)

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Declaration

I hereby declare that I have done this thesis entitled Nutritional content of Zambian underutilized species: The case of mahwahwa (*Strychnos pungens* Soler) and mahuluhulu (*Strychnos cocculoides* Baker) independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 16. 4. 2021

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Pavla Přinosilová

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Abstract

Strychnos pungens Soler and *Strychnos cocculoides* Baker are tropical fruit species originating in southern parts of Africa with the status of neglected/underutilized crops. Even though these plants are a valuable source of food during the dry season when famine usually occurs, they have been overlooked by science. Local people mainly consume the fresh fruit pulp while all parts of the trees are used for medicine. Therefore, the species play a crucial role in people's everyday life - enhancing local food security, improving health, and contributing to income generation. This study aimed to determine the nutritional aspects of both *Strychnos* species. The analyses of the lyophilizate of pulp for ash, dry matter, crude fat, crude fibre, crude fat, crude protein, NFE, vitamins B, and vitamin C were done by AOAC methods. The nutritional values of macronutrients for *S. pungens* were determined as follows: dry matter – 94.82 %, ash – 9.48 %, crude fibre – 13.78 %, crude protein – 2.37 %, crude fat – 17.16 %, NFE – 57.75 %. The content of vitamin C in *S. pungens* was 1772.76 mg/100 g, whereas the highest value from B vitamins was reached by pyridoxine (979.56 mg/100 g), followed by pantothenic acid (805.40 mg/100 g) and thiamine (778.02 mg/100 g), respectively. The nutritional values for *S. cocculoides* were determined as: dry matter – 89.65 %, ash – 8.97 %, crude fibre – 12.97 %, crude protein – 3.10 %, crude fat – 10.47 %, NFE – 56.78 %. The content of vitamin C in *S. cocculoides* was 703.04 mg/100 g, whereas the highest value from B vitamins was also reached by pyridoxine (240.00 mg/100 g), followed by pantothenic acid (238.52 mg/100 g) and thiamine (206.15 mg/100 g), respectively. Results showed that the lyophilizate of the edible pulp of both fruit species is a great source of crude fat, crude fibre together with vitamins, mainly vitamin C and pyridoxine (B_6). However, further nutritional analyses with a determination of secondary metabolites, anti-nutritional factors, and digestibility are recommended to extend the knowledge about the dietetic potential of these African indigenous trees.

Key words: corky-bark monkey orange, freeze-drying, indigenous trees, neglected and underutilized species, spiny-leaved monkey orange, tropical fruit

Abstrakt

Strychnos pungens Soler a *Strychnos cocculoides* Baker jsou tropické ovocné druhy pocházející z jižní Afriky, které patří mezi opomíjené / méně využívané plodiny. Přestože jsou tyto rostliny cenným zdrojem potravy v období sucha, kdy obvykle dochází k hladomoru, zůstávají mimo hlavní proud vědeckého zájmu. Místní lidé konzumují čerstvou dužinu plodů a zároveň využívají všechny části stromu k léčebným účelům. Ovoce hraje klíčovou roli v každodenním životě místních obyvatel – zvyšuje potravinovou bezpečnost, zlepšuje zdraví a přispívá k lepším příjmům. Cílem této práce bylo zjistit nutriční hodnoty obou druhů rodu *Strychnos*. Analýzy byly provedeny z lyofilizátu ovocné dužiny, stanoveny byly popeloviny, sušina, hrubá vláknina, hrubý tuk, hrubé bílkoviny, NFE, vitaminy skupiny B a vitamin C pomocí metod AOAC. Nutriční hodnoty makroživin pro *S. pungens* byly stanoveny takto: sušina – 94,82 %, popeloviny – 9,48 %, hrubá vláknina – 13,78 %, hrubé bílkoviny – 2,37 %, hrubé tuky – 17,16 %, NFE – 57,75 %. Obsah vitaminu C pro *S. pungens* byl 1772,7 mg/100 g, zatímco nejvyšších hodnot vitaminů skupiny B dosáhl pyridoxin (979,56 mg/100 g), následován kyselinou pantothenovou (805,40 mg/100 g) a thiaminem (778,02 mg/100 g). Hodnoty pro *S. cocculoides* byly stanoveny jako: sušina – 89,65 %, popeloviny – 8,97 %, hrubá vláknina – 12,97 %, hrubé bílkoviny – 3,10 %, hrubé tuky – 10,47 %, NFE – 56,78 %. Obsah vitaminu C pro *S. cocculoides* byl stanoven na 703,0 mg /100 g, kdežto nejvyšších hodnoty vitaminů skupiny B dosáhl také pyridoxin (240,00 mg/100 g), kyselina pantothenová (238,52 mg/100 g) a thiamin (206,15 mg/100 g). Výsledky ukázaly, že lyofilizát jedlé dužiny obou ovocných druhů má vysoký obsah hrubého tuku a hrubé vlákniny, stejně jako vitaminů, zejména vitaminu C a pyridoxinu (B_6). Pro rozšíření znalostí o dietetickém potenciálu těchto původních afrických dřevin doporučujeme provést další nutriční analýzy se zaměřením na stanovení sekundárních metabolitů, antinutričních látek a stravitelnosti.

Klíčová slova: corky-bark monkey orange, lyofilizace, původní stromy, opomíjené a méně využívané druhy, spiny-leaved monkey orange, tropické ovoce

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List of the abbreviations used in the thesis

CAGR – Compound Annual Growth Rate

CF – Crude Fibre

CFF – Crops For the Future

CP – Crude Protein

CZU – Czech University of Life Sciences Prague

DM – Dry Matter

EE – Ether Extract

EU – European Union

FAO – Food and Agriculture Organization of the United Nations

FISP – Farm Input Support Programme

FTA – Faculty of Tropical AgriSciences

GDP – Gross Domestic Product

GFU – Global Facilitation Unit for Underutilized Species

GNI – Gross National Income

HDI – Human Development Index

ICRAF – The World Agroforestry Centre

ICUC – International Centre for Underutilized Crops

m.a.s.l. – Metres above sea level

NFE – Nitrogen Free Extract

NPGS – National Plant Germplasm System

NRCS – National Resources Conservation Service

NUS – Neglected and Underutilized Species

RDA – Recommended Dietary Allowance

SCUC – Southampton Centre for Underutilized Crops

SOA – Sustainable Organic Agriculture

USDA – United States Department of Agriculture

1. Introduction

Neglected and underutilized plant species (NUS), which are considered to be primarily valued by indigenous communities in the tropics, are nowadays getting popular as a potential source of food and medicine also in high-income countries. Even though local people of the rural areas use the species every day, the plants are classified as underutilized because not much attention is paid to them by scientists, farmers, and policymakers (Padulosi et al. 2013). Consequently, these indigenous crops are mainly in the wild or semi-domesticated phase. The species usually bear the best adaptation skills to local climate and natural conditions like high elevation, drought, water scarcity, and sandy or saline soils, where other crops would survive with difficulties (Mwamba 2006; Maroyi 2009).

NUS are also important from the dietic point of view, providing nutrients such as proteins, vitamins, and minerals thus contributing to diversified and balanced nourishment unlike an oversimplified diet usually composed of maize (*Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) which supplies over 50 % human's energy intake. The biggest disadvantage of simplified food is one or more forms of malnutrition which can even occur in developed countries, not only in developing ones. In this perspective, the NUS seem to be a future strategic way to prevent avitaminosis, malnutrition, and other health problems in a natural, and affordable way. Another advantage is the resilience of these plants against climate change, due to their adaptability to the local conditions compared to exotic species (FAO 2018b).

Based on the research of the International Federation of Red Cross and Red Crescent Societies (IFRC) (2021), Zambia has faced extreme drought and floods due to climate change and climate variability previous couple of years. Since the 2019 drought alternating with sudden floods are still facing acute food insecurity, shortage of clean and potable water, high chronic malnutrition, energy deficit, and livestock diseases. It has led to reduced food availability and access to food across the country because there have been limited stocks of cereals that are dependent on rains in most households. Analysis of acute food insecurity in Zambia for 2020 showed that over 1.42 million people faced high levels of acute food insecurity. Indigenous fruit species may particularly help with this problem as they are more drought resistant compared to exotic crops.

While most of the African population suffers from hunger and malnutrition, NUS have the potential to be a novel food with many benefits (Nyirenda et al. 2007). The local plant species also improve the role of women as they are often the main producers, processors, and traders of NUS (Kasolo et al. 2018). Additionally, indigenous crops can be used in the agroforestry systems, usually in traditional homegardens or intercropped with other species. The cultivation of most NUS is not known as they mainly grow wildly, and harvest is done manually from these wild stands by women and children (Mwamba 2006). Unlike the more famous NUS as quinoa (*Chenopodium quinoa*) or baobab (*Adansonia digitata*), the most minor species are not sold on the international market because they have not a big promotion (Mwamba 2006; Maroyi 2009). Despite of that, sales of fruits, processed fruit products, timber, or medicines of NUS in rural and urban markets bring additional income to subsistence farmers.

Therefore, this thesis focuses on the nutritional content determination of two African NUS – mahwahwa (*Strychnos pungens* Soler) and mahuluhulu (*Strychnos cocculoides* Baker) which are of high importance in rural areas of Zambia, but their scientific value has not yet been fully examined. To the best of our knowledge, there has not been any nutritional analysis done by lyophilization in the case of *S. pungens* and *S. cocculoides.*

2. Literature Review

2.1. NUS as a future source of food

NUS are classified as local and traditional plant species with low external input requirements and no commercial cultivation (Kour et al. 2018). They are also called orphan, abandoned, lost, underused, local, minor, traditional, alternative, niche, or underdeveloped species (Padulosi 2017). The plants have great adaptability to extreme climatic conditions as drought or floods and contribute to keeping stable biodiversity (Dansi et al. 2012). Even though NUS do not have such high yields as major crops, they can give a guarantee of harvest when commercial crops fail (Padulosi et al. 2013). According to Kour et al. (2018), the new research discovers the potential of NUS as a source of food that helps to fight against malnutrition and hidden hunger caused by micronutrient deficiencies resulting from uniform diets because many underutilized fruits and vegetables contain more vitamins, elements, and nutrients than widely available major crop species and varieties.

From the more popular NUS, there are moringa (*Moringa oleifera*), baobab (*Adansonia digitata*) noni (*Morinda citrifolia*), oca (*Oxalis tuberosa*), mangosteen (*Garcinia mangostana*), or Indian jujube (*Ziziphus mauritiana*) (Orwa et al. 2009). One of the best examples of the NUS popularization is quinoa (*Chenopodium quinoa*) which is native to South America and is known for its perfect adaptation to high altitudes. This species contains all the essential amino acids together with a high content of protein and therefore got worldwide popularity as one of the superfoods (FAO 2017). African NUS are mainly used by rural inhabitants, from Zambian edible fruit plants sugar plum (*Uapaca kirkiana*), water berry (*Syzygium guineense*), large sour plum (*Ximenia caffra*), marula (*Sclerocarya birrea*), eembe (*Berchemia discolour*) or corky-bark monkey orange (*Strychnos cocculoides*) are reported as valuable sources of nutrients and vital natural antioxidants (Orwa et al. 2009; Bille et al. 2013; Mapunda & Mligo 2019).

The NUS are also getting more popular due to a rising international inquiry for natural ingredients for food, cosmetic, pharmaceutical, nutritional, and health products (Rudebjer et al. 2014). For this purpose, some NUS species are already under the label “superfood” or “novel food”. By Regulation of European Union (EU), novel food is defined as “food that had not been consumed to a significant degree by humans in the EU before 15 May 1997, when the first Regulation on novel food came into force.” It can be e.g., chia seeds, argan oil, noni fruit, and baobab seeds. This label guarantees among other things safety for consumers because every novel food has to be approved and added to the Union list of authorized Novel Foods (European Commision 2021). Unlike novel food, there is not any regulation for the term superfood, it can be every food with a higher content of macro-/micronutrients, compared to normally consumed crops e.g., goji berry (*Lycium chinense*), maca (*Lepidium meyenii*), spirulina (*Arthrospira platensis*), quinoa (*Chenopodium quinoa*), curcuma (*Curcuma longa*), and many more. Claiming that superfood is, in most cases, just a marketing strategy (Wohlfahrt 2017).

There is a big potential for local people to take the advantage of eco-labelling, certification of origin, Fairtrade certification, and slow food initiatives but simultaneous, integrated support along value chains from farms to markets is needed (Rudebjer et al. 2014). Main problems with future utilization and cultivation are missing or uncompleted botanical data, absence of scientific information on nutrition value, inadequate promotion, stigma attached to the resources being food for the poor, and high rate of ecosystem degradation and habitat destructions (Kasolo et al. 2018).

Most diets in Africa consist mainly of cereals or starchy root staple crops, so protein and vitamin deficiency (mostly vitamin C and A) commonly occur. Consequently, this deficiency can be the cause of stunting, poor musculature, oedema, and skin lesions, threatening especially children and pregnant women (Conti et al. 2019). Awareness-raising of well-balanced nutrition and better food preparation are also the key to decrease malnutrition and vitamin deficiency in Zambia, in many cases adding more protein and fat in form of nuts such as mongongo (*Schinziophyton rautanenii*) to the traditional maize porridge nshima can contribute to the better development of children (Vítková 2020).

The important way how to keep a traditional knowledge of the NUS is farmer training especially for women who are the main farmers and sellers at markets. They should understand the benefits of sustainable production of indigenous fruit species because many urban inhabitants consider eating the wild fruits to being backward or they eat them only in times of drought and hunger (Mwamba 2006). Another way to raise awareness of indigenous species is conservation *in-situ* and *ex-situ* but complete documentation with relevant data of indigenous species is needed (Kour et al. 2018).

According to Kasolo et al. (2018), most of the neglected and underutilized fruits and nuts can be cultivated as it has been done with wild forms of mango (*Mangifera indica*), sweet orange (*Citrus sinensis*), papaya (*Carica papaya*), avocado (*Persea americana*), custard apple (*Annona squamosa*), and sapodilla (*Manilkara sapota*). They became popular all over the world and have brought significant income.

Many international organizations have been created to support the NUS – Crops for the Future (CFF) in the UK, which has followed the International Centre for Underutilized Species (ICUC) in Sri Lanka or Global Facilitation Unit for Underutilized Species (GFU) in Italy (Mwamba 2006). The vision of the CFF (2020) is to be a "world leader producing excellent, innovative research on underutilized crops that is responsive to societal demands." ICUC is a scientific research and training centre for increasing the use of underutilized crops for food, medicinal and economic use. For the development and research of genus *Strychnos* in Zambia, there are institutions such as the Division of Forestry Research, Tree Improvement Research Centre, or Zambia/ICRAFT Agroforestry Project (Mwamba 2006).

2.2. The genus *Strychnos*

Kingdom: Plantae, plants

Subkingdom: Tracheobionta, vascular plants

Superdivision: Spermatophyta, seed plants

Division: Magnoliophyta, flowering plants

Class: Magnoliopsida, dicotyledons

Subclass: Asteridae

Order: Gentianales

Family: Loganiaceae, Logania family

Genus: *Strychnos*

Species: *S. pungens* Soler, *S. cocculoides* Baker

By the old Tachtadžjan classification, the Strychnaceae family is classified as a separate family but nowadays is a part of the Loganiaceae family (USDA, NRCS 2021).

The genus *Strychnos* is distributed mainly in tropical Africa, South America, Southeast Asia, and Australia and contains around 200 species. It is the biggest genus of Loganiaceae family (Rajesh et al. 2011). In Africa, there are 75 known species, among them, the most widely distributed is *S. spinosa* which is used as a source of food, medicine, and wood (Leeuwenberg 1969).

Many species of the *Strychnos* genus are recognized under the common name Monkey orange, reflecting the fact that monkeys, together with forest antelopes – duikers, are usually the biggest consumers of the fruits (National Research Council 2008). The common names of species that are on the focus of this thesis are spiny-leaved monkey-orange, mahwahwa (Lozi language), mumbumi, umgwadi, botter klapper, shiny-leaved mukwakwa, wild orange, or swart klapper for *S. pungens* (National Research Council 2008); and corky–bark monkey orange, kavango lemon, morapa, mahuluhulu (Lozi language), mohoruhoru, omukwakwa, geelklapper, gifklapper, grysklapper, bitter bush orange, corky monkey-orange, corky-bark monkey-orange for *S. cocculoides* (Stellenbosch University Botanical Garden 2021).

2.2.1. Distribution and ecology of *S. pungens* and *S. cocculoides*

S. pungens and *S. cocculoides* are native to Southern Africa but can be found from the Democratic Republic of the Congo to the South African Republic (Figure 1). Unlike *S. pungens*, *S. cocculoides* has been recorded in Burundi, Gabon, Kenya, Mozambique, and Uganda and it was also introduced to India and South America (PROTA 2021a; PROTA 2021c; USDA, NPGS 2021a; USDA, NPGS 2021b).

Both plants species have similar ecological requirements, they grow on dry, deep, acidic sandy soils with pH in the range of 5.5–7.5, tolerating 4.5–8. Annual precipitation should be in the range of 600–1,200 mm but *S. pungens* can survive with a mean annual rainfall of 250 mm and it is also salt tolerant. The ideal daytime temperature is within the range of 14–25 °C. Both species can be found in open woodlands, mixed forests, deciduous woodlands, rocky slopes, and even on the fringes of the Kalahari Desert (Mogotsi et al. 2019; Ngwako et al. 2019; PROTA 2021a; PROTA 2021c). The trees are commonly sharing the open space with the genus *Brachystegia*. An interesting adaptation strategy was reported in the case of *S. cocculoides*, which can survive in very poor soils due to the presence of arbuscular mycorrhizal fungi which is enhancing the plant's uptake of soil nutrients (Mwamba 2006; PROTA 2021a). However, if the trees grow on shallow soils, wind can cause stem breakages. Also, light frosts were reported in some areas of the natural distribution of species, so the growth of plants might be a problem there (Mwamba 2006).

The woodlands with *Strychnos* sp. are annually burned so the plants had to create a survival strategy. In the case of *S. cocculoides*, the corky bark became an insulation layer and does not burn easily. According to Timberlake et al. (2010), *S. pungens* is semi-tolerant species to fire, stable by early dry season fires but does not tolerate late dry season fires. During the fire, the fruits of both species stay on the tree and later can help with propagation (Glen 2009). The plants of *S. cocculoides* are adapted to dry tropical regions by growing only in places with moist soil. If there is a drought, *S. cocculoides* becomes dormant and loses leaves because of high transpiration efficiency (Mwamba 2006).

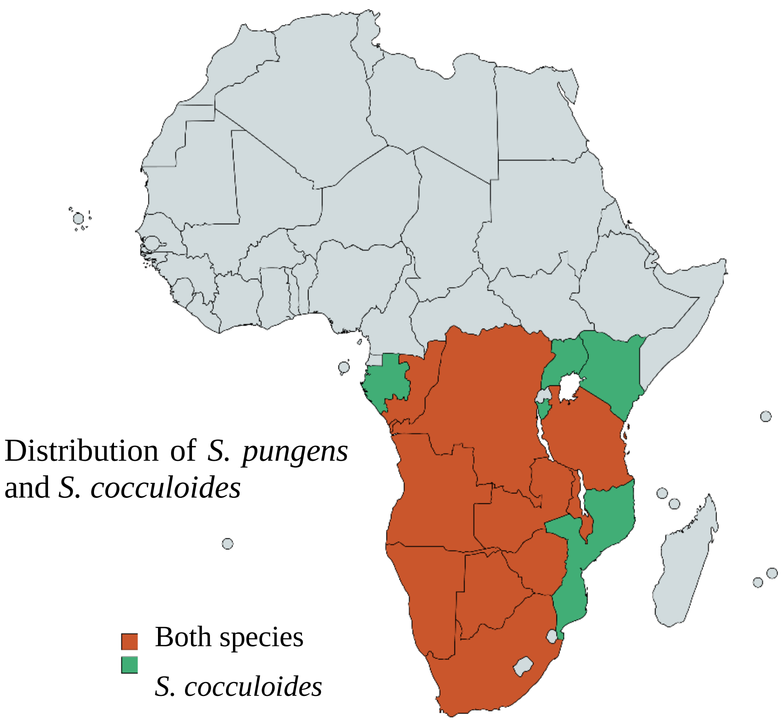

Figure 1. Distribution map of *S. pungens* and *S. cocculoides* in Southern Africa.

Source: Author (2021), based on data of PROTA (2021a); PROTA (2021c); USDA, NPGS (2021a); USDA, NPGS (2021b)

2.2.2. Botanical description

***Strychnos pungens* Soler**

S. pungens is a deciduous shrub or tree up to 8 m tall having a grey or brown bark which is smooth in smaller trees, becoming rough and shallowly reticulate, but not corky with age. The inner bark is yellow with yellowish wood. Branches are pale to dark brown with conspicuous lenticels and with short rigid lateral glabrous branchlets (Figure 3). Leaves are also glabrous, shining, and dark green above. The length of the leaves is 3–8 cm, the width is 3–5 cm. Leaves are elliptic, opposite, smooth but stiff, with a sharp spine-like tip, the glabrous petiole is 1–4 mm short. Flowers have greenish creamy white colour and usually are <9 mm long, in axillary or ramiflorous inflorescences of 2–5 cm long and 1–2 cm wide. Trees flower from September to October depending on ecological zones and countries of their occurrence (Mogotsi et al. 2019; PROTA 2021c). Pictures of *S. pungens* are presented in Appendix 1.

The shape of the fruit is round, 5–10 cm in diameter with a smooth, hard, woody shell. Fruits are bluish-green, turning yellow when ripe (Figure 2). The edible pulp is juicy and usually contains 20–30 disk-shaped hard seeds (Mwamba 2006). The seeds are 20–24 mm long, 7–12 mm wide, and flattened, they can be pale brownish yellow or whitish, and covered in very short, erect yellowish hairs (Mogotsi et al. 2019). It is known that *S. pungens* can hybridize with *S. innocua* and the new species could be seedless. The weight of one fruit is around 0.5 kg (National Research Council 2008).

Figure 2. Fruits of *S. pungens*. Unripe fruit (left), ripe fruit (right). Source: JMK (2012; JMK 2013)

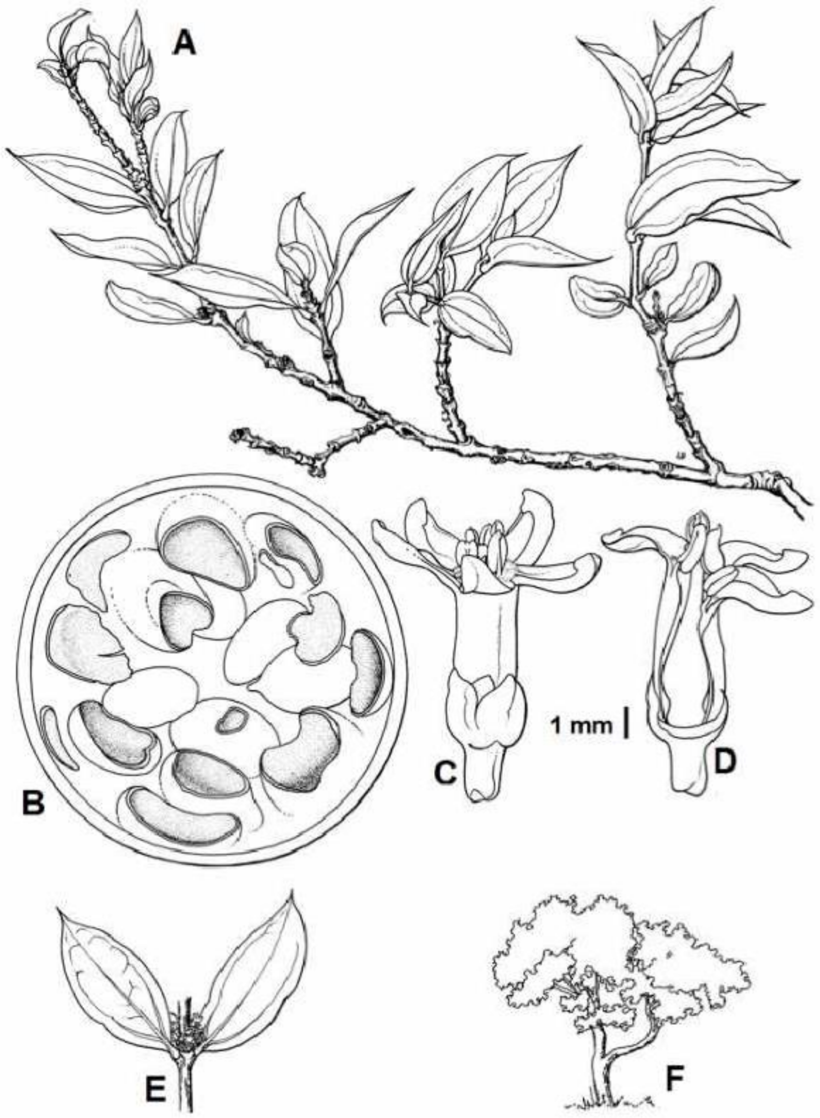

Figure 3. *Strychnos pungens*. A = branchlet with leaves, B = cross-section of fruit showing some seeds, C & D = flowers, E = leaf attachment to stem/branch, axillary inflorescence, F = canopy shape drawing, not to scale. Source: Deysel (2015)

***Strychnos cocculoides* Baker**

S. cocculoides is a semi-deciduous shrub or tree, occasionally semi-lianoid, up to 8 m tall with an open rounded crown. The bark is thin, corky, pale grey to pale brown, rough with large lenticels (Ngwako et al. 2019). The wood of this species is very hard, usually with bark-islets (Leeuwenberg 1969). Branches are pale or dark brown, fissured, corky and spiny. The length of the spines on branches is 1–1.5 cm and they are usually straight. The opposite leaves are up to 6 cm long and 4 cm wide with hairs on both sides, leaves on old fire-cut stumps are smaller (Mwamba 2006; SCUC 2006). Pale to dark green leaves are coriaceous, orbicular to ovate-elliptic with 1–3 pairs of secondary veins from the base curved along the margin. Small greenish male and female flowers of the terminal inflorescence are carried on the same plant in short dense compact heads on main branches (Figure 5). Flowers are pentamerous, sepals and petals are pale green, white or greenish-yellow. The tree blooms mainly from September to December depending on ecological zones and countries. It takes 8–9 months from flower fertilization to fruit ripening (Ngwako et al. 2019). Pictures of *S. cocculoides* are presented in Appendix 2.

The fruit is of the size of grapefruit, having on average 6–12 cm in diameter. The colour of unripe fruit is bluish, often speckled with green, dark green when nearly mature, and orange when ripe (Figure 4). The edible ripe brown pulp of sweet taste contains 10–100 irregularly curved seeds up to 2 cm in diameter (Mwamba 2006; SCUC 2006). Seeds are light brownish yellow, flattened and pubescent (Ngwako et al. 2019).

Figure 4. Fruits of *S. cocculoides*. Unripe fruit (left) and ripe fruit (right). Source: Schmidt (2008a); Glen (2009)

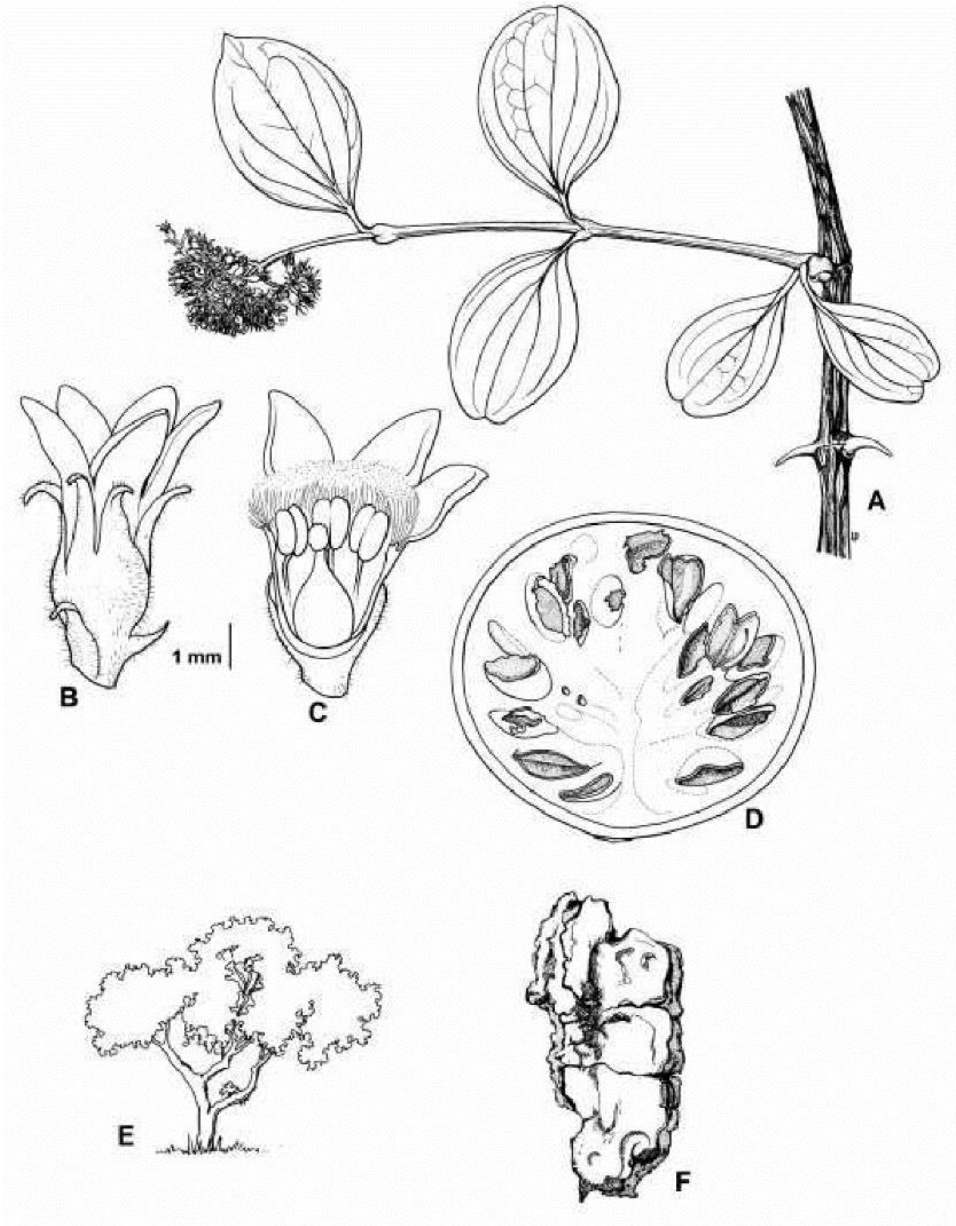

Figure 5. *Strychnos cocculoides*. A = branchlet, leaf and inflorescence, B & C = flowers, D = cross-section of fruit, E = canopy shape drawing, not to scale, F = corky bark. Source: Deysel (2015)

2.2.3. **Propagation and management**

Propagation of *S. cocculoides* is possible by seeds, coppicing, or root suckers. However, it is usually done by seeds that are reported to be physiologically dormant. The germination could be done by soaking the seeds in hot water and allowing them to cool for 24–48 hours before sowing. The seeds have short viability of two months maximum when stored at room temperature (Ngwako et al. 2019; Fern 2019a). The seeds are sown in a depth of 2–3 cm in pots or seedbeds and germinate after about a month. They have to be watered twice a day for the first weeks. After germination, the seedlings should be kept in shade, before being exposed to full light after 14 days. Direct sowing of seeds in the field leads to patchy germination and poor seedling survival. It is also possible to graft scions of *S. cocculoides* onto *S. spinosa* rootstocks in faster growth of *S. cocculoides*. According to National Research Council (2008), grafted trees grow twice as fast as ungrafted ones and start to produce fruits in 3^{rd} year instead of 4^{th} or 5^{th} as usual.

For the cultivation of *S. cocculoides,* it is necessary to find a place ideally with deep sandy soils or well-drained slopes according to the ecological requirement mentioned above. The prepared site should be dug over to 20–30 cm depth and without weed and stones. The seedlings are prepared when they are 6 months old or at least 10–20 cm in height. They have to be watered the day before planting. The seedbeds raised above ground level should be 1 m wide and 5–10 m long and the paths between the beds 60 cm wide. The pots with young trees are transplanted to seedbeds with holes 60 cm deep and 60 cm wide on cloudy days or in the morning and they are covered with frames from reeds, bamboo, or wood to have sun protection. The plants are watered three times a week during the first weeks in the early evening or as needed. Mature trees normally do not need irrigation. The weed is regularly taken away once a month otherwise, it competes with plants for light, water, space, and nutrients. Normally, *Strychnos* species grow and produce good crops without fertilizers but small-scale farmers can fertilize the trees with organic matter as leftovers from the kitchen or manure to increase yields (SCUC 2006). As reported by Mwamba (2006), less than 1 % of local farmers apply inorganic or organic fertilizers to farm forestry trees.

The agroforestry system of *S. cocculoides* grown together with marula (*Sclerocarya birrea*), wild medlar (*Vangueria infausta*), sorghum (*Sorghum* sp.), cowpea

(*Vigna unguiculata*), and watermelon (*Citrullus lanatus*) brings higher yields. Trees can also be planted as a boundary or barrier (Mwamba 2006; SCUC 2006).

Savanna species such as *S. pungens* and *S. cocculoides* flower once per year before the bushfire in the dry season (Leeuwenberg 1969). The fruit production starts between the 4th and 6th year and the tree can bear about 300 to 700 fruits per season. Fruits ripen from June to December depending on the ecological area (SCUC 2006).

No information is available about the propagation and cultivation of *S. pungens* (Mogotsi et al. 2019; Fern 2019c), but it seems that the cultivation methods are similar to *S. cocculoides.*

Even though the species do not generally suffer from pests and disease infestation, termites of *Isoptera* genera can damage old trees and the ones in poor condition. However, they usually do not penetrate the live stem and stay in a dead bark. The powdery mildew of the genera *Uncinula*, *Erisiphe*, *Spaerotheca*, or *Leveillula* attacks the outer skin of fruit or seeds during storage but it cannot damage the pulp inside because of the hard shell about 2–5 mm wide, or the seed coat (Mwamba 2006; SCUC 2006).

2.2.4. **Harvesting and storage**

Harvesting can be done by climbing the tree or by picking the fallen fruits from the ground, which is also an indicator of fruit maturity. Only ripe fruits should be eaten to avoid abdominal pains. The fruits are collected mainly by women and children from homegardens, forests, farmer's fields, or along the main roads where trees of *Strychnos* also grow. These people are, in most cases, poor or from marginalized groups. After the harvest, the fruits are immediately eaten or sold in local markets (Mwamba 2006).

The hard shells of the fruits are cracked open by a gentle tap on a stone or by hitting with a stick (Mwamba 2006). Seeds are easily removed from the pulp and then cleaned and sorted by mixing with sand and sieving. It is also possible to harvest unripe fruits and bury them in the sand where they can ripe slowly. If the fruits are going to be stored, they must be air-dried to prevent surface infection and stored in grain bags, cloth packs, or boxes (PROTA 2021a; PROTA 2021c). The shell stores the juicy pulp even for a few months in untouched condition (Mwamba 2006; National Research Council 2008).

2.2.5. Fruit chemical composition of *S. pungens* and *S. cocculoides*

Nutritional content

Not much information is available on the nutritional values of *S. pungens* whereas the fruits of *S. cocculoides* have been analysed few times by various scientists on both bases – dry weight and wet weight. The fruits of *S. cocculoides* were obtained in Tanzania, Malawi, Namibia, Botswana, and Angola. Results of broad literature research are shown in Table 1. Macronutrients of *S. cocculoides* vary as following: carbohydrates 18.2–90.8 g/100 g, protein 0.6–11.5 g/100 g, moisture 72.2–82.28 %, fat 0.0013–6.0 g/100 g, fibre 1.9–17.9 g/100 g, ash 2.2–5.4 g/100 g, energy 1390–1477 kJ/100 g. These big deviations can be caused by different analysis methods, climatic and soil conditions, habitats of plants, maturity of the fruits, etc.

Table 1. Nutritional content of *S. pungens* and *S. cocculoides* by various authors

	S. pungens	***S. cocculoides***			
	Ngadze (2018)*	Ngadze (2018)*	Mapunda & Mligo (2019)	Ndabikunze et al. (2006)	Saka et al. (2008)**
g/100 g	mean	mean	mean	mean	mean
Carbohydrates	21.3	18.2	56.01	90.8	61.0
Protein	4.3	3.5	0.94	0.6	11.5
Moisture	68.2	78.2	82.28	74.9	72.2
Fat	1.8	0.4	0.0013	1.3	6.0
Fibre	13	4.6	14.45	1.9	17.9
Ash	2	2.2	NE	5.4	3.7
Energy kJ/100 g	1,363	1,477	NE	NE	1,390

NE – not examined, *based on the research of Arnold et al. (1985) and Malaisse & Parent (1985); ** fresh sample analysis; data were measured using dry matter basis; all data were converted to g/100 g

Based on available data of the authors mentioned in Table 2 and van Rayne et al. (2020), the species of *S. cocculoides* was reported to have the highest content of iron of the genus *Strychnos*. The mean content of Fe of dry matter in mg/100 g was defined as following: *S. spinosa* – 3.3, *S. cocculoides* – 34.0, *S innocua* – 11.4, *S. pungens* – 6.1, *S. madagascariensis* – 15.78, when *S. cocculoides* has been identified to contribute more than 100 % of the recommended daily intake for Fe, especially for children between four and eight years and for pregnant women. There were 39 % of young women affected by anaemia in sub-Saharan Africa in 2016 (Ngadze 2018) and the source of these available fruits could help to handle it in the future. *S. pungens* significantly contributes to the Recommended Dietary Allowance (RDA) of vitamins thiamine and riboflavin (Table 2). According to the mineral analysis made by Mapunda & Mligo (2019), *S. cocculoides* contained 0.73 ± 0.08 mg/100 g Na and 0.01 ± 00 mg/100 g Zn.

Table 2. Mineral composition of *S. pungens* and *S. cocculoides* by various authors

	S. pungens	***S. cocculoides***				
	Ngadze (2018)*	Ngadze (2018)*	Mapunda & Mligo (2019)	Ndabikunze et al. (2006)	Saka et al. (2008)**	RDA National Academy of Sciences (2019)
mg/100 g	mean	mean	mean	mean	mean	mg/day
Fe	6.1	70.5	5.65	0.07	60	18.0
P	133.6	116.5	61.70	0.02	2,106	700.0
Ca	67.5	46.5	58.96	1.04	60	1,000
Mg	136.6	137.2	NE	0.25	1,633	310.0
K	1,718	959.2	476.66	1.68	28,670	2,600
Cu	1.28	0.36	NE	NE	NE	0.9
Thiamine	1.40	0.03	NE	NE	NE	1.1
Riboflavin	1.30	NE	NE	NE	NE	1.1
Vitamin C	38.6	34.2	93.03**	NE	22.9	75.0

NE – not examined, *based on the research of Arnold et al. (1985) and Malaisse & Parent (1985); **analysis of fresh sample; RDA is based on an adult female; data were measured on a dry matter basis

Analysis of fresh pulp of *S. cocculoides* by Saka et al. (2008) shows that the sample covers RDA for Fe, P, Mg, and K, whereas the fresh sample determination by Mapunda & Mligo (2019) covers RDA for vitamin C.

Secondary metabolites

Genus *Strychnos* is famous for a high content of alkaloids, saponins, and tannins in the seeds (Mwamba 2006). One of the most known toxic alkaloids, strychnine, was reported to be a component of *S. cocculoides* seeds but the exact content is unknown, only a small amount is mentioned (Mwamba 2006; Orwa et al. 2009; Fern 2019a). It is a colourless, odourless compound with a bitter taste. LD_{50} of strychnine for an adult male is 0.47 mg/kg (Philippe et al. 2004). Clinical signs of strychnine poisoning usually start 10–30 minutes after ingestion by apprehension, muscle tremors, and muscle tics when people under seizures are extremely sensitive to light and sound. The antidote for poisoning are barbiturates (National Center for Biotechnology Information 2021). Strychnine is also known as a part of South American arrow poison called “curare” (Mwamba 2006), but it can either save a life after a snake bite such as a mamba because it is a great stimulant for the human nervous system (Grahl 2017). The dimethoxylated analogue of strychnine is brucine, another alkaloid found in the seeds of *S. nux-vomica* (Figure 6) (Phillipe et al. 2004).

Although seeds are not usually consumed, when swallowed, they pass the body safely but if chewed they may cause vomiting and headaches. As far as it is known, there is no scientific evidence of a presence of the strychnine in the seeds of *S. pungens,* but it is possible that toxic alkaloids like strychnine degrade with maturity, and because of that they do not cause any damage to the digestive tract (Ngadze 2018). There are different data in many publications, and further research is suggested. Eleven indole alkaloids have been found by Thépenier et al. (1990) in leaves, stem bark, and root bark of *S. pungens*: O-acetylretuline, diaboline, 11-methoxydiaboline, 11-methoxyneo-oxydiaboline, 12-hydroxy,11-methoxydiaboline, henningsamine, 11-methoxyhenningsamine, 12-hydroxy,11-methoxyhenningsamine, sitsirikine, 16(R)-isositsirikine, and 16(S)-isositsirikine which are also known as curare alkaloids. They accelerate nerve transmission and are poisonous in bigger amounts.

It was recorded that the pulp of *S. cocculoides* contains phenolic compounds such as caffeoylquinic acid (CQA), quinic acid, kaempferol, quercetin, caffeic acid, protocatechuic acid, and iridoids. Phenolics are an important group of bioactive compounds in plant materials that have antioxidant properties for human health (Ngadze 2018). There are also saponins in the pulp of both studied species (Mwamba 2006).

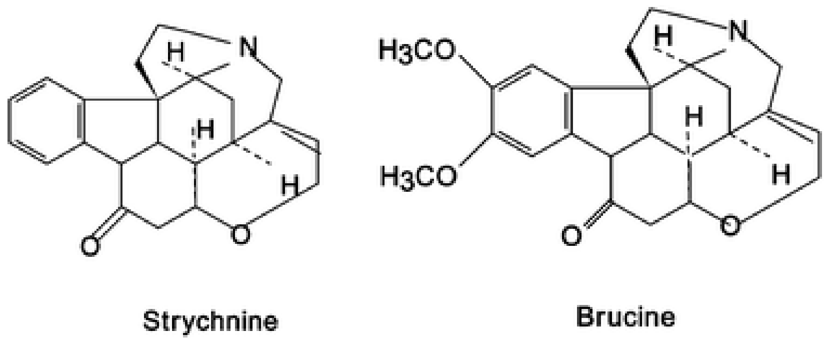

Figure 6. Chemical structure of strychnine and brucine. Source: Mishra et al. (2013)

2.2.6. **The use of *S. pungens* and *S. cocculoides***

Both *Strychnos* species are mainly used as fruit trees, while the fresh pulp hidden in a woody shell is consumed. The taste of the pulp of *S. cocculoides* has been described as a "mixture of citrus and banana or a combination of citrus and pineapple" (National Research Council 2008). Fruits of *S. pungens* are reported to be less tasty than *S. cocculoides* or *S. spinosa*. Due to the content of saponins, the pulp is also used for soap production and rounded woody shells of *Strychnos* sp. are being used as containers, rattles, or musical instruments (ICUC 2004; Mwamba 2006). Both *Strychnos* species provide shade, shelter, timber, firewood, and are grown in agroforestry systems, especially in homegardens, or harvested from the wild (SCUC 2006; Fern 2019a; Fern 2019c). The wood of *S. cocculoides* is soft and fine, used to make posts and tool handles (Mwamba 2006). Because of the presence of the enzyme pectinase, the edible pulp of *S. cocculoides* is used in food processing to make jams, jellies, muffins, and fritters. The pulp is also processed into juices, wine, beer, or local spirits such as Namibian kashipembe (Mwamba 2006; Bille et al. 2013; Ngadze 2018). Both species have local medicinal uses, crushed roots of *S. cocculoides* cure abdominal pains, gonorrhoea, sore throat, and infertility in men (Maroyi 2011). Unripe green fruits induce vomiting and have a laxative effect. Fresh mashed leaves are used for the treatment of wounds to prevent infection and promote healing and they could be used as insect repellent. By people of the Ivory Coast, the decoction of the bark is drunk and used as a lotion for elephantiasis of the scrotum and abortions (Neuwinger 1996). However, no clinical information is available to confirm these effects (SCUC 2006).

2.2.7. **Socio-economic value**

In Botswana, the fruits of *S. cocculoides* are sold by women along the roadsides or at local markets, which brings the important additional cash income for their households (Mwamba 2006). In Zambia, indigenous fruits such as *S. cocculoides* and *S. pungens* can be collected from forests and public land for free. The supply chain is usually short and consists mainly of collectors of fruits and consumers which can be even collectors themselves. The fruits are transported on foot, using the bicycle or public transport if needed, however, the roads to the markets are usually in bad conditions so it can contribute to the damage of fruits (Karaan et al. 2005). By the research of Elago &

Tjaveondja (2015) in Namibia, *S. cocculoides* has big socio-economic importance because the sale of these fruits has positively changed the standard of living. Local people have reported that indigenous fruits not only contribute to food security but also to additional income generation because from their sale they can buy more food, clothes, some livestock and even pay school fees for their children or hospital bills.

2.2.8. **Related species**

The most widely distributed species of Loganiaceae family is *Strychnos spinosa* Lam. It is a deciduous shrub or tree about 5 meters, and it can be found in tropical and subtropical Africa, especially in savannas but it also tolerates light frosts and elevations up to 2,200 metres above sea level (m.a.s.l.) (Leeuwenberg 1969). The species has also been cultivated in Madagascar and the southern part of Florida (Fern 2019d). The common name is green monkey orange because of the green colour of the shell when unripe and orange when mature (Figure 7). The round fruit can be up to 15 cm in diameter and is called "one of the best native fruits" due to edible acid-sweet, tasty pulp. Two fruiting forms are known – sweet and bitter. Seeds and bark contain the alkaloid strychnine. Smooth ridged branchlets ending in a straight spine are a distinguishing sign from *S. cocculoides*. The usage is similar to previously described species (National Research Council 2008). *S. spinosa* has been introduced to Israel as a potential commercial crop (Sitrit et al. 2003).

Another popular *Strychnos* species is *Strychnos nux-vomica* L. commonly named as a strychnine tree. This spined tree grows in Southeast Asian rainforests, especially in Indonesia but it can also be found in tropical Africa. The fruit is a globose berry 2–6 cm in diameter, orange, glabrous with 1–4 orbicular silky-haired seeds (Figure 7) (PROTA 2021b). Unlike *S. cocculoides*, this species starts to produce fruits after 10–15 years (Fern 2019b). All parts of the plant contain strychnine which is used by native Americans as the arrow poison curare – it paralyzes the muscles of prey. To reduce the content of strychnine and brucine in seeds, they are heated in oil. In India, the fruits are used in traditional Ayurvedic and Unani medicine, and seeds are used for dying cloth pale brown or as a component of liquors. The wood is very hard, and it is used for tool handles (PROTA 2021b).

Besides the previously described species, there are *S. innocua*, *S. lucens*, *S. madagascariencis*, *S. potatorum,* and many more, while *S. lucens* is typical for Zambia and *S. innocua* hybridizes with *S. pungens* (Mwamba 2006).

Figure 7. *S. spinosa* (left), *S. nux-vomica* (right). Source: Schmidt (2008b); Garg (2009)

2.3. Zambia

Zambia is a landlocked country in the tropics of Southern Africa. It borders on the Democratic Republic of the Congo in the north, Tanzania in the north-east, Malawi in the east, Mozambique in the southeast, Zimbabwe and Botswana in the south, Namibia in the southwest, and Angola in the west (Figure 8). Zambia was known as Northern Rhodesia in the 20th century, and it had been a British colony until 1964, therefore English became the official language. The capital city is Lusaka (Roberts et al. 2020). The state is one of the least developed countries in the world with high chronic poverty (People in Need 2021).

Although Zambia lies within the tropical zone, its climate is modified by the altitude of the country – between 1,000 and 1,600 m.a.s.l. We can find here humid subtropical climate, tropical savanna climate, hot-summer Mediterranean climate, hot semi-arid climate, warm-summer Mediterranean climate, and subtropical highland oceanic climate. The dominated is the humid subtropical climate, Cwa, by Köppen-Geiger classification (Climate-Data 2021).

Two main seasons are changing over the year, the rainy season lasts from November to April and the dry season from May to October. The hottest month is usually October in which the harvest of many tropical plants is done, including *Strychnos* sp., while June is the coldest period of the year (Roberts et al. 2020).

The estimated land area of Zambia is 752,610 km^2, whereas the agricultural land comprises 32 %. However, only 16 % of the agricultural land is currently under cultivation (FAO 2018a). Zambia has many water resources such as lakes and rivers but only 12 % of this surface water is used for irrigation systems, so the farms mainly depend on rain-fed growing cycles (FAO 2004). Agriculture employed 50 % of the Zambian population in 2019 (World Bank 2021). Food crops production is mainly composed of maize, soybeans, sugar cane, rice, groundnuts, and cassava. From the livestock, the most numerous were cattle, goats, and pigs, followed by chickens, sheep, donkeys, and bees in 2018 (FAO 2021). Zambian agriculture is divided into three sections: small, medium, and large, while small–scale farmers create more than 70 % of the farming population but cultivate less than 5 ha and use basic tools (FAO 2004).

The population of Zambia grows rapidly at 2.8 % per year, every 25 years is doubled, and the current population is about 18.7 million inhabitants (World Bank 2020). The high population growth is given by immense fertility e.g., live births per woman in 2020 were 4.7 (Worldometer 2021). GDP per capita in 2019 was 1,305 USD while in Ghana it was 2,200 USD and 2,790 USD in Angola. GDP shows the country's economic output divided per person (World Bank 2021). HDI in Zambia in 2020 was 0.58 while being 0.46 in Mozambique, in Angola was the same as in Zambia, in Ghana 0.61, in Botswana 0.74 and in the Czech Republic 0.9. The more developed country, the higher the number is. HDI measures long and healthy life, knowledge, and a decent standard of living (The Global Economy 2021). Another economic indicator GNI per capita in Zambia was measured as 1,430 USD in 2019 unlike Angola, where it was 2,960 USD and in Botswana 7,650 USD. GNI calculates the total income earned by the nation's people and business including the money from abroad (World Bank 2021).

The country is divided into 10 provinces and has more than 70 ethnics groups which make around 70 different dialects. There are seven main tribes: Lozi, Bemba, Ngoni, Tonga, Lunda, Luvale, and Kaonde (Nyirenda et al. 2007). The ethnicity of the people has a big influence on their eating habits. The most popular meal is nshima – a thick porridge from maize. In the Global Hunger Index (measures and tracks hunger at the global, regional, and country levels) in 2019, Zambia ranked 113th out of 117 qualifying countries and suffered from an alarming level of hunger (Grebmer et al. 2019). According to World Bank (2021), poverty in Zambia reached 54.4 % in 2015 which means the percentage of the population living below the national poverty line. Malnutrition is a common problem with fatal consequences when combined with other factors such as malaria, diarrhoea, tuberculosis, or HIV/AIDS (Nyirenda et al. 2007). Zambia's lower life expectancy of 63.5 years in 2018 (World Bank 2021) and higher death rates are attributable in part to the prevalence of HIV/AIDS in the country (Roberts et al. 2020). Access to basic drinking water has a positive trend, it raised from 49.5 % of the Zambian population in 2000 to 60 % in 2017 (FAO 2021).

Figure 8. Map of Zambia. Source: Wikipedia Commons (2011); Cornall (2016)

2.4. Lyophilization

Lyophilization, also known as freeze-drying, is a new popular method of drying in a vacuum at very low temperatures to remove moisture content (about 95 %) from the food, especially fruits. It works on the sublimation of ice that removes water from previously frozen material. The process has three main phases – freezing, primary drying, and secondary drying. In the beginning, the material is crushed and filled with liquid nitrogen (N_2). After freezing, the samples are dehydrated in a freeze dryer under the vacuum. In this second phase, approximately 90 % of water is removed from the material. The third phase repeats drying to remove as much residual moisture as possible (Marques et al. 2006; Ivančević et al. 2012).

The advantages of this process are higher nutritional quality (Table 3) as well as texture, taste, smell, fast rehydration, and colour as fresh food, which is not common in solar or hot-air drying. According to the analyses of Ivančević et al. (2012), freeze-dried raspberries better preserved L-ascorbic acid (54 %), anthocyanins (51 %), had a lower loss of total flavour (66 %), lower volume reduction (92 %), and higher porosity (49 %) compared to convectively dried fruits. Also, the transportation of lyophilized fruits is easier as freeze-dried products do not need to be stored in freezers and the durability of storage is 5–15 years with residual humidity up to 2–5 %, which contributes to the prevention of wasted food. However, the price of the lyophilized products is much higher than solar or hot-air dried due to the duration of the whole freeze-drying process which takes around three days (Marques et al. 2006).

Table 3. Change of the nutritional content of *Psidium guajava* depending on the drying method

Psidium guajava	**Fresh fruit**	**Hot-air drying**	**Freeze-drying**
Analyses	Vasquez-Osorio et al. (2014)		Moreno et al. (2014)
Protein %	1.02	6.48	20.0
Carbohydrates %	13.82	87.75	29.87
Fibre %	5.57	35.37	12.7
Fat %	0.11	0.70	0.05
Vitamin C (mg/100 g)	87.18	553.52	143.49

2.4.1. The impact of freeze-drying on nutrients

By regular methods using high temperatures to dry fruits, the amount of vitamins, antioxidants, flavour, aroma, and other organic compounds normally decrease e.g., vitamin C is relatively unstable to heat, oxygen, and light so the better way how to determine it is the lyophilization (Ivančević et al. 2012). The content of vitamin C in fresh and freeze-dried chosen fruits varies as mentioned in Table 4 so there has not been found any common principle about increase or decrease the content of vitamin C and it is probably specific for each fruit species. In the research of Hawlader et al. (2006) was found that the freeze-drying method retains more vitamin C in papaya (88 %) than other drying methods such as drying by vacuum (86 %), normal air (75 %), or CO_2 (82 %). Freeze-dried fruits have usually higher sugar content than their fresh equivalent due to the absence of water e.g., fresh strawberries contain 4.9 % sugar, and lyophilized form contains 71 % sugar in 100 g, taking into account the regular portion of lyophilized fruits stated at 20–30 g (Mellor et al. 2017).

2.4.2. **Trade of freeze-dried products**

According to the research of Pulidindi & Prakash (2019), it is estimated that the freeze-dried fruits and vegetables market reached 40 billion USD in 2018 and continues with 7.18 % of the Compound Annual Growth Rate (CAGR) from 2019 to 2025. The most traded fruits are strawberry, raspberry, blueberry, blackberry, and cranberry because the lyophilization is the best process how to keep nutritional and sensoric values for these berries. Based on application, freeze-dried products are mostly used in bakery and confectionery, breakfast cereal, soups and snacks, ice cream, and desserts. Major companies of these products are European Freeze Dry, Döhler, Mecer Foods LLC, Van Drunen Farms, Paradise Fruits, and Sleaford Quality Foods Ltd (Mordor Intelligence 2020).

The forecast for 2025 is to reach more than 60 billion USD (Pulidindi & Prakash 2019). The fastest growing market of freeze-dried products are regions in Asia-Pacific (China, Japan, Australia, India, and rest of Asia-Pacific) while the United States is the largest market (Mordor Intelligence 2020).

Table 4. Content of vitamin C in fresh and lyophilized form

	carambola (*Averrhoa carambola*)	**mango (*Mangifera indica*)**	**muskmelon (*Cucumis melo*)**	**acerola *(Malpighia emarginata*)**	**papaya (*Carica papaya*)**	**watermelon (*Citrullus lanatus*)**	**guava (*Psidium guajava*)**
Vitamin C (mg/100 g)	Serna-Cock et al. (2015)*				Shofian et al. (2011)		Moreno et al. (2014); Vasquez-Osorio et al. (2014)
Fresh weight	4.99	8.36	2.24	1,021	16.57	1.75	87.18
Lyophilizate	4.67	8.34	2.75	153.4	16.84	2.38	143.49

*Based on data from Marquez et al. (2007); Shofian et al. (2011)

3. Objectives

In this thesis, three main objectives were determined:

1. To systematize available information on the *Strychnos pungens* Soler and *Strychnos cocculoides* Baker species and make an overview of their current and potential use,
2. to evaluate the nutritional content of the fruit pulp from the lyophilizate (ash, crude fibre, crude protein, crude fat, and vitamins B + vitamin C) and
3. to compare the results with other existing studies on the same/similar fruits.

4. Materials and methods

4.1. Study site and data collection

The data collection was conducted in November 2019 in Mongu and surrounding areas by CZU specialists (Figure 9). The ripe fruits were obtained from the local farmers as well as from the fruit market directly in Mongu. Voucher specimens of *S. pungens* and *S. cocculoides* were taken and stored in the Herbarium of FTA, CZU. The species identification was proved by botanical experts from the University of Barotseland, Mongu. The city of Mongu is the capital of Western Province, one of ten provinces in Zambia. The province is divided into 16 districts including Mongu (Milupi et al. 2020).

Figure 9. Map of Barotse floodplain with the main cities. Source: Kaminski et al. (2020)

4.1.1. **Climate**

Western Province has three different climates, the Mongu area is classified as a Tropical savanna climate as Aw by Köppen and Geiger. It is also called the tropical wet and dry climate. Precipitation in Mongu town is around 1,070 mm per year, and the average annual temperature is 23.1 °C, while July is the coldest month with an average of 19.2 °C. The highest temperatures occur just before the rainy season in October when the average temperature in Mongu is 28 °C. The wet season is between November and March with the highest rainfall in January when the number of rainy days is 18 with 80 % humidity. During the dry season, there is almost no precipitation at all. Town of Mongu lies in the elevation of 1,035 m.a.s.l. (Climate-Data 2021).

4.1.2. **Agriculture**

The major economic activities of Western Province are cattle rearing, fishing, and subsistence farming. Mongu East and the Barotse floodplains are the main agricultural production areas. About 90 % of the population are farmers, and they use the moist and fertile land to grow maize, cassava, millet, and rice, the Barotse floodplain is the biggest producer of rice in Zambia. The sandy soils in the east of the province are also good for plantations of cashew nuts (*Anacardium occidentale*), which became a key cash crop in Western Province (Milupi et al. 2020). Mongu is on the edge of the Barotse floodplain of the Zambezi river (Figure 9) so the typical farming system of this area is a wetland agricultural system (Baidu-Forson et al. 2014). The plain floods from January up to May or June (Zambia Alliance For Agroecology & Biodiversity 2019). Agricultural techniques used by small-scale farmers include hand hoes, animal draft power, and hired labour for farm operations. Poorly developed infrastructure is the main problem for agriculture because farmers cannot easily access their fields and enter the urban markets to sell their products. During October to January known as a “hungry season”, crops from the last season get exhausted, new ones are usually not available, resulting in food insecurity. Based on the research of the Soil Survey Unit of Mount Makulu Central Research Station, the predominant soil type in Western Province are aerosols with seasonally waterlogged gleysols and associations along the river courses. The aerosols are acidic and not rich in nutrients, but these conditions suit *Strychnos* species, especially the mahwahwa and mahuluhulu (Baidu-Forson et al. 2014).

Since 2002, the Government of Zambia has been funding a farm input support programme (FISP) that gives access to inorganic fertilisers and seeds for monoculture cash crops, especially seeds of hybrid maize. Though, maize is not suited for sandy conditions on flood plains. This kind of farming contributes to the degradation of native ecosystems because the plant diversity together with soil fertility declines. Some of the farmers in the Mongu district try to switch to sustainable organic agriculture (SOA) but appropriate training of farmers, farming equipment, irrigation, policy changes, and protection of indigenous forests instead of burning them are needed. The idea of SOA is "not to bring something totally foreign to farmers, but to build on existing farmers' knowledge and traditional practices and combine these with modern scientific understanding" (Zambia Alliance For Agroecology & Biodiversity 2019).

4.2. Samples preparation

Samples were prepared in laboratories of the Department of Food Science and the Department of Microbiology, Nutrition, and Dietetics at the Faculty of Agrobiology, Food and Natural Resources CZU in June 2020. Fruits were taken from the freezer, where they have been stored until the time of the analyses. The first step was to break the hard woody shell of both *Strychnos* species. Then the still frozen fruit pulp was removed from the shell and carefully cut and scraped from the seeds and filled with liquid nitrogen (N_2). Later, the material was ready for lyophilization which took three days. Followingly, the lyophilized fruits were ground in a kitchen blender, resulting in a homogenized yellow powder of a nice smell of tropical fruits. Pictures of obtaining the lyophilizate are presented in Appendix 3.

4.3. Chemical analyses and materials

The content of dry matter, ash, crude fibre, crude protein, crude fat, and vitamins B + vitamin C was determined from the fruit pulp of both *Strychnos* species. All the laboratory analyses were performed at least in three duplicates according to the AOAC standard methods (AOAC 2000). The results were calculated as an arithmetic average of measurements in complying with standard deviation in MS Excel spreadsheets.

Laboratory devices that were used for the analyses:

Drying oven – UN55 Single DISPLAY

Laboratory scales – AE200

Crude fibre analysis – ANKOM 200 Fiber Analyzer

Crude protein analysis – Kjeltec 2400, Digestion unit – MB 442

Crude fat analysis – SER 148 Solvent Extractor

Muffle furnace – LH 15/13

High performance liquid chromatography (HPLC) – UV/Vis analysis – UltiMate 3000

Centrifuge – ROTANTA 460 R

4.3.1. Dry matter determination

Small porcelain crucibles of a known weight and with the unique number were filled by 2–3 g of the lyophilized sample and weighted. All the crucibles with samples were put into the drying oven under 105 °C for 3 hours. After that, the samples were weighed, and the final weight of the dried sample was determined by the formula:

$$DM = \left(\frac{w3-w0}{w2}\right) \times 100$$

w_0 – weight of empty crucible (g)

w_2 – weight of sample (g)

w_3 – weight of crucible with sample after drying (g)

4.3.2. Ash determination

From the last step of dry matter determination, the crucibles with dried samples were put into a muffle furnace for calcination under 550 °C for 5 hours. The results were calculated using the formula:

$$A = \left(\frac{w3-w0}{w2}\right) \times 100$$

w_0 – weight of empty crucible (g)

w_2 – weight of sample (g)

w_3 – weight of crucible with sample after burning (g)

4.3.3. Crude fibre analysis

Determination of crude fibre was done using Filter Bag Technology on ANKOM 200 Fiber Analyzer. Filter bags of known weight were pre-dried at 103.5 °C and filled about 1 g of sample. After weighting, the bags were put on the welder to seal and given to the dryer for one day. Weighted bags were placed into the carrier pre-heat at 100 °C, three bags only in one layer and an additional blank one as a control bag on the top. Carrier was put into the vessel, loaded with the additional weight, and poured by 1.45 litres of distilled water with 12 ml of 98 % H_2SO_4. After 55 minutes of stirring and heating, the sulphuric acid solution was drained, and the carrier rinsed three times for five minutes with hot distilled water. The same procedure was repeated with a solution of 25 g NaOH poured by 2 litres of distilled water. The bags were dried with filter paper and placed in 250 ml of acetone. After three minutes, bags were put in the oven for four hours (103.5 °C). The weight of each bag was recorded, and the samples were put in the porcelain crucibles of known weight. The cups were annealed in a muffle furnace under 550 °C for 5.5 hours. The weight of burned content was noted and crude fibre calculated using the formula:

$$CF = \left(\frac{w1+w2-w3-(w4\times c)}{w5}\right) \times 100 \qquad c = \frac{w1}{w4}$$

w_1 – weight of filter bag after drying (g)

w_2 – weight of empty crucible (g)

w_3 – weight of crucible after annealing (g)

w_4 – weight of empty filter bag (g)

w_5 – weight of sample (g)

4.3.4. Crude protein analysis

Crude protein determination was performed by Kjeldahl method on Kjeltec 2400 apparatus. 0.5 g of sample was given into a glass digestion tube with four repetitions for each species. One mineralization tablet Kjeltabs CK (3.5 g K_2SO_4, 0.4 g $CuSO_4.5H_2O$) was put into each glass tube because it works as a catalyst. Then, 10 ml of H_2SO_4 (96 %)

and two times 5 ml of H_2O were added to each glass tube. After cooling for 45 minutes, samples were prepared for Kjeltec apparatus. 10 ml of distilled water was poured into each tube and it got blue colour. Glass tubes were put to Kjeltec machine one by one. After four minutes, the testing was done, and results were provided in % N × 6.25.

4.3.5. **Crude fat analysis**

The fat content of samples was determined by continuous extraction with petrol ether using SER 148, Solvent Extractor – Soxhlet apparatus. At first, extraction glasses of known weight were filled with paper test tubes and tared on the scale. After that, 2–3 g of sample were added into test tubes, weighted, and pressed by cotton wool. A magnetic ring was given on the top of each paper test tube because the magnet was magnetized to the apparatus. For the first measuring, 70 ml of petrol ether was added to each sample, put inside to the beaker, then pushed the recover position and opened the back faucet. For the second measuring, only 50 ml of petrol ether was sufficient. Paper test tubes and beakers with petrol ether got together. After pressing a start button, it got hot at 100 °C. Then these three steps followed: Immersion (25 minutes), Washing (45 minutes), and Recover (30 minutes). After the whole process, extraction glasses with test tubes were given to the dryer for 30 minutes and finally placed to the desiccator. The crude fat content was calculated according to the formula:

$$F = \left(\frac{w3-w0}{w2}\right) \times 100$$

w_0 – weight of empty extraction glass (g)

w_2 – weight of sample (g)

w_3 – weight of extraction glass after the analysis (g)

4.3.6. **Nitrogen Free Extract analysis**

Nitrogen Free Extract (NFE) is a soluble carbohydrate such as starch and sugar. Crude fibre represents an insoluble carbohydrate. NFE was calculated by the formula:

$$\%\ \mathrm{NFE} = \%\ \mathrm{DM} - (\%\ \mathrm{EE} + \%\ \mathrm{CP} + \%\ \mathrm{ash} + \%\ \mathrm{CF})$$

DM – dry matter

EE – ether extract or crude fat

CP – crude protein

CF – crude fibre

4.3.7. Vitamin analyses

The analyses of vitamins B and C meant to take 50 mg of the sample together with 50 mg of vitamin C tablet and gave it into the conical test tube. For the best results, it had to be repeated nine times. 1 ml of hydrochloric acid 0.1 M was poured into the first six conical test tubes and the last three were filled with 0.1 hydroxide natrium 0.1 M. Three test tubes with hydrochloric acid were placed in the vortex. Last test tubes were given to the autoclave at 120 °C. After 30 minutes, they were taken out and cooled with water. Then each test tube was shaken in vortex for 30–60 seconds and then put into the ultrasonic – cleaner for 5 minutes. After that, all of them were placed in the centrifuge for 15 minutes at 9000 rpm. The last step in this process was to transfer the clear liquid from the test tube to the HPLC vials using an automatic pipette. Results for vitamins B and C were determined by HPLC – UV analysis together.

5. Results and Discussion

The contents of macronutrients as analysed by AOAC methods from the lyophilizate of *S. pungens* and *S. cocculoides* are presented in Table 5. The values of dry matter were 94.82 g/100 g and 89.65 g/100 g for *S. pungens* and *S. cocculoides*, respectively. Ash was higher in the lyophilized form of *S. pungens* (9.48 g/100 g) compared to *S. cocculoides* (8.97 g/100 g). The lyophilized sample of *S. cocculoides* contained less crude fibre (12.97 g/100 g) than *S. pungens* (13.78 g/ 100 g). The protein content was higher in the case of *S. cocculoides* (3.10 g/100 g) unlike *S. pungens* (2.37 g/100 g), but crude fat reached a higher value in the sample of *S. pungens* (17.16 g/100 g) compared to *S. cocculoides* (10.47 g/100 g). The content of NFE was quite similar in both species.

Table 5. Nutritional content of *S. pungens* and *S. cocculoides*

	***S. pungens* (g/100 g)**	***S. cocculoides* (g/100 g)**
Dry matter	94.82 ± 0.03	89.65 ± 0.30
Ash	9.48 ± 0.13	8.97 ± 0.60
Crude fibre	13.78 ± 0.45	12.97 ± 0.19
Crude protein	2.37 ± 0.15	3.10 ± 0.03
Crude fat	17.16 ± 0.08	10.47 ± 3.67
NFE	57.75	56.78

± Standard deviation, data were measured on the basis of lyophilizate

S. pungens reached higher values from all measured micronutrients from lyophilizate than *S. cocculoides* (Table 6). Pyridoxamine, pyridoxal, and pyridoxine are three forms of vitamin B_6. Vitamin C, also known as ascorbic acid, was significantly higher in the sample of *S. pungens* with a value of 1,772.76 mg/100 g compared to *S. cocculoides* (703.04 mg/100 g). Thiamine was detected more than three times higher in *S. pungens*, with an amount of 778.02 mg/100 g compared to *S. cocculoides* (206.15 mg/100 g). Riboflavin was also in higher amount in the sample of *S. pungens* (547.18 mg/100 g) than in *S. cocculoides* (181.43 mg/100 g). The amount of nicotinic acid in *S. pungens* was 2.42 mg/100 g, whereas only trace amount was detected in the sample of *S. cocculoides.* The content of pantothenic acid was 805.40 mg/100 g in *S. pungens* and 238.52 mg/100 g in *S. cocculoides.* There was also trace amount of pyridoxal in both fruits. While pyridoxamine reached almost the same values in both species, around 7 mg/100 g, pyridoxine, determined in *S. cocculoides* was about four times lower (240.00 mg/100 g) than in *S. pungens* (979.56 mg/100 g). The lyophilizate of *S. pungens* contained about 99 mg/100 g folic acid, whereas in the case of *S. cocculoides*, it was 21 mg/100 g only.

Table 6. Vitamin composition of *S. pungens* and *S. cocculoides*

	***S. pungens* (mg/100 g)**	***S. cocculoides* (mg/100 g)**
Vitamin C	1,772.76 ± 464.89	703.04 ± 132.24
Thiamine (B_1)	778.02 ± 106.26	206.15 ± 13.04
Riboflavin (B_2)	547.18 ± 56.53	181.43 ± 13.71
Nicotinic acid (B_3)	2.42 ± 0.21	tr
Pantothenic acid (B_5)	805.40 ± 216.51	238.52 ± 86.10
Pyridoxal (B_6)	tr	tr
Pyridoxamine (B_6)	7.58 ± 1.14	6.74 ± 0.56
Pyridoxine (B_6)	979.56 ± 257.34	240.00 ± 36.79
Folic acid (B_9)	99.03 ± 1.58	21.09 ± 0.57

± Standard deviation, tr – trace amount; data were measured on the basis of lyophilizate

5.1. Comparison of macronutrients in tropical fruits

To the best of our knowledge, there has not been any nutritional analysis done by lyophilization in the case of *S. pungens* and *S. cocculoides,* so we decided to compare macronutrients and vitamins with similar studies on various tropical fruits. For macronutrients, we chose guava (*Psidium guajava*), carambola (*Averrhoa carambola*), banana (*Musa acuminata*, Cavendish AAA variety), and a Zambian indigenous fruit species mumosomoso (*Vangueriopsis lanciflora*).

Table 7. Nutritional comparison of lyophilizates of various tropical fruits

	Psidium guajava	***Averrhoa carambola***	***Musa acuminata***	***Strychnos pungens***	***Strychnos cocculoides***	***Vangueriopsis lanciflora***
	Moreno et al. (2014)	Yan et al. (2013)	Khoozani et al. (2019)	Author (2021)		Taušnerová (2021)
Crude fibre %	12.70	NE*	NE	13.78	12.97	5.02
Crude fat %	0.05	1.36	0.92	17.16	10.47	1.74
Crude protein %	20.00	7.88	3.97	2.37	3.10	4.80
Carbohydrates %	29.87	24.22	84.61	57.75	56.78	76.65
Ash %	NE	3.87	5.21	9.48	8.97	2.55

NE – not examined, *only dietary fibre was detected as 7.89 %

Table 7 shows macronutrients obtained from lyophilizate of tropical fruit species. Macronutrients are products of primary metabolism and they are also essential for plant growth. The highest value of crude fibre reached *S. pungens* with 13.78 %, while *S. cocculoides* came in second place with 12.97 %. The least fibre was in the sample of *V. lanciflora* (5.02 %). Both *Strychnos* species were much richer in crude fat compared to the rest of the fruits, showing an interesting nutritional value of 17.16 % in *S. pungens* and 10.47 % in the case of *S. cocculoides*. These values were much higher, especially in comparison to *M. acuminata* (0.92 %) and *P. guajava* (0.05 %). Because crude fat has the highest energy content (9 kcal/1 g) from all the macronutrients compared to protein (4 kcal/1 g) and carbohydrates (4 kcal/1 g), it makes it an indispensable source of energy for proper body functions and physical activity (Cotton 2019), and the fruits of *Strychnos* species can be added to the traditional maize porridge in the form of juice, fresh fruit or lyophilizate.

On the other hand, in terms of protein content, both *Strychnos* species disposed only 2.37 % and 3.10 % in *S. pungens* and *S. cocculoides* respectively, which were the least amounts compared to the other fruit crops such as *A. carambola* (7.88 %), *V. lanciflora* (4.80 %), and *M. acuminata* (3.97 %). The highest content of carbohydrates showed *M. acuminata* (84.61 %), followed by *V. lanciflora* (76.65 %). Carbohydrates of *Strychnos* species were almost in the balance around 57 %. The last detected macronutrient was ash, the highest value had *S. pungens* (9.48 %) and *S. cocculoides* (8.97 %) followed by *M. acuminata* (5.21 %), *A. carambola* (3.87 %) and *V. lanciflora* (2.55 %).

5.2. Comparison of vitamins in tropical fruits

For the comparison of vitamin C content among tropical species, mango (*Mangifera indica*), acerola (*Malpighia emarginata*), carambola (*Averrhoa carambola*), guava (*Psidium guajava*), and mumosomoso (*Vangueriopsis lanciflora*) were chosen to verify if the *Strychnos* species are a good potential source of vitamin C.

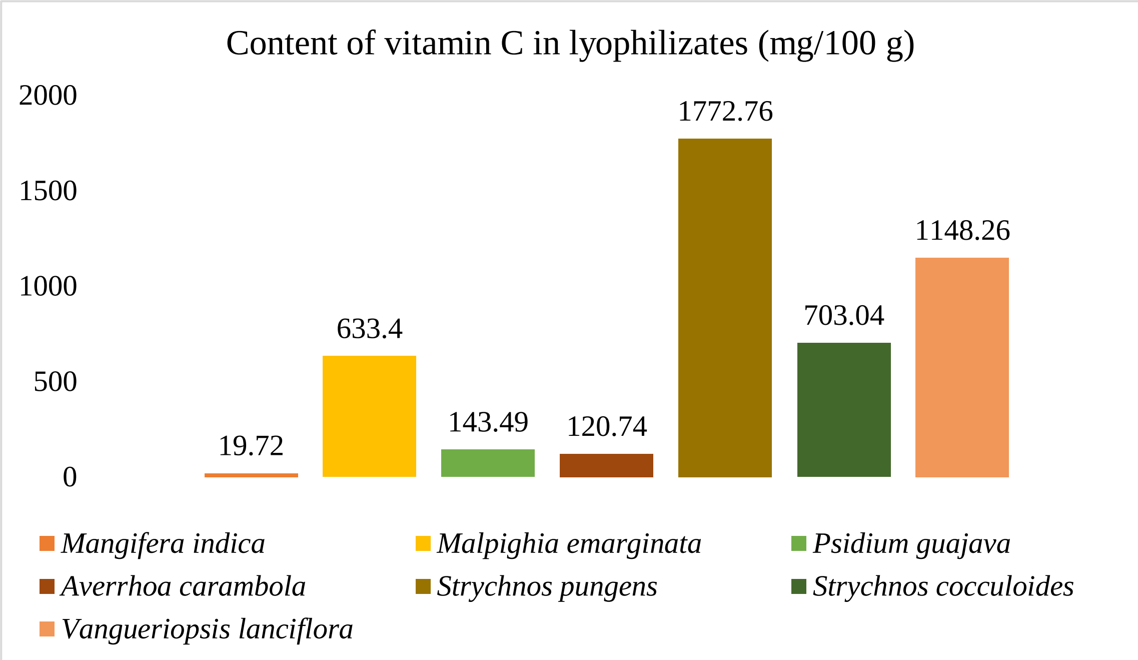

Figure 10. Comparison of vitamin C of fruit species from lyophilizate. Based on data of Marques et al. (2006); Yan et al. (2013); Moreno et al. (2014); Author (2021); Taušnerová (2021)

S. pungens reached the highest content of vitamin C in mg/100 g from the lyophilizates (Figure 10). To consume the daily recommended dietary allowance (RDA) of vitamin C, equivalent to 75 mg, only 4.23 g of lyophilized *S. pungens* fruits and 10.67 g of *S. cocculoides* is needed, which is much lower portion compared to guava, acerola, and mango where 52.27 g, 11.84 g and even 380.3 g of freeze-dried fruits is needed. Even though *V. lanciflora* contained more vitamin C in mg/100 g than *S. cocculoides*, the species was still richer in vitamin C than the other fruit crops (mango, acerola, guava, and carambola). However, in each of the studies, the analysis of lyophilizate was done under different temperature which may influence the vitamin C content in the fruits. Vitamin C is essential for the immune system because of its antioxidant activity. It also improves the absorption of iron and lowers blood pressure. Vitamin C deficiency is called scurvy and can lead to anaemia, exhaustion, ulceration of the gums, loss of teeth, and spontaneous bleeding. Fruits such as orange, kiwi fruit, guava, mahwahwa, and mahuluhulu can prevent this deficiency (Crosta 2017).

Comparing the vitamin B content in *Strychnos* species and *V. lanciflora*, the highest amount of thiamine was reached by *V. lanciflora* (909.24 mg/100 g) and there was a significant difference found between *S. pungens* (778 mg/100 g) and *S. cocculoides*

(206 mg/100 g). The level of riboflavin was the highest in *S. pungens* (547.18 mg/100 g) compared to *V. lanciflora* (473.72 mg/100 g) and *S. cocculoides* which had the least riboflavin in the sample (181.43 mg/100 g). Nicotinic acid was detected only in *S. pungens* fruits (2.42 mg/100 g). Pantothenic acid was in the balance in the case of *S. pungens* and *V. lanciflora* (805 mg/100 g) but the value in *S. cocculoides* was almost four times lower (238.52 mg/100 g). Pyridoxal was found only in trace amount in all the species. *S. cocculoides* contained the least value of pyridoxamine (6.74 mg/100 g) unlike *V. lanciflora* (30.47 mg/100 g) and *S. pungens* (7.58 mg/100 g). A great source of pyridoxine was found to be *S. pungens* with 979.56 mg/100 g in lyophilizate compared to 240.00 mg/100 g in *S. cocculoides.* The highest level of folic acid was detected also in *S. pungens* (99 mg/100 g) and *S. cocculoides* was in the last place with 21.09 mg/100 g compared to *V. lanciflora* (27.50 mg/100 g).

Table 8. Vitamin B comparison of lyophilizates

	***Strychnos pungens* (mg/100 g)**	***Strychnos cocculoides* (mg/100 g)**	***Vangueriopsis lanciflora* (mg/100 g)**
	Author (2021)		Taušnerová (2021)
Thiamine (B_1)	778.02 ± 106.26	206.15 ± 13.04	909.24 ± 199.82
Riboflavin (B_2)	547.18 ± 56.53	181.43 ± 13.71	473.72 ± 118.47
Nicotinic acid (B_3)	2.42 ± 0.21	tr	tr
Pantothenic acid (B_5)	805.40 ± 216.51	238.52 ± 86.10	805.07 ± 98.03
Pyridoxal (B_6)	tr	tr	tr
Pyridoxamine (B_6)	7.58 ± 1.14	6.74 ± 0.56	30.47 ± 8.04
Pyridoxine (B_6)	979.56 ± 257.34	240.00 ± 36.79	769.94 ± 113.00
Folic acid (B_9)	99.03 ± 1.58	21.09 ± 0.57	27.50 ± 7.47

± Standard deviation; trace amount

Because no other vitamin B analysis of tropical fruits from lyophilizate were found, we compared *Strychnos* species with for *V. lanciflora* which was done by the same methods as the *Strychnos* fruits (Taušnerová 2021).

Vitamins B are essential for the proper growth of cells, mainly in children. They also influence red blood cells production, eyesight, brain and hormonal system functions, cholesterol production and much more. Vitamin B deficiencies are dangerous especially for small children and pregnant women as they can lead to anaemia, peripheral neuropathy and compromised immune system (Cronkleton 2019). Table 9 presents amounts of lyophilizate (in grams) covering RDA of B vitamins. According to the results, more or less 1 g of lyophilizate is enough to cover this allowance except for nicotinic acid, pyridoxamine, and folic acid where a higher amount is needed.

Table 9. Amount of lyophilizate needed to cover RDA in grams

	Strychnos pungens	***Strychnos cocculoides***	**RDA** mg/day**
Thiamine (B_1)	0.14	0.53	1.1
Riboflavin (B_2)	0.20	0.61	1.1
Nicotinic acid (B_3)	578.51	*	14
Pantothenic acid (B_5)	0.62	2.10	5.0
Pyridoxal (B_6)	*	*	1.3
Pyridoxamine (B_6)	17.15	19.29	1.3
Pyridoxine (B_6)	0.13	0.54	1.3
Folic acid (B_9)	0.40	1.90	0.4

*trace amount, **RDA is based on an adult female, National Academy of Sciences (2019)

5.3. Data comparison in *S. cocculoides*

Even though there are no nutritional data available, except for one from the year 1985, for *S. pungens*, few studies have been done already on *S. cocculoides* (Table 10). Therefore, we compared the nutritional values of different bases (dry weight, wet weight and lyophilizate) in *S. cocculoides* fruits to see whether there are some major differences/similarities.

Table 10. Comparison of nutritional content of *S. cocculoides* fruits

Strychnos cocculoides					
	Saka et al. (2008)	Ngadze (2018)*	Mapunda & Mligo (2019)	Ndabikunze et al. (2006)	Author (2021)
	wet weight basis	dry weight basis			lyophilizate
g/100 g	mean	mean	mean	mean	mean
Carbohydrates	61.0	18.2	56.01	90.8	56.78
Protein	11.5	3.5	0.94	0.6	3.10
Moisture	72.2	78.2	82.28	74.9	10.35
Fat	6.0	0.4	0.0013	1.3	10.47
Fibre	17.9	4.6	14.45	1.9	12.97
Ash	3.7	2.2	NE	5.4	8.97
Thiamine (mg/100 g)	NE	0.03	NE	NE	206.15
Vitamin C (mg/100 g)	22.9	34.2	93.03**	NE	703.04

NE – not examined, *based on the research of Arnold et al. (1985) and Malaisse & Parent (1985); **analysis of the fresh sample

The content of carbohydrates increased with lower water content, however, the value of freeze-dried fruits was very close to the content of fresh pulp, and dry weight basis analysed by Mapunda & Mligo (2019). The crude protein reached a higher value in the lyophilizate compared to the dry weight basis of Mapunda & Mligo (2019) and Ndabikunze et al. (2006). However, the fresh pulp analysed by Saka et al. (2008) was significantly richer in protein (11.5 g/100 g) compared to a freeze-dried pulp (3.10 g/100 g). It was impossible to compare moisture content as the lyophilizate was firstly dried by freeze and then by hot air. The content of crude fat was the highest in lyophilizate compared to the other forms. The freeze-dried sample had a similar value of crude fibre as reported by Mapunda & Mligo (2019) but lower than the fresh form of fruit. Ash value was the highest in the lyophilizate. Very interesting is the comparison of vitamin C content because vitamins normally reach the highest value in fresh fruits and decrease after heat processes and by time. There was even a big variation of vitamin C between the two fresh bases of fruits. This can result from differences in soil conditions, ecological area, time of harvest or amount of precipitation. For example, Tembo et al. (2008) examined the effect of time of harvesting and storage on vitamin C content in fruits of *S. cocculoides* from Malawi, and the vitamin C in unripe fruit decreased from 306.25 mg/100 g to 285.07 mg/100 g after six days while the ripe fruit contained only 53.91 mg/100 g of vitamin C and decreased to 35.24 mg/100 g after six days. The time of harvesting also affected the content of vitamin C, the unripe and ripe fruits harvested in December had 25.95 mg/100 g and 20.02 mg/100 g respectively, while unripe and ripe fruits collected in October reached 20.20 mg/100 g and 15.53 mg/100 g.

However, *S. cocculoides* is not the only case when lyophilizate contained more vitamins than the fresh form. Guava analysed by Moreno et al. (2014) had a higher content of vitamin C in freeze-dried fruit compared to the fresh form and the same pattern was observed by Serna-Cock et al. (2015) in muskmelon (*Cucumis melo*) or the samples of papaya (*Carica papaya*) and watermelon (*Citrullus lanatus*) by Shofian et al. (2011).

Unfortunately, there was only one nutritional analysis of *S. pungens* by hot-air drying from the year 1985 so we do not have more comparisons, although this species reached higher amounts of macronutrients and vitamins than *S. cocculoides*. However, it is reported that the pulp of *S. cocculoides* is tastier than *S. pungens* so this might be the reason for the absence of other analyses. The results of our analyses could be affected by external factors such as soil conditions, temperature, precipitations, light intensity etc.

Also, the small-scale farmers could join to create a cooperative for growing *S. pungens* and *S. cocculoides* in orchards and transport the fruits to a company possessing a lyophilizer. After that, lyophilized fruits might be ground, vacuumed and exported to Europe or the USA which are the main consumers of lyophilized products. The freeze-dried powder is getting popular, especially as a food supplement in porridges and smoothies. The transportation of this food is much easier because the weight of lyophilizates is up to 90 % lower than fresh food and the risk of mould infestation is reduced. Eventually, the principle of Fairtrade could be applied to ensure sustainable production and fair prices for fruits.

6. Conclusion and recommendations

This study provides an overview of the cultivation, management, use, and nutritional content of *Strychnos pungens* and *Strychnos cocculoides* which are native Zambian fruit trees. To determine the nutrients of edible pulp, lyophilization as a method was chosen. The lyophilizates of our samples reached very high dietetic values, especially in crude fat, crude fibre, vitamin C, and pyridoxine (B_6). Although most of the nutritional values were higher in the fruits of *S. pungens,* scientific data on this species are generally missing. Compared to *S. cocculoides*, *S. pungens* was reported to be less tasty, however, this information has not been verified. Further testing is thus recommended to see the real preference of the fruit by local people and to confirm its nutritional benefits.

Even though the process of freeze-drying is more expensive than other drying methods, the lyophilizate is, on the other hand, needed in much lower amounts compared to the fresh fruits. Both *Strychnos* species cover the daily intake of vitamins B and C in more or less 10 grams of lyophilizate.

Because the sample collection was done just in one province of Zambia, it would be interesting to analyse if lyophilizates of the pulp of *S. pungens* and *S. cocculoides* from other countries display some significant changes in nutritional content.

Considering that both fruit species proved to be rich in vitamins as well as macronutrients, their consumption can be recommended to improve the diet of local communities, enhancing food security, while reducing the risk of malnutrition. Additionally, further research is needed to determine the secondary metabolites content (e.g. strychnine), anti-nutritional factors, and digestibility to extend the knowledge about these African indigenous trees which have the potential to become a novel food.

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Appendices

List of the Appendices:

Appendix 1. *Strychnos pungens* Soler

Habitat of *S. pungens* in South Africa (Rotational 2019)

Leaves (Wursten 2010)

Flowers (Stevens 2007)

Fruits (Stevens 2006b)

Ripe fruit (Fourie 2020)

Appendix 2. *Strychnos cocculoides* Baker

Habitat of *S. cocculoides* in the open woodland on Kalahari sand (Derennes 2019)

Corky bark (Wursten 2004)

Seedlings (Séleck 2018)

Leaves (Stevens 2006a)

Harvest on market (Séleck 2018)

Appendix 3. Process to obtain lyophilizate

(Author's personal photo documentation)


Pulp with seeds of *S. pungens*


Pulp with seeds of *S. cocculoides*


Scraped pulp of *S. pungens*


Scraped pulp of *S. cocculoides*

Lyophilizate of *S. pungens*

Vitamin analysis