Article

Southern African Soap Plants and Screening of Selected Phytochemicals and Quantitative Analysis of Saponin Content

Mpho Mohlakoana and Annah Moteetee *

Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park, Johannesburg 2006, South Africa; mr.mohlakoana@gmail.com

* Correspondence: amoteetee@uj.ac.za; Tel.: +27-11-559-3781

Abstract: In southern Africa, several plants are used ethnobotanically as soap substitutes, however, this information resides in different literature sources. The foaming and cleansing properties of such plants are attributed mainly to the presence of saponins, but other compounds such as alkaloids and terpenoids are also implicated. This study aimed to compile a comprehensive list of plants used traditionally as soap substitutes in southern Africa and to assess the chemical properties of selected species. Qualitative phytochemical analysis was done using five solvents (ethanol, methanol, water, chloroform, and acetone) to determine the presence of saponins, alkaloids, and terpenoids in selected soap plants. Quantitative analysis of the saponin content was done employing spectrophotometric tests of methanol extracts. There are thirty-seven (37) known southern African soap plants from twenty-four (24) different families, with the Fabaceae having the highest number of species (eight). Saponin concentrations of nine previously unstudied selected soap plants are reported for the first time in this study, whereby *Calodendrum capense* had the highest saponin concentrations are at 107.89 ± 4.89 mg/g, followed by *Noltea africana* (52.65 ± 6.81 mg/g), *Crinum bulbispermum* (35.43 ± 4.25 mg/g), and *Merwillia plumbea* (25.59 ± 0.83 mg/g). The knowledge of plant composition gives a better understanding of plant chemistry and possible use of plants medicinally, industrially and as soap substitutes. Furthermore, this allows the verification and the justification of traditional plant use. Soap plants have been used traditionally for many years, the potential to commercialise the use of these plants has been realised with the increase in the use of organic products by conscious consumers hence, the purpose of this investigation can have bearing on future projects and products.

Citation: Mohlakoana, M.; Moteetee, A. Southern African Soap Plants and Screening of Selected Phytochemicals and Quantitative Analysis of Saponin Content. *Resources* **2021**, *10*, 96. <https://doi.org/10.3390/resources10100096>

Academic Editors: Ben McLellan and Damien Giurco

Received: 14 June 2021

Accepted: 9 September 2021

Published: 28 September 2021

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Keywords: saponin; soap substitutes; soap plants

1. Introduction

Plants have been used as soap substitute by indigenous people for decades, however, the era in which this began is not known [1]. Plants have been used as soap substitutes by several ethnic groups and cultures in different parts of the world, for example *Yucca schidigera* Roezl ex Ortgies in America [2], *Dicerocaryum eriocarpum* (Decne.) Abels in southern Africa [3], and several other plants in Angola [4,5], Uttarakhand State, including Himalayas [6] and Thailand [7]. Soap plants are considered as such because of the natural formation of foam and lather when agitated in water. Soaps are amphiphilic surfactants that contain hydrophobic and hydrophilic parts that reduce the surface tension of water. They are known for their cleansing ability and are used for washing and cleaning purposes.

It has been argued that the cleansing effect of plants that are used as soap substitutes is due to the presence of saponins. Saponins, so-called because like soap they have foaming properties when mixed with water, derive their name from the Latin word 'sapo', which means soap. *Saponaria officinalis* L., also known as soapwort, is one of the best-known soap plants native to Europe and still in use as a soap up to date [8]. In the past, this plant was grown near wool mills to wash wool using soapy extracts from the roots and leaves [9].

Soap plants have different uses, and this is not only attributed to the saponin content of the plant but also the presence of other compounds such as alkaloids, triterpenoids, steroids, flavonoids, etc.

Saponins are amphiphilic glycosides composed of hydrophobic aglycones attached to varying numbers of hydrophilic sugar chains. The aglycone structure which includes triterpenoids, steroids or steroidal glycoalkaloids [10]. Saponins have a connection to an array of compounds which brings about their vast classifications. The classification of saponins may be difficult to determine due to the wide range of polarities because of the type of aglycone and the number of saccharide chains (moieties) [11], however, this complexity is simplified by characterizing them based on the number of the sugar chains. Besides the wide variety of saponins that can be found in soap plants, individual bioactive compounds found in these plants have significant effects on plant use. Terpenoids share many similarities to saponins as they share a biological precursor and can be converted into saponins by glycosylation, i.e., triterpene glycosides. Alkaloids play an important role in the production of basic chemically produced soaps. In the past, these alkaloids were obtained from lakes, but the main source was from plants in the form of ash [1]. Such types of plants can often be confused as soap plants based on their soapy texture due to the presence of alkaloids and alkalies. Hence it is also beneficial to determine the alkaloid, terpenoid, and saponin presence in soap plants which could assist in identifying true soap plants which are plants that naturally form foam and lather when agitated in water, as opposed to plants with high alkali and terpenoid content.

The isolation and characterisation of bioactive compounds such as saponins can be achieved using different methods and their extraction from plants is regarded as one of the more sustainable approaches [12]. Conventional methods used for extraction include infusion, digestion, maceration, reflux, and Soxhlet extractions. However, these methods have several disadvantages, for example, maceration takes long periods of extraction time which requires continuous stirring of the solution, as well as large amounts of solvent. [13]. Modern methods include enhanced/accelerated solvent extraction systems, also known as pressurized liquid extraction, involve the use of high pressure and temperature whereby elevated temperatures increase the diffusion of minimal solvent. The advantage of utilizing this technique is the speed efficiency in the extraction of the component [14]. This extraction is therefore widely used in food and pharmaceutical research. Other faster and environmentally friendly extraction methods used include ultrasound assisted extraction and microwave-assisted extraction [14]. Spectrophotometry can be used to quantify saponins. Spectrophotometric methods commonly use vanillin-sulfuric acid assay to determine total saponin content. Vanillin reacts with saponins and sterol bile acids that have an OH group at the C-3 position, in an acidic medium [15]. This gives chromogens which are independent from the nature of the sugar moieties [15]. These chromogens can be analysed using an absorbance of 455 to 460, 460 to 480, or 544 nm, these absorbance values are dependent on the nature of the saponins [15].

Recently, the use of extraction of natural products has become popular partly because of “the move away from synthetic flavours and fragrances towards plant extracts particularly for the food, drink and cosmetic industries” [16]. In 2021, the global plant extracts market is estimated at \$30.8 billion and is projected to increase, at a compounded annual growth of 6.0% from 2021 to 2026, reaching \$55.3 billion in 2026 [17]. The plant extracts market is driven mainly by industries such as food and beverages (e.g., colourants, flavourants), cosmetic (i.e., hair and skin products, as well as perfumes), pharmaceutical, and herbal medicine industries. This has therefore pushed the interest towards greener plant extraction methods. The development of soaps has been evolving for thousands of years. This was done to combat microbes that may be detrimental to the human health [2]. For example, in the past one and half years, the importance of washing hands with soap in combating the spread of the COVID-19 pandemic has become evident. However, these industrialised soaps have come with some drawbacks such as the negative impact on the skin and the environment. Synthetic surfactants contain chemicals that cause the human

body to react in different ways, sometimes causing allergies and skin irritations [2]. Therefore, in recent developments it has been acknowledged that there is a need to return to a more natural form of soap.

Numerous studies reporting the ethnobotanical uses of plants in southern Africa have been published, particularly on their various medicinal uses, for example, Botswana [18,19], eSwatini [20,21], Lesotho [22,23], Namibia [24,25], and South Africa [26–29], etc. However, no studies have specifically focused on the traditional use of plants as soaps/soap substitutes, and the available information is scattered in different literature sources. The aim of this study is therefore to investigate the current knowledge on the ethnobotanical uses of soap plants in southern Africa and to assess the plants for the presence of selected chemical constituents (alkaloids, saponins, terpenoids) and their saponin content.

2. Materials and Methods

Five grams of the ground material was extracted with 50 mL of each solvent namely, distilled water, ethanol (UnivAR, Downers Grove, IL, USA), acetone (UniLab, Mandaluyong, Philippines), methanol (Sigma Aldrich, Kempton Park, South Africa, HPLC grade), and chloroform (UnivAR, USA) at room temperature for 24 h. The solution was then filtered using a Whatman No. 1 filter paper. The filtrate was then used for different phytochemical screening protocols.

2.1. Qualitative Phytochemical Analysis

2.1.1. Detection of Saponins

Saponin analysis was done using the foam test, 2 g of each powdered sample was brought to a boil in 20 mL of distilled water, placed in a water bath, and filtered. A total of 10 mL of filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth that can be observed for at least 10 min. The frothing was mixed with a few drops of olive oil and shaken vigorously; it was then observed for the formation of an emulsion [30].

2.1.2. Detection of Alkaloids

Wagner's test was used following the methods of Tiwari et al. [31] and Zohra et al. [32]. The filtrates were treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide, both from Sigma Aldrich, Kempton Park, South Africa) in 100 mL of water. The presence of alkaloids was indicated by the formation of brown/reddish precipitate.

2.1.3. Detection of Terpenoids

The Salkowki's test was used whereby 1 mL of chloroform was added to 2 mL of each extract, then a few drops of concentrated sulphuric acid (Rochelle Chemicals, Johannesburg, South Africa) were added, a reddish-brown precipitate showed the presence of terpenoids [31].

2.2. Quantitative Analysis: Saponins

2.2.1. Plant Extraction

The methods of Makkar et al. [15] and Kaur et al. [33] were followed. Plant samples were dried and ground into a fine powder using a grinder (Mellerware, Cape Town, South Africa). Ten grams of the ground sample was then defatted in a Soxhlet apparatus for 3 h using hexane (Sigma–Aldrich) as a solvent. The content was allowed to dry and then immersed in 100 mL of methanol: dH₂O (4:1 ratio) for 24 h with continuous stirring using a magnetic stirrer. An additional 100 mL of methanol:dH₂O was added to the extract for an additional 24 h maceration. After 48 hrs, the sample was centrifuged at 3000 × g for 10 min at 5 °C to obtain the supernatant. The supernatant was then filtered using Whatman No. 1 filter paper. Methanol from the extract was then evaporated under vacuum at 40 °C using a rotary evaporator. The remaining aqueous extract was centrifuged at 3000 × g for 10 min at 5 °C to obtain the supernatant. The aqueous extract was washed three times with

chloroform at a 1:1 ratio in a separatory funnel to remove pigments. Thereafter, aqueous extracts were washed two times with butanol (Rochelle Chemicals), then the bottom layer was taken and evaporated in a rotary evaporator at 5 °C until dry. This dry extract was dissolved in 40 mL of distilled water and placed in a pre-weighed 50 mL falcon tube. The content was placed at −80 °C for 24 h and thereafter lyophilised until dry. The lyophilised product was placed in a dark cupboard until use.

The samples were prepared by dissolving 800 mg of vanillin powder in 10 mL of 99.5% ethanol (Rochelle Chemicals). Other solutions included a 72% (v/v) sulphuric acid (Rochelle Chemicals), whereby 72 mL of analytical grade sulphuric acid (Rochelle Chemicals) was added to 28 mL of distilled water. A standard saponin solution was also prepared by dissolving 10 mg of diosgenin (Sigma Aldrich) in 16 mL of methanol, with the addition of 4 mL distilled water, this solution was mixed thoroughly. The final concentration of the solution was 0.5 mg/mL of 80% aqueous methanol.

2.2.2. Preparation of Calibration Curve

An increasing volume of diosgenin standard solution from 0, 50, 150, 200 to 250 µL was added to five test tubes, which were topped up to 0.25 mL with 80% aqueous methanol. A volume of 0.25 mL of vanillin reagent was added. Then, 2.5 mL of 72% (v/v) sulphuric acid was slowly added to the inner side of the tube wall. The solutions were mixed thoroughly using a vortex mixer and transferred into a water bath adjusted to 60 °C for 10 min. The tubes were then transferred into ice-cold water to cool for 3 to 4 min. The absorbance values were then read using a spectrophotometer at an absorbance of 544 nm against the reagent blank which had 0 µL of diosgenin standard solution.

A known amount of freeze-dried saponin residue was dissolved in 80% methanol to a concentration of 1 mg/mL and from this 0.25 mL was taken to determine the standard saponin, this was done in triplicate. The regression equation obtained $y = 0.0042x + 0.0302$; $R^2 = 0.9531$, was used to calculate the concentrations of saponin content in the selected plants. The concentrations were expressed as diosgenin equivalents (DE) calculated from a standard curve [15].

3. Results

3.1. Ethnobotanical Information

Thirty-seven plant species used as soap in southern Africa were recorded as shown in Table 1. The plants are distributed in 24 different families, with the Fabaceae having the highest number of species (eight). Other families that are commonly used as soap substitutes are Rhamnaceae, Aizoaceae, Cucurbitaceae and Acanthaceae, with two species each.

3.2. Medical Uses of Soap Plants

In addition to the plants being used traditionally as soap substitutes (Figure 1a), they are also used for many medicinal purposes. Interestingly, the most common medicinal use of these plants is for the treatment of gastrointestinal problems (e.g., diarrhea, dysenteries, emetics, enemas, and purgatives), making up 57% of plant use. The treatment of various skin conditions makes up 38% of soap plants used for this purpose as depicted in Figure 1b. The treatment of respiratory tract infections (e.g., coughs and asthma) as well as the use of the plants for the treatment of sexually transmitted infections (e.g., syphilis and gonorrhea), each account for 19% of plant use. Soap plants used in southern Africa for urinary and bladder problems constitute 16% of use. Other medicinal uses of soap plants include the treatment of headaches, malaria, anxiety, etc., which together make up 57.6% of soap plant use. It is important to note that many of these plants have multiple uses, which is why the total number of plant use does not equate to 100%.

Figure 1. (a) Different usages of soap plants as soap substitutes in southern Africa; (b) percentage of plant uses.

3.3. Study of Selected Bioactive Compounds Found in Soap Plants

The various uses of soap plants can only be justified with the presence of bioactive compounds that help facilitate in the treatment of different ailments such as those listed in Table 1. Previous and current studies of three phytochemical classes namely, alkaloids, terpenoids and saponins, usually associated with cleansing qualities of soap plants, are also recorded in Table 1. Six plants have previously been investigated for their saponin quantity namely, *Aloe maculata* [32], *Merwillia plumbea* [34], *Phytolacca dodecandra* [35], *Piliostigma thonningii* [36], *Sphenostylis stenocarpa* [37] and *Vachellia nilotica* [38]. Qualitative screening of saponin content has been carried out in 46% of the soap plants recorded. A higher number of plants, i.e., 54%, has been investigated for the presence alkaloids, while 43% were previously screened for the presence of terpenoids. Some southern African soap plants have previously been extensively studied, but not specifically based on their soap use, these are *M. plumbea*, *P. dodecandra*, *P. thonningii* and *V. nilotica*. *Merwillia plumbea* has long been used for clinical experimentation and even tested for its anti-cancer properties [39]. This plant has been overly harvested due to the vast medicinal uses and is vulnerable in the wild [40].

3.4. Phytochemical Screening

All soap plants listed in Table 2 showed the presence of alkaloids except *Cyathula uncinulata* and *Merwillia plumbea*. The negative result for *Merwillia plumbea* corresponds to a previous study by Sparg et al. [41]. All the soap plants displayed the presence of saponins when using the foam test. Furthermore, an emulsification test was done to confirm the presence of saponins, this is because saponins are surfactants that make good emulsifiers. All soap plants tested showed relatively good emulsification activity. The soap plants listed in Table 2 all showed the presence of terpenoids. The five highest concentrations obtained in decreasing order were *Calodendrum capense* (107.89 ± 4.89 mg/g), *Noltea africana* (52.65 ± 6.81 mg/g), *Crinum bulbispermum* (35.43 ± 4.25 mg/g), *Merwillia plumbea* (25.59 ± 0.83 mg/g) and *Cyathula uncinulata* (20.17 ± 1.70 mg/g).

Table 1. Quantitative analysis of saponin and qualitative analysis of saponin, alkaloids, terpenoids and plant uses of selected southern African.

Species Name and Common Name	Family Name	Uses	Plant Part	Quantitative Saponin Content	Qualitative Saponin Content	Alkaloids	Terpenoids
<i>Adenodolichos rhomboideus</i> (O. Hoffm.) Harms Pumbulu	Fabaceae	Powder of the leaves heals severe wounds, used as a detergent [5].	Unknown	No records	No records	No records	No records
<i>Albizia amara</i> (Roxb.) B. Boivin Bitter Albizia	Fabaceae	Used as an astringent, treating piles, diarrhoea, gonorrhoea, leprosy, leucoderma, erysipelas and abscesses. The leaves and flowers have been applied to boils, eruptions, and swellings; used as an emetic and a remedy for coughs, ulcer, dandruff and malaria, used as soap [42].	Root	No records	Present [43]	Present [43]	Present [43]
<i>Aloe maculata</i> All. Soap aloe	Asphodelaceae	Used for ‘blood scours’ in calves and enteritis and indigestion in poultry [24]; crushed leaves used as an enema and ingested for fevers and colds; roots used to treat skin disorders, used as soap [44].	Leaves	Yield = 1.712 ± 0.051 mg/g, [45]	Present [45]	No records	Present [45]
<i>Calodendrum capense</i> (L.f.) Thunb. Cape chestnut	Rutaceae	Bark used externally to lighten skin, used as a moisturizer and to treat pimples, used for building material, used in soap, tied around wrists for skill and luck in hunting [46].	Seeds	Yield = 107.89 ± 4.89 Current study	Present [47], 2004	Present [47]	Present [47]
<i>Carica papaya</i> L. Pawpaw	Caricaceae	Root used as an anthelmintic and an infusion as a remedy for syphilis, used as a soap substitute [3].	Leaves	Yield = 6.32 ± 1.20 Current study	Present [48]	Present [48]	Current study
<i>Crinum bulbispermum</i> (Burm.f) Milne- Redh. and Schweick Orange river lily	Amaryllidaceae	To treat rheumatism, septic sores, varicose veins and kidney and bladder infections [49]; used as a soap substitute [44]; applied to sores, haemorrhoids, and abscesses [3].	Bulb	Yield = 35.43 ± 4.25 Current study	Present (current study)	Present [50]	Present [51]),
<i>Cyathula uncinulata</i> (Schrad.) Schinz Burweed	Amaranthaceae	Cleanses stomach and intestines by enabling voluntary vomiting, unguent, love charm, syphilis, urinary tract infection [3,52,53] rheumatism, aching joints, septic wounds, varicose veins, kidney, and bladder infections [50]; used as a soap substitute [3].	Root	Yield = 20.17 ± 1.70 Current study	Present (current study)	Absent (current study)	Present [54]

Table 1. Cont.

Species Name and Common Name	Family Name	Uses	Plant Part	Quantitative Saponin Content	Qualitative Saponin Content	Alkaloids	Terpenoids
<i>Deinbollia oblongifolia</i> (E. Mey.ex Arn.) Radlk. Dune soapberry	Sapindaceae	Treatment of diarrhoea, used as a soap substitute [55].	Seed, fruit (berry) and leaves	Yield = 16.02 ± 2.26 Current study	Present [55]	Present (current study)	Present [55]
<i>Dianthus crenatus</i> Thunb	Caryophyllaceae	The root infusion with <i>Tephrosia lurida</i> Sond. Is used as an emetic, used to wash the face [3].	Root	No records	No records	No records	No records
<i>Dicerocaryum eriocarpum</i> (Decne.) Abels Devil's thorn (Eng) seepbos (Afr.)	Pedaliaceae	Southern and Eastern Africans, used as a soap substitute [3] used for treating measles, treats STIs [56].	Leaves	No records	No records	Present [56]	Present [56]
<i>Dissotis princeps</i> (Kunth) Triana Purple dissotis	Melastomataceae	Leaf infusions are administered as enemas for dysentery and diarrhoea [27]. Used as a soap substitute [3].	Root	No records	Present [57]	Present [57]	No records
<i>Entada phaseoloides</i> Merr. Box bean	Fabaceae	Used for its anti-inflammatory activity, used as a soap substitute [58].	Bark	No records	Present [58]	Present [58]	No records
<i>Helinus integrifolius</i> (Lam.) Kuntze Soap bush	Rhamnaceae	Treats leg pains and stroke [59,60], treats hysteria, soothes irritation of the sand worm, used as an emetic and a soap substitute [3].	Root	No records	No records	No records	Present [59]
<i>Hibiscus cannabinus</i> L. Lu	Malvaceae	Remedy for eye diseases and dysenteries, used as a soap substitute [5].	Root	No records	Present [61]	Present [61]	Present [61]
<i>Ilex mitis</i> (L.) Radlk. African holly	Aquifoliaceae	An enema for colic in children. The leaves and the bark are grounded in water whereby a lather is formed to wash the body [62].	Leaves and Bark	8.37 ± 0.78 Current study	Present (current study)	Present (current study)	Present [63]
<i>Kedrostis capensis</i> (Sond.) A. Meeuse sesepa-sa-linoha	Cucurbitaceae	The Southern Sotho use the root as a purgative for the relief of constipation, used as a remedy for colic, and as a soap substitute [3,64].	Root	No records	No records	No records	No records

Table 1. Cont.

Species Name and Common Name	Family Name	Uses	Plant Part	Quantitative Saponin Content	Qualitative Saponin Content	Alkaloids	Terpenoids
<i>Merwillia plumbea</i> (Lindl.) Wild squill	Hyacinthaceae	Extracts of the bulb are known for their antibacterial, anthelmintic, anti-inflammatory, medicinal activity and used as ointment for wounds, scarification, as a laxative, and as an enema; it is also considered as a soap plant [39].	Bulb	20 mg/g [34]	Present [39]	Absent [41]	Present [55]
<i>Mesembryanthemum crystallinum</i> L. Crystalline ice-plant	Mesembrythamaceae	Leaf juice used to soothe inflammation of the mucous membranes of the respiratory or urinary system. In Europe, the fresh juice has been used to treat water retention, painful urination and to soothe lung inflammation [65]; antiseptic for burns sores and mouth infections [66]; used as a soap substitute [3].	Stem bark	No records	Present [67]	Present [67]	No records
<i>Morella serrata</i> (Lam.) Killick Lance-leaved waxberry	Myricaceae	Used for headaches, TB, mental illness. Powdered roots sniffed to cause sneezing to get rid of headache [68,69]; used to treat chest-related problems such as asthma, coughing and shortness of breath [70]; used as a soap substitute [3].	Fruit (berry)	No records	Present [69]	Absent [69]	Present [69]
<i>Noltea africana</i> (L.) Endl. Soap glossy-leaf	Rhamnaceae	Xhosa people use for washing clothes, used to treat “quarter-evil” (sponssiekte) in livestock [3].	Leaves and twigs	52.65 ± 6.81 Current study	Present (current study)	Present (current study)	Present (current study)
<i>Phytolacca dodecandra</i> L’ Herit African soapberry	Phytolaccaceae	Emetic in febrile conditions, treatment of genito-urinary conditions, purgative, herpes, and epilepsy remedy [3] used as a soap to wash clothes [71].	Leaves	120 mg/g [71]	Present [71]	Present [72]	Present [71]
<i>Piliostigma thonningii</i> (Schumach.) Milne- Redh. Camel’s foot	Fabaceae	Treats of dysentery, wounds, respiratory ailments, snake bites, hookworms and skin diseases, chronic ulcers, diarrhoea, toothache, gingivitis, cough and bronchitis, used as soap [73].	Stem bark	2.1 mg/g [36]	Present [73]	Present [73]	Absent [73]

Table 1. Cont.

Species Name and Common Name	Family Name	Uses	Plant Part	Quantitative Saponin Content	Qualitative Saponin Content	Alkaloids	Terpenoids
<i>Plectranthus ciliatus</i> E.Mey. Speckled spur-flower	Lamiaceae	Medicinal (for general pain); in the olden days used as a substitute for soap to wash sheepskin garments [3,52,74].	Whole plant	18.88 ± 1.36 Current study	Present (current study)	Present (current study)	Present [75]
<i>Pouzolzia mixta</i> Sohms Soap nettle	Urticaceae	Treats painful uterus, use for expulsion of retained placenta, treat venereal diseases, used as soap [76].	Leaves	No records	No records	Absent [76]	No records
<i>Psilocaulon absimile</i> N.E. Br Asbos	Aizoaceae	Used in rural areas as soap and for crop poisoning due to the alkaloid piperdene [77].	Leaves	No records	No records	Present [77]	No records
<i>Rhynchosia caribaea</i> (Jacq.) DC. Monya-a-mali	Fabaceae	Headaches, rheumatism; soap substitute [52,68,78,79].	Unknown	No records	No records	No records	No records
<i>Salsola aphylla</i> L. f. Asbos	Chenopodiaceae	With high alkali used in making soaps and glass [3].	Leaves	No records	No records	No records	No records
<i>Sceletium tortuosum</i> N. E. Br. Kanna	Aizoaceae	The Hottentot chew the leaf for the relief of toothache, used as soap [3].	Unknown	No records	No records	Present [80]	No records
<i>Sesbania bispinosa</i> Fawc. and Rendle Prickly sesban	Fabaceae	Suitable for rope-making and used as soap [5].	Unknown	No records	No records	No records	No records
<i>Solanum aculeastrum</i> Dunal var. albifolium Bitter-apple	Solanaceae	Fruit used as a remedy for ringworm in cattle and horses, remedy for scarifications over the knees for the relief of rheumatism in that situation [3]; used as a soap [81].	Unknown	No records	No records	Present [82]	No records
<i>Sphenostylis stenocarpa</i> (A. Rich.) Harms	Fabaceae	Used for food and nutrition for both humans and livestock, used as a soap [83].	Unknown	5.76 ± 0.30 mg/g [37]	Present [37]	Present [84]	No records
<i>Talinum caffrum</i> (Thunb.) Eckl. and Zeyh. Porcupine-root	Anacampserotaceae	Used for nervousness and stomach-aches, also used as a soap substitute [3].	Leaves	No records	No records	No records	No records
<i>Telfairia pedata</i> (Sm. ex Sims) Hook. Oysternut	Cucurbitaceae	Used in soap production, used for biodiesel [85]; used as an ornamental [86].	Seed oil	No records	Present [85]	Present [86]	No records

Table 1. Cont.

Species Name and Common Name	Family Name	Uses	Plant Part	Quantitative Saponin Content	Qualitative Saponin Content	Alkaloids	Terpenoids
<i>Thunbergia atriplicifolia</i> E. Mey. Natal primrose	Acanthaceae	Used by the Zulu and by the Natal Indian in making a hair-wash [3].	Leaves and unripe fruits	No records	No records	No records	No records
<i>Thunbergia dregeana</i> Nees Isiphondo	Acanthaceae	Used by the Zulu people as a remedy for venereal diseases, used as a soap [3].	Fruit and leaves	10.09 ± 2.52 Current study	Present (current study)	Present (current study)	Present (current study)
<i>Vachellia nilotica</i> (L.) P. J. Hurter and Mabb Scented-pod acacia	Fabaceae	Cytotoxic bark is used as an astringent, assists in treating haemorrhaging, wound ulcers, leprosy, leucoderma, skin diseases, burning sensation, assists with seminal weakness. The trunk bark is used for colds, bronchitis, nausea, piles [87–90], soap making [91].	Seed oil	105.600 ± 9.994 mg/g [92]	Present [38]	Present [92]	Present [93]
<i>Zygophyllum foetidum</i> Schrad. and J.C.Wendl. Scrambling twinleaf	Zygophyllaceae	Used for washing clothes [3].	Unknown	No records	No records	No records	No records

Table 2. Qualitative analysis of alkaloids and terpenoids using different polar and non-polar solvents, and determined saponin concentration for selected southern African soap plants.

Plant Species	Alkaloids	Terpenoids	Saponins	Emulsion	Saponin Concentration (mg DE/g DW)
<i>Calodendrum capense</i>	+	+	+	+	107.89 ± 4.89
<i>Carica papaya</i>	+	+	+	+	6.32 ± 1.20
<i>Crinum bulbispermum</i>	+	+	+	+	35.43 ± 4.25
<i>Cyathula uncinulata</i>	—	+	+	+	20.17 ± 1.70
<i>Deinbollia oblongifolia</i>	+	+	+	+	16.02 ± 2.26
<i>Ilex mitis</i>	+	+	+	+	8.37 ± 0.78
<i>Merwillia plumbea</i>	—	+	+	+	25.59 ± 0.83
<i>Noltea africana</i>	+	+	+	+	52.65 ± 6.81
<i>Plectranthus ciliatus</i>	+	+	+	+	18.88 ± 1.36
<i>Thunbergia dregeana</i>	+	+	+	+	10.09 ± 2.52

(+) Present (—) absent, (DE) diosgenin equivalents; (DW) dry weight.

4. Discussion

4.1. Ethnobotanical Information

Over 100 plant families have been stated to contain saponins and the legume family (Fabaceae) is specifically reported to have terpenoid saponins, with soybeans, beans and peas indicated to be rich sources of saponins [94]. The list includes well-known plants such as *Carica papaya* (better known for its nutritious fruits), *Crinum bulbispermum* (floral emblem of the Free State Province of South Africa, known for its large, attractive flowers), *Ilex mitis* (the only species found in southern Africa in a genus of about 200 species), and *Sceletium tortuosum* (better known for its medicinal use as an anti-depressant). Only one species of the family Sapindaceae, i.e., *Deinbollia oblongifolia*, was recorded as being used as a soap substitute in the region. This is surprising as members of this family are particularly known to have high concentrations of saponins, which are commonly located in the vegetative tissues [95]. For example, *Sapindus saponaria* L. (soapberry), used traditionally in Brazil, India, and the USA for making soap and *S. mukorossi* Gaertn. (soapnut, soapberry, washnut, etc.), utilized as a detergent and shampoo [7].

4.2. Soap Plant Names and Description

It is interesting to note that the common names (mostly English) of several species make some reference to the soap property and/or use of the plant. For example, *Aloe maculata* is known as soap aloe and is commonly used by various ethnic groups in the region as a soap substitute [44], although its vernacular names make no reference to this particular use. In fact, its Sesotho (South Sotho) name, *lekhala-la-bafu* (=the aloe of the dead), alludes to its use as part of funeral rituals. The succulent leaves are broken into pieces and added to the water in which people returning from the burial site must wash their hands before entering the yard [96]. The apparent cleansing effect of the plant can perhaps justify this usage, apart from its perceived magical powers as a protective charm [96]. However, the saponin content of *A. maculata* was found to be very low, i.e., 1.712 ± 0.051 mg/g in a study by Choi et al. [45]. *Helinus integrifolius* is known as soap bush, *Noltea africana* as soap glossy leaf, *Phytolacca dodecandra* as African soapberry, while *Pouzolzia mixta* is called soap nettle. *Kedrostis capensis* is known in Sesotho as *sesepe-sa-linoha* (soap of the snakes or snake soap), since it is used to chase away snakes, in addition to its use as a soap. *Dicerocaryum eriocarpum*, known in Afrikaans as seepbos (soap bush), was used by the Khoisan people in spa treatments as well as a shampoo and soap [97].

4.3. Saponin Concentrations in the Selected Plants

All the plants studied are being screened for their saponin concentration for the first time in this current study except for *M. plumbea*. In this study, quantification of saponin content was done using the spectrophotometric technique because of its fastness, simplicity, and affordability. A standard curve was generated using the absorbance values from an increasing concentration of diosgenin (Figure A1; Table A1). However, the technique determines total saponin content as opposed to chromatographic methods such as HPLC, UHPLC, GC-MS, and LC-MS, which give a specific saponin compound [12]. Using HPLC-ELSD analysis, *Saponaria officinalis* was found to contain a saponin concentration of 623 mg/g [98], which is significantly higher than any of the southern African soap plants evaluated in the current study. It should be noted that the extraction process also contributes to saponin yield, for example a study by Wang et al. [99] showed that the pressurised liquid extraction produced a slightly higher yield (7.36%) than other green extraction methods and the conventional Soxhlet (6.99%) and maceration (6.00%) methods. Therefore, these methods should be considered in future. *Calodendrum capense*, is used in commercialised products such as creams and lotions. It is worth noting that the seed oil is utilised as it is high in vitamin E and fatty acids known to have beneficial effects on the skin, this suggests that the traditional use of the seed oil from the plant in soap could be for this reason, and not necessarily for cleaning due to the presence of saponins. The lowest saponin content amongst the soap plants listed in Table 2 was found to be that of *Carica papaya* at a concentration of 6.32 ± 1.20 mg/g. According to Watt and Breyer-Brandwijk [3], the leaves are used as a soap substitute and are said to remove stains. *Carica papaya* is commercially used as the main ingredient in beauty products such as skin care products, ointments, shampoos, and soaps. However, it appears that the reason for the use of *Carica papaya* in soap-making might be due to the cleansing effect of the enzyme papain which dissolves dead cells from the skin [100]. Previous studies have shown that plant part, tissue type, age, physiological state, and genetic profiles can affect the concentration of chemical constituents in plants, including saponins. Furthermore, the quantifiable amount of saponins also depends on the solvent used in the extraction [101].

The importance of determining the saponin concentration in soap plants can allow for further use of the plant in different industries. For example, diosgenin is a well-known steroidal saponin that has been isolated mainly from the genera *Dioscorea* and *Costus* [2]. This steroid is useful in the synthesis of semisynthetic sex hormones and corticosteroids and functions as a natural precursor [102]. The beneficial use of plants containing diosgenin can also be seen in the traditional use for its pharmacological potential and has been studied further for its novel use as an anticancer therapeutic agent [102]. Hence, the known research on this biological compound makes it a good reference in determining unknown saponin concentrations.

4.4. Saponins and Environmental Factors

Soap plants should genotypically produce saponins, but the quantity of saponins within these soap plants can be affected by different biotic and abiotic factors. Environmental factors that can affect the production of saponins are habitat, light, temperature, soil composition, CO₂, oxygen and water levels, seasonal changes and the growth stage when harvesting [103]. Different geographical regions or cultivation can affect the degree of saponin accumulation in the plant. According to Szakiel et al. [103], a study on the quantity of ginsenoside in cultivated and wild ginseng plants showed that wild plants have saponin concentrations that are two-fold higher than the cultivated ginseng. A study by Ncube et al. [34] analysed the saponin content of *Merwillia plumbea* collected from the University of KwaZulu-Natal Botanical Gardens during different seasons, whereby the highest quantity was observed in plants harvested in winter. In the current study, although seasonality was not considered, *M. plumbea* collected from the Walter Sisulu Botanical Gardens displayed a concentration of 25.59 ± 0.83 mg/g, which is slightly higher than that of Ncube et al. [34] at 20 mg/g. Hence, there are many other factors that can affect the accumulation of saponins.

The extraction process for saponins is very challenging. Many parameters were considered so as not to affect the correct outcome for the saponin concentrations. Saponins are often found in low concentrations in plants and are highly polar, non-volatile compounds [104]. This study utilized alcohol extraction; however, this method may bring about esterification of acidic saponins. In some cases, extraction with methanol brings about the formation of methyl-derivatives that are not found naturally in the plant [105]. Therefore, a purification step was undertaken using chloroform which ensures the removal of pigments (methyl-derivatives) that would interfere with the assay [15]. If these pigments (methyl derivatives) are not removed it would ultimately bring about an over-estimation of saponin values [15].

Solvent extraction is the commonest method to extract plant material. Chloroform has been used in extraction processes for many years, but it has been a cause for concern due to its environmental, health, and safety problems. The chemical has been discontinued in different areas such as medicine, this is due to the toxic nature of the chemical which has been discovered to be carcinogenic by nature [106]. Alternatives for chloroform which are less hazardous is a colourless liquid dimethoxyethane (DME) which is known as a green chemical with the same dielectric as chloroform but is miscible in water [107]. The correct solvent selection is crucial in plant extraction hence the use DME as an alternative may not necessarily be effective in the removal of methyl derivatives and impurities, therefore this process would need further analysis. Based on the twelve principles of chemistry less hazardous chemical synthesis should be used to combat environmental, health, and safety problems [108]. Further analysis should be done on the use of environmentally friendly alternative chemicals. Extraction methods that are modern and environmentally viable can still be used these procedures include super critical fluid extraction, ultrasound assisted extraction and microwave assisted extraction, these procedures allow for lower organic solvent consumption and extraction time with a higher precision [109]. The use of chloroform in this experiment was based on availability and limited exposure to other modern and environmentally viable extraction processes.

5. Conclusions

A total of 37 plants are used in southern Africa for such cleaning purposes as washing the body and face, clothes, sheepskin, and hair, as recorded in the literature. Of these, only six species have been investigated for their saponin content in previous studies. Qualitative screening using the classical foam test, indicated that all the selected plants contain saponins in the parts studied. This observation may justify the use of these plants in soap or as soap substitute. Although there is no set standard for a good saponin content from plants, the content for most of the tested plants was found to be low, with the highest being *Calodendrum capense* at 107.89 ± 4.89 mg/g and *Noltea africana* at 52.65 ± 6.81 mg/g. These plants are also used for a variety of medicinal purposes including skin conditions, gastrointestinal problems, and urinary, bladder, respiratory tract, and sexually transmitted infections. The efficacy was not tested in this study; however, the therapeutic activity could be from the properties of soap plants based on the bioactive compounds, specifically saponins. The knowledge of plant composition gives a better understanding of plant chemistry and possible use of plants medicinally, industrially, and as soap substitutes. This allows the verification and the justification of plant use. Further studies should be done to analyse the critical micelle concentration to determine the efficiency of a surfactant which is the potential of efficient dirt removal.

Author Contributions: Conceptualization A.M.; methodology, M.M. and A.M.; validation, M.M. and A.M.; investigation, M.M. and A.M.; writing—original draft preparation, M.M.; writing—review and editing, M.M. and A.M.; visualization, M.M. and A.M.; supervision, A.M.; funding acquisition, A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Research Foundation and the University of Johannesburg.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work is based on the research supported in part by the National Research Foundation of South Africa for the Grant Number 93625. The authors are grateful to The University of Johannesburg for logistical support.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Figure A1. Calibration curve for diosgenin standard.

Table A1. Calculation of saponin concentration for selected southern African soap plants.

Plant Species	Weight of Dry Extract per mL m(gm)	Sample Solution (μg/mL)	Absorbance Values	DE Conc. C (μg/mL)	DE Conc. C(mg/mL)	TSC as DE A = CXV/m (mg/g)	Mean (mg/g)	STDev
<i>Calodendrum capense</i>	0.001	0.25	1.927	458.78	0.45878	114.695	107.8893	4.88689
	0.001	0.25	1.738	413.78	0.41378	103.445		
	0.001	0.25	1.773	422.112	0.422112	105.528		
<i>Carica papaya</i>	0.001	0.25	0.123	29.2555	0.029256	7.313875	6.321808	1.198715
	0.001	0.25	0.078	18.5412	0.018541	4.6353		
	0.001	0.25	0.188	28.065	0.028065	7.01625		
<i>Crinum bulbispermum</i>	0.001	0.25	0.677	161.1603	0.16116	40.29007	35.42896	4.252054
	0.001	0.25	0.503	119.7317	0.119732	29.93293		
	0.001	0.25	0.606	144.2555	0.144256	36.06388		
<i>Cyathula uncinulata</i>	0.001	0.25	0.379	90.2079	0.090208	22.55198	20.17102	1.701038
	0.001	0.25	0.324	77.1126	0.077113	19.27815		
	0.001	0.25	0.314	74.7317	0.074732	18.68293		
<i>Deinbollia oblongifolia</i>	0.001	0.25	0.322	76.636	0.076636	19.159	16.02415	2.259419
	0.001	0.25	0.234	55.684	0.055684	13.921		
	0.001	0.25	0.252	59.9698	0.05997	14.99245		
<i>Ilex mitis</i>	0.001	0.25	0.145	34.4936	0.034494	8.6234	8.365458	0.775076
	0.001	0.25	0.123	29.2555	0.029256	7.313875		
	0.001	0.25	0.154	36.6364	0.036636	9.1591		

Table A1. Cont.

Plant Species	Weight of Dry Extract per mL m(gm)	Sample Solution (µg/mL)	Absorbance Values	DE Conc. C (µg/mL)	DE Conc. C(mg/mL)	TSC as DE A = CXV/m (mg/g)	Mean (mg/g)	STDev
<i>Merwillia plumbea</i>	0.001	0.25	0.447	106.3983	0.106398	26.59958	25.58768	0.826213
	0.001	0.25	0.43	102.3508	0.102351	25.58769		
	0.001	0.25	0.413	98.3031	0.098303	24.57578		
<i>Noltea africana</i>	0.001	0.25	0.981	233.5412	0.233541	58.3853	52.65117	6.806973
	0.001	0.25	0.949	225.9221	0.225922	56.48053		
	0.001	0.25	0.724	172.3507	0.172351	43.08768		
<i>Plectranthus ciliatus</i>	0.001	0.25	0.29	69.017	0.069017	17.25425	18.88131	1.362031
	0.001	0.25	0.316	75.2079	0.075208	18.80198		
	0.001	0.25	0.346	82.3508	0.082351	20.5877		
<i>Thunbergia dregeana</i>	0.001	0.25	0.181	43.065	0.043065	10.76625	10.09165	2.524122
	0.001	0.25	0.215	51.1602	0.05116	12.79005		
	0.001	0.25	0.113	26.8746	0.026875	6.71865		

Key: TSC—total saponin content; C—concentration; V—volume; STDev—standard deviation.

References

- Levey, M. The early history of detergent substances. *J. Chem. Educ.* **1954**, *31*, 521–524. [\[CrossRef\]](#)
- Oleszek, W.; Hamed, A. Saponin-based surfactants. In *Surfactants from Renewable Resources*; Kjellin, M., Johansson, I., Eds.; John Wiley & Sons, Ltd.: Chichester, UK, 2010; pp. 244–246.
- Watt, J.M.; Breyer-Brandwijk, M.G. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd ed.; E. & S. Livingstone LTD: Edinburgh, UK, 1962.
- Routh, H.B.; Bhowmikk, R.; Parishl, C.; Witkowski, J.A. Soaps: From the Phoenicians to the 20th century—A historical review. *Clin. Dermatol.* **1996**, *1*, 3–6. [\[CrossRef\]](#)
- Bossard, E. Angolan medicinal plants used also as piscicides and/or soaps. *J. Ethnopharmacol.* **1993**, *40*, 1–19. [\[CrossRef\]](#)
- Mehta, P.S.; Bhatt, K.C. Traditional soap and detergent yielding plants of Uttaranchal. *Indian J. Tradit. Knowl.* **2005**, *6*, 279–284.
- Wisetkomolmat, J.; Suppakittpaisarn, P.; Sommano, S.R. Detergent plants of northern Thailand: Potential sources of natural saponins. *Resources* **2019**, *8*, 10. [\[CrossRef\]](#)
- Sadowska, B.; Budzynska, A.; Wieckowska-Szakiel, M.; Paszkiewicz, M.; Stochmal, A.; Singh, B.N.; Singh, B.R.; Singh, R.L.; Prakash, D.; Sarma, B.K.; et al. Antioxidant and anti-quorum sensing activities of green pod of *Acacia nilotica* L. *Food Chem. Toxicol.* **2009**, *47*, 778–786.
- Osborn, A. Saponins and plant defence—A soap story. *Trends Plant Sci.* **1996**, *1*, 4–9. [\[CrossRef\]](#)
- Moses, T.; Papadopoulou, K.K.; Osborn, A. Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Crit. Rev. Biochem. Mol. Biol.* **2014**, *49*, 439–462. [\[CrossRef\]](#) [\[PubMed\]](#)
- Oleszek, W.; Kapusta, I.; Stochmal, A. TLC of triterpenes (including saponins). In *Thin Layer Chromatography in Phytochemistry*; Waksmondzka-Hajnos, M., Sherma, J., Kowalska, T., Eds.; CRC Press, Taylor & Francis Group, LLC: New York, NY, USA, 2008; pp. 519–541.
- Gupta, A.; Naraniwal, M.; Kothari, V. Modern extraction methods for preparation of bioactive plant extracts. *Int. J. Appl. Nat. Sci.* **2012**, *1*, 8–26.
- Bart, H.J. Extraction of natural products from plants: An introduction. In *Industrial Scale Natural Products Extraction*, 1st ed.; Wiley-VCH: Weinheim, Germany, 2011; pp. 1–26.
- Cheok, C.Y.; Salman, H.A.K.; Sulaiman, R. Extraction and quantification of saponins: A review. *Food Res. Int.* **2014**, *59*, 16–40. [\[CrossRef\]](#)
- Makkar, H.P.S.; Sidhuraju, P.; Becker, K. *Plant Secondary Metabolites*; Humana Press Inc.: Totova, NJ, USA, 2007; pp. 93–101.
- Odeh, A.O.; Alshammari, G.A.A.; Almulgabsagher, K.S.R.; Andrew, P.A. Effect of solute polarity on extraction efficiency using deep eutectic solvents. *Green Chem.* **2021**, *23*, 5097–5105.
- Markets and Markets. Available online: <https://www.marketsandmarkets.com/Market-Reports/plant-extracts-market-942.html> (accessed on 10 July 2021).
- Hedberg, I.; Staugaard, F. *Traditional Medicinal Plants: Traditional Medicine in Botswana*; Ipeleng Publishers: Gaborone, Botswana, 1989.
- Motlhanka, D.M.T. Free radical scavenging activity of selected medicinal plants of Eastern Botswana. *Pak. J. Biol. Sci.* **2008**, *11*, 805–808. [\[CrossRef\]](#) [\[PubMed\]](#)
- Amusan, O.O.; Dlamini, P.S.; Msonthi, J.D.; Makhubu, L.P. Some herbal remedies from the Manzini region of Swaziland. *J. Ethnopharmacol.* **2002**, *79*, 109–112. [\[CrossRef\]](#)
- Amusan, O.O.; Sukati, N.A.; Dlamini, P.S.; Sibandze, F.G. Some Swazi phytomedicine and their constituents. *Afr. J. Biotechnol.* **2007**, *6*, 267–272.

22. Amusan, O.O.G. Herbal medicine in Swaziland: An overview. *ACS Symp. Ser.* **2009**, *1021*, 31–49.
23. Seleteng Kose, L.E.M.; Moteetee, A.; van Vuuren, S. Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho. *J. Ethnopharmacol.* **2015**, *170*, 184–200. [[CrossRef](#)] [[PubMed](#)]
24. Seleteng Kose, L.E.M. Evaluation of Commonly Used Medicinal Plants of Maseru District in Lesotho for Their Ethnobotanical Uses, Antimicrobial Properties and Phytochemical Composition. Ph.D. Thesis, University of Johannesburg, Johannesburg, South Africa, 2017.
25. Koenan, E.V. *Medicinal, Poisonous and Edible Plants in Namibia*; Klaus Hess Verlag: Windhoek, Namibia, 1996; Volume 2.
26. Chinsebu, K.C.; Hedimbi, M.; Mukaru, W.C. Putative medicinal properties of plants from the Kavango region, Namibia. *J. Med. Plant Res.* **2011**, *5*, 6787–6797.
27. Hutchings, A.; Scott, A.H.; Lewis, G.; Cunningham, A. *Zulu Medicinal Plants. An Inventory*; University of Natal Press: Pietermaritzburg, South Africa, 1996; pp. 21–311.
28. Van Wyk, B.E. A review of ethnobotanical research in southern Africa. *S. Afr. J. Bot.* **2002**, *68*, 1–13. [[CrossRef](#)]
29. Van Wyk, B.E. The potential of South African plants in the development of new medicinal products. *S. Afr. J. Bot.* **2011**, *77*, 812–829. [[CrossRef](#)]
30. Edeoga, H.O.; Okwu, D.E.; Mbaebie, B.O. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* **2005**, *4*, 685–688. [[CrossRef](#)]
31. Tiwari, P.; Kumar, B.; Kaur, M.; Kaur, G.; Kaur, H. Phytochemical screening and extraction: A review. *Intern. Pharm. Sci.* **2011**, *1*, 99–106.
32. Zohra, S.F.; Meriem, B.; Samira, S.; Muneer, A.M.S. Phytochemical screening and identification of some compounds from Mallow. *J. Nat. Prod. Plant Resour.* **2012**, *2*, 512–516.
33. Kaur, R.; Arora, S.; Thukral, A.K. Quantitative and qualitative analysis of saponins in different plant parts of *Chlorophytum borivillianum*. *Intern. J. Pharma. Bio Sci.* **2015**, *6*, 826–835.
34. Ncube, B.; Finnie, J.F.; van Staden, J. Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. *S. Afr. J. Bot.* **2011**, *77*, 387–396. [[CrossRef](#)]
35. Ndamba, J.; Lemmichi, E.; Molgaard, P. Release of molluscicidal saponins from *Phytolacca dodecandra* aqueous berry extracts as influenced by the male plant and the extraction procedure. *Biochem. Syst. Ecol.* **1994**, *22*, 249–257. [[CrossRef](#)]
36. Ighodaro, M.O.; Omole, J.O.; Fadipe, S.O. Effects of *Piliostigma thonningii* ethyl acetate leaf extract on aluminium-cum extract treated wistar rats. *Anim. Res. Int.* **2012**, *9*, 1579–1584.
37. Onyeike, E.N.; Omubo-Dede, T.T. Effect of heat treatment on the proximate composition, energy values, and levels of some toxicants in African yam bean (*Sphenostylis stenocarpa*) seed varieties. *Plant Foods Hum. Nutr.* **2002**, *57*, 223–231. [[CrossRef](#)]
38. Ali, A.; Akhtar, N.; Khan, B.A.; Khan, M.S.; Rasul, A.; Zaman, S.; Khalid, N.; Waseem, K.; Mahmood, T.; Ali, L. *Acacia nilotica*: A plant of multipurpose medicinal uses. *J. Med. Plant Res.* **2012**, *6*, 1492–1496.
39. Street, R.A.; Prinsloo, G. Commercially important medicinal plants of South Africa: A review. *J. Chem.* **2012**, *2013*, 1–16. [[CrossRef](#)]
40. Williams, V.L.; Cunningham, A.B.; Raimondo, D. *Merwillia Plumbea* (Lindl.) Septa. Available online: <http://redlist.sanbi.org/species.php?species=7485-2> (accessed on 19 December 2019).
41. Sparg, S.G.; van Staden, J.; Jager, A.K. Pharmacological and phytochemical screening of two Hyacinthaceae species: *Scilla natalensis* and *Ledebouria ovatifolia*. *J. Ethnopharmacol.* **2002**, *80*, 95–101. [[CrossRef](#)]
42. Mar, W.; Tan, G.T.; Cordele, G.A.; Pezzuto, J.M. Biological activity of novel macrocyclic alkaloids (budmunchiamines) from *Albizia amara* detected on the basis of interaction with DNA. *J. Nat. Prod.* **1991**, *54*, 1531–1542. [[CrossRef](#)] [[PubMed](#)]
43. Praveen, P.; Thippeswamy, S.; Mohana, D.C.; Manjunath, K. Antimicrobial efficacy and phytochemical analysis of *Albizia amara* (Roxb.) Boiv. an indigenous medicinal plant against some human and plant pathogenic bacteria and fungi. *J. Pharm. Res.* **2011**, *4*, 832–835.
44. Felhaber, T.; Mayeng, I. *South African Traditional Healers' Primary Health Care Handbook*, 1st ed.; Kagiso Publishers: Cape Town, South Africa, 1997; p. 195.
45. Choi, S.; Supeno, D.; Byun, J.; Kwon, S.; Chung, S.; Kwon, S.; Kwon, D.; Choi, W. The identification of saponin to obtain the maximum benefit from *Aloe saponaria*. *Adv. Sci. Technol. Lett.* **2015**, *120*, 558–563.
46. Philander, L.A. An ethnobotany of Western Cape Rasta bush medicine. *J. Ethnopharmacol.* **2011**, *138*, 578–594. [[CrossRef](#)] [[PubMed](#)]
47. Ing'ahu, O.R. Isolation of Metabolites and Screening for Antimicrobial Activity of *Calodendrum capense* Thunb. (Rutaceae). Master's Thesis, Kenyatta University, Nairobi, Kenya, 2004; pp. 156–172.
48. Yushau, M.; Onuorah, F.C.; Murtala, Y. In vitro sensitivity pattern of some urinary tract isolates to *Carica papaya* extracts. *Bayero J. Pure App. Sci.* **2009**, *2*, 75–78. [[CrossRef](#)]
49. Roberts, M. *Indigenous Healing Plants*; Southern Book Publishers: Johannesburg, South Africa, 1990.
50. Elgorashi, E.E.; Taylor, J.L.S.; Verschaeve, L.; Maes, A.; van Staden, J.; de Kimpe, N. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. *Toxicol. Lett.* **2003**, *143*, 195–207. [[CrossRef](#)]
51. Glasby, J.S. *Dictionary of Plants Containing Secondary Metabolites*; Taylor & Francis: London, UK, 1991.
52. Moffett, R. *Sesotho Plant and Animal Names and Plants Used by the Basotho*; Sun Press: Bloemfontein, South Africa, 2010.
53. Ibrahim, B.; Sowemimo, A.; van Rooyen, A.; van de Venter, M. Anti-inflammatory, analgesic, and antioxidant activities of *Cyathula prostrata* (Linn.) Blume (Amaranthaceae). *J. Ethnopharmacol.* **2012**, *141*, 282–289. [[CrossRef](#)]

54. Bisi-Johnson, M.A.; Obi, C.L.; Samuel, B.B.; Eloff, J.N.; Okoh, A.I. Antibacterial activity of crude extracts of some South African medicinal plants against multidrug resistant etiological agents of diarrhoea. *BMC Complement. Altern. Med.* **2017**, *17*, 1–9. [CrossRef]
55. Wurger, G. A Rational in In Vitro Evaluation of 53 Medicinal Plants Used in the Treatment of Diarrhoea and the Potential Use of *Deinbollia oblongifolia* (Sapindaceae) Extracts. Ph.D. Thesis, University of Pretoria, Pretoria, South Africa, 2010; p. 148.
56. Du Preez, I.; Mumbengegwi, D. Phytochemical investigation on Namibian plants for anti-malaria compounds. *J. Human. Soc. Sci.* **2012**, *1*, 147–158.
57. Fawole, O.A.; Ndhkala, A.R.; Amoo, S.O.; Finnie, J.F.; van Staden, J. Anti-inflammatory and phytochemical properties of twelve medicinal plants used for treating gastro-intestinal ailments in South Africa. *J. Ethnopharmacol.* **2009**, *123*, 237–243. [CrossRef]
58. Iwamoto, Y.; Sugimoto, S.; Harinantenaina, L.; Matsunami, K.; Otsuka, H. Entadosides A–D, triterpene saponins and a glucoside of the sulphur-containing amide from the kernel nuts of *Entada phaseoloides* (L.) Merrill. *J. Nat. Med.* **2012**, *66*, 321–328. [CrossRef] [PubMed]
59. Shai, L.J.; Chauke, M.A.; Magano, S.R.; Mogale, A.M.; Eloff, J.N. Antibacterial activity of sixteen plant species from Phalaborwa, Limpopo Province, South Africa. *J. Med. Plants Res.* **2013**, *7*, 1899–1906.
60. Ntini, V.P. Inhibitory Capabilities of Ten Medicinal Plants Used by Traditional Healers on Mammalian Carbohydrate Digesting Enzymes (Alpha-Amylase and Alpha-Glucosidase). Master's Thesis, University of Limpopo, Limpopo, South Africa, 2013; pp. 19–23.
61. Kai, N.S.; Nee, T.A.; Ling, E.L.C.; Ping, T.C.; Kamariah, L.; Lin, N.K. Anti-hypercholesterolemic effect of kenaf (*Hibiscus cannabinus* L.) seed on high-fat diet Sprague Dawley rats. *Asian Pac. J. Trop. Med.* **2015**, *1*, 6–13. [CrossRef]
62. Forrester, J. *Ilex Mitis*. Available online: <http://pza.sanbi.org/ilex-mitis> (accessed on 29 April 2020).
63. Sabela, M.I.; Makhanya, T.; Kanchi, S.; Shahbaaz, M.; Idress, D.; Bisetty, K. One-pot biosynthesis of silver nanoparticles using *Iboza Riparia* and *Ilex Mitis* for cytotoxicity on human embryonic kidney cells. *J. Photochem. Photobiol. B Biol.* **2018**, *178*, 500–567. [CrossRef]
64. Van den Bergh, C.K. The healing practices of Bishop Joseph Matuwane: Example of healing practices. *Res. Natl. Mus.* **1995**, *11*, 386–394.
65. Bouftira, I.; Abdelly, C.; Sfar, S. Characterization of cosmetic cream with *Mesembryanthemum crystallinum* plant extract: Influence of formulation composition on physical stability and antioxidant activity. *Int. J. Cosmet. Sci.* **2008**, *30*, 443–452. [CrossRef] [PubMed]
66. Hanen, F.; Riadh, K.; Samia, O.; Sylvain, G.; Christian, M.; Chedly, A. Interspecific variability of antioxidant activities and phenolic composition in *Mesembryanthemum* genus. *Food Chem. Toxicol.* **2009**, *47*, 2308–2313. [CrossRef]
67. Rahman, S.M.A.; Abd-Ellatif, S.A.; Deraz, S.F.; Khalil, A.A. Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. *Afr. J. Biotechnol.* **2011**, *10*, 10733–10743.
68. Moteetee, A.; van Wyk, B.-E. The medical ethnobotany of Lesotho: A review. *Bothalia* **2011**, *41*, 209–228. [CrossRef]
69. Ashafa, A.O.T. Medicinal potential of *Morella serrata* (Lam.) Killick (Myricaceae) root extracts: Biological and pharmacological activities. *BMC Complement. Altern. Med.* **2013**, *13*, 1–8. [CrossRef]
70. Sabiu, S.; Ashafa, O.T.A. Toxicological implications and laxative potential of ethanol root extract of *Morella serrata* in loperamide-induced constipated Wistar rats. *Pharm. Biol.* **2016**, *54*, 2901–2908. [CrossRef]
71. Parkhurst, R.M.; Mthupha, B.M.; Liang, Y.S.; Bruce, J.I.; Lambert, J.D.H.; Collier, T.L.; ApSimon, J.W.; Wolde-Yohannes, L.; Heath, G.E.; Jones, W.O.; et al. The molluscicidal activity of *Phytolacca dodecandra* L. Location of the activating esterase. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 436–439. [CrossRef]
72. Nostro, A.; Germano, M.P.; D'Angelo, V.; Marino, A.; Cannatelli, M.A. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett. Appl. Microbiol.* **2000**, *30*, 379–384. [CrossRef]
73. Dluya, T.; Dahiru, D. Antibacterial activity of *Piliostigma thonningii* methanol stem bark extract. *Int. J. Res. Pharm. Biosci.* **2018**, *5*, 15–20.
74. Phillips, W.C. *The Treatment of Labyrinthine Affections*; Medical Society: New York, NY, USA, 1916; pp. 191–195.
75. Viljoen, A.M.; Demirci, B.; Başer, K.H.C.; Potgieter, C.J.; Edwards, T.J. Microdistillation and essential oil chemistry—A useful tool for detecting hybridisation in *Plectranthus* (Lamiaceae). *S. Afr. J. Bot.* **2006**, *72*, 99–104. [CrossRef]
76. Sewani-Rusike, C.R. Antifertility effects of *Pouzolzia mixta* in female wistar rats. *Afr. J. Tradit. Complement. Altern. Med.* **2013**, *10*, 526–532. [CrossRef] [PubMed]
77. Rimington, C. *Psilocaulon absimile* N.E.Br. as a stock poison. *Onderstepoort J. Vet. Sci. Anim. Ind.* **1933**, *1*, 439–455.
78. Hutchings, A.; van Staden, J. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. *J. Ethnopharmacol.* **1994**, *43*, 89–124. [CrossRef]
79. Moteetee, A.; van Wyk, B.-E. The concept of 'Musa-pelo and the medicinal use of shrubby legumes (Fabaceae) in Lesotho. *Bothalia* **2007**, *37*, 75–77. [CrossRef]
80. Meiring, I. Notes on some experiments with the active principle of *Mesembryanthemum tortuosum* L. *Trans. S. Afr. Phi. Soc.* **1896**, *9*, 48–50.
81. Welman, M. *Solanum Aculeastrum*. Available online: <http://pza.sanbi.org/solanum-aculeastrum> (accessed on 29 April 2020).
82. Wanyonyia, A.W.; Chhabra, S.C.; Mkojib, G.; Udo Eilert, U.; Njuea, W.M. Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. *Phytochemistry* **2002**, *59*, 79–84. [CrossRef]

83. Onyeke, C.C.; Akueshi, C.O. Pathogenicity and reproduction of *Meloidogyne incognita* (Kofoid and White) chitwood on African yam bean, *Sphenostylis stenocarpa* (Hochst Ex. A. Rich) Harms accessions. *Afr. J. Biotechnol.* **2012**, *11*, 1607–1616. [CrossRef]
84. Nyananyo, B.L.; Nyingifa, A.L. Phytochemical investigation on the seed of *Sphenostylis stenocarpa* (Hochst ex A. Rich.) Harms (Family Fabaceae). *J. Appl. Sci. Environ. Manag.* **2011**, *15*, 419–423.
85. Kanyua, J.F. The Potential of Telfairia Pedata for Liquid Biofuel and Soap Production. Master's Thesis, Kenyatta University, Nairobi, Kenya, 2011; pp. 1–119.
86. Ajayi, S.A.; Dulloo, M.E.; Vodouhe, R.S.; Berjak, P.; Kioko, J.I. Conservation status of *Telfairia* spp. in sub-Saharan Africa. In *Plant Genetic Resources and Food Security in West and Central Africa, Proceedings of the Regional Conference, Ibadan, Nigeria, 26–30 April 2004*; Vodouhe, R., Atta-Krah, K., Achigan-Dako, G.E., Eyog-Matig, O., Avohou, H., Eds.; Bioversity International: Rome, Italy, 2007.
87. Kaur, K.; Michael, H.; Arora, S.; Harkonen, P.; Kumar, S. In vitro bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from *Acacia nilotica* (L.) Willd. ex Del. *J. Ethnopharmacol.* **2005**, *99*, 353–630. [CrossRef] [PubMed]
88. Singh, R.; Singh, B.; Singh, S.; Kumar, N.; Kumar, S.; Arora, S. Anti-free radical activities of kaempferol isolated from *Acacia nilotica* (L.) Willd. Ex. Del. *Toxicol. In Vitro* **2008**, *22*, 1965–1970. [CrossRef]
89. Agrawal, S.; Kulkarni, G.T.; Sharma, V.N. A comparative study on the antioxidant activity of methanol extracts of *Acacia*. *Adv. Nat. Appl. Sci.* **2010**, *4*, 78–84.
90. Kalaivani, T.; Mathew, L. Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Willd. ex Delile, an Indian medicinal tree. *Food Chem. Toxicol.* **2010**, *48*, 298–305. [CrossRef]
91. Hassan, K.J.; Zubairu, M.S.; Olayemi, O.R. Production of soap from neem seed oil and *Acacia nilotica* seed oil. *Int. J. Mod. Org. Chem.* **2015**, *4*, 70–84.
92. Abdirahman, Y.A.; Juma, K.K.; Mukundi, M.J.; Gitahi, S.M.; Agyirifo, D.S.; Ngugi, P.M.; Gathumbi, P.K.; Ngeranwa, J.J.N.; Njagi, E.N.M. The hypoglycemic activity and safety of aqueous stem bark extracts of *Acacia nilotica*. *Drug Metab. Toxicol.* **2015**, *6*, 1–9.
93. Bansa, A. Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. *J. Med. Plant Res.* **2009**, *3*, 082–085.
94. Kregiel, D.; Berlowska, J.; Witonska, I.; Antolak, H.; Proestos, C.; Babic, M.; Babic, L.; Zhang, B. Saponin-based, biological-active surfactants from plants. In *Application and Characterization of Surfactants*; Najjar, R., Ed.; IntechOpen: London, UK, 2017; pp. 183–205.
95. Acevedo-Rodríguez, P.; van Welzen, P.; Adema, F.; van der Ham, R.W.J.M. Sapindaceae. In *Flowering Plants. Eudicots: Sapindales, Cucurbitales, Myrtaceae*; Kubitzki, K., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 357–407.
96. Moteete, A. A review of plants used for magic by Basotho people in comparison with other cultural groups in southern Africa. *Indian J. Tradit. Knowl.* **2017**, *16*, 229–234.
97. Hoveka, L. *Dicerocaryum Eriocarpum*. Available online: <http://pza.sanbi.org/dicerocaryum-eriocarpum> (accessed on 29 April 2020).
98. Budan, A.; Bellenot, D.; Freuze, I.; Gillmann, L.; Chicoteau, P.; Richomme, P.; Guilet, D. Potential of extracts from *Saponaria officinalis* and *Calendula officinalis* to modulate in vitro rumen fermentation with respect to their content in saponins. *Biosci. Biotechnol. Biochem.* **2014**, *78*, 288–295. [CrossRef] [PubMed]
99. Wang, Q.Y.; Dong, X.; Yang, J.; Hu, Y.H.; Peng, L.Q.; Zheng, H.; Cao, J. Vesicle based ultrasonic-assisted extraction of saponins in *Panax notoginseng*. *Food Chem.* **2020**, *303*, 125394. [CrossRef]
100. Setiadi, S.; Putri, A.; Anindia, F. Manufacture of solid soap based on crude papain enzyme and antioxidant from papaya. In *Proceedings of the IOP Conference Series: Earth and Environmental Science, Banda Aceh, Indonesia, 26–27 September 2018*; Volume 105, pp. 1–7. [CrossRef]
101. Kunatsa, Y.; Katerere, D.R. Checklist of African soapy saponin-rich plants for possible use in communities' response to global pandemics. *Plants* **2021**, *10*, 842. [CrossRef] [PubMed]
102. Jesus, M.; Martins, A.P.J.; Gallardo, E.; Silvestre, S. Diosgenin: Recent highlights on pharmacology and analytical methodology. *J. Anal. Methods Chem.* **2016**, *2016*, 4156293. [CrossRef]
103. Szakiel, A.; Paczkowski, C.; Henry, M. Influence of environmental abiotic factors on the content of saponins in plants. *Phytochem. Rev.* **2011**, *10*, 471–491. [CrossRef]
104. Majinda, R.R.T. Extraction and Isolation of Saponins: Natural Products Isolation. *Methods Mol. Biol.* **2012**, *864*, 415–425.
105. Chaudhary, N.K.; Bhattarai, A.; Guragain, B.; Bhattarai, A. Conductivity, Surface Tension, and Comparative Antibacterial Efficacy Study of Different Brands of Soaps of Nepal. Available online: <https://www.hindawi.com/journals/jchem/2020/6989312/> (accessed on 2 June 2020).
106. Fisher Science. Available online: https://www.google.com/url?sa=t&source=web&rct=j&url=http://fscimage.fishersci.com/cmsassets/downloads/segment/ScienceEducation/pdf/green_ChemicalCrossRef.pdf&ved=2ahUKEwif4vLb8rXyAhWDiVwKHbtXB1QQFnoECAMQBg&usq=AOvVaw2jK50D97qvy8edv04GO95d (accessed on 10 August 2021).
107. Rankin, G.O.; Valentovic, M.A. Chloroform. In *Encyclopedia of Toxicology*, 3rd ed.; Wexler, P., Ed.; Elsevier Inc.: Cambridge, ME, USA, 2014.
108. Saleh, H.E.M.; Koller, M. Introductory chapter: Principles of green chemistry. In *Green Chemistry*; Saleh, H., Ed.; IntechOpen Limited: London, UK. Available online: <https://www.intechopen.com/chapters/57200> (accessed on 10 August 2021).
109. Zhang, Q.W.; Lin, L.G.; Ye, W.C. Techniques for extraction and isolation of natural products: A comprehensive review. *Chin. Med.* **2018**, *13*, 1–26. [CrossRef]