






Phytochemical screening and antioxidant evaluation of *Agave angustifolia* and *Agave sisalana*



Authors:

Ntombikayise G. Mkhize^{1,2} 
 Matthew C. Achilonu¹ 
 Idah T. Manduna² 
 Xolile V. Ngubane¹ 
 S'busiso M. Nkosi¹ 

Affiliations:

¹Technology Station in Chemicals, Faculty of Natural Sciences, Mangosuthu University of Technology, Durban, South Africa

²Life Sciences: Centre for Applied Food Sustainability and Biotechnology (CAFSaB), Faculty of Health and Environmental Sciences, Central University of Technology, Bloemfontein, South Africa

Corresponding author:

Ntombikayise Mkhize,
 gloria@mut.ac.za

Dates:

Received: 13 May 2024
 Accepted: 06 July 2024
 Published: 22 Oct. 2024

How to cite this article:

Mkhize, N.G., Achilonu, M.C., Manduna, I.T., Ngubane, X.V. & Nkosi, S.M., 2024, 'Phytochemical screening and antioxidant evaluation of *Agave angustifolia* and *Agave sisalana*', *Journal of Medicinal Plants for Economic Development* 8(1), a263. <https://doi.org/10.4102/jomped.v8i1.263>

Copyright:

© 2024. The Authors.
 Licensee: AOSIS. This work is licensed under the Creative Commons Attribution License.

Read online:



Scan this QR code with your smart phone or mobile device to read online.

Background: *Agave angustifolia* (AA) and *Agave sisalana* (AS) are used by local communities for medicinal purposes to treat skin conditions. Small companies add *Agave* leaf extracts to their cosmetic products, claiming calming and skin-rejuvenating qualities.

Aim: The study aimed to assess the phytochemical profiles and antioxidant activities of AA and AS verifying the traditional therapeutic claims about the species and accordingly establishing the assertions of cottage industries.

Setting: The AA leaves were collected from the eThekweni Metropolitan Municipality, while AS leaves were sourced from the iLembe district municipality.

Methods: The phytochemical extracts were obtained by gradient solvent maceration of the leaves. Qualitative phytochemical screening established the presence of bioactive phytochemicals in the extract. The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and the ferric reducing and/or antioxidant power (FRAP) methods measured the bioactive phytochemicals' antioxidant activity.

Results: Qualitative phytochemical evaluation confirmed the presence of secondary metabolites in both plants. AS extracts also contained alkaloids. The DPPH antioxidant activity indicated that *Agave* extracts had 20% to 80% scavenging activity. AS methanol extract had the maximum antioxidant activity among all the extracts. AA methanol and AS hexane extracts had no antioxidant activity. AA ethyl acetate extract had higher antioxidant activity (64%) than AS (52%). AA hexane extract had 70% activity while AS hexane extract had 30%.

Conclusion: The detected phytochemicals indicate potential use for emulsifying, antioxidant, anti-ageing, anti-inflammatory, and broad-spectrum antimicrobial activities.

Contribution: This study contributes to the existing knowledge of the therapeutic properties of AA and AS plants.

Keywords: *Agave*; *angustifolia*; *sisalana*; bioactive phytochemicals; therapeutic value; bioactivity; cosmetics.

Introduction

The *Agave* genus is indigenous to America (Cruz-Magalhães et al. 2020; Rivera-Lugo et al. 2018), but has spread throughout the world and is now found in South Africa, Swaziland, Angola, Brazil, China, and other countries (Cruz-Magalhães et al. 2020; Villanueva-Rodríguez et al. 2016). The *Agave* genus is made up of more than 200 species (Ahumada-Santos et al. 2013; Cruz-Magalhães et al. 2020; Salazar-Pi et al. 2017; Villanueva-Rodríguez et al. 2016), and examples include *Agave angustifolia*, *Agave salmiana*, *Agave sisalana*, *Agave americana*, *Agave tequilana*, and others (Rivera-Lugo et al. 2018). The *Agave* plants have big, fibrous leaves (López-Romero et al. 2018) and are morphologically very similar, making it difficult for some local people to differentiate them.

Although alien to South Africa, the *Agave* plants have found many uses in the local KwaZulu-Natal (KZN), South African community, such as building hedges and in traditional medicine. In Mexico, *Agave* plants are used for various things, including food, construction, medicine, textiles, and decoration (Rivera-Lugo et al. 2018). *Agave*-based alcoholic beverages are trendy both in Mexico and around the world. *Agave tequilana* and *A. angustifolia* are used to manufacture alcoholic beverages, tequila and bacanora, respectively. *A. salmiana*, *A. cupreata*, *A. duranguensis*, *A. fourcroydes*, *A. angustifolia*, *A. potatorum* are used in the manufacture of mezcal (Vera-Guzmán et al. 2018). The ecological significance of *Agave* plants is derived from their capacity to prevent

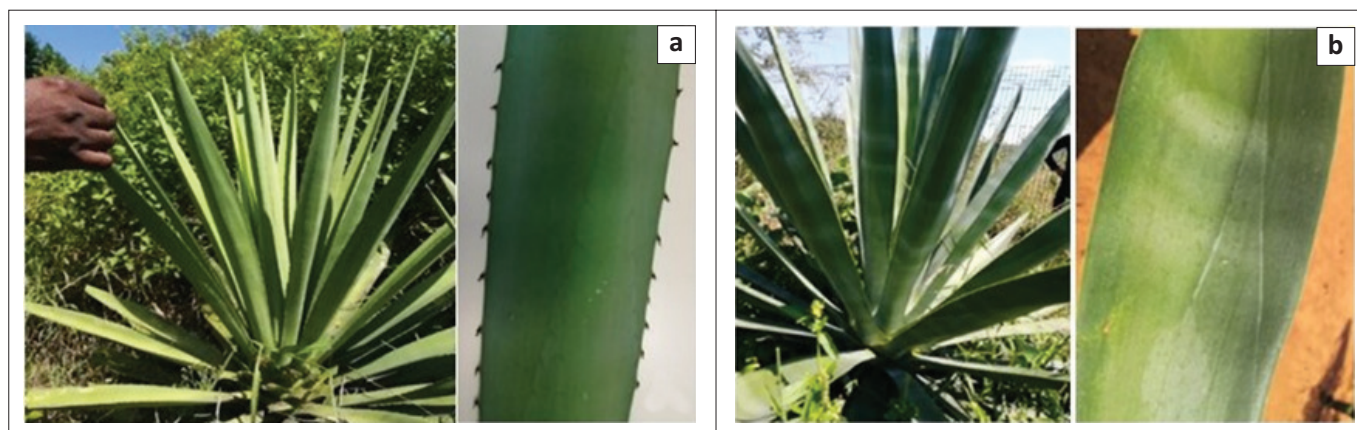
soil erosion through their root network, manage precipitation runoff, sequester carbon dioxide and provide food and shelter to various animal species and insects (Almazán-Morales et al. 2022). Traditionally, *Agave* plants have been used to treat bacterial-induced health disorders and free elemental xenobiotic-induced toxicity (Bhattacharyya et al. 2014). The disorders may include inflammations, wound infections, cancer, and others (Ahumada-Santos et al. 2013). Literature suggests that *Agave* plants have extensive, scientifically demonstrated traditional uses, such as digestive and wound infections (Villanueva-Rodríguez et al. 2016). Pharmacological research has confirmed the anti-inflammatory properties of various *Agave* species, primarily because of terpenes and steroidal saponins (Monterrosas-Brisson et al. 2013). *A. americana*, *A. angustifolia*, *A. cupreata*, *A. sisalana*, and *A. tequilana* are used to treat inflammatory-related ailments and are known to display antifungal, antihypertensive, anti-inflammatory, antiparasitic, and immunomodulatory actions (Ahumada-Santos et al. 2013; Monterrosas-Brisson et al. 2013; Salazar-Pi et al. 2017). Research on the *Agave* genus has long recognised different *Agave* species as a reliable source of steroidal saponins (Mina et al. 2013).

The *Agave* species of interest in this study are two wild *Agave* plants abundantly found in KwaZulu-Natal province, South Africa: *A. sisalana* Perrine and *A. angustifolia* Haw. var. *angustifolia*. The leaves of these two species are used traditionally by the community in treating various ill health, particularly skin conditions. The study, therefore, investigates the leaf extracts' phytochemical screening and their antioxidant activities to substantiate the plants' folk therapeutic claims. The problems in this study are that several *Agave* plant species are physiologically too similar, but exhibit different biological traits. An example is the similarities of *A. angustifolia* and *A. americana*, as well as *A. tequilana* and *A. sisalana*. The close resemblance makes it difficult for local users to differentiate the species. The species' close physiological similarity often confuses selecting the appropriate species for the treatments, resulting in poisoning of the patient rather than curing. Another problem is the lack

of literature on safe and effective doses for agave use in personal care applications.

Agave angustifolia Haw is an evergreen, perennial shrubby, succulent plant. It has stiffly erect, slender leaves with spaced-apart spikes and a sharp prickle at the end (Verloove et al. 2019) (Figure 1a). According to Verloove and Pascual (2021), *A. angustifolia* is a varied species that is endemic to Central America and is mainly used to make mezcal (Vera-Guzmán et al. 2018), as well as ropes, fuel, and decorations (Franck 2012; García-Mendoza & Chiang 2003). *Agave angustifolia* has a wide range of applications in conventional medicine. While the boiled leaves, sap, and root infusions have been used to make bandages for injuries and inflammation and to heal 'internal injuries', the fibre is used to treat urticaria (García-Mendoza & Chiang 2003). According to García-Mendoza and Chiang (2003), the roots have diuretic and diaphoretic properties. Therefore, a remedy made from boiling the root has been used to treat dysentery. *Agave angustifolia* is also employed for sprains and broken bones in people and animals, as the roasted *A. angustifolia* leaves are applied topically to treat sprains and fractured bones, as well as to reduce rheumatic pain (Jiménez-Ferrer et al. 2022). It is suggested that applying fresh *A. angustifolia* leaves to wounds can help reduce bleeding and pain (Hernández-Valle et al. 2014; Monterrosas-Brisson et al. 2013). Food supplements containing agavins from *A. angustifolia* are utilised (Velázquez-Martínez et al. 2014) because the leaves of the *A. angustifolia* plant have antioxidant qualities and are rich in phytosterols and steroidal saponins (Ahumada-Santos et al. 2013; Hernández-Valle et al. 2014). Coughs and skin problems, such as acne, can be treated with fresh *A. angustifolia* leaves, and the roasted leaves are applied topically to treat sprains and fractured bones and reduce rheumatic pain (Jiménez-Ferrer et al. 2022). Literature suggests that the sap from *A. angustifolia* is applied topically to humans and animals to treat sprains and fractured bones (Monterrosas-Brisson et al. 2013).

Agave sisalana Perrine (AS), popularly known as sisal, belongs to the Agavaceae family (Chigodi et al. 2013; Zwane, Dlamini & Nkambule 2010). It has sword-shaped,



Source: Photograph taken by Ntombikayise G. Mkhize

FIGURE 1: *Agave angustifolia* (a) and *Agave sisalana* (b) plants.

stiff, smooth-edged grey-green leaves with a sharp spike at the tip (Figure 1b). It was first brought to Tanzania in 1893, and then it spread to other regions of East Africa for fibre production from the sisal leaves (Chigodi et al. 2013). It is currently found in numerous tropical nations, such as South Africa, Tanzania, Uganda, Mozambique, and Namibia (Zwane et al. 2010). The researchers have reported that *A. sisalana* has anthelmintic, antibacterial, analgesic, and anti-inflammatory properties (Guimarães de Oliveira et al. 2016). Wu et al. (2021) reported that the pharmaceutical industry uses hecogenin, a sapogenin isolated from *A. sisalana* leaves, for pharmaceutical developments. The images of *A. angustifolia* and the *A. sisalana* plants taken by the researcher from the research site are shown in Figure 1.

Plants contain naturally occurring physiologically active chemical compounds, known as phytochemicals, that offer human health advantages beyond those of macro- and micro-nutrients (Araldi et al. 2018). They are found in a variety of foods, including fruits, vegetables, whole grains, legumes, nuts and seeds, tea, and dark chocolate, and only a small number have been isolated and identified from the plants (Xiao & Bai 2019). These phytochemicals, found in various plant parts, are well-known to possess anti-ageing, anti-inflammatory, and antioxidant properties (Michalak 2022; Roy et al. 2022). Scientific research has demonstrated the effectiveness and safety of these properties in preventing skin health issues such as photoaging and treating atopic dermatitis (Michalak 2022; Wu et al. 2021). These bioactive phytochemicals include carotenoids, phenolic acids, alkaloids, terpenes, tannins, and phytosterols. They are responsible for the plant's anti-inflammatory, antibacterial, antifungal, and antioxidant properties (Xiao & Bai 2019). Research has reported that polyphenols have good activity as antioxidants, anti-inflammatory, and anti-cancer agents that prevent cardiovascular diseases (Briguglio et al. 2020). Plant phenolics protect against environmental strains such as UV radiation, pathogenic infection, and predators (Kumar et al. 2020). The two most used methods to determine antioxidant activity are the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) and ferric reducing or antioxidant power (FRAP) assays. This study evaluated the two selected wild *Agave* plants' phytochemical profile and their antioxidant activity to establish the plants' extracts' suitability and potential use in cosmetic product development.

Research method and design

Plant collection and identification

The *A. angustifolia* leaves were collected from the Isipingo beach suburb (29°59'44.0"S 30°56'38.0"E), located on the southern coast of KZN, South Africa, under the eThekweni Metropolitan Municipality. In contrast, the *AS* leaves were collected from the veld along the R34 road (29°27'29.6"S

31°04'30.9"E), under the iLembe district municipality, KZN, South Africa.

The South African National Biodiversity Institute (SANBI) verified the taxonomic identity of the plants, and the two samples were added to the KZN Herbarium with the accession numbers *A. sisalana* (152364) and *A. angustifolia* (152365).

Sample preparation and extraction

To avoid injuring the sampler, the atopic, harmful spiky points of the leaves were removed before cutting both the *A. angustifolia* and the *A. sisalana* leaves. The cut leaves were transported to the laboratory, where side thorns on the *A. angustifolia* leaves were removed, and these leaves were washed with tap water to remove dirt, rinsed with distilled water, and arranged on a paper towel to remove excess water. The leaves were then cut into tiny pieces and spread in a fume hood (on a paper towel) to dry for a week. The dried *Agave* leaves were ground into a fine powder and put in a sterile, airtight plastic zip-lock bag before being placed in the cabinet to await the analysis' subsequent stage.

Cold maceration was used to extract the 2 kg of finely crushed *Agave* leaves over 72 h at room temperature, with occasional stirring. The solvents used were non-polar hexane, polar ethyl acetate, and most polar methanol (Dharajiya et al. 2017). The plant material was sequentially extracted with solvents, with increasing polarity, ranging from hexane to methanol to obtain different solvent extracts. Two kilogram of the pulverised *Agave* sample was soaked in 1.5 L of hexane and covered with foil to prevent solvent evaporation, as well as ingress of foreign matter. It was ensured that the powdered material was covered by the solvent to ensure maximum extraction.

The leaves were macerated for 72 h in the fume cupboard, with daily stirring to ensure maximum extraction, filtered, rinsed with hexane, and the filtrate was put aside. The residue was soaked in ethyl acetate for 72 hours. After, it was filtered, then washed with the same solvent, and the solvent was removed with rotatory evaporator. The residue was soaked in methanol for 72 hours, after which it was filtered and rinsed with the same solvent. The solvent was removed with rotatory evaporator.

The IKA RV10 digital rotatory evaporator was used to concentrate the filtrates under reduced pressure, and the extracts were kept at 4°C for the following experiment stage.

Phytochemical screening of *Agave* plant extracts

The *Agave* leaf extract underwent a preliminary phytochemical evaluation, employing standard methods to identify the presence of various phytochemicals with minor modifications (Dharajiya et al. 2017).

Test for flavonoids

Sodium hydroxide test: 2 mL of 2.0% NaOH solution added to 1 mL of *Agave* extract produced a prominent yellow hue that disappeared when two drops of mild acetic acid were added, indicating the presence of flavonoids.

Test for tannins

The *Agave* extract (0.5 g) and 10 mL of water were combined and placed in a test tube. After boiling and filtering the mixture, a small amount of 0.1% ferric chloride was added and stirred, and the colour of the mixture was examined for a blue-black or greenish-brown hue, which indicates the presence of tannins.

Test for phenols

Ellagic test: After adding five drops of 5% FeCl₃ to 1 mL of plant extract, a yellow-green fluorescence was observed, indicating the presence of a specific phenol called resorcinol.

Test for terpenoids

A total of 1 mL of chloroform, acetic anhydride, and undiluted sulphuric acid were sequentially added to 1 mL of the *Agave* leaf extract. The presence of terpenoids was established by the observation of a reddish-violet hue in the mixture.

Test for saponins

A total of 3 mL of distilled water and five drops of olive oil were added to 1 mL of *Agave* plant extract and shaken vigorously for 2 min. The solution produced a persistent foam, which indicated the presence of saponins.

Test for steroids

A total of 2 mL of acetic anhydride was added to 1 mL of *Agave* leaf chloroform extract followed by the addition of undiluted sulphuric acid. The appearance of green colouration indicated the presence of steroids.

Test for glycosides

Salkowski's test: The presence of steroidal aglycone (a glycoside component) was indicated by the brownish-red colour when 2 mL of concentrated sulphuric acid was combined with 5 mL of the *Agave* extract.

Test for alkaloid (Wagner's test)

A few drops of Wagner's reagent were added to 2 mL of *Agave* extract and 1.5% hydrochloric acid. The formation of a brownish precipitate demonstrated the presence of alkaloids.

2,2-diphenyl-1-picryl-hydrazyl-hydrate test for evaluating antioxidant activity

The *Agave* extract samples were reconstituted in dimethyl sulphate (DMSO) to yield a final concentration of 100 mg/mL. Sonication was used for the samples with solubility

challenges, and the extracts were kept at 4°C after being sonicated. The prepared DPPH (120 µL, made by dissolving 0.1 mM in ethanol) and 120 µL of Tris-HCl buffer (50 mM, pH 7.4) were put into a 96-well plate containing a five-microliter sample. The plate was then incubated in the dark for 20 min at room temperature. Using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA), the absorbance was measured at 513 nm, and the % radical scavenging equation was computed as follows:

$$\% \text{ Scavenged DPPH} = 100\% \times \frac{\text{blank sample} - \text{blank}}{\text{blank sample} - \text{blank}} \quad [\text{Eqn 1}]$$

The buffer was swapped for the 5 µL sample that served as the control. Two final concentrations of 250 µg/mL and 500 µg/mL of plant extracts were tested.

Ferric reducing or antioxidant power test for evaluating antioxidant activity

The FRAP method was used to evaluate the possible antioxidant activity of the *Agave* extracts. The following adjustments were made to the procedure that was published by Benzie and Strain (1996) to measure the ferric-reducing ability of the extracts and antioxidant controls. Dimethyl sulphate was used to generate stock solutions of the compounds (100 mg/mL) and the positive control, trolox (10 mM). Twenty millilitres of sodium acetate buffer (300 mM), 2 mL of freshly prepared TPTZ solution (10 mM TPTZ and 40 mM HCl dissolved at 50°C in a water bath), 2 mL of freshly prepared FeCl₃ solution (20 mM ferric chloride in distilled water), and 2 mL of distilled water comprised the FRAP reagent. A 96-well plate containing 50 µL of the samples and 200 µL of FRAP reagent was incubated for 30 min at 37°C. Using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA), the absorbance was measured at 593 nm. The results were expressed as (Fe₂SO₄ [µmol/L]/mg sample), which was calculated using a ferrous sulphate (Fe₂SO₄) (1.25 µmol/L – 200 µmol/L) standard curve.

Ethical considerations

This study followed all ethical standards for research without direct contact with human or animal subjects.

Results

Phytochemical evaluation

The results from the phytochemical screening of the *Agave* plant leaves, presented in Table 1, showed that both *Agave* species leaf extracts contained phytoconstituents with therapeutic benefits.

The qualitative analysis of *A. angustifolia* revealed that the plant contained terpenes or terpenoids, aglycones (a steroidal portion of glycosides), flavonoids, phenols, and saponins, while steroids and alkaloids were not detected in the extracts. The *A. angustifolia* hexane extract showed a strong content of terpenoids and a weak presence of flavonoids, tannins, phenolic compounds, saponins, and

TABLE 1: Phytochemical screening of *Agave angustifolia* (AA) and *Agave sisalana* (AS) leaf extracts.

Phytochemical Tests	<i>A. angustifolia</i> leaf solvent extracts			<i>A. sisalana</i> leaf solvent extracts		
	Hexane	EtOAC	MeOH	Hexane	EtOAC	MeOH
Flavonoids (<i>NaOH</i> test)	+	++	+++	+	+++	+++
Tannins	+	-	+	++	+	-
Phenolics	+	-	-	++	++	+
Terpenes/Terpenoids	+++	-	+	+	-	+
Saponins	+	+	++	+	+	++
Steroids	-	-	-	++	-	-
Alkaloids (<i>Wagner's</i> test)	-	-	-	-	+++	+++
Glycosides	-	-	-	-	-	-
<i>Salkowski's</i> test	+	+	+	+	+	+

Note: (+) = Slightly present; (++) = moderately present; (+++) = strongly present; (-) = not detected.

EtOAC= Ethyl Acetate; MeOH= Methanol.

glycosides, while steroid and alkaloid compounds were not detected. A moderate content of flavonoid compounds was observed in the *A. angustifolia* ethyl acetate extract, while saponin and glycoside compounds were weakly present, and the compounds of tannins, phenolics, terpenoids, steroids, and alkaloids were not detected. The *A. angustifolia* methanolic extract revealed a strong presence of flavonoids, moderate saponins, and a weak availability of tannins, terpenoids, and glycosides, while phenolics, steroids, and alkaloids were not detected. The hexane extract of *A. sisalana* indicated moderate content of tannins, phenolics, and steroids, and a faint presence of flavonoids, terpenoids, saponins and glycosides, but alkaloid compounds were not detected. On the other hand, *AS* ethyl acetate extract showed a high presence of flavonoid and alkaloids, while phenolics were moderately present, and tannins, saponins and glycosides were slightly detected. Flavonoids and alkaloids were strongly detected in the *AS* methanolic extract, while saponins were moderately detected. Phenolics, glycosides and terpenoids were slightly present, and tannins and steroids were not detected in the methanolic extract of *A. sisalana*.

Alkaloids and terpenoids are known to protect plants from entrants and herbivores as well as to repel pathogens (López-Romero et al. 2018) and the saponins have antimicrobial and anti-inflammatory qualities (Yu et al. 2022). The methanol extract of *A. angustifolia*, ethyl acetate and methanol extracts of *A. sisalana* are laden with flavonoids, which characteristically are high in antioxidant concentrations that neutralise reactive oxidative stresses caused by ultraviolet B (UVB) rays and preserve the integrity of overall skin quality and appearance. The UVB radiations destroy filaggrin, which maintains the protective functions of skin barriers. Studies have established that plant extracts loaded with flavonoids and alkaloids protect the skin from UVB radiation damage (Chen et al. 2022). The hexane extract of *A. angustifolia* contained a high amount of terpenoids, which are known to demonstrate good antimicrobial, anti-allergic, and anti-inflammatory activities (Masyita et al. 2022). The availability of these phytochemicals in the *Agave* plants indicates the potential use of the plant

extracts for antimicrobial, anti-fungal and anti-inflammatory properties.

The findings from this study agreed with a study by Hernandez-Valle et al. (2014), which established the extraction of steroidal saponins from *A. angustifolia* leaves.

Stepniowska et al. (2021) reported that alkaloids are used in cosmetics as antioxidants, skin-lightening or even-tone agents, antimicrobials, anti-cellulite, anti-ageing, calming, and anti-inflammatory substances. This suggests that the ethyl acetate and the methanol extracts from *A. sisalana* have a potential for use in cosmetic products claiming antimicrobial, anti-cellulite, anti-ageing, calming, and anti-inflammatory gentle soothing properties.

According to Kumar et al. (2020), plant phenolics offer protection against environmental stressors such as UV radiation, pathogenic infections, and predators. This indicates the potential use of *A. sisalana* in cosmetic products claiming UV protection. Goge et al. (2023) reported that tannins have therapeutic benefits because they facilitate the quick healing of wounds, and plants with high tannin content have strong antibacterial properties.

The antioxidant activity

The antioxidant activity of the *Agave* leaves (Figure 2) represented the antioxidant activity of the six *Agave* extracts (hexane, ethyl acetate and methanol extract from *A. angustifolia* and *A. sisalana*), with the DPPH assay reported as a percentage of DPPH scavenged. Trolox served as a favourable reference point. The error bars show the standard deviation of quadruplicate values obtained in a single experiment. The *A. angustifolia* exhibited negligible DPPH activity in the methanol extract, but more than 50% DPPH scavenging activity in the hexane and ethyl acetate extracts. These results suggest that *A. angustifolia* possesses non-polar and slightly polar antioxidant compounds. At the maximum treatment concentrations, the ethyl acetate and methanol extracts of *A. sisalana* demonstrated more than 50% DPPH scavenging activity, while the hexane extract exhibited negligible DPPH activity. These results indicated that only polar antioxidant phytochemicals were detected in the *A. sisalana* plant. The ethyl acetate solvent extracted the antioxidant phytochemicals from both *Agave* plants. Determining the antioxidant activity using the FRAP method (Figure 3) indicated that all plant extracts demonstrated minimal antioxidant activity at the highest treatment concentration. Both plants had more activity in the methanol extract, with the lowest activity demonstrated by the hexane extract, which had higher values in *A. angustifolia* than in *A. sisalana*. The ethyl acetate extract showed higher activity for *A. sisalana* than for *A. angustifolia*. All the outcomes were noticed at the highest treatment concentration of 2 mg/mL. Although the DPPH method claimed more potent antioxidant activity, the results from the FRAP and DPPH methods showed a similar trend. These results corresponded with Munteanu and Apetrei (2021), who wrote that, although the

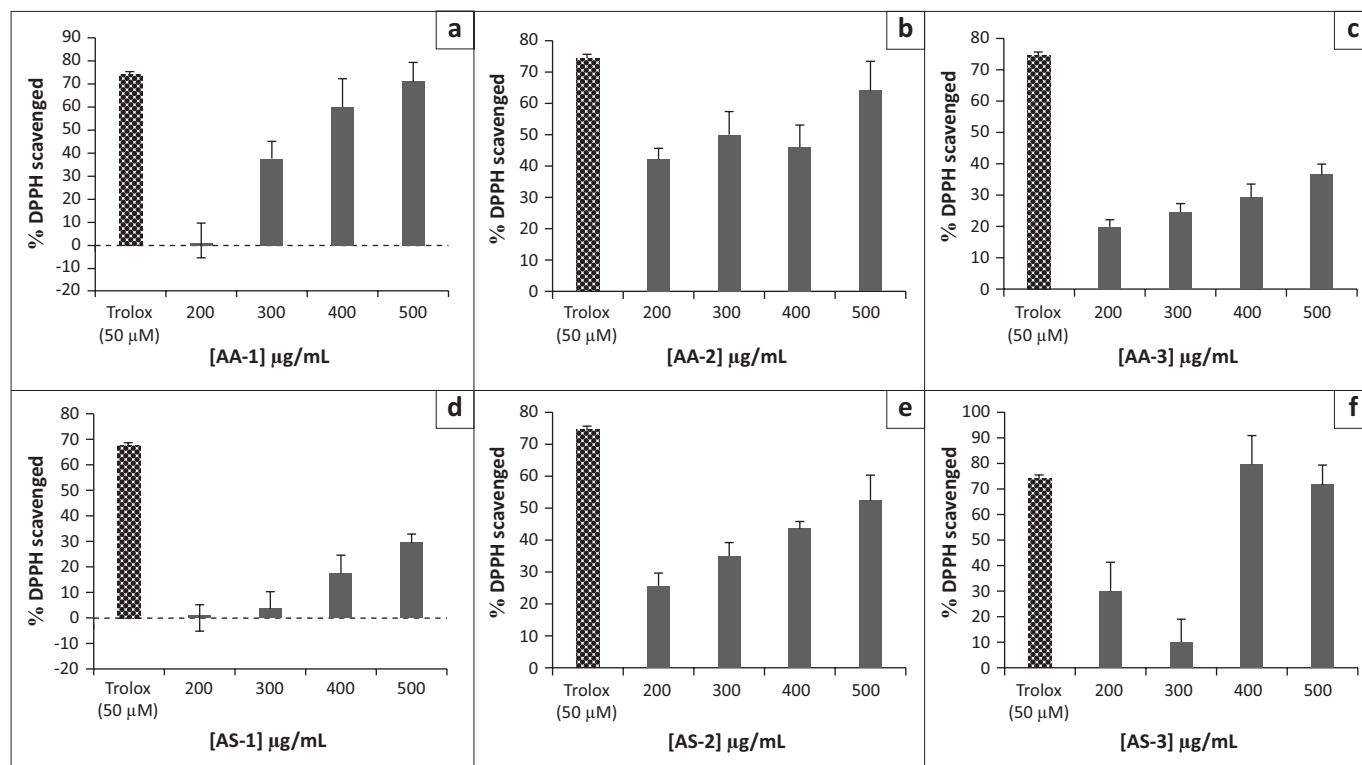


FIGURE 2: The 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay of *Agave* leaves' extracts, a & d = Hexane; b & e = Ethyl Acetate; c & f = Methanol.

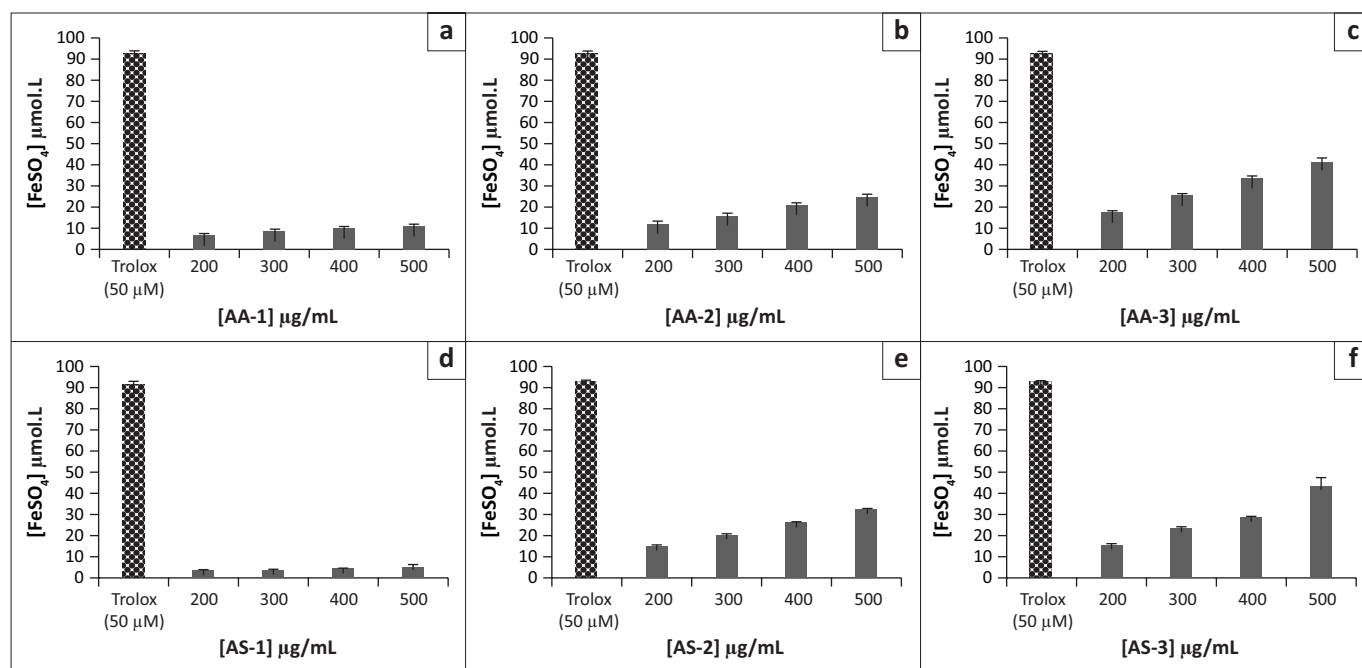


FIGURE 3: The ferric reducing or antioxidant power assay of *Agave* leaves' extracts, a & d = Hexane; b & e = Ethyl Acetate; c & f = Methanol.

kinetics and stages of the reactions differ, the single electron transfer (FRAP) and hydrogen atom transfer (DPPH) reactions produce identical outcomes because they are dependent on the solvent system, the antioxidant compound's solubility, and its structure and characteristics. These findings are consistent with Araldi's work (Araldi et al. 2018), which found that *A. sisalana* exhibited antioxidant activity. When comparing the two methods, the DPPH is the recommended approach for the antioxidant assay of the *Agave* plants.

Conclusion

This study aimed firstly to assess the phytochemical profiles and antioxidant activities of *A. angustifolia* and *A. sisalana* plants in KZN and secondly to verify the traditional therapeutic claims of the *Agave* species and, accordingly, establish the assertions of the cottage industries. The qualitative phytochemical assessment established that both the *A. angustifolia* and *A. sisalana* leaf extracts contained six phytoconstituents: flavonoids,

glycosides, phenolic acids, saponins, tannins and terpenes or terpenoids. However, the *A. sisalana* has two more phytochemical classes: alkaloids and steroids.

Both *Agave* plants showed potential to be used in cosmetic preparations, claiming antioxidant, anti-ageing, moisturising, UV protection, emulsifying, anti-inflammatory, astringent, antiseptic, anti-tumour, and antimicrobial activities. Thus, confirming the traditional therapeutic claims of the *Agave* species as asserted by the participants of the cottage industries. Further studies are required to determine the best environmentally friendly solvent for the plants' extraction, categorise the specific antioxidant compounds, and conduct antimicrobial and cytotoxic studies to confirm the safety of the extracts for use in the production of cosmetic products, as required by legislation (CTFA 2023).

Acknowledgements

The authors would like to thank the Mangosuthu University of Technology, Technology Station in Chemicals (MUT-TSC) and the Central University of Technology (CUT), Faculty of Health, and Environmental Sciences, Free State, for funding this study. Ms Samukelisiwe A. Sibiyi is highly appreciated for her assistance in the study.

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

N.G.M. carried out the research work and wrote the first draft of the article. M.C.A., I.T.M. and S.M.N. supervised and made all the necessary contributions to the study's success. X.V.N. contributed to the design of the study and revision of the draft article. All authors read and approved the final article submission.

Funding information

The Mangosuthu University of Technology, Technology Station in Chemicals, Umlazi, Durban, and the Central University of Technology, Free State, provided funding for this study.

Data availability

The authors declare that the information contained in the article supports the study's conclusions.

Disclaimer

The views and opinions expressed in this article are those of the authors and are the product of professional research. It does not necessarily reflect the official policy or position of any affiliated institution, funder, agency, or that of the

publisher. The authors are responsible for this article's results, findings, and content.

References

- Ahumada-Santos, Y.P., Montes-Avila, J., Uribe-Beltrán, M.J., Díaz-Camacho, S.P., López-Angulo, G., Vega-Aviña, R. et al., 2013, 'Chemical characterization, antioxidant and antibacterial activities of six *Agave* species from Sinaloa, Mexico', *Industrial Crops and Products* 49, 143–149. <https://doi.org/10.1016/j.indcrop.2013.04.050>
- Almazán-Morales, A., Moreno-Godínez, M.E., Hernández-Castro, E., Vázquez-Villamar, M., Mora-Aguilera, J.A., Cabrera-Huerta, E. et al., 2022, 'Phytochemical profile and in vitro activity of *Agave angustifolia* and *A. cupreata* extracts against phytopathogenic fungi', *Opatología* 40(2), 169–187. <https://doi.org/10.18781/R.MEX.FIT.2202-6>
- Araldi, R.P., Dos Santos, M.O., Barbon, F.F., Manjerona, B.A., Meirelles, B.R., De Oliva Neto, P. et al., 2018, 'Analysis of antioxidant, cytotoxic and mutagenic potential of *Agave sisalana* Perrine extracts using Vero cells, human lymphocytes and mice polychromatic erythrocytes', *Biomedicine & Pharmacotherapy* 98, 873–885. <https://doi.org/10.1016/j.biopha.2018.01.022>
- Benzie, I.F.F. & Strain, J.J., 1996, 'The Ferric Reducing Ability of Plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay', *Analytical Biochemistry* 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>
- Bhattacharyya, A., Chattopadhyay, R., Mitra, S. & Crowe, S.E., 2014, 'Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases', *Physiological Reviews* 94(2), 329–354. <https://doi.org/10.1152/physrev.00040.2012>
- Briguglio, G., Costa, C., Pollicino, M., Giambò, F., Catania, S. & Fenga, C., 2020, 'Polyphenols in cancer prevention: New insights (Review)', *International Journal of Functional Nutrition, Spandidos Publications* 1(2), 1–11. <https://doi.org/10.3892/ijfn.2020.9>
- Chen, Y.-F., Zhang, D.-D., Hu, D.-B., Li, X.-N., Luo, J.-F., Duan, X.-Y. et al., 2022, 'Alkaloids and flavonoids exert protective effects against UVB-induced damage in a 3D skin model using human keratinocytes', *Results in Chemistry* 4, 1–8. <https://doi.org/10.1016/j.rechem.2022.100298>
- Chigodi, M., Samoei, D., Mutemi, M., Chigodi, M.O., Samoei, D.K. & Muthangya, M., 2013, 'Phytochemical screening of *Agave Sisalana* Perrine leaves (waste)', *International Journal of Applied Biology and Pharmaceutical Technology* 4(4), 200–204.
- Cruz-Magalhães, V., Pereira Andrade, J., Freitas Figueiredo, Y., Arthur Santos Marbach, P. & Teodoro de Souza, J., 2020, 'Sisal Bole Rot: An important but neglected disease', *Plant Diseases – Current Threats and Management Trends*, 879. <https://doi.org/10.5772/intechopen.86983>
- CTFA, 2023, 'Bridging the gap 2023', *Cosmetic Compendium*, July 2023, 1–407.
- Dharajiya, D., Pagi, N., Jasani, H. & Patel, P., 2017, 'Antimicrobial activity and phytochemical screening of *Aloe vera* (*Aloe barbadensis* Miller)', *International Journal of Current Microbiology and Applied Sciences* 6(3), 2152–2162. <https://doi.org/10.20546/ijcmas.2017.603.246>
- Franck, A.R., 2012, 'Guide to *Agave*, *Cinnamomum*, *Corymbia*, *Eucalyptus*, *Pandanus*, and *Sansevieria* in the flora of Florida', *Phytoneuron* 102, 1–23.
- García-Mendoza, A. & Chiang, F., 2003, 'The confusion of *Agave Vivipara* L. and *A. Angustifolia* Haw.', *Two Distinct Taxa* 55, 82–87. [https://doi.org/10.1663/0007-196X\(2003\)055\[0082:TCOAVL\]2.0.CO;2](https://doi.org/10.1663/0007-196X(2003)055[0082:TCOAVL]2.0.CO;2)
- Goge, S., Singh, K., Komoreng, L.V. & Coopposamy, R.M., 2023, 'Phytochemical profile of *Aloe ferox* Mill. across different regions within South Africa', *Journal of Medicinal Plants for Economic Development* 7(1), 1–6. <https://doi.org/10.4102/jomped.v7i1.178>
- Guimarães de Oliveira, L.H., Alexandria Paiva Silva de Sousa, P., Felipe Hilario, F., Joventino Nascimento, G., Morais, J.P.S., Paulo de Medeiros, E. et al., 2016, '*Agave sisalana* extract induces cell death in *Aedes aegypti* hemocytes increasing nitric oxide production', *Asian Pacific Journal of Tropical Biomedicine, Hainan Medical University* 6(5), 396–399. <https://doi.org/10.1016/j.apjtb.2015.12.018>
- Hernández-Valle, E., Herrera-Ruiz, M., Salgado, G., Zamilpa, A., Ocampo, M., Aparicio, A. et al., 2014, 'Anti-Inflammatory Effect of 3-O-[[6'-O-Palmitoyl]-β-D-glucopyranosyl Sitosterol] from *Agave angustifolia* on Ear Edema in Mice', *Molecules*, 19(10), 15624–15637. <https://doi.org/10.3390/molecules191015624>
- Jiménez-Ferrer, E., Vargas-Villa, G., Martínez-Hernández, G.B., González-Cortazar, M., Zamilpa, A., García-Aguilar, M.P. et al., 2022, 'Fatty-acid-rich *Agave angustifolia* Fraction shows antiarthritic and immunomodulatory effect', *Molecules* 27(21). <https://doi.org/10.3390/molecules27217204>
- Kumar, S., Abedin, M.M., Singh, A.K. & Das, S., 2020, 'Role of phenolic compounds in plant-defensive mechanisms', *Plant phenolics in sustainable agriculture*, vol. 1, pp. 517–532. Springer, Singapore.
- López-Romero, J.C., Ayala-Zavala, J.F., Peña-Ramos, E.A., Hernández, J. & González-Ríos, H., 2018, 'Antioxidant and antimicrobial activity of *Agave angustifolia* extract on overall quality and shelf life of pork patties stored under refrigeration', *Journal of Food Science and Technology* 55(11), 4413–4423. <https://doi.org/10.1007/s13197-018-3351-3>
- Masyita, A., Sari, R.M., Astuti, A.D., Yasir, B., Rumata, N.R., Emran, T.B. et al., 2022, 'Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives', *Food Chemistry: X* 13, 100217. <https://doi.org/10.1016/j.fochx.2022.100217>
- Michalak, M., 2022, 'Plant-derived antioxidants: Significance in skin health and the Ageing process', *International Journal of Molecular Sciences* 23(2), 585. <https://doi.org/10.3390/ijms23020585>

- Mina, S.A., Melek, F.R., Abdel-Khalik, S.M. & Gabr, N.M., 2013, Two steroidal saponins from Agave Franzosinii and Agave Angustifolia leaves and biological activities of Agave Franzosinii', *Journal of Natural Products* 6, 188–197.
- Monterrosas-Brisson, N., Ocampo, M., Jiménez-Ferrer, E., Jiménez-Aparicio, A., Zamilpa, A., Gonzalez-Cortazar, M. et al., 2013, 'Anti-inflammatory activity of different Agave plants and the compound cantalasaponin-1', *Molecules* 18(7), 8136–8146. <https://doi.org/10.3390/molecules18078136>
- Munteanu, I.G. & Apetrei, C., 2021, 'Analytical methods used in determining antioxidant activity: A review', *International Journal of Molecular Sciences* 22(7), 3380. <https://doi.org/10.3390/ijms22073380>
- Rivera-Lugo, M., García-Mendoza, A., Simpson, J., Solano, E. & Gil-Vega, K., 2018, 'Taxonomic implications of the morphological and genetic variation of cultivated and domesticated populations of the Agave angustifolia complex (Agavoideae, Asparagaceae) in Oaxaca, Mexico', *Plant Systematics and Evolution, Springer-Verlag Wien* 304(8), 969–979. <https://doi.org/10.1007/s00606-018-1525-0>
- Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B.S., Babalghith, A.O. et al., 2022, 'Flavonoids a bioactive compound from medicinal plants and its therapeutic applications', *BioMed Research International* 2022, 1–9. <https://doi.org/10.1155/2022/5445291>
- Salazar-Pi, D.T., Castro-Ala, N., Moreno-God, M.E., Nicasio-To, M.P., Perez-Hern, J. & Alvarez-Fi, P., 2017, 'Antibacterial and anti-inflammatory activity of extracts and fractions from Agave cupreata', *International Journal of Pharmacology* 13(8), 1063–1070. <https://doi.org/10.3923/ijp.2017.1063.1070>
- Stepniowska, A., Cieplińska, P., Fac, W. & Gorska, J., 2021, 'Selected Alkaloids Used in the Cosmetics Industry', *Journal of Cosmetic Science* 72(2), 229–245.
- Velázquez-Martínez, J.R., González-Cervantes, R.M., Hernández-Gallegos, M.A., Mendiola, R.C., Aparicio, A.R.J. & Ocampo, M.L.A., 2014, 'Prebiotic potential of Agave angustifolia haw fructans with different degrees of polymerization', *Molecules* 19(8), 12660–12675. <https://doi.org/10.3390/molecules190812660>
- Vera-Guzmán, A., Guzmán-Gerónimo, R., López, M. & Chávez-Servia, J., 2018, 'Volatile compound profiles in mezcal spirits as influenced by Agave species and production processes', *Beverages* 4(1), 9. <https://doi.org/10.3390/beverages4010009>
- Verloove, F. & Pascual, M.S., 2021, 'Notes on genuine Agave vivipara (Agavaceae), a poorly known Caribbean species, recently introduced in the Canary Islands (Spain)', *Bradleya* 2021(39), 259–264. <https://doi.org/10.25223/brad.n39.2021.a28>
- Verloove, F., Thiede, J., Rodríguez, Á.M., Salas-Pascual, M., Reyes-Betancort, J.A., Ojeda-Land, E. et al., 2019, 'A synopsis of feral agave and furcraea (Agavaceae, asparagaceae s. lat.) in the Canary Islands (Spain)', *Plant Ecology and Evolution, Societe Royale de Botanique de Belgique* 152(3), 470–498. <https://doi.org/10.5091/plevevo.2019.1634>
- Villanueva-Rodríguez, S.J., Rodríguez-Garay, B., Prado-Ramírez, R. & Gschaedler, A., 2016, 'Tequila: Raw material, classification, process, and quality parameters', *Encyclopedia of Food and Health* 283–289. <https://doi.org/10.1016/B978-0-12-384947-2.00688-7>
- Wu, S., Pang, Y., He, Y., Zhang, X., Peng, L., Guo, J. et al., 2021, 'A comprehensive review of natural products against atopic dermatitis: Flavonoids, alkaloids, terpenes, glycosides and other compounds', *Biomedicine and Pharmacotherapy* 140, 111741. <https://doi.org/10.1016/j.biopha.2021.111741>
- Xiao, J. & Bai, W., 2019, 'Bioactive phytochemicals', *Critical Reviews in Food Science and Nutrition* 59(1), 827–829. <https://doi.org/10.1080/10408398.2019.1601848>
- Yu, B., Patterson, N. & Zaharia, L.I., 2022, 'Saponin biosynthesis in pulses', *Plants* 11(24), 3505. <https://doi.org/10.3390/plants11243505>
- Zwane, P.E., Dlamini, A.M. & Nkambule, N., 2010, 'Antimicrobial properties of Sisal (Agave sisalana) used as an ingredient in petroleum jelly production in Swaziland', *Current Research Journal of Biological Sciences* 2(6), 370–374.