Journal of Medicinal Plants for Economic Development
ISSN: (Online) 2616-4809, (Print) 2519-559X

Pytochemical profile of Aloe ferox Mill. across different regions within South Africa

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Dates:
Received: 27 July 2022
Accepted: 20 Sept. 2022
Published: 17 Mar. 2023

How to cite this article:
Goge, S., Singh, K., Komoreng, L.V. & Coopoosamy, R.M., 2023, 'Pytochemical profile of Aloe ferox Mill. across different regions within South Africa', *Journal of Medicinal Plants for Economic Development* 7(1), a178. https://doi.org/10.4102/jomped.v7i1.178

Background: *Aloe ferox* is an indigenous medicinal plant that is widely used for its various medicinal and pharmacological properties. Despite the medicinal importance and various applications of the species, it is surprising that little is known about the extent of geographical differences in its major chemical compounds. Also, the correlation between different geographic regions and variations in plant phytochemicals has received less attention.

Aim: This study sought to investigate the presence of biologically active compounds in the leaf extracts of *A. ferox* from different geographical regions across South Africa.

Setting: This study was set in different regions within South Africa.

Methods: Phytochemical screening was performed qualitatively using established standard procedures involving chemical reagents such as hexane, chloroform and methanol and a series of reactions to determine the presence of phytocompounds of biological importance.

Results: The study revealed that *A. ferox* leaves possess several classes of phytocompounds such as alkaloids, tannins, terpenoids, glycosides, phenolics, flavonoids, saponins and fixed oils and fats across various samples. Mucilage was absent across the samples.

Conclusion: The study revealed eight classes of phytochemical compounds present on *A. ferox* leaves in three different geographic regions, which is consistent with the previous studies; however, further research is needed to enhance the study through qualitative research, gas chromatography–mass spectrometry and high-performance liquid chromatography analyses to validate phytochemical variations and their therapeutic effects.

Contribution: This study contributes to the existing knowledge of the therapeutic Aloe genus.

Keywords: *Aloe ferox*; aloin; phytocompounds; traditional medicine; Xanthorrhoeaceae.

Introduction

The genus *Aloe* in the family Xanthorrhoeaceae comprises over 560 species ranging from small shrub-like plants to large trees (Singh, Ajao & Sabiu 2021). *Aloe ferox*, commonly known as Cape aloe or bitter aloe, is regarded as an important medicinal plant indigenous to South Africa and has many medicinal benefits. *A. ferox* has been used primarily as a purgative and to treat skin disorders (Kanama et al. 2015). Nalimu et al. (2021) reported that *A. ferox* has pharmacological activities which include anti-inflammatory, immunomodulatory, antibacterial, antifungal, antiviral, antiproliferative, antidiabetic, laxative, wound healing, moisturising, antiaging and skin protection activities. Andrea et al. (2020) indicated that numerous *in vitro* and *in vivo* studies support *Aloe*'s biological properties. Kumar et al. (2019) alluded that *Aloe* species are increasingly being incorporated into different cosmetic products, health drinks, foods and beverages due to the beneficial biological activities of the phytochemicals found mainly in their leaves.

The biologically active compounds are known as phytochemicals, which are derived from all parts of the plant including roots, stems, leaves, flowers, fruits and seeds (Balamurugan, Fatima & Velurajan 2019). They have been reported to defend plants against harmful agents like insects and microbes as well as stressful events like ultraviolet (UV) radiation and extreme temperatures (Saivinayak et al. 2022).

Aloe ferox has been reported to contain a wide range of phytochemical classes, including anthraquinones, chromones, anthrones, phenolic compounds, flavonoids, tannins, steroids and alkaloids, all of which contribute to its various pharmacological activities (Nalimu et al. 2021). Alkaloids and phenolic compounds are probably the two most important phytochemical compounds that are of medicinal value (Singh et al. 2021). Aloin and anthraquinone have been reported to be present in *A. ferox* (Nalimu et al. 2021).

According to the literature, biological properties may be attributed to several classes of compounds in the phytochemical profile of *Aloe* extracts rather than a single class of compounds (Andrea et al. 2020). Medicinal plants do not consistently produce the same chemicals in the same quantities; therefore, the effectiveness of medicinal plants may be affected not only by the biochemical factors within the individual species such as plant part extracted but also by the external factors such as climate, geographical location, season and growth conditions (Buwa & Van Staden 2006).

Aloe products require quick, sensitive and dependable raw material quality control methods due to increased commercialisation (Kanama et al. 2015). Several studies on *Aloes* as medicinal plants have primarily focused on the plant's medicinal properties, with little emphasis on the effects of geographical variations on secondary metabolites.

Research method and design

Study area and plant collection

The study sites were selected in the Eastern Cape (EC), Free State (FS) and KwaZulu-Natal (KZN) provinces, Republic of South Africa (RSA). The study sites represent different geographic locations or regions where the natural population of *A. ferox* exists (Figure 1). Matured plant leaves from three provinces of South Africa, namely EC, FS and KZN, were collected during the summer, autumn, spring and winter seasons. Samples were marked with unique tag numbers for identification and further processing.

The collected plants were deposited at the University of Fort Hare, Agriculture and Environmental Science Herbarium for identification, voucher specimens: GOG EC 1, GOG EC 2, GOG EC 3, GOG EC 4, GOG EC 5, GOG EC6, GOG KZN1, GOG KZN 2, GOG KZN 3, GOG KZN 4, GOG KZN 5, GOG KZN 6, GOG FS 1, GOG FS 2, GOG FS 3, GOG FS 4, GOG FS 5.

Plant preparation

Fresh *Aloe ferox* leaves were rinsed and subsequently chopped into 4 cm pieces, weighed and air-dried for 30 days. Then, the dried plant leaves were ground to a fine powder and subsequently stored in a sealed clear plastic container in the dark at room temperature (25 °C) until further processing.

Plant extraction

Extractions were conducted for 48 h on 1 g of each of the stored powders using 10 mL of three different solvents, namely water, ethanol and acetone. The resulting solution in each case was filtered and subsequently dried in a stream of air to generate aqueous, ethanol and acetone extracts, respectively. The three extracts were stored at 4 °C until further processing.

Phytochemical screening of Aloe ferox

Phytochemical screening methods were adapted and modified using the protocols by Yadav et al. (2011).

Detection of alkaloids

Dragendorff's test: Two drops of Dragendorff's reagent were added to 1 mL of extract. The formation of orange or reddish-brown-coloured precipitate indicated the presence of alkaloids.

Mayer's test: Two drops of Mayer's reagent were added to 1 mL of extract. The formation of yellow-coloured precipitate confirmed the presence of alkaloids.

Wagner's test: Wagner's reagent was added to 1 mL of extract. The formation of brown or reddish precipitate indicated the presence of alkaloids.

Detection of glycosides

3,5-dinitro benzoic acid test: To the alcoholic solution of the sample, a few drops of NaOH followed by 2% solution of 3,5-dinitro benzoic acid were added. The formation of a pink colour indicated the presence of cardiac glycosides.

Detection of flavonoids

Lead acetate test: To 5 mL of extract, 1 mL of 5% lead acetate solution was added. The formation of yellow-coloured precipitate indicated the presence of flavonoids.

Detection of fixed fats and oils

Sudan III test: In a test tube, 0.5 mL of chloroform was placed. A 0.5 mL sample was added drop by drop until the sample was fully dissolved, and then one drop of Sudan III reagent was added. The formation of a red colour indicated the presence of fixed oils and fats.

Detection of mucilage and gums

Ruthenium red test: Two drops of 0.5% ruthenium red solution were added to 1 mL of extract. A pink-to-red colour change indicated the presence of mucilage.

Detection of phenols

Ferric chloride test: Two drops of 0.5% ferric chloride solution were added to 1 mL of extract. The formation of green or black precipitate or a colour change indicated a positive test for phenolics.

Detection of saponins

Foam test: In a test tube, 1 mL of the extract was mixed with 4 mL of water and then shaken vigorously for 15 min. A layer of foam that persisted for 10 min indicated the presence of saponins.

Detection of steroids or terpenoids

Chloroform test: To 2 mL of chloroform, 5 mL of extract were added, and then 3 mL of concentrated sulphuric acid

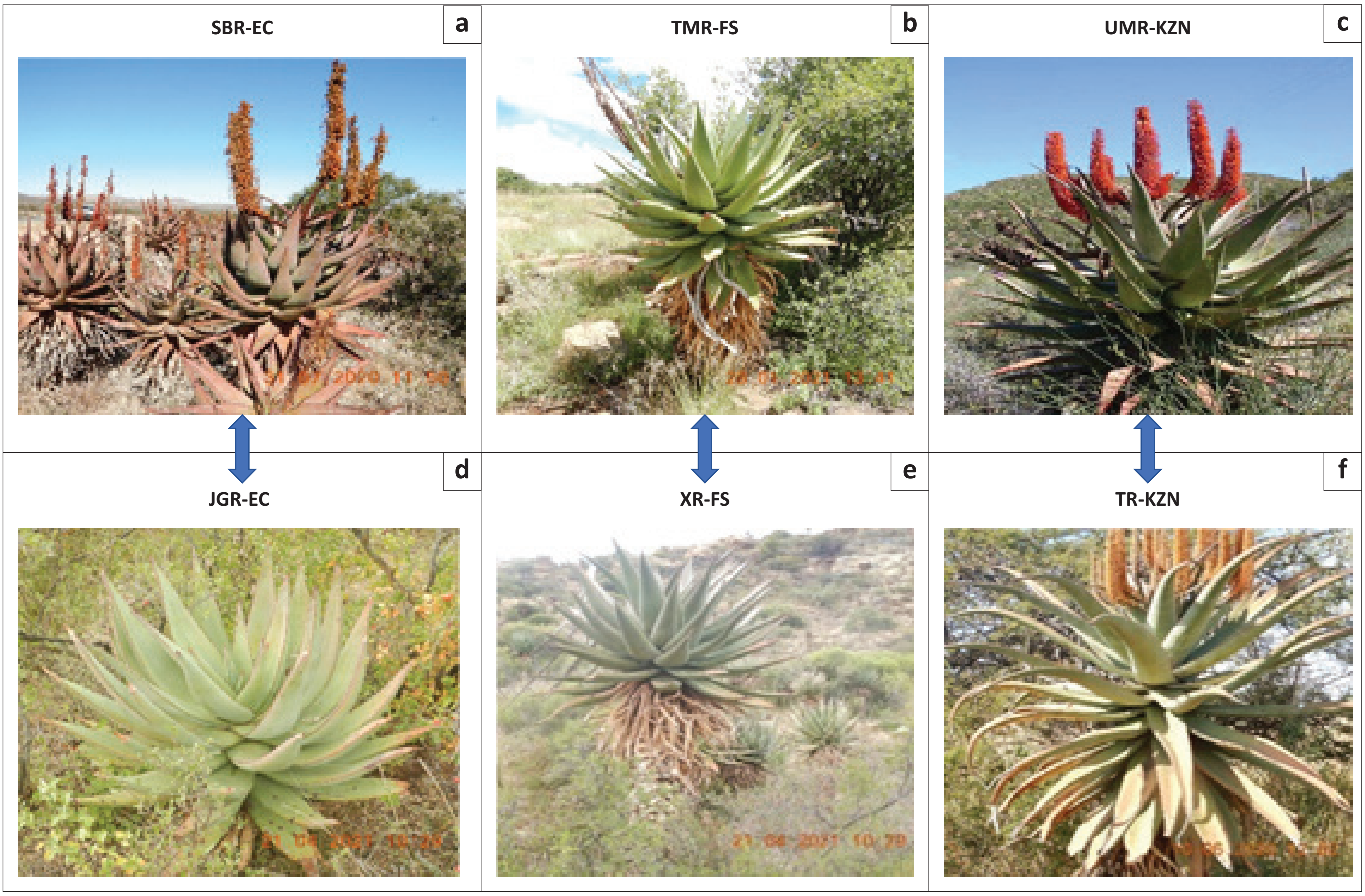

FIGURE 1: *Aloe ferox* from different regions has different physical appearances. (a) SBR-EC, Sarah Baartman Region – Eastern Cape; (d) JGR-EC, Joe Gqabi Region – Eastern Cape; (b) TMR- FS, Thabo Mofutsanyana Region – Free State; (e) XR- FS, Xhariep Region – Free State; (c) UMR-KZN, uMgungundlovu Region – KwaZulu-Natal; (f) TR-KZN, Thukela Region – KwaZulu-Natal.

were carefully added to form a layer. The reddish-brown colour change was a positive indication of steroids or terpenoids.

Ethical considerations

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

Results and discussion

The qualitative phytochemical screening results presented in Table 1, Table 2 and Table 3 revealesd that *A. ferox* extracts contain phytochemicals that are known to have therapeutic effects. Mayer's test, Wagner's test and Dragendorff's test showed the presence of alkaloids in the leaf extract of *A. ferox* across the regions. The chloroform test showed the presence of terpenoids; whereas the 3,5-dinitro benzoic acid test showed the presence of glycosides. The lead acetate test revealed the presence of flavonoids. The ferric chloride test exhibited the presence of phenols, whereas the foam test showed the presence of saponins. Eight classes (alkaloids, flavonoids, glycosides, saponins, sterols, terpenoids, phenols and fixed oils and fats) of phytocompounds were found in the leaf extract of *A. ferox* across the study sites, which was consistent with the previous studies (Nalimu et al. 2021; Singh et al. 2021; Svitina, Hamman & Gouws 2021). The leaf extracts also revealed the presence of fixed oils and fats. Fixed oils and fats are a rich source of energy that aids in growth and development by supplying essential fatty acids and fat-soluble vitamins (Kazeem & Ogunwande 2012). The ruthenium red test showed the absence of mucilage across the sample sites (Table 1, Table 2 and Table 3). The purpose of this study was to examine and screen the phytochemical constituents of *A. ferox* leaf extracts from various geographic regions in South Africa.

The qualitative phytochemical results presented in the study indicate that the leaves or latex of *A. ferox* could be used in the traditional and pharmacological markets as they contain compounds with a wide range of biological activities. Tannin-rich plant extracts have shown significant antimicrobial activity; however, this activity is affected by environmental factors such as pH, temperature, solvent type and action time (Kaczmarek 2020). Falaro and Tekle (2020) indicated that tannins are used in the treatment of various ulcers, haemorrhoids, minor burns, frostbite and inflammation of the gums. Tannins have medicinal properties because they promote rapid healing and the formation of new tissues in wounds and inflamed mucosa (Ibrahim et al. 2022). Previous studies on *Aloe vera* indicate that anthraquinones, including chrysophanol, aloe-emodin, aloeresin, aloin A and B,7-O-methylaloeresin, 9-dihydroxyl-2′-O-(z)-cinnamoyl-7-methoxy-aloesin and isoaloeresin, are potential SARS-CoV-2 3CLpro protease inhibitors (Nalimu et al. 2021).

TABLE 1: Phytochemical screening of *Aloe ferox*, Free State regions.

Study Sites	Solvents: Hexane chloroform methanol	Alkaloids: Mayer's test, Wagner's test, Dragendorff's test	Glycosides: 3,5-dinitro benzoic acid test	Flavonoids: Lead acetate test	Saponins: Foam test	Sterols: Chloroform test	Terpenoids: Chloroform test	Phenols: Ferric chloride test	Mucilage: Ruthenium red test	Fixed oils and fats: Sudan III test
TMR-FS	+	+	+	+	+	+	+	+	_	+
FDR-FS	+	+	+	+	+	+	+	+	_	+
MR-FS	+	+	+	+	+	+	+	+	_	+
XR-FS	+	+	+	+	+	+	+	+	_	+
LR-FS	+	+	+	+	+	+	+	+	_	+

–, indicated absence; +, indicated presence.

Free State Regions: TMR-FS, Thabo Mofutsanyana Region; FDR-FS, Fezile Dabi Region; MR-FS, Mangaung Region; XR- FS, Xhariep Region; LR-FS, Lejweleputswa Region.

TABLE 2: Phytochemical screening of *Aloe ferox*, KwaZulu-Natal regions.

Study Sites	Solvents: Hexane chloroform methanol	Alkaloids: Mayer's test, Wagner's test, Dragendorff's test	Glycosides: 3,5-dinitro benzoic acid test	Flavonoids: Lead acetate test	Saponins: Foam test	Sterols: Chloroform test	Terpenoids: Chloroform test	Phenols: Ferric chloride test	Mucilage: Ruthenium red test	Fixed oils and fats: Sudan III test
TR-KZN	+	+	+	+	+	+	+	+	_	+
UMR-KZN	+	+	+	+	+	+	+	+	_	+
ER-KZN	+	+	+	+	+	+	+	+	_	+
HG-KZN	+	+	+	+	+	+	+	+	_	+
IR-KZN	+	+	+	+	+	+	+	+	_	+
UR-KZN	+	+	+	+	+	+	+	+	_	+

–, indicated absence; +, indicated presence.

KwaZulu-Natal Regions: TR-KZN, Tukela Region; UMR-KZN, uMgungundlovu Region; ER-KZN, eThekwini Region; HG-KZN, Harry Gwala Region; IR-KZN, iLembe Region; UR-KZN, Ugu Region.

TABLE 3: Phytochemical screening of *Aloe ferox,* Eastern Cape regions.

Study Sites	Solvents: Hexane chloroform methanol	Alkaloids: Mayer's test, Wagner's test, Dragendorff's test	Glycosides: 3,5-dinitro benzoic acid test	Flavonoids: Lead acetate test	Saponins: Foam test	Sterols: Chloroform test	Terpenoids: Chloroform test	Phenols: Ferric chloride test	Mucilage: Ruthenium Red test	fixed oils and fats: Sudan III test
SBR-EC	+	+	+	+	+	+	+	+	_	+
AR-EC	+	+	+	+	+	+	+	+	_	+
CHR-EC	+	+	+	+	+	+	+	+	_	+
JGR-EC	+	+	+	+	+	+	+	+	_	+
ANR-EC	+	+	+	+	+	+	+	+	_	+
OTR-EC	+	+	+	+	+	+	+	+	_	+

–, indicated absence; +, indicated presence.

Eastern Cape Regions: SBR-EC, Sarah Baartman Region; AR-EC, Amathole Region; CHR-EC, Chris Hani Region; JGR-EC, Joe Gqabi Region; ANR-EC, Alfred Nzo Region; OTR-EC, Oliver Tambo Region.

Saponins are toxic chemicals that protect healthy plants from insects, fungi and bacteria (Zaynab et al. 2021). Studies have explored the uses of saponins derived from plants to control invasive worm species (Adomaitis & Skujienė 2020). Excess ruminal ammonia production increases the risk of pollution from ammonia, nitrous oxide and nitrate emissions; therefore, natural plant phytocompounds such as tannins, saponins and essential oils are promising feed additives for reducing enteric methane and ammonia formation (Jayanegara et al. 2020). Nguyen et al. (2020) reported that saponins have been discovered in abundance in a variety of *Aloe* plants, which was consistent with the study. Ibrahim et al. (2022) revealed that saponins have antioxidant effects on the skin and protect it from UV damage by inhabiting extracellular matrix degradation, as well as being anti-irritation due to their anti-inflammatory action.

Flavonoids have been shown to help regulate cellular activity and eliminate free radicals which cause oxidative stress in the human body (Jamshidi-Kia et al. 2020). Agati et al. (2020) alluded that flavonoids help the human body to function more efficiently while protecting against toxins and stressors. Makhaik, Shakya and Kale (2021) pointed out that flavonoids are powerful antioxidant agents with free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory and anticancer activities. Literature reports that some flavonoids in plants do exhibit potential antiviral activities (Wang et al. 2020). Aida et al. (2022) found the methanolic extract of *Aloe vera* to exhibit the highest polyphenol content, while ethanolic extract showed the highest flavonoid content. Flavonoids were found to be present in *A. ferox* across the samples, which supports the use of *A. ferox* as a medicinal plant.

Yang et al. (2020) claimed that terpenoids are the most abundant compounds in natural products. Terpenes are frequently used as fragrances and flavours in consumer goods such as perfumes, cosmetics, cleaning products, food and beverages (Sharmeen et al. 2021). Terpenoids are a class of secondary metabolites that play an important role in plant growth and development, environmental response and physiological processes (Yang et al. 2020). Terpenoids have been confirmed to have a wide range of medicinal uses, the most notable of which is antiplasmodial activity, as its mechanism of action is identical to the widely used antimalarial drug chloroquine (Cox-Georgian et al. 2019).

Glycosides are used to treat heart conditions such as congestive heart failure (Ayogu & Odoh 2020). Li and Jiang (2018) reported glycosides to have anthraquinone, which has laxative and purgative effects. Glycosides are used in cosmetic preparations to treat hyperpigmentation induced by UV radiation, owing to their role in the inhibition of tyrosinase enzyme. Amen et al. (2021) alluded that those significant biological activities, including antiviral, anti-inflammatory, antitumor and antimicrobial activities, have been discovered for chromone glycosides, suggesting their potential as drug leads.

Alkaloids protect plants from predators and regulate growth (Heinrich, Mah & Amirkia 2021). Therapeutically, they are well known as anaesthetic, cardioprotective and inflammatory agents (Heinrich et al. 2021). Studies indicate that alkaloids are useful as diet ingredients, supplements and pharmaceuticals in medicine and other human applications (Chen & Lin 2019). Alkaloids are important compounds in organic synthesis because they can be used to find new semisynthetic and synthetic compounds with higher biological activity than parent compounds (Ghirga et al. 2021).

According to the literature, phenolic compounds are plant substances that share an aromatic ring with one or more hydroxyl groups (Kumar et al. 2020). González- Sarrías, Tomás-Barberán and García- Villalba (2020) pointed out that phenolic compounds are most widely distributed in the plant kingdom and are the most abundant phytocompounds of plants. Studies claim that phenol derivatives have been found to possess antimicrobial, anti-inflammatory, antioxidant, anticonvulsant, anticancer, anaesthetic, antiseptic, bioinsecticides and analgesic (Kumar & Mishra 2018).

Even though little is known about the extent of geographical differences in the phytochemical composition of *Aloe ferox*, generalisations about product quality persist. Previous research has found that the phytochemical composition of *Aloe vera* varies geographically (Kumar et al. 2019). However, the current investigation has shown that the phytochemical constituents of *A. ferox* are similar across different geographical locations.

Conclusion

The qualitative phytochemical analysis revealed eight classes of phytocompounds such as alkaloids, flavonoids, glycosides, saponins, sterols, terpenoids, phenols and fixed oils and fats present on *A. ferox* leaves across different geographic regions, which is consistent with the previous studies. This study aimed to examine and screen the phytochemical constituents of *A. ferox* leaf extracts from various geographic regions in South Africa. The presence of these active phytocompounds demonstrated that *A. ferox* leaf has prominent biological and therapeutic activities. The leaf extracts showed the absence of mucilage across the sample sites. Several studies on phytochemicals found in *Aloe* species, a traditional medicinal plant, support the findings of this study.

Finally, it goes without saying that differences in active compound concentrations may result in products with incongruent chemical and physical properties, making product comparison difficult; however, additional research is needed to enhance the study through qualitative research, GCMS and HLPC analyses to validate phytochemical variations and their therapeutic effects.

Acknowledgements

The authors would like to acknowledge the University of Fort Hare, Mangosuthu University of Technology and the University of KwaZulu-Natal for providing resources and guidance and the Department of Economic Development, Environmental Affairs and Tourism for funding. Lastly, they would like to thank their family, friends and work colleagues who have provided continued support and encouragement.

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

All authors have contributed equally to this work.

Funding information

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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