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Aloe ferox Leaf Gel Phytochemical Content, Antioxidant Capacity, and Possible Health Benefits

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This study identified, quantified, and compared the phytochemical contents and antioxidant capacities
of Aloe ferox lyophilized leaf gel (LGE) and 95% ethanol leaf gel extracts (ELGE) using GC-MS and
spectrophotometric methods. Analytically, 95% ethanol is less effective than ethyl acetate/diethyl
ether or hexane (in the case of fatty acids) extractions in separating phytochemicals for characterization
purposes. However, although fewer compounds are extracted in the ELGE, they are approximately
345 times more concentrated as compared to the LGE, hence justifying ELGE use in biological efficacy
studies in vivo. Individual phytochemicals identified included various phenolic acids/polyphenols,
phytosterols, fatty acids, indoles, alkanes, pyrimidines, alkaloids, organic acids, aldehydes, dicarboxylic
acids, ketones, and alcohols. Due to the presence of the antioxidant polyphenols, indoles, and
alkaloids, the A. ferox leaf gel shows antioxidant capacity as confirmed by ORAC and FRAP analyses.
Both analytical methods used show the non-flavonoid polyphenols to contribute to the majority of the
total polyphenol content**. Due to its phytochemical composition, A. ferox leaf gel may show promise
in alleviating symptoms associated with/or prevention of cardiovascular diseases, cancer, neurodegeneration, and diabetes.**

KEYWORDS: Aloe ferox leaf gel; phytochemical; polyphenols; antioxidant capacity; gas chromatography-
mass spectrometry; spectrophotometry; leaf gel extract; ethanol extract; ORAC; FRAP

INTRODUCTION

The utilization of plants in various parts of the world is receiving more and more prominence, due to not only their health benefits but also the opportunities they present to rural-based economies. Mainly due to economic constraints, the populations of developing countries worldwide continue to rely heavily on the use of traditional medicine as their primary source of healthcare. Apart from Aloe being used extensively in the cosmetic industry, it has been described for centuries for its laxative, anti-inflammatory, immunostimulant, antiseptic (1), wound and burn healing (2), antiulcer (3), antitumor (4), and antidiabetic (5) activities. The majority of the scientifically based research on this topic was done on Aloe Vera (or Aloe barbadensis) and Aloe arborescens. However, in the rural communities, the type of Aloe that is chosen as a traditional medicine would depend on its immediate availability to the specific community. Hence, various communities in different parts of the world would use the species of Aloe indigenous to their immediate surroundings. In South Africa, for instance, various traditional communities and local industries are using a variety of location-specific Aloe species, for example, Aloe

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ferox in the Eastern and Western Cape provinces and Aloe greatheadii var. DaVyana in the northern regions of South Africa. These Aloe species are used in the treatment of arthritis, skin cancer, burns, eczema, psoriasis, digestive problems, blood pressure problems, and diabetes. These treatments are based on anecdotal evidence or research findings done almost exclusively on A. Vera. Different Aloe species would have various phytochemical contents, health benefits, and possible toxicities. Hence, it is of relevance for scientists, industry, and rural communities not only to research the relevant medicinal uses of their indigenous Aloe species but also to determine the active components and their individual or combined mechanisms of biological function. The use of 95% ethanol extracts of various Aloe species is extensively described in the literature for determining biological activity in the treatment and prevention of a variety of health conditions (6, 7), in particular, diabetes (8-11). In this study we determined and compared the phytochemical contents and antioxidant capacities of A. ferox lyophilized leaf

gel and 95% ethanol leaf gel extracts using gas chromatography-mass spectrometry (GC-MS) and spectrophotometric methods of analysis. This was done not only to describe A. ferox leaf gel extracts with regard to phytochemical contents and possible health benefits, but to compare various extraction methods for both analytical efficacy and possible biological relevance.

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MATERIALS AND METHODS

Samples. Whole, freshly cut, A. ferox leaves (100 kg) were kindly supplied by the Aloe Ferox Trust. These leaves were harvested in the month of September from farms in the Albertinia region in the Western Cape of South Africa. The inner leaf gel was removed, homogenized, freeze-dried, and stored at -20 °C until analysis. This was termed the leaf gel extract (LGE) for the purpose of this study. Approximately half of the LGE was used for preparation of a 95% ethanol extract as described previously (11). This was termed the 95% ethanol leaf gel extract (ELGE).

Materials. All analytical standards were purchased from Sigma-Aldrich (St. Louis, MO), and all of the organic solvents used were of ultrahigh purity purchased from Burdick and Jackson. Folin-Ciocalteu's phenol reagent and other reagent chemicals were purchased from Merck (Darmstadt, Germany).

Ethyl Acetate/Diethyl Ether Extraction. The internal standard, 3-phenylbutyric acid (25 mg/50 mL), was added to 25 mg of finely ground LGE and ELGE, followed by the addition of 1 mL of sodium acetate buffer (0.125 M). â-Glucuronidase (30 íL) was added, and the sample was vortexed and incubated overnight at 37 °C. The sample was extracted with 6 mL of ethyl acetate followed by 3 mL of diethyl ether. The organic phase was collected after each extraction via centrifugation. The organic phase from each extraction was pooled and dried under nitrogen. The dried extract was derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA; 100 íL), trimethylchlorosilane (TMCS; 20 íL), and pyridine (20 íL) at 70 °C for 30 min. After cooling, 0.1 íL of the extract was injected into the GC-MS via splitless injection.

Fatty Acid Extraction. Heptadecanoic acid (72 mM), as an internal standard, was added to 25 mg of LGE and ELGE followed by 100 íL of a 45 mM solution of butylated hydroxytoluene and 2 mL of methanolic HCl (3 N). The samples were then vortexed and incubated for4hat90 °C. After cooling to room temperature, the sample was extracted twice with 2 mL of hexane, dried under a nitrogen stream, and finally resuspended with 100 íL of hexane, 1 íL of which was injected onto the GC-MS via splitless injection.

GC-MS. An Agilent 6890 GC ported to a 5973 mass selective detector was used for the identification and quantification of individual fatty acids. For the acquisition of an electron ionization mass spectrum, an ion source temperature of 200 °C and electron energy of 70 eV were used. The gas chromatograph was equipped with an SE-30 capillary column (Agilent), a split/splitless injection piece (250 °C), and direct GC-MS coupling (260 °C). Helium (1 mL/min) was used as the carrier gas. The oven temperature program for analyzing the ethyl acetate/diethyl ether extract was an initial oven temperature of 40 °C and was maintained for 2 min, followed by a steady climb to 350 °C at a rate of 5 °C/min. For the fatty acid analysis, an initial oven temperature of 50 °C was maintained for 1.5 min and then allowed to increase to 190 °C at a rate of 30 °C/min. The oven temperature was maintained at 190 °C for 5 min and then allowed to increase to 220 °C at a rate of 8 °C/min. The oven temperature was again maintained for 2 min and finally ramped to 230 °C at a rate of 3 °C/min and maintained for 24 min at this temperature.

Total Polyphenol Assay. The total polyphenol content of the extracts were determined according to the Folin-Ciocalteu procedure (12). Briefly, 10 mg of finely ground LGE or ELGE was dissolved in 200 íLofH2O in a test tube followed by 1 mL of Folin-Ciocalteu's reagent. This was allowed to stand for 8 min at room temperature. Next, 0.8 mL of sodium carbonate (7.5%, w/v) was added, mixed, and allowed to stand for 30 min. Absorption was measured at 765 nm (Shimadzu UV-1601 spectrophotometer). The mean total phenolic content (n) 3) was expressed as milligrams of gallic acid (Sigma-Aldrich) equivalents per 100 g of wet and dry mass (mg of GAE/100 g) (standard deviation (SD).

Total Flavonoid Assay. The total flavonoid content was measured by using the AlCl3 colorimetric assay (13) with some modifications. Briefly, 10 mg of LGE or ELGE was dissolved in 1 mL of H2O, to which 60 íL of 5% (w/v) NaNO2 was added. After 5 min, 60 íLofa 10% (w/v) AlCl3 was added. In the sixth min, 400 íLof 1 MNaOH was added, and the total volume was made up to 2 mL with H2O. The

solution was mixed well and the absorbance measured at 510 nm against a reagent blank. Concentrations were determined using a catechin (Sigma-Aldrich) solution standard curve. The mean total flavonoid content (n) 3) was expressed as milligrams of catechin equivalents (CE) per 100 g of wet and dry mass (mg of CE/100 g) (SD.

Oxygen Radical Absorbance Capacity (ORAC). ORAC analyses of hydrophilic and lipophilic compounds in LGE and ELGE were performed as described previously (14). The analysis of lipophilic compounds was aided by the addition of randomly methylated

â-cyclodextrin as a solubility enhancer as described before (15). Briefly, in a volume of 200 íL, the reaction contained 56 nM fluorescein (Sigma-Aldrich) as a target for free radical attack by 240 nM 2,2¢ azobis(2-amidinopropane) dihydrochloride (Sigma-Aldrich). A BioTEK fluorescence plate reader (FL-600) was used, and the decay of fluorescence of fluorescein (excitation, 485 nm; emission, 520 nm) was measured every 5 min for2hat37 °C. Costar black opaque (96 well) plates were used in the assays. Trolox (Sigma-Aldrich) was used as standard at a range between 0 and 20 íM with a polynomial (second order) curve fit analysis. Mean values (n) 3) of antioxidant capacities were expressed as micromoles of Trolox equivalents (TE) per gram of wet and dry mass (SD.

Ferric Reducing Antioxidant Power (FRAP). FRAP values were determined essentially as described previously (16). Briefly, the reduction of a Fe^{3+}-2,3,5-triphenyltetrazolium (Sigma-Aldrich) complex in the assay by the antioxidants in the samples was monitored at 593 nm. As a standard, $FeSO_4$ (Sigma-Aldrich) was used, and the FRAP activities of the samples were expressed as the mean (n) 3) micromoles of Fe^{2+} per gram of wet and dry mass (SD.

RESULTS AND DISCUSSION

The compounds identified and their quantities in the A. ferox LGE and ELGE are summarized in Table 1. Of all the compounds identified, the groups of compounds best described for their health benefits are the phenolic acids/polyphenols, sterols, fatty acids, and indoles. Apart from these, various alkanes, pyrimidines, alkaloids, organic acids, aldehydes, dicarboxylic acids, ketones, and alcohols were also identified.

Although the extraction methods used in this study were not selected to target alcohols, a few of these were also identified. One would, however, expect a far larger variety of alcohols to occur in Aloe and in far higher concentrations. For better extraction of these, headspace isolation by simultaneous purging

should be used as described previously (17). However, by employing this method one would extract far less of the other biologically important health-associated compounds. Therefore, to accomplish the aims of our study, alternative extraction procedures were used as described under Materials and Methods, using ethyl acetate/diethyl ether and hexane.

A general comparison of the phytochemical contents of the LGE and ELGE, calculated per LGE dry mass, shows that, with the exception of a few compounds, far fewer compounds and at lower concentrations are extracted from 95% ethanol extracts than directly from the LGE using ethyl acetate/diethyl ether or hexane. The occurrence of higher concentrations of a few compounds from the ELGE is most probably due to matrix protein conformation changes and precipitation by the ethanol, hence making extraction of these protein-associated compounds easier (18). However, when the concentrations are quantified for the individual compounds occurring in the ELGE per dry mass of ELGE, the concentrations for the compounds extracted are approximately 345 times higher than those for the same compounds occurring in the lyophilized LGE. Similarly, higher concentrations of total polyphenols, total flavonoids, and total non-flavonoids, as well as higher antioxidant capacities using ORAC and FRAP analyses (Table 2), are seen in the ELGE extracts. Additionally, these values are again far less when

Table 1. Concentrations of GC-MS Identified Compounds from Lyophilized Aloe ferox Leaf Gel (LGE) and 95% Ethanol Leaf Gel Extract (ELGE)

compound	concentration (ppm) LGE (per dry mass LGE)	concentration (ppm) ELGE (per dry mass ELGE)	compound	concentration (ppm) LGE (per dry mass LGE)	concentration (ppm) ELGE (per dry mass ELGE)
Phenolic Acids/Polyphenols			Organic Acids		
phenol	15.37	38.87	1.3 ´ 104	isovaleric	150.4	151.34	5.2 ´ 104
gentisic	1.99			lactic	149.84	204.92	7.1 ´ 104
vanillic	60.27	24.57	8.5 ´ 103	glycolic	92.40		
homovanillic	19.66	14.36	5.0 ´ 103	pyruvic	88.86	3.1 ´ 104	
o-hydroxycinnamic	56.21			furoic	59.23		
protocatechuic	169.42	45.56	1.6 ´ 104	3-hydroxypropionic	1.36		
3,4-dihydroxyphenylacetic	8.54			2-hydroxyvaleric	24.13	80.52	2.8 ´ 104
5-methoxyprotocatechuic	2.94			cyclohexanone-3-carboxylic	1.55		
syringic	26.59			3-hydroxyisovaleric	41.53	225.97	7.8 ´ 104

sinapic 35.94 3-methyl-1,3-hydroxybutanoic 21.59
p-coumaric 453.38 methylbenzyl acetic 16.52 5.7 ´ 103
caffeic 13.84 2-hydroxycaproic 6.78
isoferulic 53.12 phosphoric 342.11 1.2 ´ 105
ferulic 89.76 4.43 1.5 ´ 103 methylcrotonic 7.3 2.5 ´ 103
4-methoxycinnamic 2.18 2-ketoisovaleric 0.63 58.62 2.1 ´ 104
aloe emodin 87.79 3-methylglutyric 1005.90 3.5 ´ 105
4-phenyllactic 11.83 succinic 385.10 118.77 4.1 ´ 104
4-ethylphenol 10.15 33.25 1.2 ´ 104 2-methylsuccinic 63.94
p-toluic 841.63 picolinic 280.80 9.7 ´ 104
hydrocinnamic 37.68 methylmalic 22.14
p-salicylic 189.54 51.74 1.8 ´ 104 malic 47.52
benzoic 880.36 5506.5 1.9 ´ 106 3,4,5-trihydroxypentanoic 20.10 5.79 2.0 ´ 103
phenylpyruvic 6.56 2.3 ´ 105 D-ribonic 7.09
mandelic 9.83 84.37 2.9 ´ 104 2-hydroxyglutyric 20.21 7.0 ´ 103
phenylpropionic 26.55 9.2 ´ 103 3-hydroxy-3-methylglutyric 20.66 7.2 ´ 103
m-hydroxymandelic 141.93 4.9 ´ 104 2-ketoglutyric 17.25 6.0 ´ 103
phenylpyruvic 6.58 2.3 ´ 103 tartaric 18.82 6.3 ´ 103
hydroxyphenylacetic 113.56 45.34 1.6 ´ 104 suberic 12.19
pyrocatechuic 4.67 3-hydroxypicolinic 61.82
hydro-p-coumaric 15.63 isonicotinic 40.68
6,7-hydroxycoumarin 38.40 hydantoinpropionic 15.15 5.2 ´ 103
2-hydroxybutyric 2.40 829.92
Alkanes 3-hydroxybutyric 71.65 2.5 ´ 104
1,3-dihydroxybutane 10.48 10.77 3.7 ´ 103
hexacosane 6.11 2.1 ´ 103 Fatty Acids
lauric (C12:0) 0.33
Pyrimidines myristic (C14:0) 0.75
uracil 697.65 pentadecenoic (C15:0) 1.14 1.71 5.91.32
thymine 429.33 181.65 6.3 ´ 104 palmitoleic (C16:1) 1.35 0.19 65.70
palmitic (C16:0) 45.55 0.20 69.16
Indoles stearic (C18:0) 3.56 0.83 287.01
indole-5-acetic acid 11.61 linoleic (C18:2 n-6) 104.06 0.42 145.24
indole-3-acetic acid 2.88 oleic (C18:1) 0.17
hexahydrobenzoindole 20.59 7.1 ´ 103 linolenic (C18:3 n-3) 1.53
5-indole carboxylic acid 12.09 4.2 ´ 103 erucic (C22:1 n-9) 0.90
nonadecenoic (C19:0) 0.14
Alkaloids arachidic (C20:1) 0.73
hypoxanthine 28.41 heneicosanoic acid (C21:0) 0.50
xanthine 1333.2 behenic (C22:0) 2.89
tricosanoic (C23:0) 3.08
Sterols lignoceric (C24:0) 9.03
cholestanol 24.82 13.42 4.6 ´ 103 pentacosanoic (C25:0) 2.87
campestrol 13.73
â-sitosterol 1602.7 Dicarboxylic Acids
stigmasterol 69.34 azelaic 0.03

undecanedioic 0.04
Alcohols 2-hydroxyadipic 6.72 2.3 ´ 103
2-butanol 13.97
1-propanol 161.16 196.66 6.8 ´ 104 Ketones
2,3-butanediol 339.28 4,6-dimethyl-2-heptanone 40.91
2-methyl-1,3-propanediol 355.58 39.14 1.4 ´ 104 acetophenone 8.06
benzyl alcohol 163.43 305.66 1.1 ´ 105 2,4-dimethyl-4-heptanone 129.50
2,3-pentanediol 8.82 heptanone 177.40
glycerol 342.75
octadecanol 3.76
phenylethanol 87.31
Aldehydes
benzaldehyde 57.46 73.57 2.5 ´ 104
m-tolualdehyde 18.46

Table 2. Concentrations of Total Polyphenols, Flavonoids, and Non-flavonoids as well as Antioxidant Capacity via Oxygen Radical Absorbance
Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) Analyses in Lyophilized Aloe ferox Leaf Gel (LGE) and 95% Ethanol Leaf Gel
Extracts (ELGE)

compound	LGE (dry mass)	LGE (wet mass)	ELGE (expressed as dry mass)	ELGE (expressed as dry mass LGE)	ELGE (expressed as wet mass LGE)
total polyphenols (mg of GAE/100 g ± SD)	79.2 ± 4.03	2.74 ± 0.14	413 ± 9.89	26.8 ± 0.64	0.94 ± 0.02
total flavonoids (mg of CE/100 g ± SD)	5.5 ± 0.38	0.19 ± 0.01	33.6 ± 1.99	2.18 ± 0.13	0.08 ± 0.004
total non-flavonoids (by calculation)	73.7 ± 0.45	2.55 ± 0.23	379 ± 6.78	24.6 ± 1.5	0.87 ± 0.02
ORAC, hydrophilic (ímol of TE/g)	53 ± 1.2	1.83 ± 0.04	136 ± 2.5	8.83 ± 0.16	0.31 ± 0.006
ORAC, lipophilic (ímol of TE/g)	NDa	ND	ND	ND	ND
ORAC, total (ímol of TE/g)	53 ± 1.2	1.83 ± 0.04	136 ± 2.5	8.83 ± 0.16	0.31 ± 0.006
FRAP (ímol/g)	4.9 ± 0.25	0.17 ± 0.08	19.0 ± 0.3	1.23 ± 0.02	0.05 ± 0.001

a Not detected.

quantified per LGE dry mass. This indicates that from an analytical perspective, 95% ethanol is in general less effective than direct ethyl acetate/diethyl ether or hexane extractions (in the case of fatty acids) for the phytochemical characterization of Aloe species. However, the results also indicate the ELGE

allows for effective concentration of a number of biologically active ingredients from LGE, confirming its popularity for use for testing biological activity for certain components in vivo and in vitro. Additionally, polyphenols are generally classified into flavonoids and non-flavonoids (19). In Table 1, GC-MS analyses indicate the majority of the polyphenol compounds identified in the A. ferox leaf gel belong to the non-flavonoid group of polyphenols. This was confirmed by the spectrophotometic analysis of polyphenols summarized in Table 2, indicating the non-flavonoid components to contribute to 93% of the total polyphenols in the LGE and 92% in the ELGE.

Over the past 10 years there has been a growing interest in the value of polyphenols among researchers and food manufacturers. This is mainly because of their antioxidant properties, their abundance in our diet, and their role in the prevention of various diseases associated with oxidative stress such as cancer, cardiovascular disease, neurodegeneration (20), and diabetes (21). Polyphenols constitute a large class of molecules containing a number of phenolic hydroxyl groups attached to ring structures allowing for their antioxidant activities. These compounds are multifunctional and can act as reducing agents, hydrogen-donating antioxidants, and singlet oxygen quenchers (19). All of the individual A. ferox leaf gel antioxidant polyphenols identified in Table 1 may contribute to the prevention of the above-mentioned diseases to a greater or lesser extent. The individual contributions of these to disease prevention would, however, depend on their concentrations, antioxidant capacities, bioavailabilities, and specific mechanisms of action. Although the individual phenolic acids/polyphenols occurring in the highest concentrations were benzoic acid, p-toluic acid, p-coumaric acid, p-salicylic acid, protocatechuic acid, hydroxyphenylacetic acid, ferulic acid, aloe emodin, and vanillic acid, it is well-known that the protective health benefits of polyphenols are mainly through a combination of additive and/or synergistic effects between the individual compounds (22). Consequently, those polyphenol/phenolic compounds identified in lower concentrations may also be of value.

Due to the fact that the majority of the phenolic acids/polyphenols identified in A. ferox leaf gel in Table 1 are antioxidants

(19) and these compounds as a group occur in the highest concentrations, one would expect these to contribute to the majority of the antioxidant capacity measured in these extracts (Table 2). However, apart from these polyphenols, the indoles

(23) and alkaloids identified (24) are also known to possess antioxidant activities and may consequently also contribute to the ORAC and FRAP values of these extracts. When interpreting data of this nature, one should keep in mind that using the concentrations of these antioxidant compounds alone is insufficient criteria for making predictions of individual contributions to oxidative stress. As previously described, this is due to the fact that the concentrations of individual polyphenol antioxidants are not the only factor influencing antioxidant capacity; the structural arrangements (number and position of hydroxyl groups, double bonds, and aromatic rings) of these compounds also play a role (19). Additionally, their individual contributions to ORAC and FRAP may also differ. Due to the FRAP analysis being an indication of the ferric ion reducing power of a compound or mixture and the ORAC analysis indicating the ability of a compound or mixture to scavenge free radicals, the various individual polyphenol components of the mixture may have stronger free radical scavenging abilities than reducing power, or vice versa, dependent on their chemical structures (25).

Phytosterols are another group of compounds well-known for their health benefits. Of the four phytosterols identified in Table 1, â-sitosterol occurred in by far the highest concentrations in the LGE, contributing to 93% of the total phytosterols identified. The ELGE was once again less effective in extracting these compounds, and only cholestanol was identified. However, the levels normalized to dry mass ELGE were not insignificant. Phytosterols are best described for their total cholesterol and low-density lipid cholesterol (LDL-C) lowering effects, consequently associated with reducing the risk for cardiovascular disease (26). As summarized by Devaraj and Jialal (31), evidence for this has been observed in hypercholesterolemic, diabetic, and healthy volunteers. The mechanism proposed by which phytosterols accomplish this is by lowering cholesterol absorption due to the structural similarities these compounds share with cholesterol (27-29). Apart from lowering cardiovascular risk factors associated with diabetes, phytosterols (â sitosterol in particular) have been shown to positively affect diabetes by directly lowering fasting blood glucose levels by cortisol inhibition (30). Additionally, phytosterols have been shown to reduce biomarkers for oxidative stress and inflammation (31), as well as to reduce cancer development by enabling antitumor responses by increasing immune recognition of cancer, influencing hormonal-dependent growth of endocrine tumors, and altering sterol biosynthesis due to the structural

similarities of the phytosterols with these compounds and their substrates (32). Phytosterols have also been shown to directly inhibit tumor growth by slowing cell cycle progression, by induction of apoptosis, and by the inhibition of tumor metastasis (32).

Long-chain polyunsaturated fatty acids (PUFAs) also have important biological functions noted to modulate risks of chronic degenerative and inflammatory diseases, of which the essential PUFAs, linolenic (C18:3 n-3) and linoleic (C18:2 n-6) acids, are best described (33, 34). Both these were present in the A. ferox leaf gel extracts, with linoleic acid being the major fatty acid present. However, despite this, the concentrations of these are still very low in comparison to the other compounds identified with possible health benefits and were not even detectable in the lipophilic ORAC analysis. These fatty acids may probably be too low for the A. ferox leaf gel to contribute to health through its fatty acid composition.

In conclusion, the results of this study show that from an analytical perspective, 95% ethanol is a less efficient solvent for the extraction of the phytochemical components of A. ferox leaf gel for descriptive purposes as compared to ethyl acetate/ diethyl ether or hexane (in the case of fatty acids). Although the 95% ethanol extracts contain a smaller variety of extracted compounds, their concentrations are, however, approximately 345 times higher than those of the lyophilized A. ferox leaf gel when quantified as dry mass ELGE extract. This justifies the popularity of the ELGE for applications testing biological efficacy in vivo and in vitro. For the purpose of determining possible biological application, A. ferox leaf gel was characterized. Various phenolic acids/polyphenols, phytosterols, fatty acids, indoles, alkanes, pyrimidines, alkaloids, organic acids, aldehydes, dicarboxylic acids, ketones, and alcohols were identified and quantified. Due to the presence of the antioxidant polyphenols, indoles, and alkaloids, the A. ferox leaf gel shows antioxidant capacity as confirmed by ORAC and FRAP analyses. Both GC-MS and spectrophotometric analyses show the non-flavonoid polyphenols to contribute to the majority of the total polyphenol content. Due to the occurrence of the polyphenols, phytosterols, and perhaps the indoles present,A. ferox leaf gel may show promise in alleviating or preventing the symptoms associated with cardiovascular diseases, cancer,

neurodegeneration, and diabetes. This may be due to the well-documented lowering effects of these compounds on total cholesterol, LDL-C, and fasting blood glucose. These results support the current use of A. ferox by both industry and traditional healers for the treatment of the above-mentioned diseases. However, further clinical trials regarding these claims are necessary before accurate conclusions regarding these heath benefits can be made.

ABBREVIATIONS USED

LGE, leaf gel extract; ELGE, ethanol leaf gel extract; GCMS, gas chromatography coupled mass spectrometry; ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing antioxidant power; LDL-C, low-density lipid cholesterol; BSTFA, bis(trimethylsilyl)trifluoroacetamide; TMCS, trimethylchlorosilane; GAE, gallic acid equivalent; CE catechin equivalent; TE, Trolox equivalent.

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