

Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill.

Olubunmi Abosede Wintola, Anthony Jide Afolayan

Department of Botany, University of Fort Hare, Alice 5700, South Africa

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ABSTRACT

Background: *Aloe ferox* Mill. (Asphodelaceae) is used in South Africa for the treatment of constipation among various ailments. Despite the extensive studies conducted on the antioxidant activities of the leaf gel and pulp extract of the plant, there is no information on the antioxidant properties of the whole leaf extract of the species. **Materials and Methods:** The antioxidant activities of ethanol, acetone, methanol and aqueous extracts of *A. ferox* were investigated spectrophotometrically against 1,1 diphenylpicrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) diammonium salt, hydrogen peroxide (H₂O₂), nitric oxide (NO), lipid peroxidation and ferric reducing power. Total phenols, flavonoids, flavonols, proanthocyanidins, tannins, alkaloids and saponins were also determined using the standard methods. **Results:** The percentage compositions of phenols (70.33), flavonols (35.2), proanthocyanidins (171.06) and alkaloids (60.9) were significantly high in the acetone extract, followed by the ethanol extract with values of 70.24, 12.53, 76.7 and 23.76 respectively, while the least composition was found in the aqueous extract. Moreover, both flavonoids and saponins contents were appreciably high in both methanol and ethanol extracts, while others were very low. Tannins levels were, however, not significantly different ($P > 0.05$) in all the solvent extracts. At 0.5 mg/ml, the free radical scavenging activity of the methanol, acetone and ethanol extracts showed higher inhibition against ABTS, hydrogen peroxide and nitric oxide radicals. Whereas, scavenging activity of the extracts against DPPH* and lipid peroxidation were observed at a concentration of 0.016 and 0.118 mg/ml respectively in comparison to the butylated hydroxytoluene (BHT), gallic acid and rutin. The ferric reducing potential of the extracts was concentration dependent and significantly different from that of vitamin C and BHT. **Conclusion:** The present study showed high level of radical scavenging activity by ethanol and methanol whole leaf extracts of *A. ferox* with higher antioxidant activities than acetone and aqueous extracts. The significant differences show that the whole leaf extract could be used as a potent antioxidant in medicine and food industries.

Key words: *Aloe ferox*, antioxidant, asphodelaceae, free radicals, phytochemicals

INTRODUCTION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons produced from various biochemical reactions.^[1,2] They occur continuously in the cells as a result of enzymatic and non-enzymatic reactions to different molecules in the body.^[3] They are generated as a result of imbalance between formation and neutralization of pro-oxidants in the body metabolic process.^[4,5] Examples of these are reactive oxygen species (ROS) or reactive nitrogen species (RNS) radicals which

include superoxide anions, singlet oxygen, hydrogen peroxide and hydroxyl radicals. These radicals can cause variety of pathological effects such as protein damage, DNA mutation, carcinogenesis, aging, cardiovascular and neuro-degenerative diseases. They are implicated as a result of oxidative stress, occasional leakages from continuous exposure to chemicals, contaminants and exogenous factors.^[2,6,7] The pathological disruption of these radicals in the human cells could be prevented by quenching the upshot of catalytic activities.^[5,7]

Synthetic drugs such as butylated hydroxytoluene (BHT), rutin, and butylated hydroxyl anisole (BHA) are commonly used. However, they have been reported to cause adverse side effects such as toxicity, cell damage, inflammations, and atherosclerosis in animals and humans.^[2,8] In view of

Address for correspondence:

Prof. A. J. Afolayan, Department of Botany, University of Fort Hare, Alice 5700, South Africa. E-mail: aafolayan@ufh.ac.za

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the increasing risk factors, there has been a global trend toward the use of natural substances present in medicinal plants as therapeutic antioxidant agents.^[3] Several studies have therefore demonstrated that plants produce potent phytochemicals with strong antioxidant activities and thus represent an important source of natural antioxidants.^[9] Moreover, the use of natural products of plant origin has been proposed because they are natural antioxidants with rich sources of metabolites. They contain bioactive chemicals such as phenols, alkaloids and lignin which are potent radical terminators that can help in reducing the risk of cancer, toxicity, inflammation and cardiovascular diseases.^[10,11]

Aloe ferox (Cape aloe), locally called ikhala in Xhosa, is widely distributed in Free State, Lesotho, Western and Eastern Cape of South Africa. It is a perennial, single-stemmed shrub, reaching 2–3 m in height, with thick succulent leaves bearing brown spines on the margin. For centuries, the leaf exudate of *A. ferox* has been used therapeutically for the treatment of constipation.^[12] Today, the plant is reputed for its antiseptic, laxative, moisturizing, cleansing and anti-inflammatory properties. Traditionally, the fresh and dried whole leaf extract of the plant is used directly as an infusion and decoction for the treatment of various diseases such as skin cancer, gastrointestinal disorder, inflammation, burns and psoriasis.^[13,14] The leaf gel has been extensively documented to have significant laxative,^[15] anti-inflammatory,^[16] wound healing,^[13] sexually transmitted diseases cure,^[17] control of gastrointestinal parasite,^[18] immunostimulant, antioxidant, antitumor and antidiabetic activities.^[19] Furthermore, the leaf gel and pulp extract of the plant have several pharmacological effects such as antimicrobial and antitumor properties.^[20,21] We previously reported the laxative and toxicological effect of the aqueous whole leaf extract of *A. ferox* in the treatment of constipation.^[22,23] Despite the extensive studies conducted on the antioxidant activities of the leaf gel and pulp extract of *A. ferox*,^[19,24] there is no information in the scientific literature on the antioxidant properties of the whole leaf extract of the species. Therefore, the present study was aimed at determining the quantitative phytochemical constituents, free radical scavenging and antioxidant activities of the whole leaf extract of *A. ferox* using various solvents.

MATERIALS AND METHODS

Collection of plant materials and preparation of extracts

Fresh mature whole leaves of *A. ferox* were collected in June 2009 at Ntselamanzi area of the Eastern Cape Province of South Africa. The plant was authenticated by Prof. D. S.

Grierson at the Department of Botany, University of Fort Hare, and a voucher specimen (Wintola Med. 2009/01) was deposited at the Giffen herbarium of the University. The leaves were oven dried to constant weight at 40°C and milled to a homogeneous powder. The powdered plant material (200 g) was extracted separately in distilled water, acetone, methanol and ethanol on a shaker (Stuart Scientific Orbital Shaker, Essex, UK) for 48 h. Each extract was filtered using a Buchner funnel and Whatman No. 1 filter paper. The filtrate obtained with water was frozen at –40°C and dried for 48 h using a freeze dryer (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY, USA) to give a yield of 31.2 g of dried extract. The other extracts were individually concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. Acetone, methanol and ethanol extracts yielded 19.5 g, 25.6 g and 20.6 g, respectively. The resulting extracts were reconstituted with their respective solvents to give the desired concentrations used in the study.

Chemicals used

The following chemicals were used for the various experiments: 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), vanillin, aluminium chloride (AlCl₃), potassium acetate (CH₃CO₂K), ferric chloride (FeCl₂), BHT, ascorbic acid, rutin, Folin-Ciocalteu reagent, sodium carbonate (Na₂CO₃), phosphate buffer, potassium ferricyanide [K₃Fe(CN)₆], trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), glacial acetic acid (CH₃COOH), sodium nitroprusside (Na₂[Fe(CN)₅NO]2H₂O). They were purchased from Merck, Gauteng, South Africa. All other chemicals used were of analytical grade.

Determination of total phenols

The amount of phenol in the whole leaf extracts of *A. ferox* was determined spectrophotometrically using the modified method of Wolfe *et al.*,^[25] with Folin-Ciocalteu reagent. An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water at a concentration of 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and left to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the AJI-C03 UV-VIS spectrophotometer. Results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y = 0.121x$, $R^2 = 0.936512$, where x is the absorbance and Y is the tannic acid equivalent.

Estimation of flavonoids

Total flavonoid content was determined using the method of Ordon Ez *et al.*^[26] A volume of 0.5 ml of 2% AlCl₃ ethanol solution was added to 0.5 ml of the sample solution. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the

presence of flavonoids. Plant extracts were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as mg/g of quercetin using the following equation based on the calibration curve: $Y = 0.0255x$, $R^2 = 0.9812$, where x is the absorbance and Y is the quercetin equivalent.

Total flavonols

Total flavonol content was determined by adopting the procedure described by Kumaran and Karunakaran.^[27] The reaction mixture consisted of 2.0 ml of the sample, 2.0 ml of $AlCl_3$ prepared in ethanol and 3.0 ml of (50 g/l) sodium acetate solution. The absorbance at 440 nm was measured after 2.5 h at 20°C. Total flavonol content was calculated as mg/g of quercetin equivalent from the calibration curve using the equation: $Y = 0.0255x$, $R^2 = 0.9812$, where x is the absorbance and Y is the quercetin equivalent.

Determination of total proanthocyanidin

Total proanthocyanidin was determined based on the procedure of Oyedemi *et al.*^[5] To 0.5 ml of 1 mg/ml of the extract solution was added 3 ml of vanillin-methanol (4% v/v) and 1.5 ml of hydrochloric acid and vortexed. The mixture was allowed to stand for 15 min at room temperature and the absorbance was measured at 500 nm. Total proanthocyanidin content was evaluated at a concentration of 0.1 mg/ml and expressed as catechin equivalent (mg/g) using the calibration curve equation: $Y = 0.5825x$, $R^2 = 0.9277$, where x is the absorbance and Y is the catechin equivalent.

Determination of tannins

Tannin determination was done according to the method of AOAC^[28] with some modifications. To 0.20 g of the sample was added 20 ml of 50% methanol. This was shaken thoroughly and placed in a water bath at 80°C for 1 h to ensure uniform mixing. The extract was filtered into a 100-ml volumetric flask, followed by the addition of 20 ml of distilled water, 2.5 ml of Folin-Denis reagent and 10 ml of 17% aq. Na_2CO_3 and was thoroughly mixed. The mixture was made up to 100 ml with distilled water, mixed and allowed to stand for 20 min. The bluish-green color developed at the end of the reaction mixture of different concentrations ranges from 0 to 10 ppm. The absorbance of the tannic acid standard solutions as well as sample was measured after color development at 760 nm using the AJI-C03 UV-VIS spectrophotometer. Results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y = 0.0593x - 0.0485$, $R^2 = 0.9826$, where x is the absorbance and Y is the tannic acid equivalent.

Determination of alkaloids

Alkaloids were quantitatively determined according to the method of Harborne.^[29] Two hundred milliliters of 10%

acetic acid in ethanol was added to 5 g powdered plant sample, covered and allowed to stand for 4 h. The filtrate was then concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using the formula: % alkaloid = final weight of the sample / initial weight of the extract $\times 100$.

Determination of saponins

Quantitative determination of saponins was done using the method of Obadoni and Ochuko.^[30] The powdered sample (20 g) was added to 100 ml of 20% aqueous ethanol and kept in a shaker for 30 min. The samples were heated over a water bath for 4 h at 55°C. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 ml over the water bath at 90°C. The concentrate was transferred into a 250 ml separatory funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 ml *n*-butanol was added. The *n*-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven at 40°C to a constant weight. The saponin content was calculated using the formula: % saponin = final weight of sample / initial weight of extracts $\times 100$.

Antioxidant assay

The antioxidant activities of the whole leaf extracts of *A. ferox* were determined using DPPH, ABTS, reducing power, lipid peroxidation, nitric oxide and hydrogen peroxide.

Determination of ferric reducing power of the extracts

The reducing power of the whole leave extract of *A. ferox* was evaluated according to the method described by Aiyegoro and Okoh.^[31] The mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of the extracts and standards (0.025–0.5 mg/ml) prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10% w/v), which was then centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was then measured at 700 nm against blank sample. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

DPPH radical scavenging assay

The method of Liyana-Pathiana and Shahidi^[32] was used for the determination of scavenging activity of DPPH free radical. DPPH (1 ml, 0.135 mM) prepared in methanol was mixed with 1.0 ml of aqueous extract ranging from 0.025 to 0.5 mg/ml. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the plant extract was calculated using the equation: DPPH scavenging activity (%) = [(Abs control – Abs sample)/(Abs control)] × 100, where Abs control is the absorbance of DPPH + methanol and Abs sample is the absorbance of DPPH radical + sample (sample or standard).

ABTS radical scavenging activity

The method described by Adedapo *et al.*^[1] was adopted for the determination of ABTS activity of the plant extract. The working solution was prepared by mixing two stock solutions of 7 mM ABTS and 2.4 mM potassium persulfate in equal amounts and allowed to react for 12 h at room temperature in the dark. The resulting solution was further diluted by mixing 1 ml ABTS⁺ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm after 7 min using a spectrophotometer. The percentage inhibition of ABTS⁺ by the extract was calculated from the following equation: % inhibition = [(Abs control – Abs sample)/(Abs control)] × 100.

Nitric oxide scavenging activity

The modified method described by Oyedemi *et al.*^[5] was used to determine the nitric oxide radical scavenging activity of aqueous and other solvent extracts of *A. ferox*. A volume of 2 ml of 10 mM of sodium nitroprusside prepared in 0.5 mM phosphate buffered saline (pH 7.4) was mixed with 0.5 ml of plant extracts, gallic acid and BHT individually at 0.025–0.5 mg/ml. The mixture was incubated at 25°C for 150 min. 0.5 ml of the incubated solution was mixed with 0.5 ml of Griess reagent [1.0 ml sulfanilic acid reagent (0.33% prepared in 20% glacial acetic) acid at room temperature for 5 min with 1 ml of naphthylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min, followed by the measurement of the absorbance at 540 nm. The amount of nitric oxide radicals inhibited by the extract was calculated using the following equation: NO radical scavenging activity (%) = [(Abs control – Abs sample)/(Abs control)] × 100, where Abs control is the absorbance of NO radicals + methanol and Abs sample is the absorbance of NO radical + extract or standard.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of the plant extract was estimated using the modified method described by

Oyedemi *et al.*^[5] Plant extract (4 mg/ml) prepared in distilled water at various concentrations was mixed with 0.6 ml of 4 mM H₂O₂ solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was measured at 230 nm using the Biomate thermospectronic, against a blank solution containing the plant extract without H₂O₂. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation: H₂O₂ radical scavenging activity = [(Abs control – Abs sample)/(Abs control)] × 100, where Abs control is the absorbance of H₂O₂ radicals + methanol and Abs sample is the absorbance of H₂O₂ radical + sample or extract or standard.

Estimation of lipid peroxidation

A modified thiobarbituric acid-reactive species (TBARS) assay described by Dasgupta and De^[33] was used to measure the lipid peroxide formed, using egg-yolk homogenates as lipid-rich media. Egg homogenate (0.5 ml, 10% in distilled water, v/v) and 0.1 ml of the whole extract of *A. ferox* were mixed in a test tube and the volume was made up to 1 ml with distilled water. Finally, 0.05 ml FeSO₄ (0.07 M) was added to the mixture and incubated for 30 min to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulfate) and 0.05 ml 20% TCA were added, vortexed and heated in a boiling water bath for 60 min. After cooling, 5.0 ml of n-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. For the blank, 0.1 ml of distilled water was used in place of the extract.

Statistical analysis

Where applicable, the results were expressed as mean ± standard deviation (SD) of three replicates and were subjected to analysis of variance using the student Minitab release version 12, Windows 95. Significant levels were tested at *P* < 0.05.

RESULTS

Phytochemical constituents

Phytochemical analysis conducted on the whole leaf extracts of *A. ferox* revealed the presence of phenols, flavonoids, flavonols, proanthocyanidins, tannins, alkaloids and saponins [Table 1]. Most plants are used for the treatment of diseases because of the presence of these phytochemicals which have been reported to have high medicinal values. The concentrations of phenols in the different solvent extracts were significantly different from each other. This gives an indication that solvents possess different extracting capacity for phenol. The high content

Table 1: Phytochemical analysis of different solvent extracts of the whole leaf of *A. ferox*

Phytochemicals (mg/g)	Extracts			
	Aqueous	Acetone	Methanol	Ethanol
Total phenol	37.29 ± 0.19 ₁ ^a	70.33 ± 0.07 ^b	25.01 ± 0.07 ^c	70.24 ± 0.07 ^b
Total flavonoids	0.81 ± 0.04 ₂ ^a	0.88 ± 0.55 ^a	5.22 ± 0.05 ^b	2.30 ± 0.04 ^c
Total flavonols	5.45 ± 0.01 ₂ ^a	35.20 ± 0.05 ^b	8.57 ± 0.03 ^a	12.58 ± 0.01 ^a
Total proanthocyanidin	12.04 ± 0.89 ₃ ^a	171.06 ± 1.87 ^b	11.65 ± 0.58 ^a	76.70 ± 1.21 ^c
Tannins	0.027 ± 0.01 ₁ ^a	0.027 ± 0.01 ^a	0.014 ± 0.01 ^a	0.026 ± 0.01 ^a
Alkaloids (%)	1.20 ± 0.03 ^a	60.90 ± 0.02 ^b	13.63 ± 0.01 ^c	23.7 ± 0.01 ^d
Saponins (%)	8.34 ± 0.01 ^a	10.00 ± 0.15 ^a	15.60 ± 0.01 ^a	35.20 ± 0.01 ^b

Data expressed as means ± SD; n = 3; values along a row with different superscripts are significantly different ($P < 0.05$), Mean with the same subscript in the same column are: ₁Expressed as mg tannic acid/g of dry plant materials, ₂ expressed as mg quercetin/g of dry plant materials, ₃ expressed as mg catechin/g of dry plant materials

of phenol in the whole leaf extracts of *A. ferox*, observed in the acetone and ethanol extract, may be a contributing factor toward its antioxidant activity.

The flavonoid content in the whole leaf extracts in the various solvents was in the order: methanol > ethanol > acetone > aqueous extracts. The flavonoid content in the methanol extract was significantly higher than the ethanol extract. Meanwhile, the aqueous and acetone extracts were not significantly different from each other in this aspect. The acetone extract possessed high flavonol content which was significantly different from the other solvent extracts evaluated in this study, and this may be linked to the polarity of the solvent used. This was followed by the ethanol, methanol and aqueous extracts in this order, but these were not significantly different from each other. A moderate amount of flavonols was observed in the methanol and aqueous extracts, but there was an appreciable amount in the acetone and ethanolic extracts. The acetone extract contained high proanthocyanidin content, followed by the ethanol, methanol and aqueous extracts [Table 1]. Tannin content was generally low in all the solvent extracts of *A. ferox* compared to all other phytochemicals. Alkaloid content of the whole leaf extract of *A. ferox* followed the trend: acetone > ethanol > methanol > aqueous extracts, as shown in Table 1. The acetone extract exhibited high concentration of alkaloids which was significantly different from other solvent extracts. The percentage concentration of saponins in the ethanol extract was significantly higher than other solvents, but lower in the aqueous extract.

Antioxidant activities of the extracts

The extracts showed high percentage inhibition activity in all the free radical scavenging models evaluated in the present study.

Ferric reducing power

The potential of the plant extracts to reduce Fe^{3+} to Fe^{2+} by electron transfer is an indication of their antioxidant ability. The reducing power of the extracts in comparison

with the standards (vitamin C and BHT) is presented in Figure 1. The observed change of yellow color of test solution to various shades of blue and green, depending on the concentration of the extract, is an indication of its antioxidant activity. The ferric reducing activities of the extracts were significantly lower than the standard drugs used in this order: vitamin C > BHT > ethanol > acetone > methanol > aqueous extracts. At 0.5 mg/ml, the absorbance values of acetone, methanol and ethanol extracts were comparable with vitamin C and BHT as depicted in Figure 1.

DPPH radical scavenging activity

The DPPH scavenging potential of the whole leaf extracts of *A. ferox* was concentration dependent as shown in Figure 2. The percentage inhibition of DPPH by the various solvent extracts and the standard drugs was recorded in decreasing order: BHT > rutin > methanol > ethanol > acetone > aqueous extract. The % inhibition of BHT and rutin was significantly different from the extracts. Though the aqueous extract showed the least inhibition, it was not significantly different from the other solvents. The IC_{50} value of methanol extract was 0.086 mg/ml, acetone and ethanol extracts with similar value of 0.288 mg/ml and that of aqueous extract was 0.517 mg/ml.

ABTS radical scavenging activity

The percentage inhibition of ABTS radical by the various solvent extracts of *A. ferox* is shown in Figure 3. The % inhibition of ABTS by the various solvent extracts was concentration dependent and compared favorably well with the BHT and rutin. The scavenging activity in the various solvents was in the order: rutin > methanol > acetone > ethanol > BHT > aqueous extracts. The acetone and ethanol extracts compared favorably well with BHT but lower than the methanol extract [Figure 3]. There was a significant difference in the % inhibition of $ABTS^{\cdot+}$ at the different concentrations and solvents used. The methanol extract and BHT had IC_{50} values of 0.02 and 0.024 mg/ml, respectively, followed by acetone and ethanol extracts at

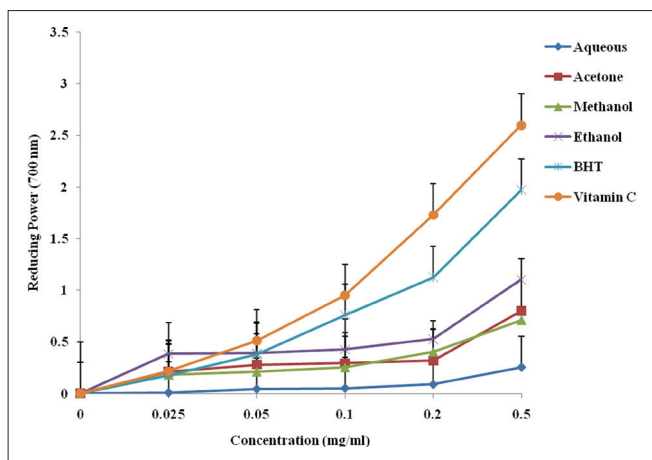


Figure 1: Reducing power of different solvent extracts of *A. ferox*

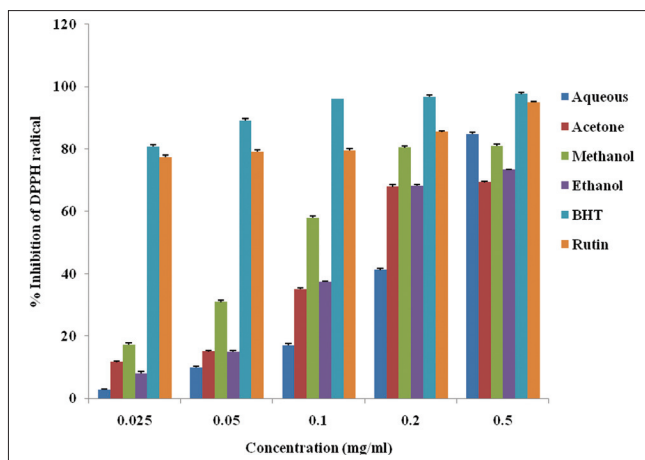


Figure 2: DPPH radical scavenging activity of the whole leaf extract of *A. ferox* obtained using different solvents

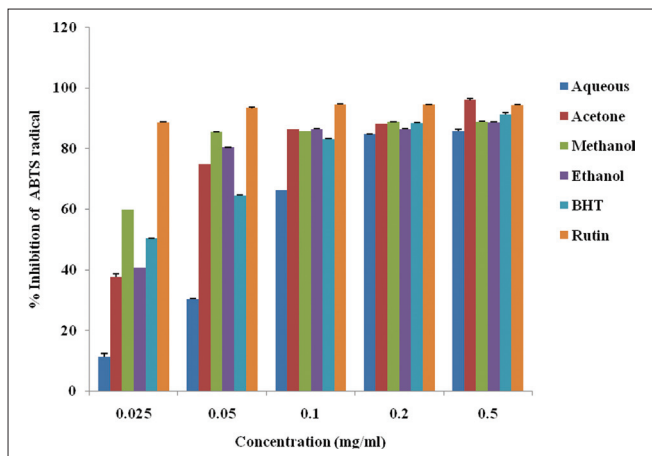


Figure 3: ABTS radical scavenging activity of the whole leaf extract of *A. ferox* obtained using different solvents

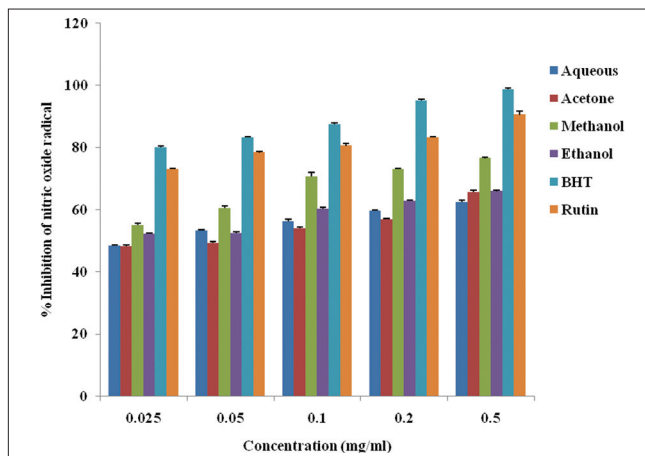


Figure 4: Nitric oxide radical scavenging activity of the whole leaf extract of *A. ferox* obtained using different solvents

0.033 and 0.062 mg/ml, respectively. The aqueous extract exhibited the least scavenging activity (0.173 mg/ml) in comparison with other solvent extracts.

Nitric oxide inhibition

The radical scavenging activity of the whole leaf extract of *A. ferox* was reflected as the % inhibition of nitric oxide [Figure 4]. The activities of the various extracts were in the order: BHT > rutin > methanol > ethanol > aqueous > acetone extracts. The % inhibition of nitric oxide by the various solvent extracts differed significantly from those of BHT and rutin. The IC₅₀ values of 0.023, 0.024, 0.016 and 0.017 mg/ml were recorded for the methanol extract, ethanol extract, BHT and rutin respectively, while acetone and aqueous extracts had their activities at 0.077 and 0.074 mg/ml respectively.

Hydrogen peroxide inhibition

The scavenging activity of the whole leaf extract of

A. ferox against hydrogen peroxide is shown in Figure 5. The scavenging activities of the plant in various solvents were in the order: acetone < ethanol < gallic acid < methanol < BHT < aqueous extract. The percentage inhibitions of hydrogen peroxide by the various solvents were concentration dependent and differed significantly from each other.

Lipid peroxidation

The scavenging of lipid peroxides by the whole leaf extract of *A. ferox*, BHT and gallic acid is presented in Figure 6. The % inhibition of lipid peroxides by the various solvent extracts was recorded in increasing order: gallic acid > BHT > methanol > aqueous > ethanol > acetone extract. The % inhibition of lipid peroxidation by the various solvents did not differ significantly from the standard BHT as compared to gallic acid. The data showed a significant difference from the solvent extract used. Gallic acid had the highest inhibitory activity against lipid peroxidation, with an

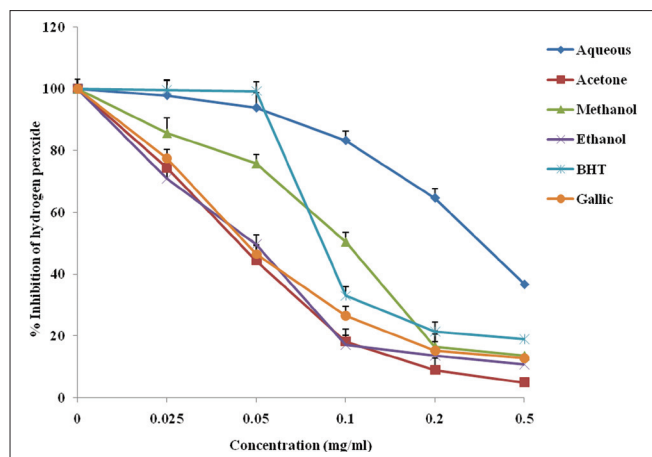


Figure 5: Hydrogen peroxide radical scavenging activity of the whole leaf extract of *A. ferox* obtained using different solvents

IC_{50} value of 0.118 mg/ml, while BHT, aqueous, acetone, methanol, ethanol extracts were active with IC_{50} values of 1.186, 1.837, 1.492, 0.930 and 1.270 mg/ml, respectively.

DISCUSSION

Phenolic compounds are known to exhibit strong antioxidant activities, which have direct antioxidant properties due to the presence of hydroxyl groups, which act as hydrogen donor.^[34] Additionally, they are found to be effective in scavenging free radicals as a result of their redox properties that allow them to act as reducing agents.^[5] The phytochemicals investigated, as shown in this study, have been reported to possess strong antioxidant activities due to their ability to adsorb, quench free radicals and decompose peroxides generated in the system.^[1]

Flavonoids are hydroxylated phenolics and are potent water-soluble antioxidants which help in radical scavenging and prevention of oxidative cell damage. They have been reported to possess strong antioxidant activities.^[35,24] The concentration in the whole leaf extracts of *A. ferox* was low. However, methanol extracts had more flavonoids than the other solvent extracts investigated in this study.

Proanthocyanidins are group of polyphenolic bioflavonoids which have a protective effect in eliminating hydroxyl radicals.^[36] Proanthocyanidin content was relatively high in the acetone extract. This is in agreement with the findings of Loots *et al.*^[24] who reported higher concentration of proanthocyanidins (polyphenols) in the leaf gel extract of *A. ferox*. According to Loots *et al.*^[24], *A. ferox* that contains phenolic compounds may serve as a potential source of bioactive agents in the treatment of cancer and other degenerative diseases. Several phenolic compounds including tannins are inhibitors of many hydrolytic enzymes

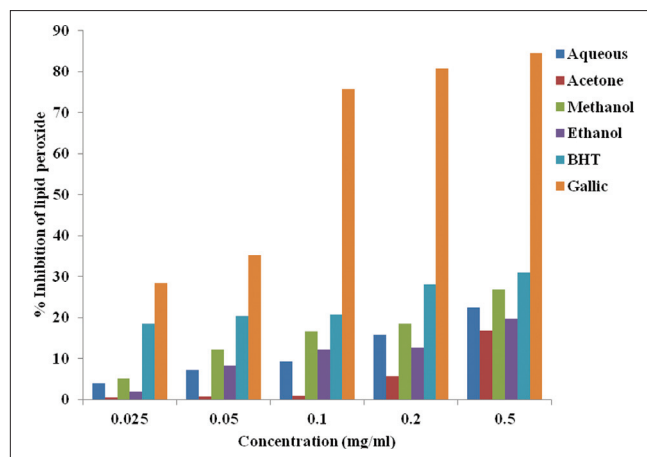


Figure 6: Lipid peroxidation scavenging activity of the whole leaf extract of *A. ferox* obtained from different solvents

such as proteolytic macerating enzymes used by plant against pathogens.^[37]

Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues.^[38,39] However, tannin level was low and not significantly different in comparison to other solvent extracts used in this study. Alkaloids have been associated with medicinal purpose for centuries as agents possessing analgesic, antimalarial, antiseptic and bactericidal activities, but could be toxic to cells.^[10] In this study, the high content of alkaloids in the acetone, ethanol and methanol extracts of *A. ferox* could be responsible for its much acclaimed medicinal value. Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation.^[38,40] The level of saponin content in the solvent extracts of the whole leaf of *A. ferox* was quite moderate. However, ethanol extract produced more saponins than other solvents. There was no significant difference in saponin content in all the various solvent extracts investigated in this present study.

The ability of a substance to act as an antioxidant depends on its strength to reduce ROS by donating hydrogen atom.^[41] The reducing capacity of a compound generally depends on the presence of reductants which exhibit antioxidative potential by breaking the free radical chain and donating hydrogen atom.^[42] It has been found that the transformation of Fe^{3+} to Fe^{2+} occurred in the presence of plant extract.^[43] *A. ferox* whole leaf extracts showed the reductive capabilities when compared to ascorbic acid and BHT. The reducing power of the extracts was concentration dependent and the antioxidant activities in all the solvents used were comparable with vitamin C and BHT.

DPPH is a relatively stable free radical scavenger which converts the unpaired electrons to paired ones by hydrogen proton donation. Scavenging of DPPH radical in this study indicates the potency of the plant extracts in donating hydrogen proton to the lone pair electron of the radicals. Because the inhibition was more at a higher concentration in all the solvent extracts, it could be suggested that the plant extracts contain compounds capable of donating protons to the free radicals. The methods have proven the effectiveness of the extracts in a concentration-dependent manner.^[41] The weak activity observed in the DPPH radical scavenging assay may be as a result of the low level of flavonoid contents in the plant extracts.^[5] However, the ability of the plant extracts to scavenge DPPH radical could also reflect its ability to scavenge and inhibit the formation of ABTS⁺.

The scavenging activity of ABTS⁺ by the plant extracts was found to be higher than that of DPPH radical. The solubility of extracts in different testing systems and radical reactivity confirming the removal of odd electron are believed to be responsible for the higher scavenging activity of ABTS. The scavenging activity of ABTS⁺ radical by the plant extract could imply that the extracts may be useful for treating radical related pathological damage, especially at higher concentrations.^[11] Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages and neurons and is involved in the regulation of various physiological processes. Excess concentration of nitric oxide is associated with several diseases.^[41,44] Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals.^[45] In the present study, the extract showed a moderate activity by competing with oxygen to react with nitric oxide and thus inhibits the generation of the anions.

Hydrogen peroxide is an important ROS because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell.^[46] The hydroxyl radical is an extremely reactive free radical formed in biological systems. It has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. Among the oxygen radicals, the hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules.^[47] Scavenging of H₂O₂ by the extracts may be attributed to their phenolics, which donate electron to H₂O₂, and thus reduce it to water. The solvent extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. This indicates the potency of the plant extracts in preventing the joining of nucleosides in the DNA and possible breakage leading to carcinogenesis and cytotoxicity.^[48] Free radical induced peroxidation in polyunsaturated lipids occurs

either through ferryl–perferryl complex or through OH* radicals.^[49] In this study, lipid peroxidation was brought about by egg-yolk homogenates as lipid-rich media. The tested plant extracts showed weak antioxidant activity which is indicated by their high absorbance values. This study shows concentration-dependent prevention of lipid peroxide generation.

The present study shows the scavenging activity of the whole leaf extracts of *A. ferox* in ethanol, methanol, acetone and aqueous extracts. The activity was high in ethanol, acetone and methanol extracts, but low in aqueous medium, indicating their antioxidant potential. The observed results suggest further analyses to confirm its prophylactic effect in the treatment of free radical-mediated diseases. Most antioxidant activities depend on the amount of the phytochemicals present in the plants. Although the contents of most phytochemicals evaluated are not very high but synergistically boost the antioxidant activity of the whole leaf extracts of *A. ferox*. Thus the plant had potent antioxidant properties to curtail progression of radical related diseases and thereby give credence to the traditional usage of *A. ferox* extract.

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