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Evaluation of antioxidant activity and the fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa

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Abstract

The leaves of *Vernonia amygdalina* were examined for antioxidant activity and analysed for their fatty acids content. Using transesterification and GC–MS analysis, 12 fatty acids were identified, which accounted for 74.1% of the lipid content. Two essential fatty acids (EFA), linoleic and α-linolenic acid were found in abundance in the oil. Using DPPH and a ABTS radical scavenging experiments, acetone, methanol and water extracts were screened for their antioxidant activity. The methanol extract exhibited high activity, by scavenging 75–99.3% of the DPPH radicals and 96.2–100% of the $ABTS^{\cdot+}$radicals. These values are higher than those of butylated hydroxytoluene and were less than or equal to those of catechin. The water extract was the least active; its activity ranged from 29% to 88% for DPPH radicals and 76.8–98.3% for $ABTS^{\cdot+}$. The presence of EFA and the high antioxidant activity of the leaf extracts have validated the importance of *V. amygdalina* in the diet.

Keywords: *Vernonia amygdalina*; Fatty acids; Antioxidant activity; Free radical scavenging

1. Introduction

Vernonia amygdalina Del. (synonym: *V. randii* S. Moore) is probably the plant most used both for food and medicinal purposes throughout tropical Africa. It is a small tree growing between 1 and 3 m high. The plant is popularly known for its astringent bitter taste, which is associated with its non-nutritional phytochemical constituents (Igile, Oleszek, Burda, & Jurzysta, 1995). Despite the bitter taste, *V. amygdalina* is used as a vegetable in west and central African countries (Ajebesone & Aina, 2004; Igile et al., 1995). Besides its use as a vegetable, the plant is used in the treatment of malaria, diabetes, diarrhoea, venereal disease, hepatitis, gastrointestinal problems, skin diseases and wounds (Akah & Ekekwe, 1995; Bullough & Leary, 1982; Hamill et al., 2000; Johns, Faubert, Kokwaro, Mahunnah, & Kimanani, 1995; Kambizi & Afolayan, 2001; Otshudi, Vercruysse, & Foriers, 2000; Riley, 1963).

Due to its medicinal and nutritional uses, several phytochemical studies have been conducted on *V. amygdalina*. Nutritional analyses revealed high level of crude protein, fibre, vitamin A and C, minerals and resin (Ajebesone & Aina, 2004; Igile et al., 1995). Some of the reported phytochemicals from *V. amygdalina* include flavonoids, sesquiterpene lactones and steroidal saponins (Igile et al., 1994; Igile et al., 1995; Jisaka, Ohigashi, Takegawa, Huffman, & Koshimizu, 1993; Koshimizu, Ohigashi, & Huffman, 1994; Ohigashi et al., 1991).

According to several workers, phenolic compounds (such as flavonoids, anthocyanins and phenolic acids) are responsible for the antioxidative activities of edible and non-edible plant products. These natural products offer protection against harmful free radicals and have been known to reduce the risk of certain types of cancer, coronary heart diseases, stroke, atherosclerosis, and other

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degenerative diseases associated with oxidative stress (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Mazza, Fukumoto, delaquis, Girard, & Ewert, 1999).

There is relatively no information on the fatty acid profile of *V. amygdalina*. Also, little is known about the antioxidant activity of this plant species. This paper presents the fatty acids profile and the antioxidant activity of extracts from the leaves of *V. amygdalina*, with a view to validating its nutritional and medicinal potential.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate ($K_4S_2O_8$) were purchased from Sigma–Aldrich. All analytical grade solvents were supplied by Merck Chemicals, S. Africa.

2.2. Collection and extraction of plant materials

The leaves of *V. amygdalina* were obtained from a cultivated population in East London, South Africa. The plant was authenticated by Prof. Afolayan and a voucher specimen (Erasto12) deposited in the herbarium of the University of Fort Hare. Leaves were allowed to dry at room temperature before pulverisation using a blending machine. The ground material was divided into four portions. A portion was used for the extraction of fatty acids, while the rest was separately shaken in acetone, methanol and water for 24 h.

2.3. Extraction of fatty acids

The pulverised leaves of *V. amygdalina* (342.7 g) were shaken in hexane/isopropanol (3:1) for 8 h and the extract was concentrated under reduced pressure to give 1.07 g (0.31% w/w) of the oil sample. A complete removal of solvents from the oil sample was achieved by passing a stream of nitrogen through it and thereafter the oil was stored below 4 °C before derivatisation of its fatty acids.

2.4. Preparation of fatty acid methyl esters (FAMEs)

Adopting the method of Lepage and Roy (1984), 40 ml of dried methanol was transferred into a 500 ml round-bottomed flask and cooled in an ice bath for 30 min. While cooling, 14 ml of acetyl chloride was added to the dry methanol dropwise, with constant shaking. The dried oil sample was added to the cold product followed by addition of 20 ml of chloroform to dissolve the sample completely. The resulting mixture was refluxed for 2 h, after which the mixture was cooled and 4.0 M NaOH was added to make the reaction mixture alkaline. To the basic mixture 100 ml of distilled water was added and then the whole mixture was transferred to a 500 ml separating funnel, where diethyl ether (50 ml × 3) was added, in order to extract FAMEs. The organic layer was dried with anhydrous magnesium sulphate, filtered and the solvent was evaporated in *vacuo*. A complete removal of the solvent was achieved under a stream of nitrogen gas.

2.5. GC–MS analysis of fatty acid methyl esters (FAMEs)

The fatty acid methyl esters (FAME) were analysed using a Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP 6890 gas chromatograph. Electron ionisation at 70 eV with an ion source temperature of 240 °C was used. An HP-5 column was used (30 m × 0.25 mm i.d; film thickness 0.25 μm), with helium carrier gas. The oven temperature was 70–325 °C at 4 °C min^{-1} and 0.2 μl of the diluted fatty acid esters were injected into the GC–MS. Identification of constituents was done by matching their mass spectra with those in the Wiley 275 K (Wiley, New York) library. The experiment was replicated thrice.

2.6. Determination of DPPH radical scavenging activity

The method described by Liyana-Pathirana and Shahidi (2005) was used to assess the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity of *V. amygdalina* leaves. DPPH solution (0.0 ml, 0.11 mM) in methanol was separately mixed with 0.01, 0.02, 0.05, and 0.1 mg/ml of extracts and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 60 min at 10 min intervals. Catechin and butylated hydroxytoluene (BHT) were used as the reference antioxidant compounds. The absorbance of the remaining DPPH radicals was read at 519 nm using a Beckman spectrophotometer (Model DU 7400, USA). The analysis of each assay solution was replicated thrice. The scavenging of DPPH radicals was calculated according to the following equation:

$$\text{DPPH radical-scavenging activity } (\%) = [(A_{\text{control}} - A_{\text{sample}}\ (A_{\text{control}})] \times 100,$$

where A_{control} is the absorbance of DPPH radical + methanol; A_{sample} is the absorbance of DPPH radical + sample extract/standard.

2.7. Determination of ABTS radical scavenging activity

A modified method of Re et al. (1999) was used for the assessment of the scavenging activity of a pre-formed 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical monocation, ($ABTS^{\cdot+}$). The $ABTS^{\cdot+}$ was formed by reacting 7.2 mM of ABTS solution with 2.44 mM of potassium persulfate and the resultant $ABTS^{\cdot+}$solution

was diluted in ethanol to an absorbance of 0.75 at 734 nm. Finally, 1.0 ml of the solution was mixed with various amounts (containing 0.01, 0.02, 0.05 and 0.1 mg/ml, respectively) of the leaves extracts. The absorbance of the mixtures at ambient temperature was recorded for 6 min at 1 min intervals. Catechin was used as a reference antioxidant compound. The analysis of each assay solution was replicated thrice. The scavenging of ABTS radical cation was calculated according to the following equation:

$$\text{ABTS radical cation scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}})] \times 100,$$

where A_{control} is the absorbance of ABTS radical cation + methanol; A_{sample} is the absorbance of ABTS radical + sample extract/standard.

The results were analyzed using MSUTAT statistical package. Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

3.1. Fatty acids profile

The GC–MS analysis of the lipid extract from the leaves of *V. amygdalina* revealed 12 fatty acids, accounting for 74.1% of the total extract composition. Palmitic acid was the most abundant lipid (22.2%) in the extract (Table 1). The essential fatty acids (EFA), α-linolenic and linoleic acid, were found in high amounts (21.5% and 15.8%, respectively) in this plant. This is a reflection of the species significance as a good supplement in humans diet. Although the human body can manufacture most of the fats it needs, these two EFAs cannot be manufactured in the body and hence have to be obtained from dietary sources (Deferne & Pate, 1996).

Table 1
Fatty acids profile of the leaves of *V. amygdalina*

Fatty acid	% Composition
Tetradecanoic acid[a]	1.24
9-Methyldodecanoic acid	0.98
Hexadecanoic acid[b]	22.19
14-Methylhexadecanoic acid	1.3
(*Z,Z*)-9,12-octadecadienoic acid[c]	15.81
(*Z,Z,Z*)-9,12,15-octadecatrienoic acid[d]	21.5
Octadecanoic acid[e]	3.07
Eicosanoic acid	3.21
Heneicosanoic acid	0.58
(*Z,Z,Z*)-6,9,12-octadecatrienoic acid[f]	0.46
Docosanoic acid	2.26
Tetradocosanoic acid	1.52
Total fatty acids identified	74.12
Other hydrocarbon compounds identified	2.61
Unidentified compounds	23.27

[a] Myristic acid.
[b] Palmitic acid.
[c] Linoleic acid.
[d] α-Linolenic acid.
[e] Stearic acid.
[f] γ-Linolenic acid.

Linoleic acid (an omega-6 fatty acid) is the metabolic precursor of eicosanoids. Eicosanoids are a group of biologically important lipids which include prostaglandins, thromboxanes, lipoxins and leukotrienes (Bourre et al., 1993). These lipids play an important role in immunity, inflammation and blood clotting. α-Linolenic acid (an omega-3 fatty acid), is a precursor in the biosynthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DHA is one of the predominant fatty acids in the human brain and has been found to play an important role in brain development in infants (Makrides, Neumann, Simmer, Pater, & Gibson, 1995). Polyunsaturated lipids have also been reported in the reduction of coronary heart disease (Oliver, 1997).

3.2. Antioxidant activity of extracts

The antioxidant activity of the leaf extracts of *V. amygdalina* was determined from the reduction of the absorbancies of DPPH and ABTS radicals at 519 and 734 nm, respectively. At 0.01, 0.02, 0.05, and 0.1 mg/ml, the methanol extract showed high activity, with 75.9%, 93.9%, 97.1%, and 99.3% of the DPPH radicals scavenged, respectively (Fig. 2). The values are slightly less than those of catechin at the same concentrations. However, the methanol extract was relatively more active than BHT, which had a DPPH radical scavenging activity of 87.9%, 94.4%, 94.8% and 97.1%, respectively. The acetone extract was less active than the methanol extract and standard antioxidant compounds. Its activity ranged from 63.3% to 91.7% (Figs. 1 and 2). The water extract was the least active of all the extracts. The concentration-dependent curves of the DPPH radical scavenging assay (Figs. 1 and 2) showed the following general trend of activity: $0.01 < 0.02 < 0.05 \leqslant 0.1$ mg/ml for acetone and methanol extracts while in water extracts, the trend was $0.01 < 0.02 < 0.05 < 0.1$ mg/ml.

In the $ABTS^{\cdot+}$ scavenging test, a similar trend of activity was observed. Methanol and acetone extracts exhibited high activity with 100% of the radical scavenged at 0.05 and 0.1 mg/ml (Figs. 5 and 6). Again, the water extract exhibited weak activity but showed a slightly different trend of activity, *viz*. $0.01 < 0.02 < 0.05 > 0.1$ mg/ml (Figs. 5 and 6).

The leaf extracts of *V. amygdalina* have demonstrated concentration and time-dependent radical-scavenging activities. This implies that the determinant of the antioxidant activity in this study is the concentration of the sample and how long the extract and free radical are allowed to react. This is clearly shown in Figs. 1–6.

Generally, the extracts were found to possess high antioxidant activity. This indicates the ability of the leaf extracts to neutralise free radicals. Free radicals possess an unpaired electron, which makes them highly reactive (Abe & Berk, 1998). The role of antioxidants is to

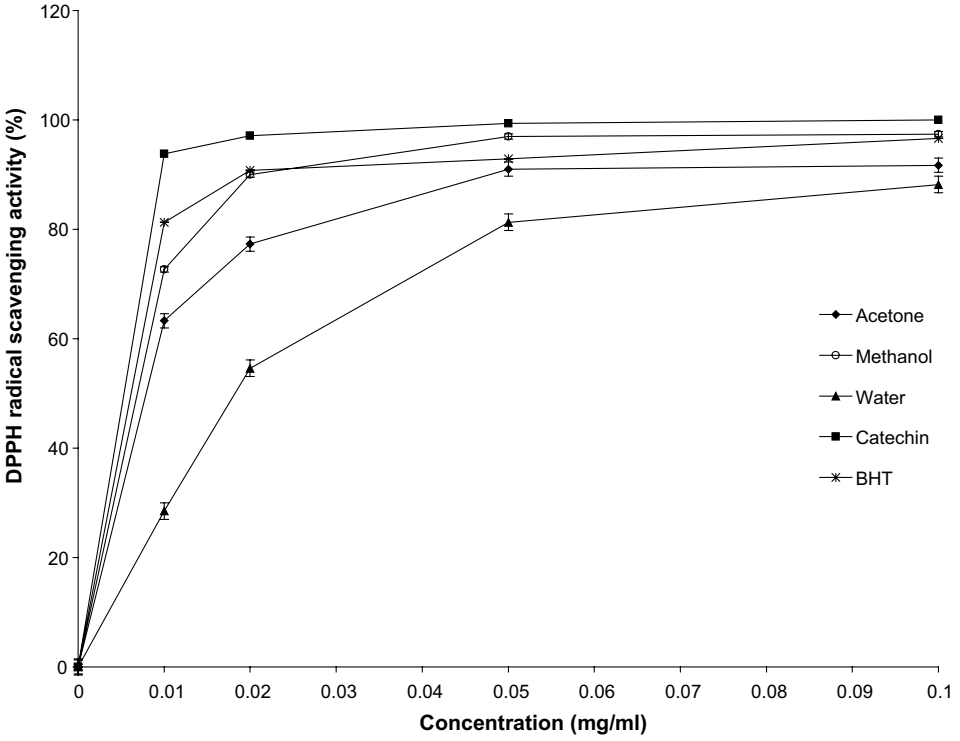

Fig. 1. DPPH radical scavenging activities after 30 min of extracts-free radical reaction. Each value is expressed as mean ± SD ($n = 3$).

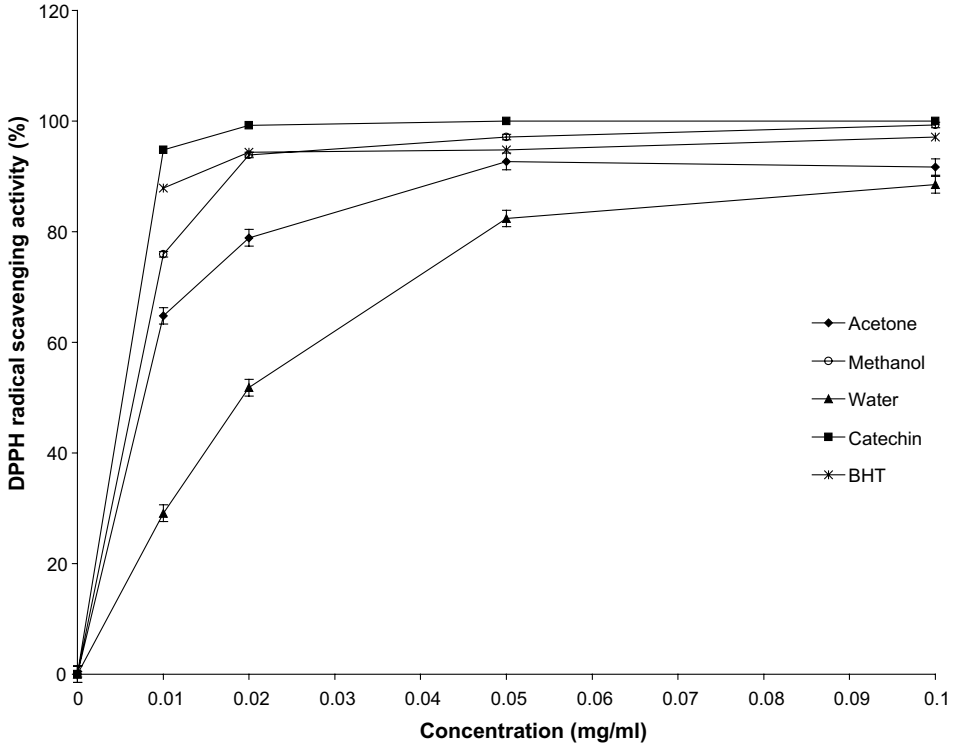

Fig. 2. DPPH radical scavenging activities after 60 min of extracts-free radical reaction. Each value is expressed as mean ± SD ($n = 3$).

neutralise these radicals by donating hydrogen atoms. Examples of such antioxidant compounds are flavonoids. Flavonoids isolated from the leaves of *V. amygdalina* have been reported to be highly antioxidative. For example, Luteolin has been found to be more active than BHT (Igile et al., 1994).

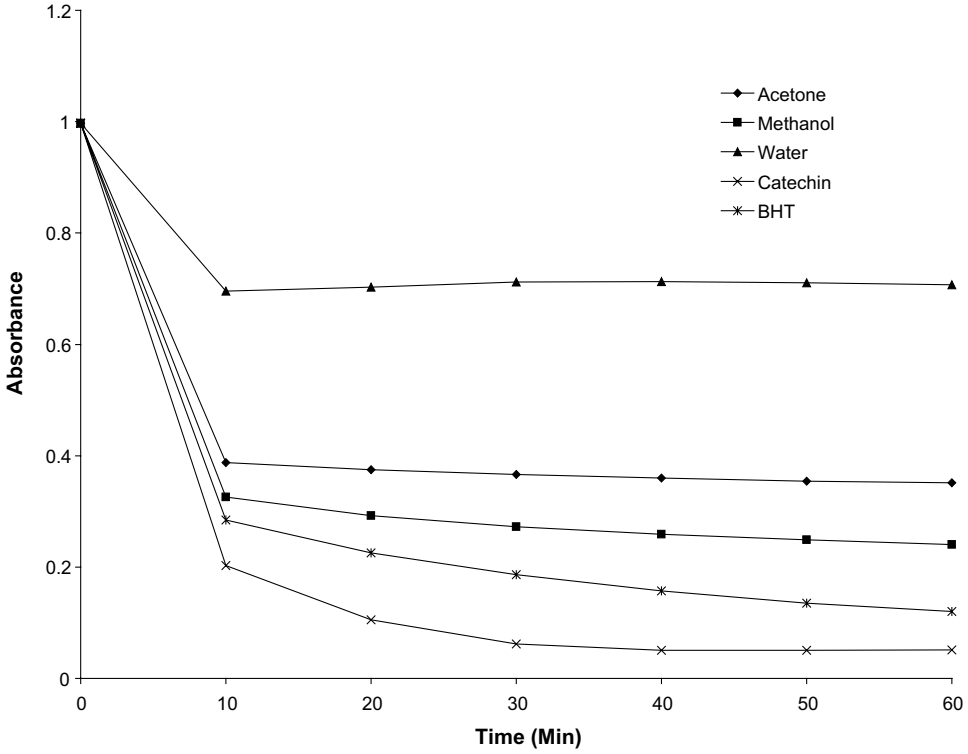

Fig. 3. The effect of time on the suppression of the absorbance of DPPH radical at 0.01 mg/ml of the extract/standard.

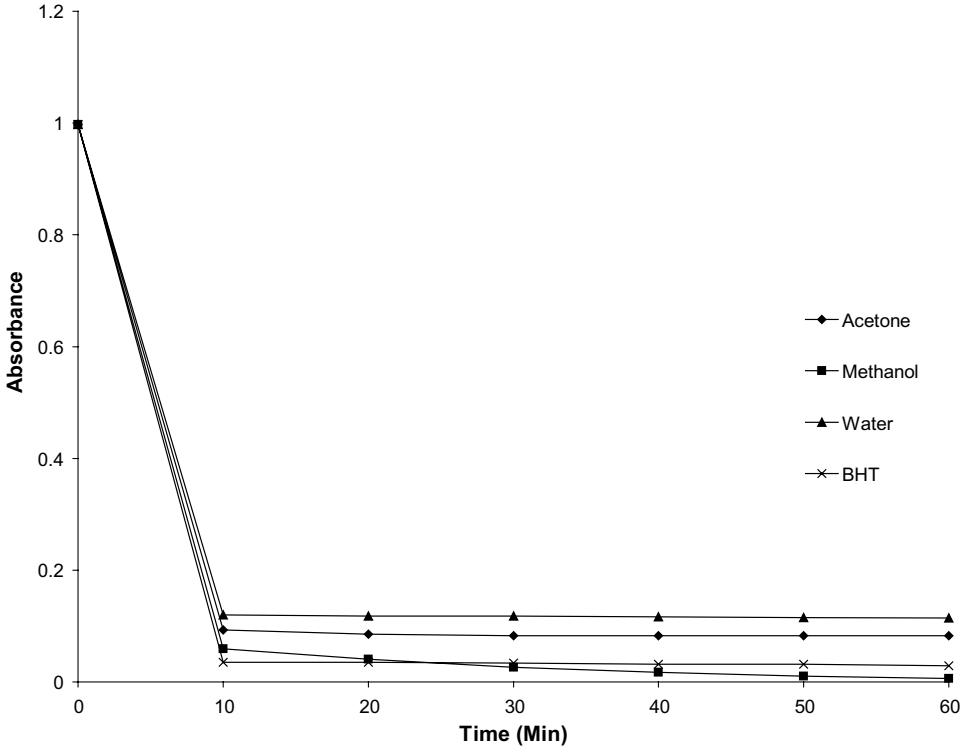

Fig. 4. The effect of time on the suppression of the absorbance of DPPH radical at 0.1 mg/ml of the extract/standard.

4. Conclusion

The presence of essential fatty acids in the leaves of *V. amygdalina* demonstrates their importance in the human diet. The role played by this plant in human health is enormous, especially its use in the treatment of diabetes (Erasto, Adebola, Grierson, & Afolayan, 2005). EFA have improved the secretion of insulin in diabetic patients and also helped

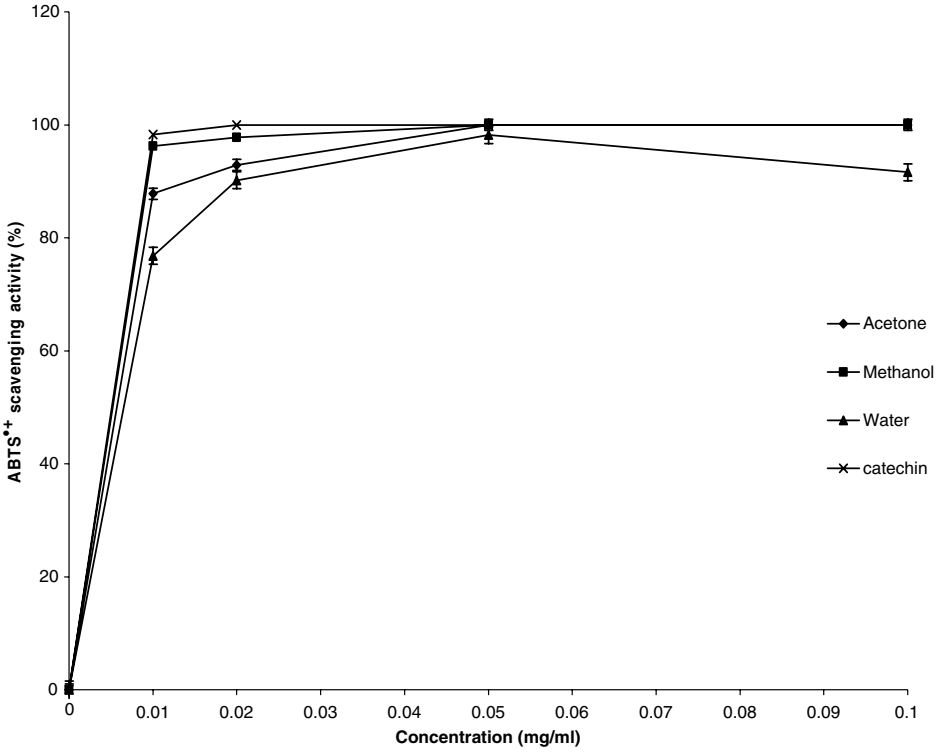

Fig. 5. $ABTS^{\cdot+}$ scavenging activities after 3 min of extracts-free radical reaction. Each value is expressed as mean ± SD ($n = 3$).

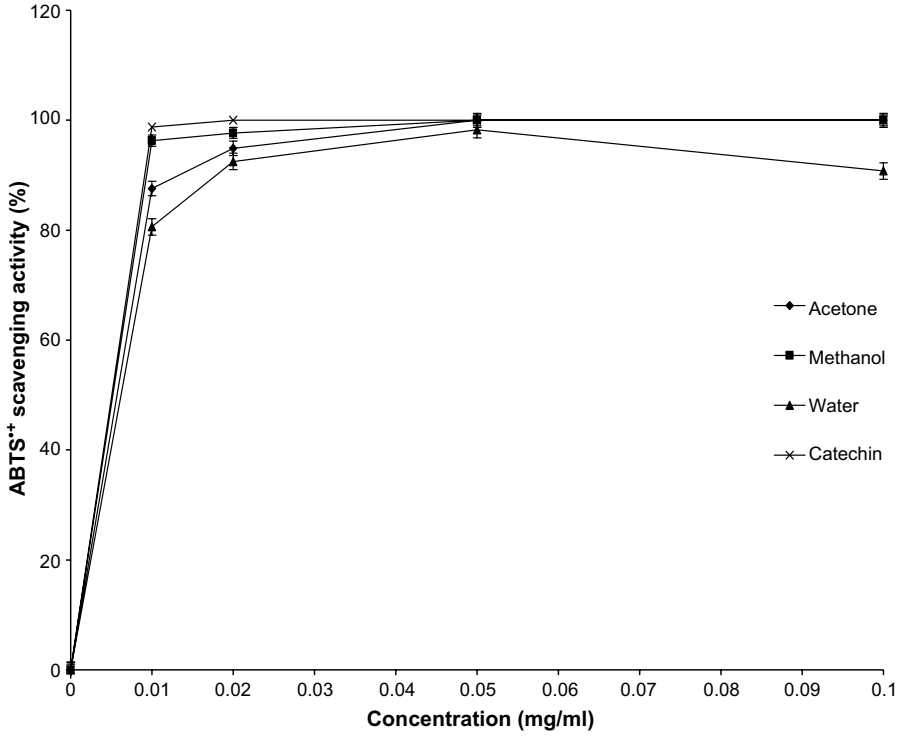

Fig. 6. $ABTS^{\cdot+}$ scavenging activities after 6 min of extracts-free radical reaction. Each value is expressed as mean ± SD ($n = 3$).

to combat some diseases associated with it, such as retinopathy and other complications (Haag, 2002). Our findings on the antioxidant activity of *V. amygdalina* have validated the medicinal importance of this plant in the human diet.

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References

Abe, J., & Berk, B. C. (1998). Reactive oxygen species as mediators of signal transduction in vascular disease. *Trend in Cardiovascular Medicine, 8*, 59–64.

Ajebesone, P. E., & Aina, J. O. (2004). Potential African substitutes for hops in tropical beer brewing. *Journal of Food Technology in Africa, 9*, 13–16.

Akah, P. A., & Ekekwe, R. K. (1995). Ethnopharmacology of some Asteraceae family used in Nigerian traditional medicine. *Fitoterapia, 66*, 351–355.

Bourre, J. M., Bonneil, M., Clement, M., Dumont, O., Durand, G., Lafont, H., et al. (1993). Function of dietary polyunsaturated fatty acids in the nervous system. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 48*, 5–15.

Bullough, C. H. W., & Leary, W. P. (1982). Herbal medicines used by traditional birth attendants in Malawi. *Tropical Geographical Medicine, 34*, 81–85.

Deferne, J. L., & Pate, D. W. (1996). Hemp seed oil: a source of valuable essential fatty acids. *Journal of the International Hemp Association, 3*, 1–7.

Erasto, P., Adebola, P. O., Grierson, D. S., & Afolayan, A. J. (2005). An ethnobotanical study of the plants used for the treatment of diabetes in the Eastern Cape Province, South Africa. *African Journal of Biotechnology, 4*, 1458–1460.

Haag, M. (2002). Polyunsaturated fatty acids: their cellular role and clinical applications. *The Medical Journal, 44*(4)www.medpharm.co.za.

Hamill, F. A., Apio, S., Murbiru, N. K., Mosango, M., Bukenya-Ziraba, R., Maganyi, O. W., et al. (2000). Traditional herbal drugs of southern Uganda. *Journal of Ethnopharmacology, 70*, 281–300.

Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., & Kromhout, D. (1993). Dietary antioxidant flavonoids and the risk of coronary heart diseas: the Zutphen elderly study. *Lancet, 342*, 1007–1011.

Igile, G. O., Oleszek, W., Burda, S., & Jurzysta, M. (1995). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. *Journal of Agricultural and Food Chemistry, 43*, 2162–2166.

Igile, G. O., Oleszek, W., Jurzysta, M., Burda, S., Fanfunso, M., & Fasanmade, A. A. (1994). Flavonoids from *Vernonia amygdalina* and their antioxidant activities. *Journal of Agricultural and Food Chemistry, 42*, 2445–2448.

Jisaka, M., Ohigashi, H., Takegawa, K., Huffman, M. A., & Koshimizu, K. (1993). Antitumoral and antimicrobial activities of bitter sesquiterpene lactones of *Vernonia amygdalina*, a possible medicinal plant used by wild chimpanzee. *Bioscience, Biotechnology, Biochemistry, 57*, 833–834.

Johns, T., Faubert, G. M., Kokwaro, J. O., Mahunnah, R. L. A., & Kimanani, E. K. (1995). Anti-giardial activity of gastrointestinal remedies of the Luo of East Africa. *Journal of Ethnopharmacology, 46*, 17–23.

Kambizi, L., & Afolayan, A. J. (2001). An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. *Journal of Ethnopharmacology, 77*, 5–9.

Koshimizu, K., Ohigashi, H., & Huffman, M. A. (1994). Use of *Vernonia amygdalina* by wild chimpanzee: possible roles of its bitter and related constituents. *Physiology and Behavior, 56*, 1209–1216.

Lepage, G., & Roy, C. C. (1984). Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *Journal of Lipid Research, 25*, 1391–1396.

Liyana-Pathirana, C. M., & Shahidi, F. (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *Journal of Agricultural and Food Chemistry, 53*, 2433–2440.

Makrides, M., Neumann, M., Simmer, K., Pater, J., & Gibson, R. (1995). Are long chain polyunsaturated fatty acids essential nutrient in infancy. *Lancet, 345*, 1463–1468.

Mazza, G., Fukumoto, L., delaquis, P., Girard, B., & Ewert, B. (1999). Anthocyanins, phenolics and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *Journal of Agricultural and Food Chemistry, 47*, 4009–4017.

Ohigashi, H., Jisaka, M., Takagaki, T., Tada, T., Huffman, M. A., Nishida, T., et al. (1991). Bitter principle and a related steroid glucoside from *Vernonia amygdalina,* a possible medicinal plant for wild chimpanzees. *Agricultural and Biological Chemistry, 55*, 1201–1203.

Oliver, M. F. (1997). It is more important to increase the intake of unsaturated fats than to decrease the intake of saturated fats: evidence from clinical trials relating to ischemic heart disease. *American Journal of Clinical Nutrition, 66*, 980S–986S.

Otshudi, A. L., Vercruysse, A., & Foriers, A. (2000). Contribution of the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in Lomela area, Democratic Republic of Congo. *Journal of Ethnopharmacology, 71*, 411–423.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Riley, H. P. (1963). *Families of flowering plants of Southern Africa*. University of Kentucky, p. 170.