Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib20160418043227

Original Research

ScopeMed

Impact of two different media extracts of *Vernonia amygdalina* on free radicals and membrane lipid stability under normal condition

Hanson O.T. Iyawe[1], Mark C. Azih[2]

[1]Department of Biochemistry Faculty of Natural Sciences, Ambrose Alli University, P.M.B. 14, Ekpoma Edo State Nigeria.
[2]Department of Chemistry Faculty of Natural Sciences, Ambrose Alli University, P.M.B. 14, Ekpoma Edo State Nigeria.

Address for correspondence:
Hanson O.T. Iyawe,
Department of Biochemistry Faculty of Natural Sciences, Ambrose Alli University, P.M.B. 14, Ekpoma Edo State Nigeria.
iyawehanson@yahoo.com

Received: September 06, 2015
Accepted: April 03, 2016
Published: April 24, 2016

ABSTRACT

Introduction: *Vernonia amygdalina* is a tropical plant commonly used in the local treatment of ailments because it is assumed that it possesses medicinal properties. Objective: The mediating role of its leaf extract in ameliorating oxidative stress is explored in this investigation. **Methods:** The leave samples were collected, air dried, homogenized and sieved to obtain particle sizes of ≤ 0.250mm. Aqueous and methanolic extracts of samples were obtained and used to assay for free radical scavenging activity, reducing power and the extent of lipid peroxidation in liver and kidneys were determined. Standard analytical methods were used in all assays. **Results:** Free radicals inhibitions and reducing powers of extracts were found to increase with increasing extracts concentrations, with highest inhibitions obtained as 98.80 ± 6.45 and 77.99 ± 6.34% in aqueous and methanol, as against 100.00 ± 0.00% respective controls of ascorbic acid and Butylated Hydroxytoluene (BHT). Reducing powers of extracts increased with progressive increases in extracts concentrations, with 1.23 ± 6.34mgRE/g for aqueous and 0.53 ± 0.04mgRE/g in methanol, as compared with respective controls of 2.13 ± 0.04 and 2.49 ± 0.03 respectively. The inhibitory concentrations (IC50) for aqueous and MeOH extracts are 2.0mg/mL and 2.3mg/mL respectively. For reducing power, it was 4.2mgRE/g and 2.2mgRE/g respectively, as against 2.8 mgRE/g and 2.96mgRE/g for Vit C and BHT controls respectively. The leave extracts of *Vernonia amygdalina* significantly ($p<0.05$) increased lipid peroxidations in liver and kidneys of rats. **Conclusion:** It is therefore assumed that *V. amygdalina* possesses properties which could act as a strong free radical quencher. However, for proper characterisation, further investigations are required to isolate the active ingredient(s) particularly influencing this likely process.

KEY WORDS:Bitter leaf; Free radicals; Lipid peroxidation.

INTRODUCTION

The most potentially harmful effects of oxygen are due to the formation and activity of a number of highly Reactive Oxygen Species (ROS), which can affect DNA integrity, or the stability of cell lipid bilayer. Free radicals and other ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to xenobiotics. Free radical formation occurs continuously in the cells as a consequence of both enzymatic and nonenzymatic reactions under normal conditions. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and in the cytochrome P_{450} system [1].

Plants have been widely accepted as one of the main sources of prophylactic and chemopreventive drug discovery and development [2]. An example of such plant is *Veronica amygdalina*. The leaves of *veronica amygdalina* are eaten, after crushing and washing thoroughly to reduce bitterness. All parts of the plant are pharmacologically applied in phyto-medicine to treat various diseases and infections in addition to antitumourigenic properties reportedly ascribed to the plant [3, 4].

Plants like the living cells of the body have antioxidants. The antioxidant activity of *Vernonia amygdalina* has been attributed to the presence of flavonoids [5]. The antioxidant mechanism of *Vernonia amygdalina* had been justified in some studies. For example, it has been reported to protect against carbon tetrachloride-induced liver injury by inducing antioxidant and phase II enzymes [6], the cytoprotective activities of boiled, cold, and methanolic extracts of the plant had been documented [7], as well as the antioxidant effects of an aqueous extract of *Vernonia amygdalina* leaves against acetaminophen-induced hepatotoxicity and oxidative stress in mice [8].

Traditionally, the leaves of *Vernonia amygdalina* are consumed by many under normal health conditions particularly in Nigeria where it is used as soup ingredient after washing the leaves to reduce its alkaloid content. In view of the wide acceptability and usefulness of this plant, in addition to efforts by researchers to elucidate the functionalities of this plant on different cell conditions, there is still a dearth of comparative information on the specific effect of *Vernonia amygdalina* consumption under normal health conditions. Particularly as it affect free radical mopping and lipid membrane integrity under normal condition. In view of this, we designed this experiment to examine the possible outcome of consuming aqueous and alcoholic extracts of *Vernonia amygdalina*, with the aim to

provide sufficient empirical information to this identified lapse.

MATERIALS AND METHODS

Experimental animals

Adult male Wistar albino rats of weight range 150- 200g. The rats were housed in cages under standard conditions (12h light / 12h dark, 25°C ±2°C) and were acclimatized for 7 days prior to experimentation. They were fed with standard diet (product of Pfizer Nigeria Ltd), with free access to tap water. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the care and use of Animals and approved by Department Committee on the Use and Care of Animals.

Study design

The study was a 4 x5x3 design of sixty (60) rats, divided into four groups of fifteen rats per group, with each group consisting of three animals. Groups I and II were controls and received graded volumes (1-5ml) of ascorbic acid (Asa) and Butylated Hydroxyl Toluene (BHT) respectively. Groups III and IV received graded volumes (1-5ml) of aqueous (Aq) leaf extract and Methanol (MeOH) extract of V. *Amygdalina* respectively. All animals in the each group received specific volume of extract irrespective of body weight (bwt). All doses were administered through gastric gavage twice daily for 28 days. At the end of the experiments, blood samples were taken from the left ventricle a day after the last dose of administration of extracts into heparinised and plain tubes. The rats were sacrificed by neck decapitation. Kidneys and livers organs were removed, processed and homogenised with acid washed sand, centrifuged at 3,000rpm for 5min to obtain organ extracts used for analysis.

Plant sample collections and extracts preparations

Leaves of V. *amygdalina* were collected from natural habitat within a community and authenticated in Botany Department of the University, and voucher specimen preserved in the laboratory for future reference. The leaves were carefully sorted and washed without squeezing to remove debris and dust particles, and then dried in an uninhabited room at room temperature to constant weight. Dried sample was pulverized using manual grinder and sieved to obtain particle sizes of ≤ 0.250mm. 10g each of sample powder were soaked in 300ml of distilled water and methanol respectively, and kept in refrigerator at 4°c for 48 hours for extractions.

The mixtures were shaken intermittently for complete extraction. The resulting mixtures were rapidly filtered through Whatman No 1 filter paper and later with cotton wool to obtain a homogenous filtrate, which were concentrated *in vacuo* at 38°C using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness, yielding 0.42g (4.20%) and 0.62g (6.20%) in water and methanol respectively. Both extracts were later reconstituted in100mL of distilled water and methanol respectively. From these stocks, 1-5mg/mL were prepared and refrigerated at 8°C until required for use.

Biochemical Analysis

Free radical scavenging activity of each plants were analysed using DPPH (1, 1-dipheny-2picrylhydrazyl) dye as described [9]. Serum and tissue MDAs were measured bythiobarbituric acid assay procedure [10], which was calibrated using 1,1,3,3, - tetraethoxypropane (Sigma Chemicals, St. Louis, MO, USA.) as a standard, and results were expressed as nanomoles of MDA.

Data Analysis

Data collected from this experiment were expressed as mean ± SD and subjected to analysis of variance (ANOVA) using computer software (Graphpad Prism 6.0 software). $p<0.05$ was considered significant and differences between means were separated by Tukey-Kramer multiple comparisons test.

RESULTS

Both MeOH and Aq extract of V. *amygdalina* showed strong free radical inhibitory ability compared with reference. Free radical inhibitory activity increases with concentration, with highest inhibition of 98.80% in aqueous extract, with significant reductions ($p<0.05$) in free radical inhibitory ability of methanolic extracts from a concentration of 3-5 mg/ml (Table 1).

Table 1. Free radical scavenging activity (% inhibition) of *V. amygdalina* leave Extracts

Conc. (mg/ml)	Aqueous	Methanol
Control	100 ± 0.00	100 ± 0.00
1	58.50 ± 7.34a	66.00 ± 6.22a
2	75.00 ± 5.00a	71.00 ± 3.11a
3	93.30 ± 5.65a	74.90 ± 8.32b
4	94.10 ± 6.02a	75.40 ± 5.08b
5	98.80 ± 6.45a	77.99 ± 6.34b

Values with different letter superscript across rows are significantly different ($p<0.05$)

Reducing powers of Aq extracts declined significantly ($p<0.05$) from reference values, with similar declines observed in methanol fractions of 3-5mg/ml (Table 2).

Lipid peroxidation markers showed membrane lipid stability in liver up to 4mg/ml of MeOH extract as compared to significant membrane lipid instability with Aq extracts on liver across the concentration line. On the other hand, kidney tissue membrane was significantly perturbed with Aq extract from 2mg/mL, and MeOH from 3mg/ml (Table 3).

Table 2. Reducing power assay (mgRE/g) of *V. amygdalina* extracts

Conc. (mg/ml)	Aqueous		Methanolic	
	Control (Asa)	Test 1	Control (BHT)	Test 2
1	0.55 ± 0.03a	0.29 ± 0.03b	0.41 ± 0.03a	0.43 ± 0.01a
2	0.86 ± 0.05a	0.32 ± 0.05b	0.47 ± 0.05a	0.47 ± 0.01a
3	1.29 ± 0.04a	0.46 ± 0.03b	1.93 ± 0.03a	0.51 ± 0.04b
4	2.10 ± 0.05a	0.56 ± 0.04b	2.00 ± 0.04a	0.51 ± 0.02b
5	2.16 ± 0.04a	1.23 ± 0.02b	2.49 ± 0.03a	0.53 ± 0.04b

Values with different letter superscript across rows are significantly different (p<0.05)

Table 3. Malondialdehyde (MDA) concentrations (nmol/mL) in organs of *V. amygdalina* treated rats.

		Extract Conentrations (mg/ml)					
		Control	1	2	3	4	5
Liver	(Aq)	0.87 ± 0.56a	2.45 ± 0.35b	1.55 ± 0.34c	2.72 ± 0.42b	2.04 ± 0.39b	3.41 ± 0.31d
	(Me)	1.43 ± 0.08a	1.58 ± 0.12a	1.45 ± 0.54a	2.28 ± 0.25a	2.04 ± 0.39a	3.55 ± 0.23b
Kidney	(Aq)	2.07 ± 0.01a	2.89 ± 0.63a	4.17 ± 0.35b	3.69 ± 0.12b	4.85 ± 0.35b	5.58 ± 0.38b
	(Me)	2.27 ± 0.02a	2.69 ± 0.05a	3.55 ± 0.46a	4.51 ± 0.08b	5.67 ± 0.30b	8.51 ± 1.43c

Values with different letter superscript across rows are significantly different (p<0.05)

DISCUSSION

The rational for using two polar solvent in this study derives from the fact that the leaf is prepared and consumed with polar solvent particularly water. Data from this work have given insight to the free radical scavenging potentials and reducing powers of V. *amygdalina* in aqueous and methanolic media against standard antioxidant references (Tables 1 and 2). Inhibition of free radicals with plant extracts measures the reduction of purple 1, 1-dipheny-2picrylhydrazyl dye to yellow. A comparison of result from this study indicate Aq fraction of plant extract to be better in free radical quenching than MeOH portion, probably as a result of extraction efficiency of the solvent. However, result and observation from this study strongly suggest both plant extracts to have good potentials at higher concentrations to inhibit free radicals comparatively better than their respective Asa and BHT references. Since plant based antioxidants are becoming preferred than synthetic ones due to their multiple mechanisms of action and non-toxic nature [11], it can be hypothesized that aqueous extract of V. *amygdalina* will provide a better benefit for the purpose of free radical quenching.

The reducing power of antioxidant determines its ability to transform Fe^{3+} to Fe^{2+} in the presence of sample extract. Both MeOH and Aq extracts were shown to exhibit concentration dependent increases in reducing power. This observation has been corroborated [12]. This study have however shown that Aq extracts have stronger ability than MeOH extract in reducing Fe^{3+} under same condition, improving in ability with increasing extracts concentrations. Though, both extracts exhibited ability to reduce Fe^{3+}, their powers fell short of their respective references of Asa and BHT. An earlier report on similar study indicated contrary, where increased extract concentration of plant extracts reduced Fe^{3+} better than BHT [13]. The implication of this result is that aqueous medium is more efficacious than methanol in extraction of phenolics from V. *amygdalina* responsible for redox properties.

The liver and kidneys are two crucial organs in vertebrates which functions must not be compromised. Free radicals have the potentials to compromise tissues by molecular abstractions from macromolecular components of cells such DNA and polyunsaturated fatty acids which are integral structural components of biological membranes present in organs under consideration.

As earlier reported V. *amygdalina* containsl luteolin, luteolin 7-O-β-glucoside, and luteolin 7-O-β-glucoronoside which may in part account for the ability of the plant to counteract lipid peroxidation caused mainly by the action of reactive oxygen species on polyunsaturated fatty acids [5]. The liver and kidneys appear to be susceptible to lipid membrane perturbation in presence of Aq fraction compared to the alternative portion. The suspected susceptibility of both organs needed to be further confirmed by histopathological studies. On the other hand, MeOH fraction of extract did not show significant perturbation of liver and kidneys cell membranes, except from a concentration of 3mg/mL. If as reported [5], the phytochemical that counteracts lipid peroxidation in V. *amygdalina* is high, then it is likely to partition higher in solvent than in Aq medium.

Conclusively, data from this study had shown that aqueous extract of V. *amygdalina* could provide a better benefit for the purpose of free radical quenching because it appears to be more efficacious than methanol, probably in the extraction of phenolics ascribed to be responsible for redox properties, while MeOH fraction would appear to more efficiently concentrate components that better stabilizes lipid membranes.

REFERENCES

1. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010. 4(8): 118–126.
2. Aruoma OI, Sun B, Fuji H, Neergheen VS, Bahorun T, Kang KS, Sung MK. Low molecular proanthocyamidin dietary biofactor oligonol: Its modulation of oxidative stress, bioefficacy, neuroprotection, food application and chemoprevention potentials. Biofactors. 2006. 27:245–265.
3. Izevbigie EB, Bryant JL, Walker AA. novel natural inhibitor of extracellular signal related kinases and human breast cancer cell growth. Expt. Biol. Med. 2004. 229: 163-169.
4. Oboh FOJ, Masodje HI. Nutritional and Antimicrobial Properties of *Vernonia amygdalina* Leaves. Int. J. Biomed & Hlth. Sci. 2009. Vol. 5, No. 2: 51-56.
5. Igile GO, Oleszek W, Jurzysta M, Burda S, Fafunso M, Fasanmade AA. Flavonoids from *Vernonia amygdalina* and their antioxidant activities. J. Agric. Food Chem. 1994. 42:2445–2448.
6. Adesanoye OA, Farombi EO. Hepatoprotective effects of *Vernonia amygdalina* (astereaceae) in rats treated with carbon tetrachloride. Exp. Toxicol. Pathol. 2010. 62: 197–206.
7. Iwalewa EO, Adewunmi CO, Omisore NOA, Adebanji OA, Azike CK, Adigun AO, Adesina OA, Olowoyo OG. Pro- and anti-oxidant effects and cytoprotective potentials of nine edible vegetables in South West Nigeria. J. Med. Foods. 2005. 8:539–544.
8. Iwalokun BA, Efedede BU, Alabi-Sofunde JA, Oduala T, Magbagbeola OA, Akinwande AI. Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. J. Med. Food. 2006. 9:524–530.
9. Ganiyu O. Antioxidant and antimicrobial properties of ethanolic extracts *Ocimum gratissimum* leaves. J. Pharm, Toxicol. 2006. 1 (1): 47-53.
10. Albro PW, Corbelt JT, Schroeder JL. Application of the thiobarbiturate assay to the measurement of lipid products in microsomes. Chem. Biol. Interact. 1986. 86: 185-194.
11. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obutor EM, Farombi EO. Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr. J. Biotechnol. 2007. 6: 1197-1201.
12. Gulcin I, Oktay M, Kirecci E, Kufrevioglu I. Screening of antioxidant and antimicrobial activities of anise (*Pimpella anisum L.*) seed extracts. Food Chem. (2003). 83: 371-382.
13. Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS. Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr. J. Biotechnol. 2009. 8 (3): 484-489.

Source of Support: Nil, Confl ict of Interest: None declared