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ORIGINAL RESEARCH

In vivo evaluation of graded fresh leave extracts of *vernoniaamygdalina* and *ocimumgratissimum* on some oxidative stress indicators in rats

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Abstract— Background: *Vernoniaamygdalina* and *Ocimumgratissimum*are widely used plants in the tropics. The leaves of these plants are popularly acclaimed to have medicinal values, with scarce information on the effect of graded extracts in mediating oxidative stress. The interest on these leaves derives from the need to have an insight to the possible effect of thesegraded leaves extracts may have on oxidative stress. Objectives: The objective of the study was to examine the effect of graded extracts of *Vernoniaamygdalina* and *Ocimumgratissimum* leaves specifically on free radical inhibition, reducing power and lipid peroxidation in rat liver and kidneys. Methods: The leave samples were collected and 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 total alkaloid and phenolic contents, free radical scavenging activity, reducing power and the extent of lipid peroxidationin liver and kidneys were determined, using standard analytical assays. Results: Phenolic contents of both leave extracts were higher in aqueous medium *vernoniaamygdalina* (0.85 ± 0.07mgGAE/g) and *Ocimumgratissimum* (1.59 ± 0.13mgGAE/g). The alkaloid contents of the leaves were higher in methanolic extracts, with *Ocimumgratissimum* highest (0.96 ± 0.04µg/g) and *vernoniaamygdalina* (0.38 ± 0.01µg/g). The reducing power of these leaves increased with increasing extract concentrations. *Ocimumgratissimum* had the highest reducing power in both methanolic (3.00 O.D) and aqueous (2.82 O.D) medium in comparison with respective butylatedhydroxytoluene (BHT) (2.49 O.D) and ascorbic acid (2.16 O.D) controls. The ability to scavenge free radicals increased with increasing extract concentrations in both leaves with highest percentages in aqueous extracts. *Ocimumgratissimum*(95.90%) and *vernoniaamygdalina*(98.80%) compared to control (100%).The leave extracts of both plants significantly ($p<0.05$) increased lipid peroxidations in liver and kidneys of rats. Conclusion: Fresh leaves of *Ocimumgratissimum*and *vernoniaamygdalina*have shown potentials to be valuable in reducing powers and free radical quenching.

Keywords— Scent leaf, Bitter leaf, iron, free radicals

INTRODUCTION

Oxidative stress plays a role in causation of several disease conditions, through the involvement of **free radicals** which produce structural damage to tissues and contribute to disease generation via activation of gene regulatory proteins (Galli et al., 2005; Van Wijk et al., 2008). Herbs are sources of various phytochemicals, many of which possess powerful antioxidant activity which have a role in antioxidant defence and redox signalling (Dragland et al., 2003).

Vernoniaamygdalina Del (VA) and *Ocimumgratissimum* (OG) are two tropical greens widely utilized primarily for their medicinal values and secondly as delicacies. Both plants have a long history of use in folk medicine in African (Hamzah et al., 2013). These plants are recognized as sources of natural antioxidants that can protect against oxidative stress and thus play an important role in chemoprevention of diseases that have their aetiology and pathophysiology in reactive oxygen species [ROS] (Atawodi, 2005; Odukoya et al., 2001).

Aqueous and ethanolic extract of VA as well as the methanolic and aqueous extract of OG have been reported to have potent antioxidant properties which compared favourablywith synthetic antioxidants: Butylatedhydroxytoluene, [BHT] and butylatedhydroxyanisole [BHA] (Owolabi et al., 2008).

Mineral element such as free iron is essential to catalyse the formation of reactive oxygen species (ROS), particularly the hydroxyl radical (·OH) in Fenton reaction. Cell damage associated with iron overload has been attributed to the emergence of excessive levels of cell labile plasma iron (LPI) which promote production of ROS exceeding cellular defence capacities (Rachmilewitzet al., 2005).

A recent study on the comparative antioxidant activities of extracts of *Vernoniaamygdalina* and *Ocimumgratissimum* leaves had substantiated the medicinal value of these vegetables and encouraged their consumption to help militate against deleterious effects of reactive species (Oriakhi et al., 2013). It was observed that the report (Oriakhi et al., 2013) was silent on the nature of the leaves used as these plants are noted to be shrubs. Whether the fresh young leaves of these plants will show the same effect on oxidative stress is not clear.

Despite the in-depth attention given to these leaves, there appears to be a gap as to the effect(s) of graded plant extracts of these leaves on *in vivo* assessment of some oxidative stress indicators, with particular reference to membrane lipid peroxidation. This study was thendesigned to investigate these leave extracts with the aim to determine the effect of graded young leave extracts of *Vernoniaamygdalina* and *Ocimumgratissimum* on free radicals and lipid peroxidation on rats liver and kidney tissues.

Material and Methods

Plant sample collections and extracts preparations

Young fresh leaves of *V. amygdalina* and O. *gratissimum* were collected from natural habitat within a community and authenticated in the Department of Botany. Voucher specimen has been preserved in the laboratory for future reference. The samples collected were sorted and washed without squeezing to remove debris and dust particles, and then dried in an uninhabited room for four weeks at room temperature to constant weight. Dried samples were pulverized using manual grinder and sieved to obtain particle sizes of ≤ 0.250 mm. 5g of each sample powder were soaked in 300ml of distilled water and methanol respectively, and kept in refrigerator at 4°c for 48 hours.

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 low temperature (37- 40°C) using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness, yielding 0.35g (7.00%) and 0.42g (8.4%) in water and methanol respectively for *V. amygdalina* and 0.33g (6.6%) and 0.38 (7.6 %) in water and methanol respectively for *O. gratissimum*. Both extracts were later reconstituted in with 100mL of distilled water and methanol respectively.From these stocks, desired concentrations (1-5mg/mL) were prepared and refrigerated at 2- 8°C until required for use.

Experimental Animals

The animals used for this study were eighty-four (84) adult male Wistar albino rats of weight range 150-200g. The rats were housed in cages under standard conditions (12h light/12h dark, 25ºC±2ºC) and were kept for 7 days for acclimation prior to experimentation, in the Departmental animal house. 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.

Table 1. Total Phenolics and Alkaloids Concentrations in Plant Extracts

	V. amygdalina		O. gratissimum	
	Aqueous	**Methanol**	**Aqueous**	**Methanol**
Phenolics (mgGAE/g)	0.85 ± 0.07a	0.60 ± 0.14c	1.59 ± 0.13b	0.15 ± 0.07d
Alkaloids (μg/g)	0.38 ± 0.01a	1.31 ± 0.15c	0.24 ± 0.02b	0.96 ± 0.04c

**Figures on same row with different letters are significantly different*

Table 2. Reducing Power Assay (mgRE/g) of Sample Extracts

Conc.	Aqueous			Methanolic		
(mg/mL)	Control (Aq)	V.A	O.C	Control (BHT)	V.A	O.C
1	0.55 ± 0.03a	0.29 ± 0.03b	1.99 ± 0.03b,c	0.41 ± 0.03a	0.43 ± 0.01a	1.85 ± 0.04b
2	0.86 ± 0.05a	0.32 ± 0.05b	2.46 ± 0.04b	0.47 ± 0.05a	0.47 ± 0.01a	2.76 ± 0.06b
3	1.29 ± 0.04a	0.46 ± 0.03b	2.68 ± 0.06c	1.93 ± 0.03a	0.51 ± 0.04b	2.78 ± 0.03c
4	2.10 ± 0.05a	0.56 ± 0.04b	2.71 ± 0.03c	2.00 ± 0.04a	0.51 ± 0.02b	2.89 ± 0.02c
5	2.16 ± 0.04a	1.23 ± 0.02b	2.82 ± 0.04c	2.49 ± 0.03a	0.53 ± 0.04b	3.00 ± 0.05a

**Figures on same row with different letters are significantly different.*

Experimental design

This study was a 6x2x3 design, making the total rats used in this to be seventy-two (72). For each extract study, the rats were randomly divided into six groups of six animals each. Each group had two sub-units of three rats each, for methanol and aqueous extracts.Groups A and B served as the controls for waterand methanol extracts.The other groups (C-G) received equal extracts (1-5mg/mL) for both *V. amygdalina and O. gratissimum*, irrespective of body weight. 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 the extracts into heparinised and plain tubes. The rats were sacrificed by neck decapitation, to remove kidneys and livers organs which were homogenised with acid washed sand, centrifuged at 3,000rpm for 5 min to obtain organ extracts used for analysis.

Analytical Procedure

Total phenolic compounds in leave samples were determined with Folin-Cioalteu reagent. Reducing power was assayed with $FeCl_3$ solution and free radical scavenging activity of each plants were analysed using DPPH (1,1-dipheny-2picrylhydrazyl) dye as described by Ganiyu (2006) (Oboh, 2006). Serum and tissue MDAs were measured bythiobarbituric acid assay procedure (Albro et al., 1986), which was calibrated using 1,1,3,3, - tetraethoxypropane (Sigma Chemicals, St. Louis, MO, USA.) as a standard, andresults were expressed as nanomoles of MDA. Alkaloids in plant samples were measured using bromocresol green reagent and atropine (Sigma Chemicals, St. Louis, MO, USA.) as standard as described by Shamsa et al., (2007) (Fadhil et al., 2007).

Data Analysis

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

RESULTS AND DISCUSSION

The effect of *Vernoniaamygdalina* (VA) and *Ocimumgratissimum* (OG) extracts on free radical scavenging activity, reducing power, lipid peroxidation as well as the phenolic and alkaloid contents in these plant extracts were monitored as an index for their antioxidant properties. Results clearly demonstrate that both plants contained phenolic compounds and alkaloids. The phenolic compounds in VA., was higher in aqueous and methanolic extracts than obtained for OG in same medium. The reverse was observed for alkaloids (**Table 1**). Previous studies (Adeniyi et al., 2010; Oboh, 2006; U. A et al., 2012) had reported presence of alkaloid and quantitative values of phytochemicals for OG. Other authors on similar study Oriakhi et al., (2013), had shown higher phenolic content values for both VA and OG (Oriakhi et al., 2013).

Alkaloids in samples appear to be more effectively extracted in solvents in both cases. Earlier workers used ethanol as extraction solvents and only qualitatively determined alkaloids in samples (Oboh, 2006; U. A et al., 2012). Total phenol values obtainedfrom this study for VA and OG are higherthan that earlier reported (Oriakhi et al., 2013). The reason for this observed disparity in total phenol contents may be attributed either to the nature of the young leaves, bioavailability of soil nutrients or plant species or combina-

Table 3. Free Radical Scavenging activity (% inhibition) of Leave Extracts

	V. amygdalina		O. gratissimum	
Conc. (mg/mL)	Aqueous	Methanol	Aqueous	Methanol
Control	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
1	58.50 ± 7.34a	66.00 ± 6.22a	28.40 ± 3.54a	50.80 ± 4.22b
2	75.00 ± 5.00a	71.00 ± 3.11a	51.90 ± 5.32a	56.30 ± 5.22a
3	93.30 ± 5.65a	74.90 ± 8.32b	72.30 ± 6.11a	59.20 ± 4.32b
4	94.10 ± 6.02a	75.40 ± 5.08b	86.00 ± 7.21a	68.80 ± 5.12b
5	98.80 ± 6.45a	77.99 ± 6.34b	95.90 ± 6.73a	77.10 ± 5.23b

**Figures on same row with different letters are significantly different.*

Table 4. Malondialdehyde (MDA) Concentrations (nmol/mL) in Extracts Treated Liver.

		Extract Concentrations (mg/mL)				
	Control	1	2	3	4	5
V. amygdalina (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
O. gratissimum (Aq)	0.94 ± 0.42a	1.17 ± 0.46a	1.78 ± 0.22a	2.51 ± 0.36a	3.63 ± 0.52b	4.03 ± 0.41b
V. amygdalina (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
O. gratissimum (Me)	1.16 ± 0.40a	1.58 ± 0.33a	2.09 ± 0.31a	3.21 ± 0.48b	4.39 ± 1.47b	5.33 ± 0.64b

**Figures on same row with different letters are significantly different.*

Table 5. Malondialdehyde (MDA) Concentrations (nmol/mL) in Extracts Treated Kidneys

		Extract Concentrations (mg/mL)				
	Control	1	2	3	4	5
V. amygdalina (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
O. gratissimum (Aq)	1.20 ± 0.66a	2.57 ± 0.14b	2.83 ± 0.26b	3.85 ± 0.23b,c	4.43 ± 0.51c	6.40 ± 1.34d
V. amygdalina (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
O. gratissimum (Me)	2.88 ± 0.61a	2.88 ± 0.12a	3.33 ± 0.06a,b	4.02 ± 0.06b	4.43 ± 0.17b	6.76 ± 0.76c

**Figures on same row with different letters are significantly different*

tion of one or more of these.

The reducing powers of *VA* and *OG* showed concentrations dependent increases. Both aqueous and methanolic extracts of *OG* had significantly (p<0.05) high reducing power in comparison with *VA,* with methanol extract showing highest reducing potential (**Table 2**), than the aqueous and methanolic references.

The reducing ability of an extract is an important indicator of its potential antioxidative activity. The reducing power of an antioxidant measures its ability to donate electrons. Both assays showed concentration dependent increases in reducing capacities. *O.gratissimum* extract had higher electron ability compared to the *V. amygdalina* extract in both aqueous and methanol. The high reducing power of OC over VA is in line with previous report (Oriakhi et al., 2013). An important deductionmade from this study is the high reducing ability of both aqueous and methanolic extract of OC above their respective references, which is an indication of the possible superiority of electron donating power of these plants to their synthesized reference.

Free radical inhibition by extracts significantly (p<0.05) progressed with increasing concentrations for both plant extracts, with free radical inhibition highest in aqueous extracts for *V. amygdalina* and *O. gratissimum* in comparison with methanolic extracts of both plant leaves (**Table 3**).

*Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity*is a popular method utilized in determining antioxidantactivity of biological samples. The data obtainedfrom this work do not support an earlier claim that OG extract is more effective as free radical scavengers that the AV extracts (Oriakhi et al., 2013). From the results presented (**Table 3**), the aqueous extracts of the samples (AV and OG) are significantlyhigher than methanol extracts of both plant samples and are shows higher percentages of free radical inhibited. This observation may be peculiar to the young leaves of *V. amygdalina* and *O. gratissimum*.

The extent of lipidperoxidations in liver and kidney tissues of rats were not consistent with increasing extract administration as MDA values fluctuated (**Tables 4 and 5**) with increased extract consumptions.

Other researchers have reported the abilities of OG and AV in separate studies to significantly reducelipid peroxidation and superoxide dismutase in rats (Iweala and Obidoa, 2010; Nwanjo, 2006). These reports were holistic and not specific. In specific terms the effects of extracts of OG and VA in liver and kidneys of rats as used in this study do not appear to have substantial inhibitory effects on lipid peroxidation. This may be understood on grounds that the young fresh leave extracts may not have synthesized the necessary phytochemicals required to substantially inhibit lipid peroxidations. Another important consideration may be the disposition of the cellular milieu of these organs.

Though it is asserted that vegetables are rich in antioxidants, it can be hypothesized based on the result obtained from this study, that the antioxidant contents in these plants matures with age and may have affected their ability to reduce lipid peroxidation.

CONCLUSION

Conclusively, the young leave extracts of OG and AV are valuable as free radical scavengers, with potent reducing powers but do not have same ability in reducing lipid peroxidation in rats liver and kidneys

Competing interests

The authors declare that they have no competing interests.

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