Antiradical Activities of Basella alba and Solanecio biafrae Extracts; In vitro

Borokini Funmilayo Bosede

Abstract— *Basella alba L.*, commonly called "Amunututu" and *Solanecio biafrae* called "Worowo" among the Yoruba tribe in the southwest part of Nigeria are reported to be of great ethnomedicinal importance but are among many underutilized green leafy vegetables in the country. Many studies have established the nutritional values of these vegetables, utilization are very poor and in-depth informations on their chemical profile are scarce. The polyphenol profiles of the alcoholic extracts were characterized, using high-performance liquid chromatography coupled with diode array detector. Total phenol and flavonoid contents, antioxidant activities were evaluated using *in vitro* assays to assess 2, 2-diphenyl-1-picryl hydrazyl, nitric oxide and hydroxyl radical-scavenging abilities, with the aim of assessing their nutraceutical potentials to encourage their production and utilization. The results revealed the presence of phenolic compounds: gallic acid, chlorogenic acid, caffeic acid, coumarin, rutin, quercitrin, quercetin and kaemferol, ellagic acid, rosmarinic acid, and catechin. The vegetables showed varying concentration dependent radical scavenging abilities from weak to strong compared with gallic acid, rutin, trolox and ascorbic acid used as positive controls. There was strong positive correlation with total phenol (mg GAE/g) and total flavonoid (mg RE/g) contents in the range TFC (r = 0.671 – 0.997and r = 0.724-0.998) and TPC (r= 0.594 – 0.996 and r= 0.722 – 0.991) for *B. alba* and *S. biafrae* respectively. Inhibition concentration at 50 % (IC_{50}) for each extract to scavenge DPPH, OH and NO radicals ranged from 0.346 to 14.855 compared with control (0.205 -1.926) mg/ml. The vegetables possess appreciable antioxidant properties for promoting good health, their cultivation and utilization should be encouraged especially in the face of increasing health and economic challenges and food insecurity in many parts of the world.

Index Terms— Antioxidants, *B. alba* , extracts, phytochemicals, polyphenol, utilization, *S. biafrae*

1 INTRODUCTION

A practical way of fighting against the prevailing cases of degenerative disease which has become a Global concern is to improve body anti-oxidant status through consumption of fruits and vegetables. Phytochemicals from plant vegetables have been found to exert their beneficial effect by acting as antioxidants thereby preventing damages to cell membrane and cellular oxidative processes that may give rise to diseases **[1, 2, 3].** Free radicals are highly unstable species, since they contain one or more unpaired electrons; they can damage other molecules by getting electrons from them **[10, 11, 12, 13].** Excessive generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system therefore gives rise to oxidative stress which plays a role in heart diseases, neurodegenerative diseases, cancer, and in the aging process **[14, 15].** There is considerable evidence that antioxidants could help to prevent these diseases because they have the capacity to quench free radicals and many epidemiological reports associate diets on fruits and vegetables with reduced risk due to the presence of natural substances in plants with potent *in vitro* antioxidant ability that help to protect cells against oxidative damage caused by free radicals **[14, 15]**

Many vegetables in Nigeria are in the wild, underutilized, becoming rare and going to extinction while cultivated vegetables are becoming expensive. Of more than twenty leafy vegetables consumed in South-Western Nigeria, there are several reports on routine and commercial cultivation on few while many grow wild and are under-explored **[4, 5]**. It is imperative to pay attention to these vegetables in the face of increasing food insecurity, economy and health challenges especially in Africa.

Basella (alba L. and rubra L.) commonly known as Ceylon spinach and locally called "Amunu-tutu" in southwestern Nigeria is a leafy vegetable that belongs to the family Basellaceae. It is semi domesticated and underutilized **[6].** It is high in vitamin A, vitamin C, vitamin B9 (folic acid), calcium, magnesium and several vital anti-oxidants **[5].** Senecio biafrae (with local name "worowo" or Sierra Leone bologni) grows in large quantity as undercover in tree crop plantation. Also, leaves of *S. biafrae* contain various secondary metabolites such as dihydroisocoumarins, terpenoids, sesquiterpenes or amino acids **[7, 8, 9].**

Many studies have been carried out on the nutritional values of many vegetables in Nigeria **[16]** but there is dearth of information on the antioxidant properties of many of these vegetables. In the present study, attempt was made to assess the total flavonoid content (TFC), total phenolic content (TPC), antioxidant activities and quantify the polyphenolic compounds of the ethanol extracts of the above mentioned vegetables.

- Borokini Funmilayo Bosede –A lecturer at the Department of Science Laboratory Technology, Rufus Giwa Polychtenic. P.M.B 1019, Owo, Ondo State Nigeria. **borokinif59@yahoo.com**, Tel: +234(0)8033358199

2 MATERIALS AND METHODS

2.1. Samples and Extracts Preparation

The air-dried vegetable samples from local markets in Nigeria were ground and sieve to give 40 mm mesh size powder. Bioactive extract of each powdered vegetable was obtained by weighing 20 g into clean and dried reagent bottle and 400 ml each of distilled water, methanol and ethanol was separately added and subjected to cold maceration process for 24 h to obtain the aqueous extract and 72 h to obtain the alcohol extracts. The extracts were concentrated under vacuum and evaporated using rotary evaporator at low temperature (45^{o}C). The extracts were kept for analyses **[17].**

2.2. Quantification by HPLC-DAD

Chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm x 250 mm) in reverse phase, packed with 5µm diameter particles; the mobile phase was water containing 1% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 10 min and changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by **[18]** with slight modifications. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curve for gallic acid: Y = 13480x + 1257.5 (r = 0.9998); coumarin: Y = 11983x + 1196.9 (r = 0.9997); chlorogenic acid: Y = 11786x + 1267.1 (r = 0.9991); caffeic acid: Y = 13048x + 1345.6 (r = 0.9995); rutin: Y = 12478x + 1194.9 (r = 0.9997), quercitrin: Y = 13641x + 1178.4 (r = 0.9997), kaempferol: Y = 11458x + 1269.4 (r = 0.9998) and quercetin: Y = 12783x + 1195.8 (r = 0.9996). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve **[18]**

2.3. Determination of total phenol contents

Exactly 0.5 ml of each extract was mixed with 2.5 ml 10 % Folin-Cioalteu's reagent (v/v) and 2.0 ml of 7.5% Na_2CO_3. The reaction mixture was subsequently incubated at 45^{o}C for 40 min, and the absorbance measured at 760 nm in the spectrophotometer. All tests were performed three times. Gallic acid was used as a standard phenolic compound. The amount of total phenolic compound in the extract was determined as ug of gallic acid equivalent (GAE) per g dry weight **[19].**

2.4. Determination of total flavonoid

A known volume (0.5 ml) of each extract was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5 % w/v $NaNO_2$ was added to the flask. After 5 min, 0.6 ml of 10 % w/v $AlCl_3$ was added and after 6 min, 2 ml of 1M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content expressed as mg rutin equivalent/g **[20]**.

2.5. Determination of 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging ability

Extracts of 1-5mg/ ml each was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm. Percentage of inhibition = $[(A_o - A_1) / A_o] \times 100$, where A_o is the absorbance of the trolox and A1 is the absorbance of the sample **[21].**.

2.6. Determination of OH radical scavenging activity

Exactly 1-5 mg/ml of each extract of the samples were mixed with 1 ml of reaction mixture (100 µM $FeCl_3$, 104 µM Ethylenediaminetetraacetic acid, 1.5 M H_2O_2, 2.5 M deoxyribose and 100 µM ascorbic acid in 10 mM KH_2PO_4-KOH, pH 7.4) and incubated for 1h at 37^{o}C. Thereafter, 1 ml of 0.5% thiobarbituric acid in 0.025 M NaOH and 1 ml of 2.8 % trichloroacetic acid was added to the mixture and heated for 30 min at 80 ^{o}C before reading the Absorbance at 532 nm against an appropriate blank solution**.** All tests were performed three times. Ascorbic acid was used as a positive control. Percent inhibition of OH was calculated by the following expression: Percentage of inhibition = $[(A_o - A_1) / A_o] \times 100$, where A_o is the absorbance of the ascorbic acid and A1 is the absorbance of the sample **[22, 23].**

2.7. Determination of NO scavenging activity

Briefly, 5 Mm sodium nitroprusside in phosphate-saline was mixed with different concentrations of the extracts: 1-5mg/ml, before incubation at 25^{o}C for 150 min. Thereafter, the reaction mixture was added to Greiss reagent (1 % sulfanilamide, 2 % H_3PO_4 and 0.1 % naphthylethylenediamine dihydrochloride), before measuring the Absorbance at 546 nm **[24].** Ascorbic acid was used as control. The nitric oxide radicals scavenging activity of the fractions was calculated according to the following equation: Percentage of inhibition = [(Ao – A1) / Ao] x 100, where Ao is the absorbance of ascorbic acid and A1 is the absorbance in the presence of the fractions and ascorbic acid.

Statistical analysis

Values are presented as the mean ± SD of three replicates. ANOVA and LSD and Pearson correlation analyses were performed using the commercial software SPSS 16.0

3 RESULTS AND DISCUSSION

3.1. POLYPHENOLIC COMPOSITION

Phenolic profile of the two vegetables (Table 1, Figures 1-4) revealed the presence of phenolic acids and flavonoids in higher concentrations in methanol than ethanol extracts. Gallic acid, caffeic acid, rutin quercetin and kaempferol were found in all the extracts; coumarin and quercitrin were found in the ethanol extracts at low concentrations, ellagic acid and catechin were detected only in methanol extracts of *S.biafrae* and rosmarinic acid only in methanol extract of *B.alba.* Catechins especially epigallocatechin gallate (EGCG) are very powerful antioxidants with activity about 25–100 times more potent than that of vitamins C and E **[25].** Chlorogenic acid was found in all except the methanol extract of *S.biafrae,* while isoquercitrin was detected in methanol extracts of both but not in the ethanol extracts. Phenolic compounds are responsible for

the antioxidant activities of plant materials due to their redox properties through the conjugated rings and hydroxyl groups, hydrogenation or complexing with oxidizing species; they work as reducing agents, hydrogen donors and singlet oxygen quenchers thereby serve various protective and preventive functions **[26].**

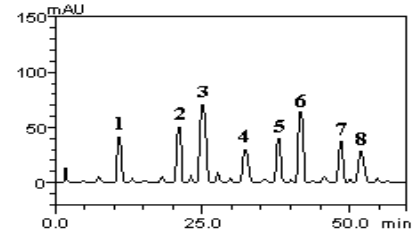


Figure 1: HPLC profile of *Basella alba* methanol extract. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rosmarinic acid (peak 4), rutin (peak 5), isoquercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8).

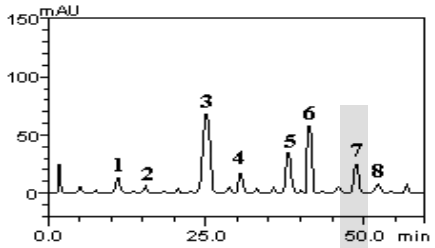


Figure 2: HPLC profile of *Senecio biafre* methanol extract. Gallic acid (peak 1), catechin (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), isoquercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8).

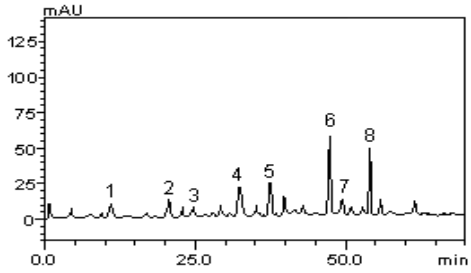


Figure 3 HPLC profile of ethanol extract of *B. alba*. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), coumarin (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8).

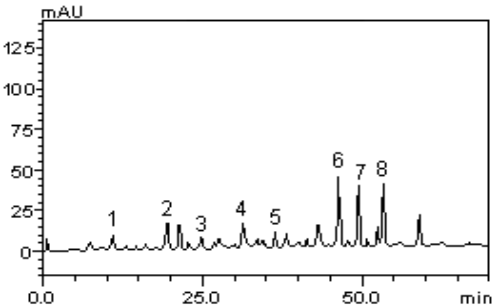


Figure 4 – HPLC profile of ethanol extract of *S. biafrae*. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), coumarin (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8).

Table 1: Phytochemical composition of the vegetables (mg/g)

	Methanol extracts		Ethanol extracts	
Compounds (mg/g)	*B.alba*	*S.biafrae*	*B.alba*	*S.biafrae*
Gallic acid	21.53±0.01	8.15±0.01	0.52 ± 0.03	0.85 ± 0.01
Chlorogenic acid	26.39±0.01	0±0.00	0.97 ± 0.02	1.06 ± 0.02
Caffeic acid	32.74±0.02	37.51±0.01	0.51 ± 0.01	0.49 ± 0.03
Coumarin	0±0.00	0±0.00	2.73 ± 0.01	1.07 ± 0.01
Quercitrin	0±0.00	0±0.00	5.86 ± 0.01	3.97 ± 0.02
Ellagic acid	0±0.00	9.48±0.03	0±0.00	0±0.00
Rosmarinic acid	18.91±0.01	0±0.00	0±0.00	0±0.00
Catechin	0±0.00	3.32±0.02	0±0.00	0±0.00
Rutin	21.95±0.03	18.63±0.01	2.78 ± 0.03	0.60 ± 0.01
Isoquercitrin	30.16±0.02	30.71±0.01	0±0.00	0±0.00
Quercetin	21.10±0.01	12.34±0.02	0.91 ± 0.02	3.65 ± 0.01
Kaempferol	19.32±0.01	7.56±0.03	4.17 ± 0.03	3.71 ± 0.01

Results are expressed as mean ± standard deviations (SD) of three determinations.

3.2. Total Phenol and Flavonoid Contents

Total phenols concentrations ranged from 8.164±0.20 and 9.023 ±0.05 to 83.788 ±0.008 and 197.654 ± 0.110 mg/100g GAE for the aqueous and the alcohol extracts. The gallic acid used as the standard gave significantly higher ($p \leq 0.05$) values of total phenol (275.972 mg GAE/100g) than all the vegetable extracts at all concentrations. All the extracts of *S.biafrae* gave higher values of TPC than *B.alba;* and methanol extracts gave higher values for TPC than the alcohol and ethanol extracts contrary to the observation of koffi *et al*., **[27]** who observed that the average total phenols contents of ethanolic extracts (9,000 mg GAE: 100 g DM) is at least 4 times higher than that of aqueous or acetonic extracts (2,000 mg GAE g/ 100 g DM), and 9 times higher than that of methanolic extracts.

A different trend was observed for the TFC as the aqueous extracts of the vegetables were found to contain higher TFC than the alcohol extracts; and TFC of aqueous extracts of *B. alba* were significantly higher ($p \leq 0.05$) than that of *S*.biafrae. The range of TFC obtained for *B. alba* extracts in this study (0.606 ± 0.014 – 139 ± 0.071 mg RE / 100g) were significantly lower than 34.849 ±0. 016 -181.818 ± 0.211mg/100 g of rutin at 1- 5mg/ml concentrations. TPC and TFC of the methanol extracts of *B. alba* in the study conducted by **Oloyede *et al.* [6]** were found to be 2817 and 3044 mg/100g respectively. Adefegha and Oboh **[28]** also reported total phenol content (mg gallic acid equivalent /100 g) and TFC (mg quercetin equivalent /100 g) of 350.0 ± 0.5 and 36.4 ± 0.4 for methanol extracts of *B. alba.* Phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the

different solvents and have capacity to trap free radicals associated with different diseases **[29].**

3.3. DPPH Scavenging Properties

The aqueous extracts of both vegetables exhibited weaker abilities than the alcohol extracts in inhibiting DPPH. Though, trolox which was used as reference standard showed higher inhibition ability than all the extracts, aqueous and alcohol extracts of both vegetables demonstrated significant concentration dependent increase from weak to strong antioxidant properties in the range; 17.279 – 62.095 and 1.703 - 45.676 % for *B. alba* and *S. biafrae* respectively (Figures 5 a-c). IC_{50} values, which are the concentration of sample required for 50 % scavenging of free radicals in the specified reaction time, were calculated from the graph plotting scavenging percentage against sample concentration and presented in Table 2. The higher the scavenging ability of extract, the lower the IC_{50} value. The IC_{50} for DPPH in this study which ranged from 3.404 to 14.452 in ethanol and aqueous extracts of *B. alba* respectively were higher than 0.511obtained for trolox. The percentage inhibitions of DPPH radical in this study were lower than 91.3% and 89.3% reported by Oloyede **[6]** for *B. rubra* and *B.alba* respectively. TPC and TFC of the two vegetables showed strong positive correlations with the DPPH at between 0.01 - 0.05 significant levels (Table 3 and 4). It has been established that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons as reducing agents and to capture the free radicals **[30].**

Table 2: IC_{50} values for DPPH, OH and NO free radicals

Table 3: Pearson's correlation coefficient between total flavonoid content and antioxidant assays

Solvents	samples	DPPH IC_{50}	OH IC_{50}	NO IC_{50}
Aqueous	*B. alba*	14.452	1.506	3.394
	S. biafrae	9.881	4.585	5.193
Methanol	*B. alba*	8.437	9.561	1.384
	S. biafrae	7.817	14.855	2.576
Ethanol	*B. alba*	3.404	7.878	10.477
	S. biafrae	5.471	8.403	5.482
Control		0.511	1.179	0.564
		Trolox	Ascorbic acid	Ascorbic acid

Table 3: Pearson's correlation coefficient between total flavonoid content and antioxidant assays

TFC		Samples	Pearson correlation		
			OH	DPPH	NO
Aqueous	r value	*B. alba*	.996**	.931*	.997**
	p value		.000	.021	.000
	r value	*S. biafrae*	.949*	.854	.958*
	p value		.014	.066	.010
Methanol	r value	*B. alba*	.949*	.876	.671
	p value		.014	.052	.215
	r value	*S. biafrae*	.724	.979**	.816
	p value		.167	.004	.092
Ethanol	r value	*B. alba*	.972**	.994**	.970**
	p value		.006	.001	.006
	r value	*S. biafrae*	.985**	.996**	.998**
	p value		.002	.000	.000
Control			.965**	.987**	.955*
			.008	.002	.011

3.4. OH Scavenging Properties

Solution of OH scavenger when added to the reaction mixture removes the hydroxyl radicals from the sugar and prevent it from degradation and the absorbance decreases with increasing concentrations of the scavenger. The results presented in Figures 6 a-c revealed that aqueous extracts showed efficient radical scavenging activities on hydroxyl radicals at concentrations 1-5 mg/ml, compared with the vitamin C standard. The high % inhibition of hydroxyl radicals demonstrated could be associated with the high values obtained for TFC of the aqueous extracts in this study. Both the aqueous and the alcohol extracts of *B. alba* were more effective scavengers than the extracts of *S. bifrae* (Figures 6 a- c). The range of IC_{50} for OH radical was 1.506 – 14.855 from aqueous extracts of *B. alba* to methanol extract of *S. bifrae*.

The correlations between the capacities of all the extracts to scavenge hydroxyl radicals and their TFC and TPC were strong and positive; TFC (r =0.949 -0.996); TPC (r = 0.928 – 0.986) and TFC(r = 0.724 – 0.985); TPC (r = 0.722 – 0.986) for *B. alba* and *S. bifrae* respectively at 0.01- 0.05 level of significance.

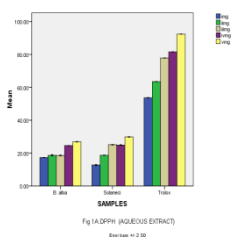
The results indicate the potential of these samples to prevent or decrease the damage in a human body caused by free radicals which according to **Aruoma *et al.* [31]** and **Valko *et al.* [32]** attack biological macromolecules such as protein and DNA.

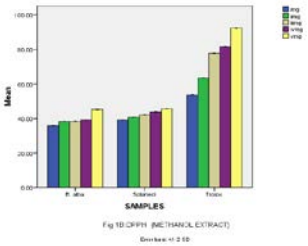
Table 4: Pearson's correlation coefficient between total phenol content and antioxidant assays

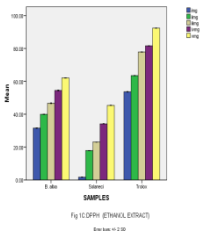
TPC		Samples	Pearson correlation		
			OH	DPPH	NO
Aqueous	r value	*B. alba*	.971**	.850	.940*
	p value		.006	.068	.017
	r value	*S. biafrae*	.953*	.894*	.966**
	p value		.012	.041	.007
Methanol	r value	*B. alba*	.928*	.787	.594
	p value		.023	.114	.291
	r value	*S. biafrae*	.722	.974**	.756
	p value		.168	.005	.139
Ethanol	r value	*B. alba*	.986**	.989**	.996**
	p value		.002	.001	.000
	r value	*S. biafrae*	.986**	.985**	.990**
	p value		.002	.002	.001
Control			.983**	.985**	.965**
			.003	.002	.008

3.5. NO Scavenging Properties

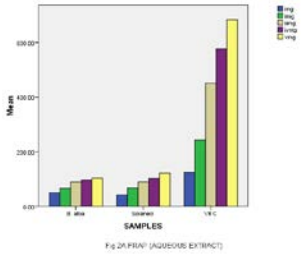
The % inhibition of NO radical by the vegetables are presented in Figures 7 a-c. The aqueous and alcohol extracts of the two vegetables possessed significant scavenging ability against NO radical when compared to the ascorbic acid standard used as a reference standard. Methanol extracts of *B. alba* and *S. bifrae* showed the strongest nitric oxide scavenging property with respective IC_{50} values of 1.384 and 2.576 and the correlation between the scavenging ability and the corresponding TFC and TPC were strong and positive as shown in Table 3 and 4.
The methanol extract of *Centella asiatica,* a medicinal plant showed maximum activity of 50 % at 200 µg /ml; whereas ascorbic acid at the same concentration exhibited 47.3% inhibition **[33].** Nitric oxide are important signaling molecules in biological processes but high levels of these radicals are toxic to tissue and contribute to molecular damage in human system **[34]**. The two vegetables understudy possesses quite appreciable effective scavenging power against NO.

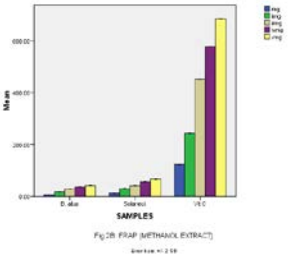


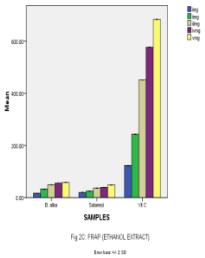




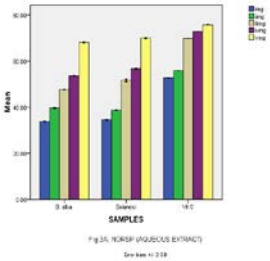
Figures 5a: DPPH scavenging property (aq) **5b: DPPH scavenging property (MeOH)** **5c: DPPH scavenging property (EtOH)**

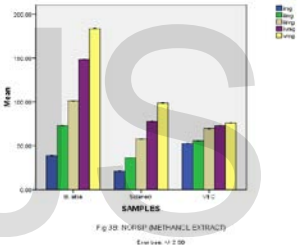


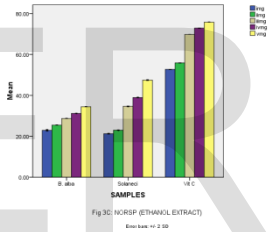




Figures 6a: OH scavenging property (aq) **6b: OH scavenging property (MeOH)** **6c: OH scavenging property (EtOH)**







Figures 7a: NO scavending property (aq) **7b: NO scavenging property (MeOH)** **7c: NO scavenging property (EtOH)**

Figure 8a: Antibacterial property **Figure 8b: Antifungal property**

IV CONCLUSION

Aqueous and alcohol extracts of *B.alba* and *S. biafrae* generally demonstrated concentration dependent effectiveness in their free radical inhibition. There was positively strong correlation between the antioxidant properties and the total phenol and total flavonoid contents of the extracts in agreement with several studies which associate the phenolic compounds with antioxidant property. Phenolic profile of the two vegetables showed the presence of phenolic acids and flavonoids which could exert their antioxidant capacity individually as well as synergistically to produce various protective and therapeutic effects. Large scale cultivation and utilization of these vegetables of great health benefit potential should be encouraged.

ACKNOWLEDGEMENT

I am grateful to Aline AugustiBoligon, Margareth Linde Athayde, both of Department of Industrial Pharmacy, Federal University of Santa Maria, Santa Maria, Brazil *and everyone who in one way or the other has contributed to the successful completion of this work.*

REFERENCES

[1] G.R. Wasson, V.J. Mckelvey-Martin, and S.C. Downes, "The Use of the Comet Assay in the Study of Human Nutrition and Cancer," *Mutagenesis*, vol. 23, no. 3, pp. 153 – 16, May, 2008 (Journal citation).

[2] E.S. Omoregie, and A.U. Osagie, "Effect of *Jatropha tanjorensis* Leaves Supplement on the Activities of Some Antioxidant Enzymes, Vitamins and Lipid Peroxidation in Rats," *J Food Biochem,* vol. 35, no. 2, pp. 409-424, Sept.. 2010; DOI: 10.1111/j.1745 – 4514.2010.00392.x (in press).xx

[3] G. Oboh, and J.B.T. Rocha, "Polyphenols in red pepper [*Capsicum annuum var. aviculare* (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver," *Eur. Food Res. Technol*, vol. 225, no. 2, pp. 239-247, June 2007.

[4] O.O. Odueso, "The Effects of Fertilizers on the Growth and Yield of Indian Spinach (Basella alba)". Bachelor of Agriculture (B.Agric) Thesis, Dept of Horticulture, College of Plant Science and Crop Production, University of Agriculture, Abeokuta, Ogun State, 2011 (Thesis)

[5] O.A. Oyewole, and O.A. Kalejaiye, "The Antimicrobial Activities of Ethanolic Extracts of *Basella Alba* on Selected Microorganisms," *scientific Journal of Microbiology,* vol.1, no. 5, pp.113-118, Oct. 2012

[6] F.M. Oloyede, F.A. Oloyede, and E.M. Obuotor, " Comparative Studies of Chemical Compositions of Two Species of Basella," *App. Sci. Report.* Vol. 3, no. 2, pp. 121-124, 2013.

[7] F.A.S Dairo, and I.G. Adanlawo "Nutritional Quality of *Grassocephalum crepidioides* and *Senecio biafrae*," *Pakistan J. Nutrit*., vol. 6, no.1, pp. 35-39, 2007.

[8] T.K. Tabopda, G.W. Fotso, J. Ngoupayo, A.C. Mitaine-Offer, B.T. Ngadjui , and M.A. Lacaille-Dubois, "Antimicrobial dihydroisocoumarins from Crassocephalum biafrae," *Plant Med*., vol. 75, no. 11 pp. 1258-1261, April 2009.

[9] I.O. Okoro, I.A. Umar, S.E. Atawodi, and K.M. Anigo, "Comparative Antihyperglycemic Effect of Petroleum Ether, Acetone, Ethanol and Aqueous Extracts of *Cleome* rutidosperma DC and *Senecio biafrae* (Oliv. and Hiern) in Streptozotocin-induced Diabetic Mice," *British Journal of Pharmacology and Toxicology* vol. 5, no. 3, pp. 115-124, June 2014

[10] H. Sies, "Oxidative Stress; Oxidants and Antioxidants," *Exp physical,* vol. 82, no. 2, pp. 291-5, Mar. 1997

[11] T. Rong, "Chemistry and Biochemistry of Dietary Polyphenols," Nutrients, vol. 2, no. 12, pp. 1231-46. Dec. 2010

[12] T. F. Lien, H.S. Yeh, and W.T. Su, "Effect of Adding Extracted Hesperetin, Naringenin and Pectin on Egg Cholesterol, Serum Traits and Antioxidant Activity in Laying Hens," *Arch. Anim. Nutr*. Vol. 62, no. 1, pp. 33–43, Feb. 2008.

[13] G. Mingjiang, R. Mingxin, L. Zhenling, and S. Xiaojun, "Free Radical Scavenging Activities of Pigment Extract from *Hibiscus syriacus*i Petals in vitro," *African Journal of Biotechnology* Vol. 11, no. 2, pp. 429-435, Jan. 2012

[14] Y.Z. Fang, S. Yang, and G. Wu, "Regulation of Physiological Systems by Nutrients; Free Radicals, Antioxidants and Nutrition," *Nutrition.* Vol. 18, no. 10, pp. 872–879, Oct. 2002.

[15] M.J.A.T. Corpuz, M.O. Osi, and L.A. Santiago, "Free Radical Scavenging Activity of *Sargassum siliquosum* J. G. Agardh," *International Food Research Journal* vol. 20, no. 1, pp. 291-297, Jun. 2013

[16] C.E. Achikanu, P.E. Eze-Steven, C.M. Ude and O.C. Ugwuokolie, "Determination of the Vitamin and Mineral Composition of Common Leafy Vegetables in South Eastern Nigeria," *Int.J.Curr.Microbiol.App.Sci.* vol. 2, no. 11, pp. 347-353, 2013.

[17] E.J. Iweala, and C.U. Okeke, "Comparative Study of the Hypoglycemic and Biochemical Effects of C*atharanthusroseus*(Linn) g. apocynaceae (Madagascar periwinkle) and *chlorpropamide* (diabenese) on Alloxan-Induced Diabetic Rats," *Biokemistri*, vol.17, no. 2, pp. 149-156, Dec. 2005.

[18] A.R.H. Silva, L.R. Moreira, E.S. Brum, M.L. Freitas, A.A. Boligon, L.A. Margareth, S.S. Roman, C.M. Mazzanti, and R. Brandão, "Biochemical and Hematological Effects of Acute and Sub-Acute Administration to Ethyl Acetate Fraction from the Stem Bark *Scutia Buxifolia* reissek in Mice," *Journal of Ethnopharmacology,* vol.153, no. 3, pp. 908-916, May 2014.

[19] V.L. Singleton, R. Orthofer, and R.M. Lamuela-Raventos, "Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent," *Methods in Enzymology*. Vol. 299, no.null pp. 152-178, 1999.

[20] J. Zhishen, T. Mengcheng, J. Wu, "The Determination of Flavonoid Contents in Mulberry and their Scanvenging Effects on Superoxide Radicals," *Food Chemistry*, vol. 64, no. 4, pp. 555-559, Mar. 1999.

[21] F. Ursini, M. Maiorino, P. Morazzoni, A. Roveri, and G. Pifferi, 1994. "A Novel Antioxidant (Idb 1031) Affecting Molecular Mechanisms of Cellular," *Free Radical Biology and Medicine*, vol.16, no. 5, pp. 547 – 553, May 1994.

[22] B. Halliwell, J.M.C. Gutteridge, and O.I. Arouma, "The Deoxyribose Method: A Simple Test Tube Assay for the Determination of Rate Constants for Reactions of Hydroxyl Radicals," *Anal.Biochem*.,vol. 165, no. 1, pp. 215-219, Aug.1987.

[23] K.S. Heo, and K.T. Lim, "Antioxidative Effects of Glycoprotein Isolated from *Solanum nigrum L.*," *Journal of Medicinal Food,* vol. 7, no. 3, pp. 349-357, Sept. 2004.

[24] G.C. Jagetia, S.K. Rao, M.S. Baliga, and K.S. Babu, "The Evaluation of Nitric Oxide Scavenging Activity of Certain Herbal Formulations *In Vitro*: A Preliminary Study," *Phytotherapy Research,* vol. 18, no. 7, pp. 561 – 565, Jul. 2004.

[25] N. Khan, F. Afaq, and H. Mukhtar, "Cancer Chemoprevention Through Dietary Antioxidants: Progress and Promise," *Antioxid Redox Signal*., vol. 10, no. 3 pp. 475-510, Jan. 2008

[26] T. Mahesh, and P.V. Menon, "Quercetin Alleviates Oxidative Stress in Streptozotocin Induced Diabetic Rats," *Phytother. Res*., vol. 18, no.2 pp. 123 – 127. [27], Feb. 2004.

[27] E. Koffi, T. Sea, Y. Dodehe, and S. Soro, "Effect of Solvent Type on Extraction of Polyphenols from Twenty Three Ivorian Plants," *Journal of Animal & Plant Sciences*, vol. 5, no. 3, pp. 550- 558, Jan. 2010.

[28] S.A. Adefegha and G. Oboh , "Cooking Enhances the Antioxidant Properties of Some Tropical Green Leafy Vegetables," *African Journal of Biotechnology,* vol. 10, no. 4, pp. 632-639, Jan. 2011. DOI: 10.5897/AJB09.761.

[29] G. Cao, and R. Prior, "Measurements of Oxygen Radical Absorbance Capacity in Biological Samples," *Methods in enzymology,* vol. 299, no. null, pp. 50-62, 1999.

[30] A.A. Dehpour, M.A. Ebrahimzadeh, S.F. Nabavi, and S.M. Nabavi, "Antioxidant Activity of Methanol Extract of *Ferula assafoetida* and its Essential Oil Composition," *Grasas Aceites,* vol. 60, no. 4, pp. 405 – 412, Jul.- Sept. 2009.

[31] O.I. Aruoma, J.P. Spencer, D. Warren, P. Jenner, J. Butler, and B. Halliwell, "Characterization of Food Antioxidants Illustrated using Commercial Garlic and Ginger Preparations," *Food Chemistry*, vol. 60, no. 2, pp. 49 – 56, Oct. 1997.

[32] M. Valko, M. Izakovic, M. Mazur, C.J. Rhodes, and J. Telser, "Role of Oxygen Radicals in DNA Damage and Cancer Incidence," *Mol Cell Biochem*. Vol. 266, no. 1-2, pp. 37-56, Nov. 2004.

[33] D. Singh, M. Mishra, M. Gupta, P. Singh, A. Gupta, and R. Nema ," Nitric Oxide Radical Scavenging Assay of Bioactive Compounds Present in Methanol Extract of *Centella asiatica," International Journal of Pharmacy and Pharmaceutical Science Research*, vol. 2, no. 3, pp. 42-44, Jul. 2012.

[34] S. Rajan, S. Mahalakshmi, V.M. Deepa, K. Sathya, S. Shajitha, and T. Thirunalasundari, "Antioxidant Potentials of *Punica granatum* Fruit Rind Extracts," *Int J Pharm Sci*, vol. 3, no. 3, pp. 82-88, May, 2011.