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ANTIOXIDANT AND ANTI-LIPIDPEROXIDATION POTENTIALS OF THE ETHYLACETATE AND CHLOROFORM EXTRACTS OF *BASELLA ALBA* LEAVES

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ABSTRACT

The search for antioxidants in various plants fractions became pertinent since they limit the oxidation of macro molecule by free radical that leads to cellular oxidative damage implicated in several chronic human diseases. The present study was undertaken to find the antioxidant and inhibitory potentials of lipid peroxidation by ethyl acetate and chloroform extracts of Basella Alba leaves. Extracts were prepared by soaking dried leaves of the selected plant in appropriate solvents using liquid-liquid chromatographic technique. Phenol and Flavonoid contents of the extracts were measured by Folin Ciocalteu and Aluminium chloride assays. Hydroxyl radicals and 1,1diphenyl 2-picrylhydrazyl (DPPH) scavenging effect were determined using deoxyribose assay and the ability of the extract to donate electron to the stable DPPH. Inhibition of lipid peroxidation was carried out using the reactions of peroxides with Thiobarbituric acid. Results revealed the total phenolic contents equivalent of gallic acid (GAE) of the ethyl acetate and chloroform extracts were 0.029±0.001mg/g and 0.030± 0.002mg/g respectively. The total flavonoids content, equivalent of quercetin standard were 0.045±0.001mg/g and 0.085±0.001mg/g. The extracts exhibited radical scavenging activity in a concentration dependent manner. Ethylacetate and chloroform extract scavenged DPPH radical by 57.29% ±0.011 and by 82.19%±0.01 at 200µg/mL concentrations. Maximum hydroxyl radical scavenging activity was obtained at 150µg/mL by ethylacetate (50.10±0.1%) and chloroform (41.10%) extracts respectively. The two fractions conferred 50% protection at maximum concentration on lipid peroxidation induced by $FeS0_4$, as inhibition was dose dependent. Results obtained shows that certain bioactive agents with antioxidant and anti-lipidperoxidation properties are present in the leaves extracts. This study give scientific support for its wide consumption as leafy vegetable in western part of Nigeria as a rich source of dietary antioxidant, while these extracts may be explored as template in drug discovery and design especially in prevention and management of diseases associated with aging.

Keywords: Antioxidants, *Basella Alba*, Ethyl acetate extract, Chloroform extracts, Aging, Lipid peroxidation

INTRODUCTION

Various biological systems naturally generate oxygen free radicals produced from various cellular activities or functions or from exposure to exogenous substances in the environment. Oxygen - centered free radicals play a central role in the pathogenesis of many human diseases resulting in oxidative stress by damaging membrane lipids, proteins and DNA molecules. (Steenkamp et al., 2005). Their involvement in the etiology and pathophysiology of human aging (Finkel, 2000) and diseases, such as cancer, coronary heart disease,

Alzheimer's disease (Ames, 1983; Smith et al., 1996) neurodegenerative disorders, atherosclerosis, cataracts and inflammation (Aruoma, 1998) are well documented.

Considerable scientific evidence suggested that under situations of oxidative stress, reactive oxygen species (ROS) such as superoxide, hydroxyl, and peroxyl radicals are generated and the balance between anti-oxidation and oxidation is believed to be a crucial concept for maintaining a healthy biological system (Davies, 2000). However, the free radicals and ROS can be removed by the body s natural antioxidant defense such as the actions of glutathione peroxidase, catalase and superoxide dismutase (Aruoma, 1994).

Interestingly, overproduction of ROS arising from either the mitochondrial electron transport chain or excessive stimulation of NAD (P) H or from exposure to environmental pollutants such as cigarette, smoke, UV-rays, radiation and toxic chemicals (Valko et al., 2006) results in a weakened body defense system, hence creating the need to provide the body with a constant supply of plant chemicals (antioxidants) through dietary supplementation (Aliyu et al., 2010).

In recent years, antioxidants derived from natural sources mainly plants have been intensively used to prevent oxidative damage because of its advantages over synthetic ones; as they are easily obtained, economical and have slight or negligible effects (Onay–ucar et al., 2006).Thus, antioxidants may offer resistance against oxidative stress by scavenging free radicals as natural antioxidants which are present in herbs and spices and are responsible for inhibiting or preventing the deleterious consequences of oxidative stress as they have free radical scavenging ability mostly due to the presence of certain bioactive agents contained in them (Halliwell ., 1997).

Basella alba is a perennial vine belonging to the family *Basellaceae*. It is known as Malabar spinach, Red vine spinach, creeping spinach and Amunututu by the Yoruba,s of the south-western Nigeria. It is commonly found in the tropical regions of the world, and is widely used as a leaf vegetable. *Basella alba* is a vigorous soft-stemmed climbing vine and grows up to 10m long. It has broad heart-shaped, thick semi-succulent green leaves, 5-12cm wide. The plant is a good source of vitamins A, and C, Iron and Calcium (Haskell et el, 2004). The leaf juice is used in Nepal to treat catarrh as its pastes are used externally to treat boils. The cooked leaves and stems are used as laxatives while the flowers are locally used as Antidote for poisons (Duke and Ayensu , 1985).

Although *Basella alba* has its various parts used for numerous purposes in folk medicines, the investigations of the antioxidant potential of its leaf extracts may help to evaluate the bioactive constituents of the plant as potential template for drug discovery using different antioxidant assays.

MATERIALS AND METHODS

Basic laboratory materials such as weighing balance ,P^H meter, micropipette, spectrophotometer and glasswares were used for this work .Reagents includes; methanol,follin–ciocalteu, gallic acid,quercetin, sodium carbonate,thiobarbituric acid ,trichloroacetic acid, 2-deoxyribose ,1,1-dipheny-2-picrylhydrazyl radical (DPPH).All the reagents used were of pure analytical grade obtained from Sigma chemical company USA

Sample Collection and Preparation

Basella alba plants were collected from the Baptist seminary garden Ogbomoso and authenticated by Drs Ogunkunle and Akintola of the botany unit of the Department of pure and applied Biology Ladoke Akintola University of Technology ,Ogbomoso .Fresh

healthy leaves of the plant were air –dried at room temperature and further powdered. About 10 grams of the powdered leaves was extracted in 100ml of methanol in the cold for 72 hours.The solvent was concentrated at temperature below 40^0C and the resulting methanol extract was subjected to liquid-liquid chromatographic separation technique using chloroform and ethylacatate as the solvent to obtained their corresponding extracts (Ogundipe et al.,2000).The resulting extracts obtained were used for determination of flavonoids, phenols,free radical scavenging activity and inhibition of lipid peroxidation.

Total flavonoids and phenol determination

Flavonoids were determined using Aluminium chloride colorimetric method (Chang et al., 2002). The calibration curve was made by preparing quercetin solutions at different concentrations in methanol. Total phenol were determined by Folin ciocalteu reagent (Mc Donald et al.,2001).The phenol values were expressed in terms of gallic acid equivalent as the extracts and the standards were treated based on the experimental procedures while the flavonoids content of the extract were expressed in term of standard quercetin equivalence.

Radical scavenging assays and inhibition of lipid peroxidation

The antioxidant activity of volatile compounds was measured in term of hydrogen donating or radical scavenging ability using the stable radical DPPH. DPPH scavenging potential of the extract was measured based on the scavenging ability of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by the extracts (Mensor et al.,2001). The hydroxyl radicals scavenging effect and inhibition of lipid peroxidation were determined using deoxyribose assay (Halliwell et al .,1987) and the reactions of peroxides with Thiobarbituric acid using egg yolk homogenate as lipid rich media (Ruberto et al.,2000).

Statistical Analysis

Statistical analysis were based on the Duncan `s experimental analysis with mean standard deviation of sample analysed using the student T test and $p< 0.05$ i.e 95% level of significance or confidence limit (Bliss , 1967).

RESULTS

Table 1. Total phenolic content of the ethyl acetate and chloroform extracts of Basella Alba leaves (mg/g. GAE).

Concentration (mg/ml)	*Total phenolic content(mg/g)Gallic acid Equivalent.(ETHYLACETATE EXTRACT)*	*Total phenolic content(mg/g)Gallic acid Equivalent.(CHLOROFORM EXTRACT)*
0.025	0.010 ± 0.001	0.001 ± 0.0001
0.050	0.014 ±0.004	0.011 ± 0.006
0.075	0.020 ±0.001	0.018 ± 0.003
0.100	0.022 ±0.004	0.022 ± 0.004
0.125	0.029 ±0.001	0.030 ± 0.002

Mean Value ± Standard deviation of three replicates.

Table 2. Total flavonoids content of the ethyl acetate and chloroform extracts of Basella Alba leaves (mg/g. QE).

Concentration (mg/ml)	*Total flavonoids content(mg/g)Quercetin Equivalent.(ETHYLACETATE EXTRACT)*	*Total flavonoids content(mg/g)Quercetin Equivalent.(CHLOROFORM EXTRACT)*
0.025	0.010 ± 0.007	0.020 ± 0.001
0.050	0.010 ± 0.004	0.025 ± 0.003
0.075	0.010 ± 0.005	0.038 ± 0.004
0.100	0.020 ± 0.001	0.060 ± 0.001
0.125	0.045 ± 0.001	0.085 ± 0.001

Mean Value ± Standard deviation of three replicates.

Table 3. % DPPH radical scavenging activity of the ethyl acetate and chloroform extracts of Basella Alba leaves

Concentration (µg/ml)	*%DPPH radical scavenging activity.(ETHYLACETATE EXTRACT)*	*%DPPHradicalscavenging activity.(CHLOROFORM EXTRACT)*
25.00	15.64 ± 0.010	40.25 ± 0.010
50.00	24.37 ± 0.015	42.13 ± 0.015
75.00	31.63 ± 0.033	65.38 ± 0.025
100.00	36.97 ± 0.065	67.60 ± 0.028
125.00	45.98 ± 0.001	70.36 ± 0.056
150.00	51.32 ± 0.170	78.74 ± 0.130
175.00	51.85 ± 0.233	81.07 ± 0.040
200.00	57.29± 0.940	82.19 ± 0.010

Mean Value ± Standard deviation of three replicates.

Table 4. % Hydroxyl radical scavenging activity of the ethyl acetate and chloroform extracts of Basella Alba leaves.

Concentration (µg/ml)	*%Hydroxylradicalscavenging activity.(ETHYLACETATE EXTRACT)*	*%Hydroxylradicalscavenging activity.(CHLOROFORM EXTRACT)*
25.00	40.20 ± 0.060	39.70 ± 0.077
50.00	42.40 ±0.050	37.70 ± 0.085
75.00	42.90 ±0.081	39.95 ± 0.100
100.00	45.66 ± 0.100	40.12 ± 0.122
125.00	47.50 ±0.120	40.76 ± 0.254
150.00	50.10±0.114	41.10 ± 0.342

Mean Value ± Standard deviation of three replicates.

Table 5. % Inhibition of lipid peroxidation by the ethyl acetate and chloroform extracts of Basella Alba leaves.

Concentration (µg/ml)	*%Inhibitionoflipidperoxidation. (ETHYLACETATE EXTRACT)*	*%Inhibitionoflipidperoxidation. (CHLOROFORM EXTRACT)*
50.00	47.80 ± 0.501	51.80 ± 1.643
100.00	55.20 ± 0.654	52.60 ± 1.901
150.00	67.20 ± 0.902	52.90 ± 1.988
200.00	69.60 ± 1.100	57.40 ± 1.001
250.00	76.30 ± 1.178	59.00 ± 1.768
300.00	78.90 ± 2.220	63.40 ± 3.021

Mean Value ± Standard deviation of three replicates.

DISCUSSION

Many plant constituents have been reported to have free radical scavenging activity (Aruoma, 1998). Interestingly, flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals and inhibitors of lipid peroxidation (Takanami et al., 2000). The medicinal properties of folk plants are mainly attributed to the presence of phenolic compounds mostly flavonoids, phenolic acids and antioxidant micronutrients (Repetto and Liesuy 2002).Therefore it is pertinent to explore these herbs and plants which are capable of producing naturally occurring antioxidants.The results of the analysis of the total phenol content in the extracts are shown in table 1.The Total phenol contents expressed as gallic acid equivalent were 0.029 mg/g and 0.030 mg/g for the ethylacetate and chloroform extracts respectively. Plant phenols present in fruits and vegetables have received considerable attention because of their potential antioxidants activities (Gulcin et al., 2002). They are regarded to be the most important antioxidative components of plants while the correlations between the concentrations of plant phenolics and their total antioxidant capabilities have been reported (McDonald et al., 2001).

Flavonoids are a group of naturally occurring compounds with powerful antioxidant properties widely distributed in the plant kingdom (Halliwell, 1997). The results of the total flavonoids expressed in Quercetin equivalent are shown in table 2. The total flavonoids expressed in the ethyl acetate and chloroform extracts are 0.450 mg/g and 0.850mg/g respectively. This result may be attributed to the presence of phenolic compounds especially flavonoids which has been shown to have antioxidant property of natural plant products (Gulein et al., 2006).

The advent of reactive oxygen species (ROS) and free radical generation from various cellular activities have been well documented. In mitochondria, part of the electrons transported by the respiratory chain is delivered to oxygen in a process generating superoxide anion, O_2^- , and subsequently H2O2 and reactive oxy and peroxy radicals (Murphy, 2009). While this fraction may be as high as 2-3% *in vitro*, chronic production of reactive oxygen species (ROS) by mitochondria *in vivo* is probably much lower (Murphy, 2009). Also under physiological conditions, oxidants and pro-oxidants such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) producing ROS have been implicated in apoptosis and other related diseases such as hypertension, arteriosclerosis, cancer and diabetes (Koren et al., 2001; Doughan et

al.,2008). DPPH is a stable free radical and can be reduced in the presence of an antioxidant molecule; its usage has been widely applied for evaluating antioxidant activity in a number of studies (Brand-williams et al., 1995). The ability of a molecule to donate a hydrogen atom to a radical determines its antioxidant potentials. DPPH accepts an hydrogen atom from an antioxidant and becomes a stable diamagnetic molecule .The percentage DPPH radical scavenging activity of the two extracts shows scavenging activity to be dose dependent (table 3). Although at the maximum concentrations, the ethyl acetate extract scavenged the radical by 52.29% while the chloroform extract scavenged the radical by 82.191% compared with vitamin C (92.50 %).

Hydroxyl radicals ($OH^{\cdot}$) are known to be the most toxic and reactive of all free radicals because of its ability to diffuse through cellular constituents. It initiates the oxidation of cell membrane lipids (Halliwell and Gutteridge 1985), yielding malondialdehyde which is mutagenic and carcinogenic (Miyake and Shibamoto , 1997). Results of the percentage hydroxyl radical scavenging activity are reported in table 4. The percentage scavenging activity increases in a dose-dependent manner with the two extracts. Maximum scavenging activities of 50.10 % and 41.10 % at 150µg/ml were obtained from the ethyl acetate and chloroform extracts respectively. An indication that the bioactive components of the extracts have the potential to scavenge hydroxyl radicals and maintain membrane integrity.

The higher burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body (Tiwari, 2001). This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases such as atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases (Doughan et al., 2008). Peroxidation of lipid is a natural phenomenon and occurs on its exposure to oxygen. Recently, free radicals-induced lipid peroxidation has gained much importance because of its involvement in several pathological conditions such as aging, wound healing, oxygen toxicity, liver disorders, inflammations etc. The results of this investigation showed that the extracts inhibited lipid peroxidation in a dose dependent manner (table 5).The ethyl acetate extract inhibited lipid peroxidation maximally by 78.90% compared with the chloroform extract with 63.40% inhibition. The two extracts conferred 50% protection at maximum concentrations while the property shown by the extracts could be attributed to the presence of phenolic compounds which has been shown to be correlated to the antioxidant property of natural plant products. (Gulcin, 2006).

CONCLUSION

Results obtained from this study shows that certain bioactive agents with antioxidant and anti-lipidperoxidation properties are present in the *Basella alba* leaves extracts. This study gives scientific support for its wide consumption as leafy vegetable in western Nigeria, while these extracts may be explored as template in drug discovery and design especially for the management and prevention of diseases associated with oxidative damage, tissue degenerations and aging.

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REFERENCES

Aliyu, A. B., Ibrahim, H., Musa, A. M., Ibrahim, M. A., Oyewale, A. O. & Amupitan, J. O. (2010). Invitro Evaluation of Antioxidant Activity of Anisopus mannii. *African Journal of Biotechnology.* 9(16):2437-2441.

Ames, B. N. (1983). Dietary Carcinogens and anticarcinogens: Oxygen Radicals and Degenerative Diseases. *Science* 221:1256-1264

Arouma, O. I. (1994). Nutrition and Health Aspect of Free Radical and Antioxidants. *Food Chem. Toxicol*. 32:671-683

Arouma, O. I. (1998). Free Radicals, Oxidative Stress and antioxidants in Human Health and Disease. *J. Am. Oil Chem. Soc.* 75:199-212

Bliss, C. I. (1967). *Statistics in Biology, Statistical Methods for research in the natural sciences*, 1, McGraw Hill Book Company, NY, pp:558.

Brand-Williams, W. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science Technology (London)* 28:25-30.

Chang, C. C., Yang, M. H., Wen, H. M. & Chern, J. C. (2002). Estimation of Total Flavonoid content of Propolis by two complementary colorimetric methods. J. *Food Drug Analysis,* 10:178-182

Davies, K. J. A. (2000). Oxidative Stress, Antioxidant Defences and Damage, removal, repair and Replacement systems. *IUBMB Life*. %50:279-289.

Doughan & Joan, E. K. (2008). The comprehensive Bio-Bibliography (review), Volume 64 Number 3. pp 514-516DOI 10.135/not 2008.0021.

Duke, J. A. & Ayensu, E. S. (1985). *Medicinal Plants of China*. Reference Publication Inc.

Finkel, T. (2000). Oxidants, Oxidative Stress and The Biology of Aging. *Nature* 408:239-248.

Gulcin, I., Buyukokuroglu, M. E., Oktay, M. & Kufrevioglu, O. I. (2002). On the invitro antioxidant properties of Melatonin. *J. Pineal Res.* 33(3):167-171.

Gulcin, I. (2006). Antioxidant and activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology* 217:213-220.

Gulein, I. (2006). Antioxidant and antiradical activities of L-carnitine. *Life Sci.,* 78:803-811.

Halliwell, B. (1997). Antioxidants and Human Disease: A General Introduction. *Nutr. Rev.*; 52:253-265.

Halliwell. B. & Guitteridge, J. M. C. (1985). Role of Free Radicals and Catalytic metal ions in Human Disease: an overview. *Method Enzymol.*; 186:1-85.

Haskel, M. J. (2004). Daily consumption of Indian Spinach (*Baselle alba*) or Sweet potatoes has positive effect on total-body vitamin A store in Bangladeshi men. *Ame. J. Clin*. 80(3):705-714.

Koren, R., Hadari-Naor, I., Zuck, E., Rotem, C., Liberman, U.A. & Ravid, A. (2001). Mitochondrial Complex I Inhibitor Rotenone Induces Apoptosis. www.adultmito.net/wpcontent/upload.i-inflammatory 12 /08/2010.

McDonald, S., Prenzler, P. D., Autolovich, M. & Roberds, K. (2001). Phenolic content and Antioxidant activity of Olive extracts. *Food Chem*. 73:73

Mensor, M. M., Menezes, F. S., Leitao, G. G., Reis, A. S. & Santos (2001). Screening of Brazillian Plants for antioxidant activity by use of DPPH free radical method. Upetd.ap.za/thesis/available/etd-01312008121838

Miyake, T. & Shibamoto, T. (1997). Antioxidant activity of Natural compounds found in plants. *J. Agric Food Hcem.* 45:1819-22.

Murphy, M. P. (2009) How mitochondria produce reactive oxygen species. *Biochem.J.* 417:1-13.

Ogundipe, O.O., Moody, J. O., Houghton, P. J. & Odelola, H. A. (2000): Bioactive chemical constituents from Alchornea Laxiflora (benth) pax and hoffman. J. *of Ethnopharmacology* 74 : 275-280.

Onay-ucar, E., Karagoz, A. & Arda, N. (2006). Antioxidant activity of *Viscium album spp*. Fitoterapia, 77:556-560

Repetto, M. G. & Llesuy, S. F. (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz J Med Biol Res*., 35:523-34.

Ruberto, G., Baratta, M. T., Deans, S. G. & Dorman, H. J. D (2000) Antioxidant and antimicrobial activity of foeniculum vulgare and crithmum maritimum essential oils. *Plant medica* 66, 687-693.

Smith, M. A., Perry, G., Richey, P. L., Sayre, L. M., Anderson, V. E., Beal, M. F. & Kowal, N. (1996). Oxidative Damage in Alzheimer's. *Nature* 382:120-121

Steenkamp, V., Stewart, M. J., Chimuka, L. & Cukrowska, E. (2005). Uranium concentration in South African herbal remedies. *Health physiol.* 89:79-83

Takanami, Y., Iwane, H., Kawai, Y. & Shimoitsu, T. (2000). Vitamin E supplementation and endurance exercises? *Are there benefits? Sports Med.*; 29(2)1381-1478

Tiwari, A. K. (2002). Imbalance in antioxidant defence and human disease: multiple approach of natural antioxidants theraphy: *Curr. Sci.*81: 1179-1187.

Valko, M., Leibfritz, D., Moncol, J., Cronn, M. T. D., Mazur, M. & Telsa, J. (2006). Free radicals and antioxidants in normal Physiological functions and human disease. *Int. J. Biochem. Cell. Biol.* 7(1):45-78.