Methanolic fruit extract of *Solanum macrocarpon (Ahara-mbe)* African eggplant is a potential *in-vitro* lipid peroxidant

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ABSTRACT

This work evaluated the anti-lipid peroxidation activities of the methanolic extracts of fruits of *Solanum macrocarpon* which was carried out based on the principle of inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS). ~25kg body weight goat brain was dissected and homogenized with polytron ice cold to produced 1:2 (w/v) which was centrifuged at 3000g for 10mins. An aliquot (0.1) of the supernatant was incubated with 0.2ml *Solanum macrocarpon* methanolic fruit extract in the presence of $FeSO_4$ (0.1ml) and ascorbic acid (0.1 ml) at 37°C for 1 hr. Trichloracetic acid (0.5ml) and thiobarbituric acid (TBA, 0.38ml) were used to stop the reaction, heated at 80^{0}C for 20mins and re-centrifuged (3000g for 10mins). The colour intensity of malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at λ = 532nm and the inhibition ratios (%) i.e. the activities (0, 13.265, 44.898, 57.143, 20.408, and 46.939) were deduced at various concentrations (0, 200, 400, 600, 800 and 1000). The effective concentration at 50% (EC_{50}) for the standard was 610µg/ml and for the sample was 300µg/ml. Results of this investigation proposes an inhibitory effect suggesting why *Solanum macrocarpon* fruit is a good antioxidant with lipid peroxidation potential.

Keywords: Lipid peroxidation, Fruit, *Solanum macrocarpon*, BHA, TBA.

INTRODUCTION

Lipid peroxidation process, which occurs following the oxidation of unsaturated fatty acids could be an enzymatic or non-enzymatic event [1,2,3,4] and occurs in three stages of initiation, propagation and termination [5,6,7,8]. During the initiation step, one atom of hydrogen is extracted from the unsaturated fatty acid (LH) by hydroxyl radical (*OH) resulting in the formation of lipid radical (L*) leading to the formation of conjugated diene [9]. The propagation step is characterized by the reaction of conjugated diene with oxygen to form peroxyl radical (LOO^*) which in turn attacks another unsaturated fatty acid to form unstable hydroperoxide and a new radical. In the termination step, a reaction between two of the formed radicals occurs to form non-radical products. In the body, endogenous antioxidant mechanisms exist to limit this formation and to scavenge free radicals [10]. These mechanisms include antioxidant enzymes like the superoxide dismutase, glutathione peroxidase, or catalase [11]. In addition to antioxidant enzymes, endogenous molecule like glutathione is an antioxidant with other exogenous products like vitamins E and C and plant-derived natural antioxidants like carotenoids and polyphenols which possess antioxidant potentials with free radical scavenging properties [12,13,14]. Phenolic compounds, important constituents in many plants, have received considerable attentions as potentially protective factors against cancer and heart diseases because of their antioxidant potency and their ubiquity in a wide range of commonly consumed foods of plants origin [15,16,17,18,19,20]. Many studies have reported that phenolic compounds possess other biological activities such as anti-inflammatory, antiulcer, antispasmodic, antisecretory, antiviral, anti-diarrhea, antitumor [21,22,23,24,25,26]. Phytochemicals, including phenolics are suggested to be the major bioactive compounds contributing to the health benefits of vegetables and fruits [27]. It was shown

that the health properties of these natural products depend on the contents of bioactive compounds, mainly phenolics, and partly on dietary fibers [26].

Aims of the Study

Despite the existence of recent studies such as nutritional composition, phytochemical analysis, antioxidant properties, etc on the fruits and leaves of *S. macrocarpon*, no study has looked at the capability of the fruits and leaves of this vegetable to inhibit lipid peroxidation. However, where any such information may exist, the samples were not necessarily the same variety as those of the present study. Thus, this research was aimed at determining the lipid peroxidation inhibiting abilities of methanolic extracts of the fruits and leaves of *Solanum macrocarpon*.

MATERIALS AND METHODS

SAMPLE COLLECTION

The sample used was fresh (*Aharambe*) *Solanum macrocarpon* African Eggplant leaves and fruits, which were purchased from New Market Enugu North LGA, Enugu State. Sample was conveyed to Industrial Biochemistry Laboratory of ESUT where it was identified and authenticated by Prof. Eze Charles of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology (ESUT).

METHODS

REAGENT PREPARATION

5% Sodium Trioxonitrate

Sodium Trioxonitrate (5%, 100ml) was prepared by weighing exactly 5.0g of $NaNO_3$ into a 100ml volumetric flask using Harvard trip balance. Measuring cylinder (100ml) was used to measure 10ml of distilled water which was used to dissolve the pellet in a 100ml volumetric flask, after which an additional quantity of distilled water was added to make up to the mark. The $NaNO_3$ solution was transferred to a 100ml reagent bottle.

Measurement of 10g Aluminum Trichloridemonohydride

Aluminum trichloride monohydrid (10g, 100ml) was prepared by weighing exactly 10.0g of $AlCl_3$ into a 100ml volumetric flask using a digital weighing balance. Measuring cylinder (50ml) was used to measure 20ml of distilled water which was used to dissolve the pellet in a 100ml volumetric flask, after which an additional quantity of distilled water was added to make up to the mark. The $AlCl_3$ solution was transferred to a 100ml reagent bottle.

1.0M Sodium Hydroxide.

Sodium Hydroxide (1.0M, 100ml) was prepared by weighing exactly 4.0g of NaOH into a 500ml volumetric flask using digital weighing balance. Measuring cylinder (100ml) was used to measure 20ml of distilled water which was used to dissolve the pellet in a 100ml volumetric flask, after which an additional quantity of distilled water was added to make up to the mark. The NaOH solution was transferred to a 100ml reagent bottle.

0.5M Sulphuric Acid.

Sulphuric acid (0.5M, 250ml) was prepared by measuring exactly 7.0ml of Sulphuric acid into a 250ml volumetric flask using a 50ml Pyrex measuring cylinder. About 30ml of distilled water was used to dilute it before an additional quantity of distilled water was used to make up to the mark. The acid solution was stored in 250ml reagent bottle.

SAMPLE PREPARATION

The sample (leaves of *Solanum macrocarpon*) was separated from pebbles and microbial infected ones by hand-picking. The sample was later washed under a running tap water, cut into smaller size (to reduce their surface area) and air-dried. Leaf extract of the sample was prepared using Soxhlet method described by [28].

SAMPLE ANALYSIS.

Anti-Lipid Peroxidation Assay (TBARS) of *S. macrocarpon*

A modified thiobarbituric acid-reactive species (TBARS) assay was used to measure the lipid peroxide formed, using pig brain (*Sus scrofa domesticus*)

homogenate as lipid rich medium. A 150kg of pig brain (*Sus scrofa domesticus*) was obtained from the slaughter house of Ogbete Main Market Enugu and homogenized with polytron in ice-cold Tris-HCl buffer (pH7.4, 20mm) to produce a 1:2(w/v) brain homogenate. The homogenate was cold-centrifuged at 3000rpm for 10mins. About 0.1ml of the supernatant was incubated with (0.2ml) of *S. macrocarpon* extract in the presence of (10µm: 0.1ml) $FeSO_4$ and ascorbic acid of (0.1mm:0.1ml) at 37°C for 1 hour. The reaction was stopped with the addition of trichloroacetic acid (28% w/v, 0.5ml) followed by thiobarbituric acid (TBA 2% w/v, 0.38ml). The mixture was heated at 80°C for 20 mins. The mixture was also centrifuged at 3000rpm for 10mins to remove precipitated protein. The colour intensity of the malondialdehyde (MDA)-TBA complex on the supernatant was measured by its absorbance at 532nm wavelength. The inhibition ratio (%) was calculated using the following formula:

Inhibition ratio (%) = [(A-B)/A] X 100%

Where A and B were the absorbances of the control and the sample respectively. The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the calibration of the antioxidant activity percentage against extract concentration. BHA was used as standard.

RESULTS

Table 1: For the sample methanolic extract of *S. macrocarpon*

Concentrations (µg/ml)	Absorbance (532nm)	Activity
0	0	0
200	0.085	13.265
400	0.054	44.898
600	0.042	57.143
800	0.078	20.408
1000	0.052	46.939.

Table 2: For the standard: Butylatedhydroxylanisole (BHA)

Concentrations (µg/ml)	Absorbance (532nm)	Activity
0	0	0
200	0.051	47.959
400	0.042	57.142
600	0.042	57.142
800	0.031	66.367
1000	0.024	75.510

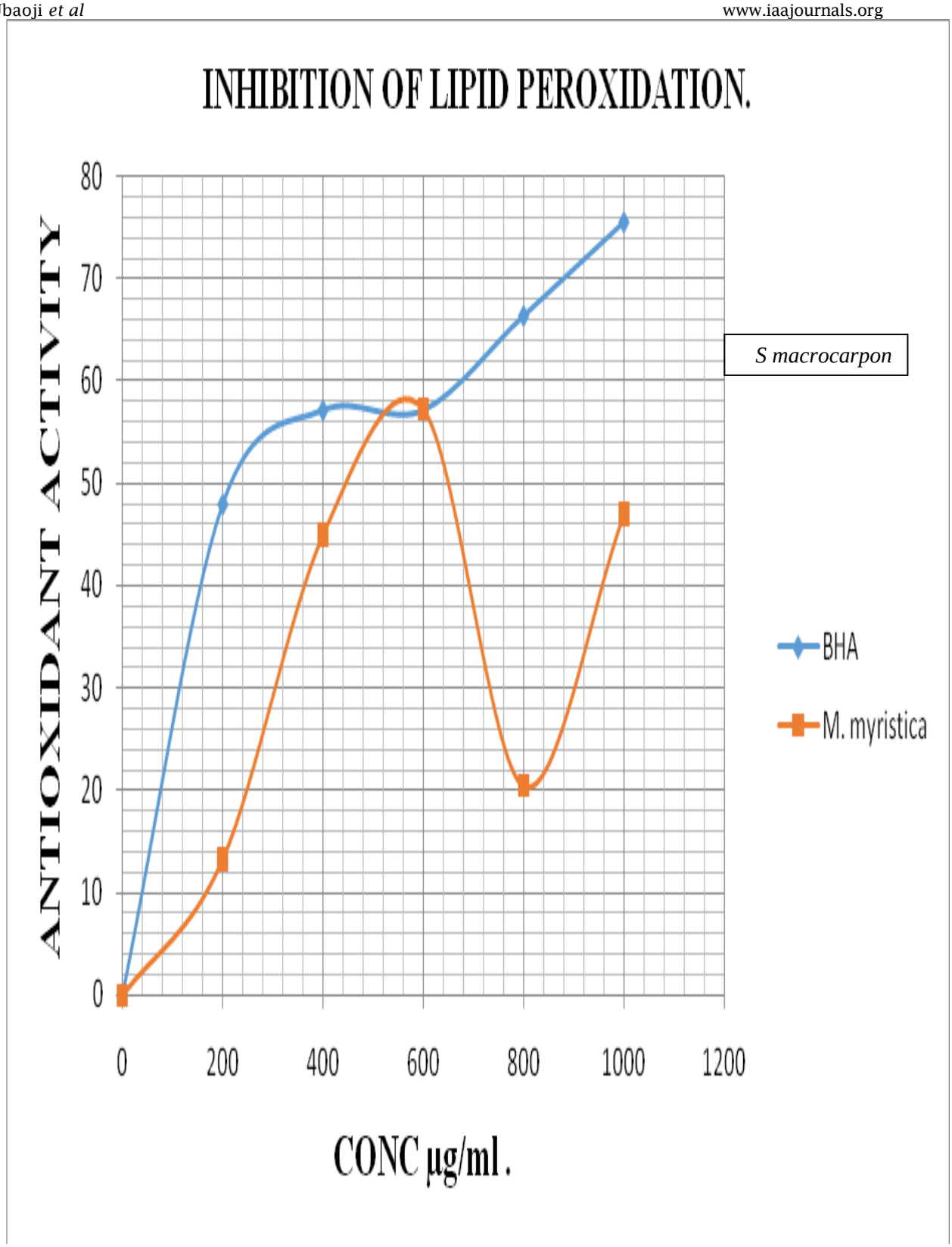

Fig 1: Graph showing the inhibition of lipid peroxidation.

DISCUSSION

Although lipids are one of the most abundant bio-molecules, its biological importance cannot be over emphasized. Therefore, it is necessary to retain healthy states of these essential lipids, by prevention and/or reduction (to clinical and physiological ranges) of these reactive oxygen species that attack and eventually distort the bioactivity of lipids. Lipid peroxidation is the most evident impact of ROSs on lipids [1,2,3]. Natural antioxidants are synthesized by plants and are present in the foods we eat, as opposed to those synthetic antioxidants that are either added to food to extend its shelf-life or prepared by extraction from plant sources to be taken as supplements in concentrated form [6]. Restriction in the use of synthetic antioxidants has caused an increased interest towards the natural counterparts [7]. Combining antioxidants may increase their effectiveness [8], because it has found that dietary synthetic antioxidants combined with α-tocopherol were more effective than rosemary, green tea, grape seed, or tomato extracts alone or in combination in sparing tocopherols oxidation and in preventing oxidation of fresh frozen chicken patties. It has been proposed that the mixed free radical acceptors involve two antioxidants: one that reacts with the peroxy radical (and is consumed) and a second that regenerates the first, effectively sparing. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not been investigate yet, and their medical activities could be decisive in the treatment of present or future studies [9].

CONCLUSION

The anti-lipid peroxidation activity of methanol extract showed a concentration-dependent increase. Value of the extract concentration providing 50% lipid inhibition (EC50) was low compared to that of BHA. These results suggested that the methanol extracts of *Solanum macrocarpon* fruit inhibited lipid peroxidation. In as much as this sample poses a more potent inhibitory effects than synthetic antioxidants such as the BHA, there is a general preference of natural to synthetic medicinal compounds. Thus, the seed methanolic extracts of *Solanum macrocarpon* can be used in food and pharmaceuticals; as drugs and food supplements, or synergism with other medicinal compounds to combat a wide range of ROSs related ailments.

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