Total Antioxidant Capacity of *Sapium ellipticum* (Hochst) Pax Leaf Extract, and Its Antioxidant Enzymes Potential in Free-Radical Systems *in vitro*

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Abstract: The total Antioxidant Capacity (TAC) of *Sapium ellipticum* (SE) leaf extract was evaluated using phosphomolybdenum and ABTS.$^{+}$ Models. The results obtained showed that SE extracts possesses 561.18 mg Ascorbic acid equivalent Antioxidant Capacity (AETAC) and 0.62 nmoles Trolox equivalents Antioxidant Capacity (TEAC) per gram of the extract. SE extract expressed high level of superoxide dismutase (SOD) and catalase (CAT) activities and moderate glutathione peroxidase (GPx) activity. However, it was relatively low in both glutathione reductase (GR) and polyphenol oxidase (PPO) activities. Overall, the data of the present study suggest that SE is apparently a good source of SOD and CAT through its ability to respectively inhibit the photochemical reduction of nitro blue tetrazolium (NBT) chloride and promote the decomposition of H_2O_2 to oxygen and water *in vitro* [Ighodaro OM, Adeosun AM, Adeoye BA, and Soetan OG. **Total Antioxidant Capacity of *Sapium ellipticum* (Hochst) Pax Leaf Extract, and Its Antioxidant Enzymes Potential in Free-Radical Systems *in vitro*.** *J Am Sci* 2017;13(7):113-118]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 10. doi:10.7537/marsjas130717.10.

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1. Introduction

Interest in free radicals in terms of their chemistry and damaging roles in various health conditions is increasing globally. Free radicals refer to any chemical species which are capable of independent existence and contained one or more unpaired electrons, which makes them unstable and highly reactive (Halliwell, 1997). The body may utilize them for beneficial purposes such as attack of pathogens. However, in excess of what the body's natural antioxidants can cope with, they cause oxidative damage to useful biological molecules such as nucleic acids, proteins, and lipids, consequently leading to compromise in cell structure and function (Bartsch and Nair, 2000; Valko *et al.,* 2006, Singh *et al.*, 2007).

The body parades an antioxidant defense mechanism grid involving enzymes (superoxide dismutase, catalase, glutathione peroxidase etc) and naturally occurring molecules (e.g glutathione and ascorbic acid), capable of scavenging free radicals and protecting the body against their damage (Gillespie *et al.,* 2009). However, in some cases, this natural protective strategy is overwhelmed; needing support or reinforcement from exogenous sources (Halliwel, 2006).

Most plants are rich in naturally occurring antioxidants which they utilize for protective biochemical functions. Many secondary compounds and enzymes of higher plants have been demonstrated with *in vitro* experiments to protect against oxidative damage induced by free radicals or reactive oxygen species (Tsai *et al.,* 2002; Zheng and Wang, 2001).

Sapium ellipticum (Hochst) Pax has a number of therapeutic usages in folk medicine (Burkill, 1985 and 1995). It belongs to the family *Euphorbiaceae* and is commonly referred to as jumping seed tree. *S. ellipticum* is widely distributed in eastern and tropical Africa. In southwest part of Nigeria, particularly among the Ilorin indigenes, the plant is popularly known as *aloko-ọgbọ.*

A few scientific investigations have been carried out on it. The antioxidant properties of the stem bark extract of the plant has been reported by Adesegun *et al.* (2008). Cytotoxicity screening of selected Nigerian plants used in traditional cancer treatment on HT29 (colon cancer) and MCF-7 (breast cancer) cell lines (HeLa cervix adenocarcinoma cells) indicated that *Sapium ellipticum* leaf extract expressed the highest cytotoxic activity among other plants with anticancer potential. Its activity was comparable to that of the reference drug, ciplastin (Sowemimo *et al.,* 2011). The phythochemical constituents, *in vitro* antioxidant capacities and antiplasmodial activities of *Sapium ellipticum* stem bark extracts were documented by Nana *et al.* (2013).

This present study sought to assess the total antioxidant capacity of the plant leaf extract and its antioxidant enzymes potential in free-radical systems, *in vitro.*

2. Materials and Methods

2.1 Collection of *Sapium ellipticum* leaves

Fresh *Sapium ellipcitum* (SE) leaves were harvested in the month of December, 2012 from a forest in a suburb of Ibadan, southwest of Nigeria. The harvested leaves were taxonomically authenticated by a botanist (Mr. T.K. Odewo) at the Lagos University Herbarium (LUH), Nigeria, were a specimen was deposited and assigned a voucher number, LUH 5423.

2.2. Preparation of *Sapium ellipticum* leaf extracts

The plant material was freed of extraneous materials; air dried at room temperature and milled to a fine powder, using a Waring blender. 300 grams of the powdered sample was macerated in 2.5 liters` of the extracting solvent (ethanol). The mixture was allowed to stand for 72 h and stirred intermittently with a glass rod to facilitate extraction. Sieving of the mixture was achieved with a muslin cloth (maximum pore size 2mm). The resulting filtrate on sieving was further filtered through Whatman filter paper (No 42) and subsequently reduced in volume with a rotary evaporator at 40 ^{o}C. Final elimination of solvent and drying was done using a regulated water bath at 40 ^{o}C.

2.3 Determination of Total Antioxidant Capacity

The Total Antioxidant capacity of SE extract was evaluated using phosphomolybdenum and ABTS.$^{+}$ Models.

2.3.1. Phosphomolybdenum model

The total antioxidant capacity of SE extract was evaluated by the phosphomolybdenum method as described by Prieto *et al.* (1999). 0.3 mL, 10 mg/mL of SE extract, ascorbic acid (0.5 to 2.5 mg/mL) used as standard and blank (ethanol) were separately combined with 3mL of the reagent mixture (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95 ^{o}C for 90 minutes. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid and was calculated using the equation: **A= (c x V)/m.** Where A = total content of antioxidant compounds, mg/g plant extract, in Ascorbic Acid Equivalent, c = the concentration of Ascorbic acid established from the calibration curve, mg/mL (Fig. 1) V = the volume of extract (mL), and m = the weight of crude plant extract (g).

2.3.2. ABTS.+ Scavenging model

The methods described by Re *et al.* (1999) with some modifications were used to assess the ABTS radical scavenging property of SE as an index of total antioxidant capacity. The ABTS.$^{+}$ radical was pre-generated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate and incubating for 12 to 16 h in the dark at room temperature until the reaction was complete and the absorbance was stable. The absorbance of the ABTS.+ solution was equilibrated to 0.70 ± 0.02 by diluting with water at room temperature. 1.0 mL of the prepared reagent was mixed with 10 µL of the SE extract (1.0 mg/mL) and the absorbance was measured at 734 nm after 6 minutes. The extract ABTS.$^{+}$ scavenging capacity was estimated in Trolox equivalent by using the following equation:

$$AC=\frac{\text{Sample (Abs)} - \text{Blank (Abs)}}{\text{Volume of Sample (µL)}} \times \text{Slope of curve}$$

Result was expressed as Trolox Equivalent Antioxidant Concentration (TEAC) (Fig. 2). AC=Antioxidant capacity.

2.4. Effects of SE crude extract on antioxidant enzyme activities *in vitro*

The *in vitro* effect of SE crude extract on selected antioxidant enzymes was carried out using the enzyme extract prepared from fresh leaves of the plant.

2.4.1. Preparations of enzyme extract for *in vitro* antioxidant enzymes assays

Enzyme extraction was carried out according to methods of Nayar and Gupta (2006) and Hakiman and Maziah (2009). Fresh leaves of SE (0.5 g) was homogenized in 8 mL solution containing 50 mM potassium phosphate buffer (pH 7.0) and 1 % polyvinylpolypyrolidone. The homogenate was centrifuged at 15, 000 rpm for 30 minutes to obtain a supernatant which was used for superoxide dismutase, catalase, glutathione-s-transferase and glutathione reductase assays.

2.4.2. Determination of protein in SE crude enzyme extract

The amount of protein in the plant extract was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard.

2.4.3. *In vitro* Superoxide Dismutase Activity of SE

The activity of SOD was measured according to the method of Giannopolitis and Ries (1977) by measuring the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) chloride. The reaction mixture (3 mL) contained 100 µL 12 mM L-methionine, 100 µL 50 mM sodium carbonate (pH 10.2), 100 µL 1 µM riboflavin, 100 µL 0.1 mM EDTA (pH 7.8), and 100 µL 75 µM nitroblue tetrazolium (NBT) in 2,300 µL 25 mM sodium phosphate buffer (pH 6.8), with 200 µL crude enzyme extract. The test tube containing the mixture was kept under a fluorescent lamp (120 W) for 15 minutes and the absorbance was read at 560 nm. The complete assay mixture without the enzyme extract served as control. One unit of SOD was defined as the amount of enzyme activity that was able to cause 50 % inhibition in the rate of NBT reduction to blue formazan.

% inhibition = ($A_{blank} - A_{sample}/A_{blank}$) x 100

Enzyme activity was expressed as SOD unit/mg protein.

2.4.4. *In vitro* Catalase (CAT) Activity of SE

Catalase activity was determined using the methods of Aebi (1984) and Luck (1974). The reaction mixture (1 mL) contained potassium phosphate buffer (pH 7.0), 250 µL of enzyme extract and 60 mM H_2O_2 to initiate the reaction. The complete assay mixture without the enzyme extract served as control. CAT activity was assayed by measuring H_2O_2 consumption at 240 nm for 3 minutes by decrease in absorbance. Enzyme activity was expressed as CAT unit/mg protein.

2.4.5. Glutathione Reductase (GR) activity

Glutathione reductase (GR) activity was assayed according to the method described by Sherwin and Farrant (1998). The reaction mixture constituted 300 mM potassium phosphate buffer (pH 7.5), 3 mM $MgCl_2$, 0.1 mM EDTA, 10 mM GSSG, 0.15 mM NADPH and 0.2 mL enzyme extract. The complete assay mixture without the enzyme served as control. The reaction was initiated by addition of NADPH and the decrease in absorbance at 340 nm for 3 minutes was recorded. GR activity was expressed GR unit/mg protein.

2.4.6. Glutathione Peroxidase (GPX) activity

Glutathione peroxidase activity (GPX) was assayed according to the method of Edwards (1996). The reaction mixture constituted of 100 mM potassium phosphate buffer (pH 7.0), 0.2 % Triton X-100, 0.24 U glutathione reductase, 1 mM GSH and 0.2 mL enzyme extract. After addition of enzyme extract, cuvettes were incubated at 30 oC for 10 minutes and 0.15 mM NADPH was added to measure the basal rate of GSH oxidation by monitoring the absorbance at 340 nm for 3 minutes. The reaction was initiated by the addition of 1 mM cumene hydroperoxide and GPX activity was expressed as unit/mg protein.

2.4.7. Polyphenol oxidase (PPO) activity

The activity of polyphenol oxidase was assayed in terms of catechol by the spectrophotometric method proposed by Esterbauer (1997). Fresh leaves of SE (5 g) was homogenized in 20 mL solution containing 50 mM Tris-Hcl, pH 7,2, 0.4 M sorbitol and 10m M NaCl. The homogenate was centrifuged at 2000 rpm for 10 minutes and the supernatant was used for the assay. The assay mixture contained 2.5 mL 0.1 M phosphate buffer and 0.3 mL of catechol solution (0.01M). The spectrophotometer was set at 495 nm. The enzyme extract (0.2 mL) was added to the same cuvette and the change in absorbance was recorded every 30 seconds up to 3 minutes. The complete assay mixture without the enzyme extract served as control. One unit of catechol oxidase is defined as the amount of enzyme that transforms 1 µmole of dihydrophenol to 1 µmole of quinine per minute under the assay conditions. Activity of PPO was calculated using the formula:

PPO activity (µmole/min) = K x ΔA/min, where K is the extinction coefficient, for catechol = 0.272, ΔA/min = change in absorbance per minute.

3. Results

3.1. Total antioxidant capacity of SE

The total Antioxidant Capacity (TAC) of SE was as estimated as 561.18 mg Ascorbic acid equivalent Antioxidant Capacity (AETAC) and 0.62 nmoles Trolox equivalents Antioxidant Capacity (TEAC) per gram of SE (Figs. 1and 2).

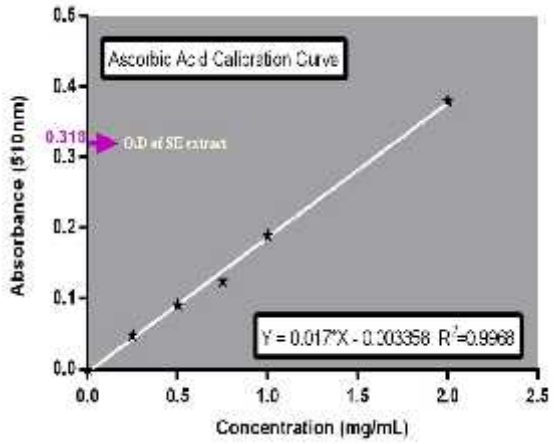

Fig 1: Estimation of TAC of SE in terms L-AA using optical density (O.D)

Optical Density of SE = 0.318 = Y, mass (m) of SE = 100mg. From the linear regression equation of the calibration curve: Y = 0.017*X - 0.003358, X = Y/0.017, X = 0.318/0.017, X = 18.706 mg and it is equivalent to the concentration of ascorbic acid established from the standard curve. Using the formula **A = (c x V) m**

Where A = Total Antioxidant Capacity (TAC), c = the concentration of ascorbic acid established from the calibration curve (mg/mL), V = the volume of extract in mL, and m = the weight of crude plant extract in gram. A (Total antioxidant capacity) = (18.706 * 0.318) * 0.01 = 561.18 mg/g AETAC.

The extract $ABTS.^+$ scavenging capacity was estimated in Trolox equivalent by using the following equation:

$$TAC = \frac{\text{Sample (Abs)} - \text{Blank (Abs)}}{\text{Volume of Sample (µL)}} \times \text{Slope of curve}$$

$$\text{Slope} = \frac{0.715 - 0.36}{1.0 - 0.8} = 0.44375$$

$\frac{0.170-0.142}{0.44} \times 10 = 0.62$

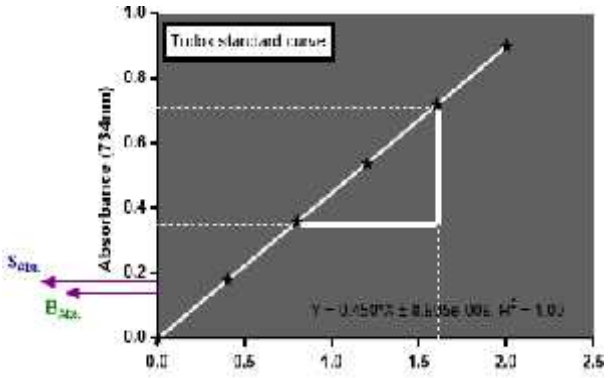

Fig. 2: Estimation of TAC of SE in terns of Trolox using optical density (O.D)

3.3. Effects of SE *in vitro* free radical systems

Figure 3 shows the result of investigation of the presence of antioxidant enzymes in fresh SE leaf extract. SE extract contains substantial amounts of superoxide dismutase (SOD) and catalase (CAT) and fair amount of glutathione peroxidase (GPx). However, it has relatively low amount of glutathione peroxidase (GR) and polyphenol oxidase (PPO).

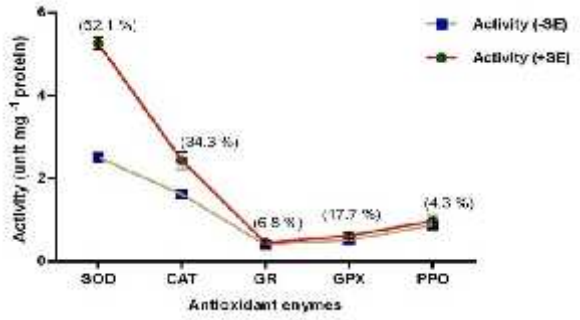

Figure 3: *In vitro* Effects of SE leaf enzyme extract

Values are mean of duplicate determinations (n=2). Activity (+SE) = Activity in the presence of SE extract, Activity (-SE) = Activity in the absence of SE extract, figures in parentheses represent the percentage difference in enzyme activity in the presence and absence of SE extract.

4. Discussion

Highly reactive compounds called free radicals or reactive species are constantly being generated as oxygen is utilized during normal cellular metabolic functions in living systems, and these molecules are capable of extensive damage via oxidative stress to tissues and biomolecules, leading to various disease conditions (Halliwell and Gutteridge, 2006).

Superoxide anion (O_2^-) is a well known toxic radical which attacks a number of cellular molecules (Korycka-Dahl and Richardson, 1978). Hydrogen peroxide on the other hand, although not a radical species but capable of directly inactivating a few enzymes, usually by oxidation of essential thiol (-SH) group components of the enzymes. Besides, its accumulation in cells contributes enormously to oxidative stress. The molecule easily diffuses across cell membranes, and once inside the cell, it is believed to reacts with ferric ion (Fe^{2+}) and copper II ion (Cu2+) to generate highly reactive hydroxyl radicals, and this usually is the origin of its numerous toxic effects (Miller *et al.*, 2000).

Conversely, intake of natural antioxidants through the consumption of botanicals, food supplements and medicinal plants appears to be offering enormous solution to the problem of oxidative-stress related ailments (Zhang *et al.*, 2003; Yazdanparast and Ardestani, 2007; Yazdanparast, 2008). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) along with some minerals (Se, Mn, Cu and Zn) constitute the first line antioxidant defense mechanism.

In this study, *Sapium ellipticum* showed significant activities of these first line defense antioxidant enzymes. SE leaf extract significantly inhibited the photochemical reduction of nitro blue tetrazolium (NBT) chloride and facilitated the decomposition of H_2O_2 to oxygen and water *in vitro*. This observation has a wide range implication on the role of the extract in human health and diseases. SOD is one of the major antioxidant enzymes that prevent biological macromolecules from oxidative damage (Zhang *et al.*, 2003). It is the first enzyme of the detoxification processes which catalyzes the breakdown of O_2^- to H_2O_2 and rendering the potentially harmful superoxide anion less hazardous. CAT is primarily responsible for the degradation of hydrogen peroxide (H_2O_2) obtained from SOD activity or other sources to water and molecular oxygen, using either an iron or manganese cofactor (Chelikani *et al.*, 2004). GPx is selenium containing intracellular enzyme which is also involved in the reduction of hydrogen peroxide (H_2O_2) to water and lipid peroxides to their corresponding alcohols, using reduced glutathione as substrate. The enzyme plays a more crucial role of inhibiting lipid peroxidation process, and therefore protects cells from oxidative stress (Gill and Tuteja, 2010). The ability of *Sapium ellipticum* leaf extract to suppress induced oxidative stress *in vitro* via the supply of first line antioxidant defense enzymes as noted in this study is similar to the previous report of Valyova *et al.* (2012) on free radical scavenging potential of variety of *Tagetes erecta* L. flowers growing in Bulgaria

5. Conclusion

The outcome of this study portends that SE extract is apparently a good source of natural antioxidants such as SOD, CAT and GPx and possesses substantial antioxidant capacity, particularly in terms of ascorbic acid. These observations suggest that SE has potential relevance in the management and treatment of oxidative-stress related ailments. Nonetheless, *in vivo* assessment of the antioxidant capacity of the plant extract is necessary to affirm its relevance in combating oxidative-stress related disorders.

Conflict of interest statement

The authors declare that there is no conflict of interest in respect to this article.

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