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# Curative anti-oxidant potential of *Psidium guajava* stem-bark extract on animal model

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**Abstract**
*Psidium guajava* elicited important functions in the management of several disease condition as proved by traditional medicine practitioner. This study investigate the curative anti-oxidant potential of *P. guajava* stem-back aqueous extract. Twenty five Wistar rats were randomly selected into ten groups (n=5). The curative study involved the untreated control that received 40 % ethanol, normal control was administered with 0.5 ml distilled water and tested groups at graded doses where administered with 50, 100 and 200 mg/kg *P. guajava* stem-back aqueous extract after 40 % ethanol where administered orally for 14 days prior to treatment across the groups. *In-vivo* antioxidant study of enzymatic and non-enzymatic antioxidant elicited a significant increase in Superoxide dismutase of plasma and tissue homogenates across the tested groups with the highest significant values at (4.93; 4.07; 2.41; 4.63; 3.51 U/ml), reduced glutathione at (4.58; 2.60; 2.49; 4.37; 2.69 U/ml), catalase at (289.87; 126.00; 126.97; 244.28;121.94 U/ml) and glutathione peroxidase (1.97; 2.10; 2.26; 2.06; 2.04 U/ml) when compared with untreated control ($p<0.05$) with decreased in the level of MDA at (18.74; 16.02; 15.81; 17.78; 14.33 U/ml) when compared with untreated control at (32.01; 35.17; 29.74; 31.85; 29.75 U/ml) of antioxidant indexes in plasma, liver, kidney, heart and lungs homogenates thereby scavenging or eliminating free radicals when compared with untreated control and vitamin C control. In conclusion, *Psidium guajava* elicited neproprotective, hepatoprotective and bio-protective effect of visceral organs via antioxidant scavenging property.

**Keywords:** *in-vivo* antioxidant, curative, *Psidium guajava*, animal model

## Introduction

Since orthodox medicine has failed in the management, treatment and diagnose of diseases due to their resistivity, harmful adverse effect, high cost of purchase, inefficient and scarcity, herbal medicine have been proven with solution to counteract these problems. Natural products with its derivatives are acquire from plant constituents with ethnomedicinal benefits in the management of several diseases globally. Since ancient times, human solely life on plants based medicine as remedy mediators [1]. Presently, orthodox medicines display a varied character in current medicine with evidences obtained from the phytochemical reports [2, 3]. Drugs discovery involved some active plant like yohimbine, vincristine, ergotamine, artemisinin, quinine, emetine etc. were achieve from medicinal plants with stipulates latent aid the finding and synthesis of novel and synthetic pharmaceuticals natural materials [4]. Natural products with their byproducts are resultant from plants sources used in the treatment of diseases universally. From ancient times, humans depend solely on herbal medicine as therapeutic mediators [1]. Currently, traditional medicines are broadly active in modern medicinal systems and it increases over the proofs obtain from phytochemical reports [2]. WHO improved herbal preparations in managing and treating health complications where the products can be simply afford and incorporated into the culture [5]. Phytomedicine display vital character in Medicare because of the metabolites synthesized by plants, which is comprises of bioactive constituents [6]. Medicinal plants showed several biologically properties basically with diverse constituents beneficial to mankind, whose lead raw materials and are mainly employ for the treatment of trivial and chronic disorders [3].

*Psidium guajava* L is commonly known as guava, it belongs to the family Myrtaceae. It is broadly establish in the globe. Teas gotten from the leaves are frequently used as diarrheal, cough, colic, uterine bleedings, gingivitis, scurvy, bronchitis, some intestinal parasitosis and arterial hypertension. The subsequent *P. guajava* extracts effects were reported: depression, slowed locomotion, retroviral exchange transcriptase inhibition, anti-malarial, cytotoxic, anti-mutagenic effect, anti-diabetic, anti-inflammatory, anti-biotic, anti-pyretic amoebicide, anti-allergic [7, 8] antioxidant [9] and myocardial defense against ischemia-reperfusion promoting injury maneuvers [10]. In Mexico it is known as an important crop cultivated in over 36,447 acres and produces approximately 192,850 tons. *Psidium guajava* is ingested as food and as a conventional medicine in sub-tropical zones in the globe due to its pharmacologic effects [11]. Therapeutic plants is of great importance to health care systems globally. These remarks revealed the traditional knowledge implicated. Also, *P. guajava* recurrently are useful in most part of the world for curative measure against sickness such as diarrhoea, fever, gastroenteritis, hypertension, dysentery, diabetes, caries, nociceptive and wounds healing. Guava is comprises of great organic and inorganic compounds such as secondary metabolites, antioxidant, anti-inflammatory,

polyphenols and antiviral properties. It has several compounds responsible for anticancerous effects. It enhances the cure of cancerous cells and serves as stem cell in the prevention of skin aging [12]. It also affect the myocardium inotropism of the heart [13].

Free radical are chemical constituents capable of free existence with one or some unpaired electrons present in atomic orbital [14]. Free radicals are commonly synthesis in the process of metabolism [15]. The cells employed oxygen to synthesize energy in the mitochondria as a by-products produced through the process. These by-products are characteristically reactive oxygen species (ROS) with reactive nitrogen species (RNS) ensuing to cellular redox movement. Free radicals possesses discrete attraction for carbohydrates, nucleic acids, lipids and proteins [16]. The word anti-oxidant is explicate as any component responsible for deferments prevention or removal of oxidative injury on target molecule [17]. Humans have developed intricate antioxidant structures (enzymatic and non-enzymatic) working synergistically with combination to one another, defensive to cells, organ and systems of the body against free radical injury. Antioxidants are categorised as endogenous and exogenous constituents e.g. part of diet or dietary supplements. Selected dietary compounds cannot neutralize free radicals, rather increase endogenous action by categorising it as anti-oxidants [18]. Endogenous antioxidants exhibited important character in supporting optimal cellular property, therefore systemic health is achievable. However, under definite conditions, endogenous antioxidants excite oxidative stress, which is insufficient to dietary antioxidants required to withstand optimal cellular effect. The extreme dexterous enzymatic anti-oxidants encompasses catalase, superoxide dismutase and glutathione peroxidase [19]. Non-enzymatic antioxidants are Vitamin E and C, thiol antioxidants (glutathione, thioredoxin and lipoic acid), natural flavonoids, melatonin, carotenoids and others [20]. The synthesis of oxidants is a characteristic event connected with aerobic metabolism. Oxygen supplied in surplus or its decrease is deficient, responsive oxygen species or free radicals includes; superoxide anions, hydroxyl radicals and hydrogen peroxide are created [21]. Increase in free radicals in the plasma and organs or tissues can stimulate oxidative injury to biomolecules and cell membranes, ultimately principal to various chronic disorders, like inflammatory, aging, cardiac malfunction, cancer, diabetes and other deteriorating diseases [22]. Antioxidants are proficient in alleviating or deactivating free radicals prior to their attack to cells [23]. Reactive oxygen species could be remove by certain amount of enzymatic and non-enzymatic antioxidant action. Enzymatic antioxidants include; glutathione peroxidase, superoxide dismutase and catalase. Non-enzymatic antioxidants include; ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), reduced glutathione, carotenoids, flavonoids and other antioxidants [24]. Conversely, in oxidative stress circumstances, enzymatic antioxidants may not be adequate while non-enzymatic antioxidants (dietary antioxidants) can be requisite to sustain maxima cellular roles [18]. Antioxidants can defend cell components against oxidative impairment and limit the peril of various deteriorating diseases linked with oxidative stress. The objectives of this study elicited the enzymatic and non-enzymatic scavenging property of the *Psidium guayava* against underline diseases.

## Method and Material

### Plant collection

*Psidium guajava* stem-back aqueous extract was obtained in Egor Local government area, Nigeria. The plant was identified and authenticated by Dr Akinibosun, in the herbarium unit of the Department of Plant Biology and Biotechnology, Life Sciences, University of Benin with the voucher number UBG-2113. The leaves were rinsed with distilled water and shade dried. It was pulverized using a mechanical grinder and stored in an airtight container.

### Preparation of Extracts

The powdered materials were subjected to successive extraction by hot Maceration Method using distilled water for 72 hours. The filtrate was evaporated at 40°C to get a dried extract. The percentage yield of the extract was 15.97 % w/w and stored for advance studies [25].

### Experimental Animals

Adult Wistar male rats weighed 180-220 were gotten from the animal house of Pharmacology, University of Benin. They were housed in clean wooden cages and maintained under regulated room temperature (25 ± 1°) with relative humidity of 45-55% under 12: 12 hours light and dark cycle for one week with free access to food and water ad libitum. All procedures using animals attained the approval of Life Sciences Institutional Animal Ethical Committee, University of Benin with LS21331 ethical number.

### Experimental Protocol

Male albino rats were obtained and randomly divided in five groups (n = 5). Treated groups received 50, 100, 200 mg/kg of *Psidium guajava* stem-back aqueous extract orally. 20 mg/kg Vitamin C and untreated control, 14 days after induction with 40% ethanol, treatment commences.

Group A: administered 50 mg/kg *P. guajava* stem-back aqueous extract

Group B: administered 100 mg/kg *P. guajava* stem-back aqueous extract

Group C: administered 200 mg/kg *P. guajava* stem-back aqueous extract

Group D: administered 20 mg/kg Vitamin C orally.

Group E: administered 2 mL/kg of 40 % ethanol orally.

These substances were given daily to the respective groups throughout the periods of 28 days. The animals were sacrifice under mild chloroforms anesthetic. Blood samples and uterus where isolated for analysis.

### Determination of Anti-oxidant Property.

Superoxide Dismutase (SOD) effect was read via methods described by Sachin and Choudhary [2] Principle; Auto-oxidation with hematoxylin (increases the absorbance at 560nm wavelength) was inhibited by SOD effect assay at pH 7.8; percentage amount of SOD present within a specific range. SOD activity in the sample is determined by measuring the amount of heamatin present. The crucial principle to assay is revealed schematically via the following equation: $O_2 + HTH_2$ SOD $H_2O_2 + HT$ (Abs560↑)

Procedure: Aliquant mixture of plasma 0.20ml of the diluted microsome was enclosed using 2.5 ml solution of 0.05 M carbonate buffer. The reactions started by additing 0.3 ml solution of 0.3 mM adrenaline. The standard was combine with 2.5 ml solution of 0.05 M carbonate buffer, 0.3 ml solution of 0.3 mM Adrenaline and 0.20 ml distilled water.

Absorbance was measured after 30 sec. to 150 sec. using wavelength of 480 nm. Calculations augment absorbance/ minute = % inhibition = 100 - Where As is increase absorbance of substrate and Ab is increase absorbance of blank 1 unit of SOD property is the sum total of SOD required to elicit 50 % inhibition of oxidation via adrenaline to adenochrome per 1 minute. 5 .21 5 A A 100 x AAxs

The assayed using Sachin and Choudhary [2] method. Test principle: Catalase scavenging hydrogen peroxide, converted into molecular oxygen and water. The action of catalase in this sample was resolute following decreased rate of absorbance using wavelength of 240nm and by monitoring the consumption of $H_2O_2$ substrate at 240 nm spectrophotometrically. 40 $H_2O_2$ Catalase $H_2O$ $+_2O_2$ Procedure; Tissue homogenate (10 µL) (100-150 µg of protein) being added to 2.8 ml solution 50 mM potassium phosphate buffer (pH 7.0) in 3 ml cuvette. Reaction was instigated via addition of 0.1 ml solution of freshly prepared 30mM $H_2O_2$ with decomposed rate of $H_2O_2$ measured at 240 nm wavelength for 300 seconds in spectrophotometer. Molar loss coefficient of 0.041 mM-1cm-1 was utilized by calculating catalase effect in $H_2O_2$ mole reduced/min/mg/protein. The enzymatic property is calculated using the formula below:

$$K = 2.303/T \times \log (A1/A2) \quad (4)$$

Where:
K: Serves as the Rate of reaction
T: The time interval (minutes)
A1: The absorbance level at time zero
A2: The absorbance level at 60 seconds interval

**Malondialdehyde**

Malondialdehyde (MDA) activity was determined using the method of Sachin and Choudhary, [2]. Test principle: The MDA assay is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm. Lipid peroxidation evaluation was done using measurement from malondialdehyde (MDA) development, with a proposed method of Okhawa *et al*. [26]. Malondialdehyde (MDA), lipid peroxidation end product, is a viable marker associated with free radical–umpired impairment and oxidative stress. The standard of this technique comprises of MDA reaction with thiobarbituric acid (TBA) in acidic state and at increase temperature from 90-100 °C to form a rosy MDA-(TBA) 2 compound that are quantified using spectrophotometer at 530 nm wavelength. The procedure, 0.5mL of 20 % TCA was added to 0.5 mL tissue homogenates, then 1 mL of 0.67% TBA was addition. The combination was incubated at $100^0$C for 15 min in water bath, allowed to cool and 4 mL of *n*butanol was added and centrifuged via the speed 3000 rpm for 15min. absorbance of clear pink supernatant was read at 532nm via spectrophotometer. The level of MDA is stated in nmol / g of the tissue.

**Reduced Glutathione activity**

Reduced Glutathione activity was measured in terms of the first order rate constant for the decomposition of tetra-butyl hydroperoxide according to [2]. The assay is based on GSH by 5, 5'- dithiobis (2-nitrobenzoic acid) (DTNB) oxidation, DTNB and glutathione (GSH) interact to synthesized 2-nitro-5-thiobenzoic acid (TNB) having yellow color. Thereby, GSH level can be measure at 412 nm wavelength. For this assay, 50 µL of the tissue homogenate was diluted in 10 mL of phosphate buffer (0.1 M, pH 8). To 3 mL of the mixture of dilution, 20 µL of DTNB (0.01 M) was added. Absorbance was read at 412 nm wavelength against the blank prepared using the same conditions.

Glutathione peroxidase activity was measured in terms of the first order rate constant for the decomposition of tetra-butyl hydroperoxide according to Sachin and Choudhary [2].

**Statistical analysis**

Obtained results were expressed as Mean ± SEM. Data were compared using one-way analysis of variance and Dunnet multiple comparison test. Differences were considered to significant different at $P < 0.05$.

**Results and Discusion**

Plasma antioxidant capacity (PAC) is a frequently used biomarkers aid in evaluating the efficiency of nutraceuticals or antioxidant treatment. The fact that plasma is comprises of endogenous antioxidants network such as (albumin, bilirubin, reduced glutathione and uric acid) as well as exogenous antioxidants found in food. These antioxidants possibly serves as complementary and synergistically deliver viable defense against ROS. The large quantity of antioxidants found in plasma, have several been improved on.

**Table 1:** Curative effect of *Psidium guajava* stem-back aqueous extract on plasma *in-vivo* antioxidant

| Group | Dose mg/kg | Mean ± SEM MDA (x10-3 mmole/ml) | Mean ± SEM SOD (U/ml) | Mean ± SEM Catalase (U/ml) | Mean ± SEM Reduced Glutathione (U/ml) | Mean ± SEM Glutathione perioxidase (U/ml) |
|---|---|---|---|---|---|---|
| Ethanol | 40 % | 32.01±0.51$^a$ | 2.04±0.14$^a$ | 149.14±1.92$^a$ | 1.57±0.21$^a$ | 5.31±0.43$^a$ |
| Ascorbic acid | 20 | 19.30±0.23$^b$ | 5.12±0.24$^c$ | 289.71±2.81$^c$ | 4.62±0.33$^c$ | 2.13±0.21$^c$ |
| *P. guajava* | 25 | 21.69±0.42$^b$ | 3.48.±0.19$^b$ | 211.93±1.99$^b$ | 2.98±0.35$^b$ | 1.97±0.28$^c$ |
| *P. guajava* | 50 | 19.21±0.30$^b$ | 4.24±0.16$^c$ | 264.13±2.82$^b$ | 3.93±0.44$^c$ | 2.69±0.34$^b$ |
| *P. guajava* | 100 | 18.74±0.37$^b$ | 4.93±0.26$^c$ | 289.87±2.25$^c$ | 4.58±0.42$^c$ | 5.45±0.41$^b$ |

Values are expressed as Mean ±SEM n=5. ($p<0.05$).

Results from the aqueous extract showed that Table 2 showed reduction in oxidative stress in *in-vivo* free radicals. Hence, MDA concentration in liver homogenates showed significantly reduced in the treatment groups and ascorbic acid when compared with untreated control. The significant reduction in liver MDA controlled oxidative stress. CAT together with SOD and GPx establish primary enzymatic defence, catalysing ROS decomposition. Acute effect of the extract increases CAT and SOD effect across the test groups.

**Table 2:** Curative effect of *Psidium guajava* stem-back aqueous extract on liver homogenate *in-vivo* antioxidant
Values are expressed as Mean ±SEM n=6. (p<0.05).

| Group | Dose mg/kg | Mean ±SEM MDA (x10-3 mmole/ml) | Mean ±SEM SOD (U/ml) | Mean ±SEM Catalase (U/ml) | Mean ±SEM Reduced Glutathione (U/ml) | Mean ±SEM Glutathione perioxidase (U/ml) |
|---|---|---|---|---|---|---|
| Ethanol | 40 % | 35.17±0.36[a] | 1.94±0.35[a] | 78.64±1.02[a] | 1.38±0.31[a] | 3.42±0.54[a] |
| Ascorbic acid | 20 | 15.11±0.22[c] | 4.13±0.46[c] | 125.22±1.78[b] | 2.63±0.25[b] | 2.15±0.39[b] |
| *P. guajava* | 25 | 19.52±0.53[b] | 2.74±0.32[b] | 111.37±1.39[b] | 2.06±0.19[b] | 2.29±0.33[b] |
| *P. guajava* | 50 | 17.73±0.42[c] | 3.79±0.30[c] | 120.21±2.04[b] | 2.37±0.26[b] | 2.24±0.41[b] |
| *P. guajava* | 100 | 16.02±0.35[c] | 4.07±0.37[c] | 126.00±1.99[b] | 2.60±0.32[c] | 2.10±0.43[b] |

Results from the aqueous extract showed that Table 3 showed reduction in oxidative stress in *in-vivo* free radicals. Hence, MDA concentration in kidney homogenates showed significantly reduced in the treatment groups and ascorbic acid when compared with untreated control. The significant reduction in kidney MDA regulates oxidative stress. CAT together with SOD and GPx establish primary enzymatic defence, catalysing ROS decomposition. Acute effect of the extract increases CAT and SOD effect across the test groups**.**

**Table 3:** Curative effect of *Psidium guajava* stem-back aqueous extract on kidney homogenate *in-vivo* antioxidant

| Group | Dose mg/kg | Mean ±SEM MDA (x10-3 mmole/ml) | Mean ±SEM SOD (U/ml) | Mean ±SEM Catalase (U/ml) | Mean ±SEM Reduced Glutathione (U/ml) | Mean ±SEM Glutathione peroxidase (U/ml) |
|---|---|---|---|---|---|---|
| Ethanol | 40 % | 29.74±1.65[a] | 1.38±0.52[a] | 84.52±1.46[a] | 1.83±0.37[a] | 1.69±0.18[a] |
| Ascorbic acid | 20 | 15.47±1.06[b] | 2.37±0.65[b] | 127.05±2.38[b] | 2.52±0.55[b] | 2.37±0.21[b] |
| *P. guajava* | 25 | 20.79±1.46[b] | 1.88±0.48[b] | 100.06±1.98[b] | 2.11±0.42[c] | 2.26±0.27[b] |
| *P. guajava* | 50 | 17.06±1.15[b] | 2.08±0.59[b] | 118.37±2.10[b] | 2.35±0.50[b] | 2.35±0.29[b] |
| *P. guajava* | 100 | 15.81±1.09[b] | 2.41±0.63[b] | 126.97±3.06[b] | 2.49±0.46[b] | 2.33±0.31[b] |

Values are expressed as Mean ±SEM n=6. (p<0.05).

Results from the aqueous extract showed that Table 4 showed reduction in oxidative stress in *in-vivo* free radicals. Hence, MDA concentration in cardiac homogenates showed significantly reduced in the treatment groups and ascorbic acid when compared with untreated control. The significant reduction in cardiac MDA aid in oxidative stress regulation. CAT together with SOD and GPx establish primary enzymatic defence, catalysing ROS decomposition. Acute effect of the extract increases CAT and SOD effect across the test groups

**Table 4:** Curative effect of *Psidium guajava* stem-back aqueous extract on heart homogenates *in-vivo* antioxidant

| Group | Dose mg/kg | Mean ±SEM MDA (x10-3 mmole/ml) | Mean ±SEM SOD (U/ml) | Mean ±SEM Catalase (U/ml) | Mean ±SEM Reduced Glutathione (U/ml) | Mean ±SEM Glutathione perioxidase (U/ml) |
|---|---|---|---|---|---|---|
| Ethanol | 40 % | 31.85±0.63[a] | 2.46±0.42[a] | 158.16±1.95[a] | 2.08±0.47[a] | 3.96±0.72[a] |
| Ascorbic acid | 20 | 19.94±0.35[b] | 4.97±0.58[c] | 245.76±2.26[b] | 4.35±0.51[c] | 2.02±0.54[c] |
| *P. guajava* | 25 | 21.00±0.48[b] | 2.99.±0.37[a] | 196.76±1.48[b] | 3.66±0.49[b] | 2.62±0.58[c] |
| *P. guajava* | 50 | 19.99±0.43[b] | 3.45±0.34[b] | 220.61±2.10[b] | 4.13±0.53[c] | 2.16±0.61[b] |
| *P. guajava* | 100 | 17.78±0.29[c] | 4.63±0.45[c] | 244.28±2.54[b] | 4.37±0.55[c] | 2.06±0.55[b] |

Values are expressed as Mean ±SEM n=5. (p<0.05).

Results from the aqueous extract showed that Table 5 showed reduction in oxidative stress in *in-vivo* free radicals. Hence, MDA concentration in the lungs homogenates showed significantly reduced in the treatment groups and ascorbic acid when compared with untreated control. The significant reduction in the lungs MDA regulate oxidative stress. CAT together with SOD and GPx establish primary enzymatic defence, catalysing ROS decomposition. Acute effect of the extract increases CAT and SOD effect across the test groups

**Table 5:** Curative effect of *Psidium guajava* stem-back aqueous extract on lungs homogenate *in-vivo* antioxidant

| Group | Dose mg/kg | Mean ±SEM MDA (x10-3 mmole/ml) | Mean ±SEM SOD (U/ml) | Mean ±SEM Catalase (U/ml) | Mean ±SEM Reduced Glutathione (U/ml) | Mean ±SEM Glutathione perioxidase (U/ml) |
|---|---|---|---|---|---|---|
| Ethanol | 40 % | 29.75±0.42[a] | 1.97±0.36[a] | 87.64±1.42[a] | 1.58±0.45[a] | 1.81±0.53[a] |
| Ascorbic acid | 20 | 15.12±0.25[c] | 3.48±0.28[b] | 124.21±2.52[b] | 2.65±0.48[b] | 2.93±0.57[b] |
| *P. guajava* | 25 | 20.11±0.39[b] | 3.12±0.32[b] | 109.35±2.26[b] | 2.17±0.42[b] | 2.04±0.48[b] |
| *P. guajava* | 50 | 18.26±0.45[b] | 3.39±0.40[b] | 120.63±2.35[b] | 2.44±0.39[b] | 2.48±0.55[b] |
| *P. guajava* | 100 | 14.33±0.40[c] | 3.51±0.43[c] | 121.94±2.67[b] | 2.69±0.46[b] | 2.86±0.47[b] |

Values are expressed as Mean ±SEM n=6. (p<0.05).

Plants used as medicine has been recorded useful far back six hundred thousand years ago and these medicinal plant provides more advantage than their synthetic counterpart by being cheaper, recommendable, safer and up to standard for chronic treatments. Medicinal plants are part and parcel of human society to combat diseases, from the dawn of

civilization (Sachin and Choudhary [2]. Plants have invariably ben a rich source for new drugs either obtained from plants or developed using their chemical structure as templates. Antioxidants scavenging property protected some cells liable for diseases occurrence [25]. Antioxidant property of *Psidium guajava* aqueous stem-bark extract designated its scavenging capacity against reactive oxygen species (ROS) in cells responsible for oxidative damages.

Orthodox application of biomarkers help in redox state evaluation of plasma and tissues using enzymatic and non-enzymatic antioxidant assay with macromolecule oxidative factor [27, 28]. Oxidation is takes place in cellular membranes damage, which is improved by thiobarbituric acid reactive constituents (TBARS) assay. This assay elucidate malondialdehyde (MDA) action in relation to lipoperoxidation (LPO) [18]. *P. guajava* impedes free radicals synthesis by regulation of normal plasma and liver, kidney, heart and lungs homogenates MDA values at graded doses of the extract when compared with the control (Table 1). *P. guajava* aqueous stem-bark extract enhances the regulation of oxidation, which possibly is as a result of the present of antioxidant deposited by the extract in the plasma and tissues samples. These ingredients are possibly involved in radical scavenging activity as adjuvant deactivation of transcriptional causes responsible for the control of expressed genes encoded by antioxidant enzymes [27, 16]. Lipid peroxidation act as an auto-catalytic, free-radical facilitated, destructive means, where by polyunsaturated fatty acids present in cell membranes undertake breakdown to form lipid hydroperoxides [29]. Reactive oxygen species disintegrate into polyunsaturated lipids, developing malondialdehyde [30]. This constituents is a reactive aldehyde, and the major reactive electrophile species causing toxic stress in cells forming covalent protein adducts known to progress lipoxidation end-products. The synthesis of aldehyde is utilized as a biomarker to investigate oxidative stress level in an organism [31]. Tissue sulfhydryl action is antagonistic to reactive oxygen species (ROS) interrelated with lethal tissues activated by certain phytochemicals in plant to triggered oxidation. NPSH is comprises of about 90 % intracellular NPSH decreased by glutathione (GSH) [28]. GSH displayed crucial role, which is associate with antioxidant protective mode of action, perhaps via direct radical-scavenging properties, elicited by glutathione peroxidase (GPx) systems, to eliminate different hydro-peroxides [32]. The non-enzymatic antioxidant, glutathione serves as an abundant tripeptides found in the liver. Its roles are primarily attributed with free radical species removal such as superoxide radicals, hydrogen peroxide, and maintenance of membrane protein thiols, alkoxy radicals and glutathione peroxidase substrate and GST [33].

Toxic plants investigated aid the depletion of GSH constituents initiated by some visceral cells, tissues and organs with pro-oxidant effect with doses dependent to subdue free-radical stress. GSH is suitable for onset defense against diseases [27] as shown in this study. Non-enzymatic defences, exhibited no significant variations in thiols content (NPSH), acting synergically as antioxidant property between NPSH and SBSB constituent in instinctual tissue [34].

Catalase (CAT) act as an enzymatic antioxidant broadly dispersed in animal tissues. It decays hydrogen peroxide and defends tissue from extremely reactive hydroxyl radicals [35]. Catalase effect differs significantly from tissue to tissue, highest effect is present in hepatic and renal tissues, whereas with lowest property observed in connective tissue [36]. Impede of this enzyme could promote sensitivity from free radical-induced cellular impairment. Thus, decrease in CAT activity could bring about poisonous effects associated with hydrogen peroxide and superoxide integration. Catalase (CAT) and super oxide dismutase (SOD) antioxidant property were estimated at graded doses of *P. guayava* aqueous stem-bark extract in plasma and liver, kidney, heart, lungs homogenates when compared with the control [20]. Comparable research designated *in vivo* model of *Psidium guayava* found to decrease oxidative stress by reducing TBARS concentration and cumulating antioxidant enzymes properties includes SOD, catalase and GPX present in hepatic and cardiac tissues in hypercholesteromic rats [37, 38] also elicited in *in vitro* study that *Ruta gravoelens* L. extract scavenges hydroxyl radical and block lipid peroxidation. Flavonoids is broadly distributed in plants possessing the potential to inhibit oxidative injury. Certainly, these flavonoids have the potential to function as *in vitro* antioxidants via scavenging superoxide anion [39] singlet oxygen [40] lipid peroxy-radicals [41, 42] and/or alleviating free radicals intricate in oxidative procedures through hydrogenation or by complex oxidizing species [43]. Flavonoids prevailing in *Ruta chalepensis* L., in addition with their free radical scavenging effects also improve the countenance of intracellular endogenous antioxidants like superoxide dismutase (SOD), catalase, and GPX [44]. CAT together with SOD and GPx institute of enzymatic defense, catalysing decay of ROS against diseases. *P. guayava* extract at graded doses elicited increased in CAT and SOD effects of plasma and homogenates of visceral organs when compared with the control (Table 2, 3, 4 and 5). Theoretically, SOD and CAT activity rises as compensatory mechanism to scavenge free-radical stress, thereby eliminate disease conditions [45].

## Conclusion

In conclusion, *Psidium guayava* aqueous stem-bark extract elicited *in-vivo* antioxidant properties perhaps liable for some biological effects recorded. Hence, this study validated the folklore report with scientific findings.

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