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Ethanol Extracts of Pterocarpus soyauxii and Pterocarpus santalinoides

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Abstract

Recently, medicinal plants are gaining considerable attention for their therapeutic antioxidant activities. Though many studies have investigated the pharmacological and medicinal activities of Pterocarpus soyauxii and Pterocarpus santalinoides, there is limited knowledge of their antioxidant potential. Hence, this study aimed to assess the polyphenol content and investigate the in vitro antioxidant activity of these plants. Aqueous-ethanol extracts of the plants? leaves were obtained by maceration. The total flavonoid content (TFdC) and total flavonol content (TFlC) of the leaf extracts were determined by standard methods, while ferric reducing power and hydrogen peroxide scavenging assays were used to assess their in vitro antioxidant potentials. The mean TFdC of P. santalinoides (1083.33 ± 35.12 mg/g) was higher than that of P. soyauxii (730 ± 40 mg/g), while the mean TFlC was higher in P. soyauxii (390 ± 60.83 mg/g) than in P. santalinoides (260 ± 45.83 mg/g). The reducing potential of extracts of P. santalinoides was significantly higher ($p < 0.05$) than that of P. soyauxii, as well as the standard compound, at all concentrations tested. The hydrogen peroxide scavenging activity of P. santalinoides was superior to that of P. soyauxii, as well as ascorbic acid. The results of this study suggest that P. soyauxii and P. santalinoides are rich in flavonoids and flavonols and exhibit potent hydrogen peroxide scavenging activity and ferric reducing capacity, with the later showing greater activities. These properties may contribute to the therapeutic potential and medicinal applications of these plants and suggests a potential drug candidacy of flavonoid compounds of these species of Pterocarpus.

Index terms— antioxidants; Pterocarpus soyauxii; Pterocarpus santalinoides; flavonoids; flavonols, ferric reducing potential, hydrogen peroxide scavenging activity

1 Introduction

xidative stress contributes to many pathological conditions and diseases including cancer, stroke, diabetes, inflammatory diseases such as arthritis, cardiovascular disorders, etc. [1][2][3][4]. It results from an overwhelming level of free radicals or reactive oxygen species (ROS) such as hydroxyl radical, various peroxidises etc. [2,5,7]. The antioxidant defense systems under normal physiological conditions are sufficient only to cope with the normal threshold of the physiological rate of free-radical generation. Therefore, any additional burden of free radicals, either from endogenous or exogenous sources on the human physiological system may lead to oxidative stress [2,7]. Hence, supplementary sources of antioxidants are needed to prevent oxidative stress. Recently, medicinal and dietary plants are gaining considerable concern, as they are rich in micronutrients such as vitamin E (?-tocopherol), vitamin C (ascorbic acid) and ?-carotene, as well as plants secondary metabolites such as phenolic compounds, flavonoids, saponins, etc. [4,5,[8][9][10][11] which have been shown to exhibit promising therapeutic antioxidant properties. Though the activity of synthetic phenolic antioxidants is often observed to be higher than

that of natural antioxidants, [12] there is evidence of increased predisposition to various fatal diseases following use of synthetic antioxidants [4,[8][9][10], hence the renewed interest in natural antioxidants.

Sierra Leone and Equatorial Guinea [13,14]. Pterocarpus soyauxii and Pterocarpus santalinoides, locally known as "oha" and "uturukpa" respectively in Igbo, are abundant and widely consumed as vegetables in South-Eastern Nigeria [13,14]. They are traditionally used in the treatment of headaches, pains, fever, convulsions, skin rashes and respiratory disorders, and as antiabortive, antidiabetic, hepatoprotective and antimicrobial agents [13][14][15]. Though many studies have investigated the pharmacological and medicinal activities of these species [16][17][18], little is known about their antioxidant potential. Hence, this study was aimed to determine the polyphenol content of these plants and investigate the in vitro antioxidant activity of Pterocarpus soyauxii and Pterocarpus santalinoides.

2 II.

3 Materials and Methods

4 a) Plant materials

Fresh leaves of Pterocarpus soyauxii and Pterocarpus santalinoides were purchased from Ahia Abapka in Enugu, Enugu State of Nigeria. The plants were identified taxonomically by Prof C. U. Okeke (Department of Botany, Nnamdi Azikiwe University, Akwa) as Pterocarpus soyauxii (P. soyauxii) and Pterocarpus santalinoides (P. santalinoides). The leaves were air-dried at room temperature (28 ± 2°C) in the Biotechnology Laboratory of Godfrey Okoye University Enugu for seven days and thereafter pulverized before further processing.

5 b) Chemicals and reagents

Ethanol and ascorbic acid were purchased from JHD, Guangdong Guanghua Sci-Tech Co., **??**td

6 c) Maceration and extraction of plant materials

Extraction was carried out according to the methods of Bothon et al. [15] with slight modifications. Hundred gram (g) of the pulverized leaves of P. soyauxii and P. santalinoides were separately macerated in 500 ml of aqueous-ethanol for 48hours. The aqueousethanol extracts were prepared by adding 500 ml of an ethanol-water mixture (70:30) to 100g plant powder and mechanically stirred for 48 hours. The resulting solutions were filtered through Whatman No. 1 filter paper and the extracts obtained were then concentrated and finally dried to a constant weight. The extraction yields of the samples were calculated using the following equation: Total extraction yield, Y t (%)=

7 Mass of extract, M t

Mass of sample, M s ×100% Extracts were stored in sterile containers at 4 °C until further use.

8 d) Estimation of polyphenol compounds

i. Total flavonoids content Total flavonoids content of the plant extracts was determined based on the formation of an aluminium-flavonoids complex, using the methods described by Ordon Ez et al. [19]. A volume of 0.5 ml (2 %) aluminium chloride-ethanol solution was mixed with 0.5 ml of plant extracts (100 mg/l). The mixture was incubated at room temperature for 1 hr and the absorbance measured at 420 nm. All determinations were carried out in triplicates. The same procedure was repeated for the various concentrations (6.25 -100 mg/l) of a standard solution of rutin, and the rutin calibration curve was constructed. The concentration of flavonoids was expressed as rutin (mg/l) equivalent from the calibration curve of rutin (Figure 1) using the equation:Y = 0.001X -0.003, R 2 = 0.991 X = Y+ 0.003 0.001

where, Y was absorbance and X was concentration of rutin (mg/l). ii.

9 Total flavonols content

The total flavonols content was estimated based on the method of Kumaran and Karunakaran [20], using rutin as a reference compound. Two milliliters of the extracts (100 mg/l) were separately mixed with 2 ml of 2% aluminium chloride-ethanol solution and 3 ml of sodium acetate solution (50 mg/ml). The resulting solution was incubated at room temperature for two and half hours, and the absorbance was read at 440 nm. All determinations were carried out in triplicates. The same procedure was repeated for the various concentrations (6.25 -100 mg/l) of standard solution of rutin and the rutin calibration curve was constructed. The concentration of flavonols was expressed as rutin (mg/L) equivalent from the calibration curve of rutin (Figure 2) using the equation: Y = 0.001X + 0.008, R 2 = 0.990X = Y-0.008 0.001

where, Y was absorbance and X was concentration of rutin (mg/l). ii. Hydrogen peroxide scavenging activity The ability of the aqueous-ethanol extracts of P. soyauxii and P. santalinoides to scavenge hydrogen peroxide was determined using the methods of Yen and Chen [22]. A solution of hydrogen peroxide (4mM) was prepared in phosphate buffer (0.1 M, pH 7.0). The hydrogen peroxide solution (0.6 ml) was separately mixed with 4

ml of various concentrations of the extracts (1.25 -10.00 mg/ml) and incubated at room temperature for 10 min. Absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing plant extracts without hydrogen peroxide. Percent scavenging activity of the plant extracts was determined by following formula:H 2 O 2 scavenging activity (%) = ?1- absorbance of sample absorbance of control ? ×100%

Where, Absorbance of control was the absorbance of hydrogen peroxide radical + solvent; Absorbance of sample was the absorbance of hydrogen peroxide radical + sample extract or standard. Ascorbic acid served as standard.

10 iii. Statistical analysis

Experimental results were reported as mean ± Standard deviation (SD) of three parallel measurements. Unpaired T-test was performed to compare the means of the total flavonoids and flavonols content of the plant extracts. For other analyses, significant differences were established by Two-way ANOVA, followed by Tukey's multiple comparisons test, using GraphPad Prism version 6.05 for Windows. A difference was considered significant at $p < 0.05$.

11 III.

12 Results

13 a) Extraction yields

The percentage yield of Pterocarpus soyauxii and Pterocarpus santalinoides aqueous-ethanol extracts was 6.63% and 5.61% respectively.

14 B

solution was incubated at 50°C for 30 min, followed by addition of 2.5 ml of 10% trichloroacetic acid, and centrifugation of the resulting mixture at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferrous chloride (FeCl 3) and the absorbance was measured at 700 nm against a blank sample using a UV-5800(PC) UV/VIS Spectrophotometer. Increased absorbance of the reaction mixture was indicative of high reducing

15 b) Estimation of polyphenol compounds

The total flavonoids content (TFdC) and total flavonols content (TFlC) of aqueous-ethanol leaf extracts of P. soyauxii and P. santalinoides is summarised in Table 1. The mean TFdC level was higher in P. santalinoides (1083.33 ± 35.12 mg/g) than P. soyauxii (730 ± 40 mg/g), while the TFlC level was higher in P. soyauxii (390 ± 60.83 mg/g) than P. santalinoides (260 ± 45.83 mg/g). 3). The reducing potential of extracts of P. santalinoides was significantly higher ($p < 0.05$) than that of P. soyauxii, as well as the standard compound (ascorbic acid) at all concentrations tested. The reducing potential of the tested compounds was greatest in P. santalinoides, followed by ascorbic acid and least in P. soyauxii (P. santalinoides > ascorbic acid > P. soyauxii).

16 d) Hydrogen peroxide scavenging activity of P. soyauxii and P. santalinoides leaf extracts

Hydrogen peroxide scavenging activity of aqueous-ethanol leaf extracts of P. soyauxii and P. santalinoides was observed to be concentration dependent (Figure 4). P. soyauxii exhibited the lowest scavenging activity at all concentrations tested, with an exception of the extracts at 10.00 mg/ml concentration, which had a higher percent inhibition of hydrogen peroxide (99.63 %) than the standard antioxidant compound at an equivalent concentration (99.23 %). Similar to its reducing power activity, the hydrogen peroxide scavenging activity of P. santalinoides was superior to that of P. soyauxii, as well as ascorbic acid, with percentage inhibitions of 98.50, 99.07, 99.33 and 99.80 at 1.25 mg/ml, 2.50 mg/ml, 5.00 mg/ml and 10.00 mg/ml respectively. The only exception of enhanced hydrogen peroxide scavenging activity of standard compound over P. santalinoides was for the starting concentration of 1.25 mg/ml, with a percent inhibition of 98.83 % as against P. santalinoides with percent inhibition of 98.50 % at similar concentration.

17 IV.

18 Discussion

Plant secondary metabolites exert important functions in living plants. Flavonoids for instance, can protect against free radicals generated in plants [23]. High content of phenolics and flavonoids in medicinal plants have been associated with their antioxidant activities that play a role in preventing the development of chronic as well as age-related diseases, particularly caused by oxidative stress [6,10,24]. Preliminary phytochemical screening of Pterocarpus soyauxii and Pterocarpus santalinoides has revealed the presence of flavonoids in these plants [13,14]. Estimation of polyphenols in this study revealed the presence of both flavonoids and flavonols in extracts

of both Pterocarpus species. The total flavonoid content of aqueous-ethanol leaf extracts of P. santalinoides was significantly higher than that of P. soyauxii (p = 0.0003), while total flavonols concentration was higher in P. soyauxii than in P. santalinoides.

Flavonoids are well known for their antioxidant activity [8]. They are thought to exert their antioxidant activity by the mechanisms of radical scavenging and metal ion chelation to inhibit lipid peroxidation [4]. Several studies in recent years have shown that flavonoids, like other polyphenols in plants, scavenge reactive oxygen species and effectively prevent oxidative cell damage [1]. The activities of antioxidants have been ascribed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging [5,21]. The reducing power of a compound may thus serve as an important marker of its possible antioxidant activity [21]. Reducing power of a plant extract correlates with phenolic constituents in the plant [10]. In this assay, the oxidation form of iron (Fe +3) in ferric chloride is converted to ferrous (Fe +2) through electron transfer ability by antioxidant compounds [10,25]. The aqueous-ethanol extracts of P. soyauxii and P. santalinoides exhibited good reducing power activity at the different concentrations tested (Figure 3), however extracts of P. santalinoides showed a higher ferric reducing power than P. soyauxii and ascorbic acid at all concentrations tested. The observed higher ferric reducing activity of P. santalinoides over P. soyauxii may be attributed to its higher flavonoids content and possibly the presence of other bioactive compounds with antioxidant properties. Bothon et al. for instance, has reported the presence of coumarins in extracts of Pterocarpus santalinoides [15]. Coumarins are well established antioxidant compounds [26][27][28], hence their presence in P. santalinoides may potentiate the reducing power activity of these plants. The trend in the reducing power of extracts from P. santalinoides was similar to those of their hydrogen peroxide scavenging activities and the total flavonoids content, indicating that there is a correlation between the total flavonoids content and the antioxidant activities of plant extracts.

The ability of extracts of P. soyauxii and P. santalinoides to scavenge free radicals in vitro strongly suggests their antioxidant activity. Percentage inhibition of hydrogen peroxide (H 2 O 2) by both extracts was comparable to that exhibited by ascorbic acid, a standard antioxidant compound. In this study, this relationship was verified by the observation that both the total flavonoids composition and the H 2 O 2 scavenging activity of species of Pterocarpus tested were in the order of P. santalinoides > P. soyauxii. Scavenging of H 2 O 2 by plant extracts may be attributed to their phenolics and flavonoids which can donate electrons to H 2 O 2 , thus neutralizing it to water [29]. Although hydrogen peroxide is itself not very reactive, it is converted to highly reactive hydroxyl radicals by Cu 2+ and Fe 2+ ions, leading to lipid peroxidation, oxidative stress and cytotoxicity [30][31][32]. Thus, removing H 2 O 2 throughout biological systems, particularly the human body, is very important.

V.

19 Conclusion

Pterocarpus soyauxii and Pterocarpus santalinoides are shown to both be rich in flavanoid and flavonols compounds and exhibit potent hydrogen peroxide scavenging activity and ferric reducing capacity. This raises the possibility that phenolic-rich plants such as Pterocarpus soyauxii and Pterocarpus santalinoides could provide beneficial antioxidant effects in disease states characterized by oxidative stress conditions. Further in vitro and in vivo studies to validate the antioxidant potential of extracts of Pterocarpus soyauxii and Pterocarpus santalinoides are however suggested, to establish the potential drug candidacy of flavonoid and flavonols compounds from these plants. [1]

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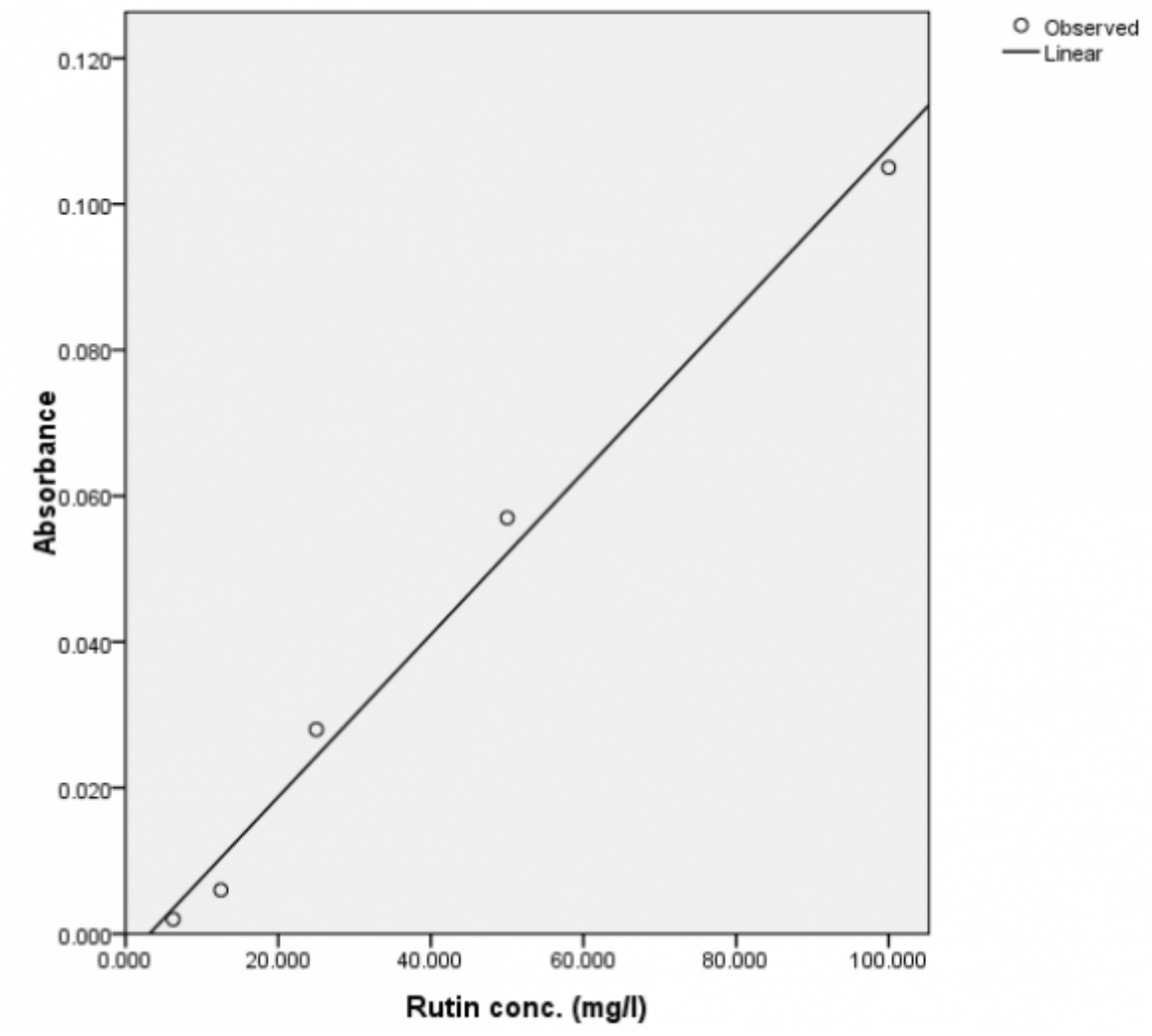

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Figure 1: Figure 1 :

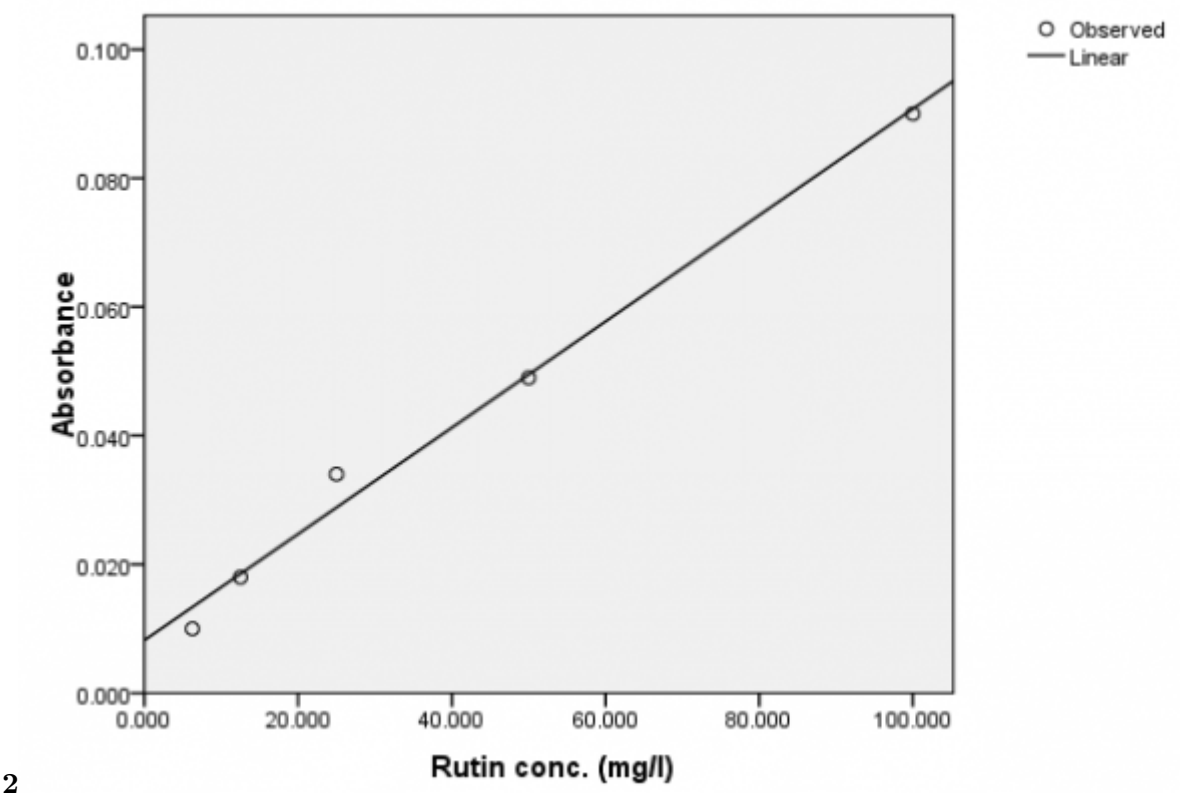

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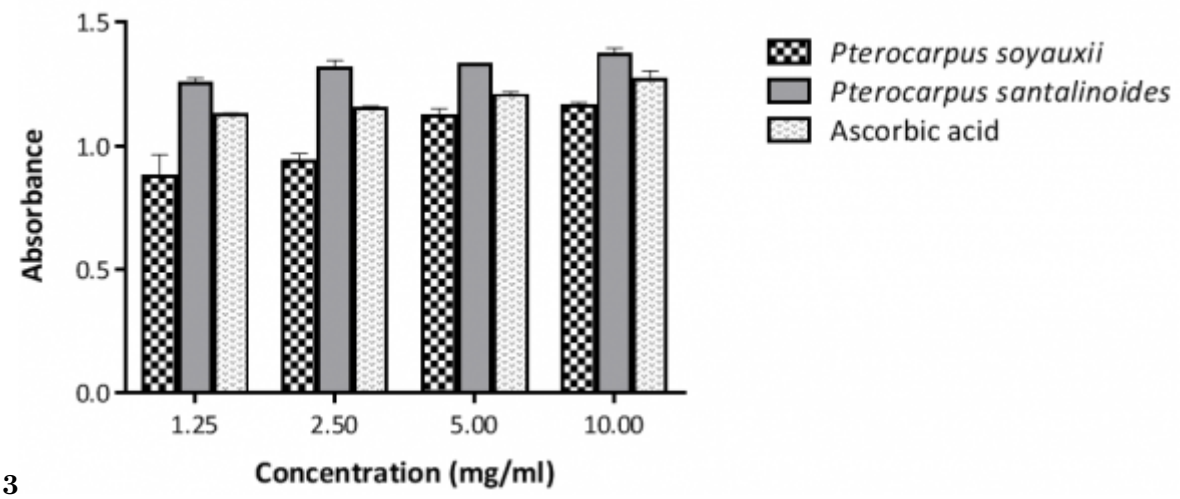
Figure 2: Figure 2 :



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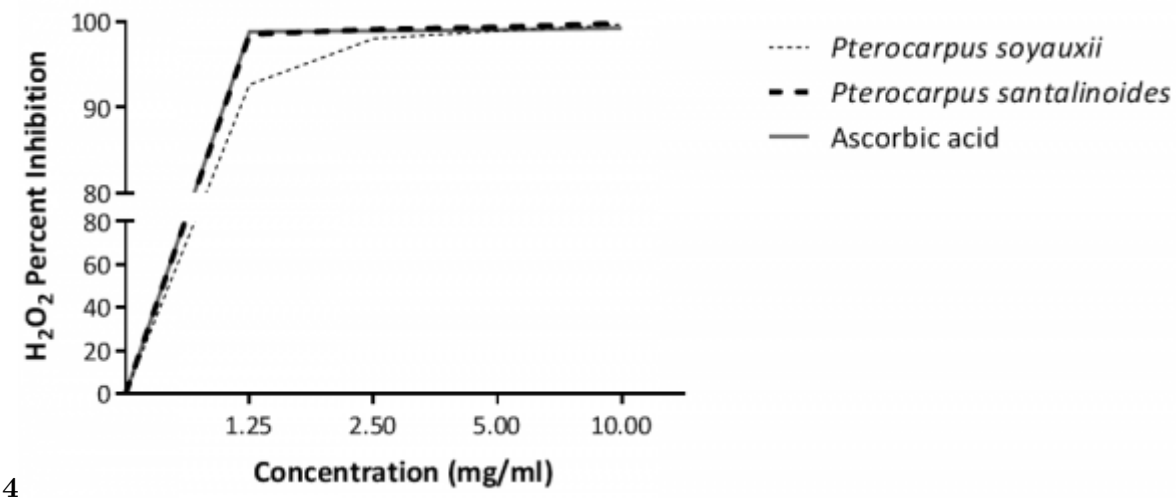

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Figure 4: PolyphenolFigure 4 :

1

Compounds	Concentration of rutin (mg/g)	
	P. soyauxii (PSO)	P. santalinoides (PSU)
Flavonoids (mg of RU/g of extract)	770	1120
	730	1050
	690	1080
Mean TFdC	730 ± 40	1083.33 ± 35.12
Flavonols (mg of RU/g of extract)	350	220
	360	310
	460	250
Mean TFlC	390 ± 60.83	260 ± 45.83

[Note: c) Reducing power activity of P. soyauxii and P. santalinoides leaf extractsThe reducing power of leaf extracts of Pterocarpus soyauxii and Pterocarpus santalinoides exhibited different degrees of electron donating capabilities, all in a concentration-dependent manner (Figure]

Figure 5: Table 1 :

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