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PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS OF *Erythrina senegalensis* STEM BARK

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ABSTRACT
Given the known medicinal value of erythrina senegalensis and presences of some phytochemicals being investigated, the stem bark of the plant was extracted using some known solvent sequentially from lower polarity (hexane) to high polarity (water) and their antioxidant activities were investigated using DPPH scavenging assay. The phytochemical compositions of some of the nutrient were also analyzed on dry weight basis. The study shows that, the plant stem bark contain 2.75 ± 0.15% alkaloids, 3.92 ± 0.06 % flavonoids, 7.42 ± 0.05 % saponnin and 1.16 ± 0.07 % phenol. The plant extracts is also observed to inhibit free radical activities as compared with standard ascorbic acid. This implies that the erythrina senegalensis stem bark has potential antioxidant agent hence it is justifies that the plant material is used as a remedy against some chronic diseases as claimed by the traditional healer.
Keywords: Anti-oxidant, phytochemical, erythina senegalensis, free radical scavenging.

INTRODUCTION
Oxidation and reduction of molecules are the common reactions in every cell leading to the formation of free radicals. These free radicals react with organic substrates within the body cells causing damage to the cells. Frequent oxidation cause an imbalance between the formation and termination of the free radical within the cell which gives rise to oxidative stress. This oxidative stress disturbs the normal functions of the cells resulting in the manifestation of variety of severe illness including cancer, heart disease, and other degenerative diseases (Neeharika, 2012).

There are evidences which suggest that by quenching the free radicals, antioxidants help reduce danger that might arise. The antioxidant may either be naturally synthized (body immune) or artificially synthesized (alternative) currently, the natural antioxidants are replacing synthetic molecules because of toxicities associated with the later (Neeharika, 2012). Erythina senegaalensis known as minjiriya in Hausa is used traditionally for the treatment of were malaria, jaundice, infections, among other diseases (Adiaratou, *et al.,* 2008).

In view of the above, the researchers' attempts to investigate the phytochemical composition and antioxidant activity of *erythrina senegalensis* stem bark.

MATERIALS AND METHODS

Sample collection and preparation
The Plant materials were collected in Biu, Borno state and identified in the biological science department of Abubakar Tafawa Balewa University. The stem bark was allowed to dry completely under shade and powdered into fine/coast powder using mortar and pistil.

Method of Extraction

The sample was extracted with hexane, ethyl acetate, acetone, methanol, and distilled water sequential in the order of increasing polarity using soxhlet extractor as described by Laurence and Christopher (1992). The extracts were concentrated in the distillation flask, typically by means of a rotary evaporator, yielding the extracted compounds which were dried in desiccators.

Quantitative determination of the chemical constituent

Determination of alkaloids

The alkaloid was determined as described by Sathya (2013). Five grams of the plant sample was placed in a 250ml beaker and 200ml of 10% CH_3CO_2H in C_2H_5OH was added. The mixture was covered and allowed to stand for 4 hours. It was then filtered, reduced to barest minimum volume. This was mixed with ammonium hydroxide until precipitate appear. The mixture was next allowed to stand the decanted. The precipitate was then collected on a fresh weighed filter paper and washed with dilute NH_4OH. The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was calculated by difference.

Determination of flavonoids

Ten grams of plant sample was repeatedly extracted using 100 ml of 80% methanol at prevailing temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was taken to a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference (Sathya, 2013).

Determination of saponins

Saponins were determination as described by Iqbal, et al. (2011). 5 g of each plant samples was weighed and was dispersed in 100 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The residue was re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage.

Determination of total phenols

The total phenol was determined as described by Iqbal, *et al.* (2011) and Mudasir, (2012). 5 g of the plant sample was weighed into a 250 ml titration flask and 100 ml n-hexane was added twice for 4 h each; the filtrates were discarded for fat free sample preparation. Then, 50 ml diethyl ether was added twice, was heated for 15 min each, was cooled up to room temperature and was filtered into a separating funnel. About 50 ml of the 10% NaOH solution was added twice and shook well each time to separate the aqueous layer from the organic layer. It was washed three times with 25 ml de-ionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10% HCl solution and 50 ml dichloro methane (DCM) twice to acidify the aqueous layer in the separating flask. Consequently, the organic layer was collected, dried and then weighed.

In-vitro Antioxidant Assay (1, 1-diphenyl-2-picrylhydrazyl) (DPPH) Radical Scavenging Assay)

DPPH free radical scarvenging activity was carried out as described by Deepika, and Rajinder, (2011), and Hemalatha *et al.,* (2010).The antioxidant activity of the plants water extracts were examined on the basis of scavenging effect on the stable DPPH free radical activity. 0.1mM of DPPH solution (0.0039g/l) was prepared in 95 % methanol. Each extract solution (25µg/cm^3, 50µg/cm^3 and 100µg/cm^3) was prepared in 95 % methanol. 1 ml of freshly prepared DPPH reagent was added to 3ml of each extract in a test tube and incubated in dark. After 30 minutes of incubation, the absorbance was measured at 517 nm using spectrophotometer. 25µg/cm^3, 50µg/cm^3 and 100µg/cm^3 of ascorbic acid solution were also prepared in 95% methanol and used as standard. A blank sample containing only same amount of methanol and DPPH was also prepared incubated for 30 minutes after then the absorbance was determined. These procedures were performed in triplicate. Free radical scavenging activities of the test sample expressed as percentage of inhibition were calculated according to the following equation.

Percentage (%) inhibition of DPPH activity

$$=.\frac{AB-AA}{AB}\ x\ 100$$

AA - absorbance value of test sample
AB – absorbance value of blank sample

RESULT AND DISCUSSION

Table 1: Phytochemicals composition of the four plant samples on dry weight basis expressed as percentage dry weight.

Plant Sample	% Alkaloids	% Flavonoids	% Saponnins	% Phenol
ESSB	2.75 ± 0.15	3.92 ± 0.06	7.42 ± 0.05	1.16 ± 0.07

ESSB = *Erythrina senegalsensis* stem bark

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Table 2: Antioxidant Activities of *Erythrina senegalensis* plant and *Datura metel* Seeds Extracts.

Sample	100µg/ml Absorbance	% Inhibition	50µg/ml Absorbance	% Inhibition
ESSB-WE	0.117	77.92	0.236	55.47
ESSB-ME	0.157	70.37	0.230	56.60
ESSB-ACE	0.152	71.32	0.250	52.83
ESSB-EAE	0.113	78.67	0.139	73.77
ESSB-HE	0.169	68.11	0.325	38.67
Control (Ascorbic acid)	0.092	89.64	0.103	80.56

Note: ESSB = Erythrina senegalensis stem bark; WE = water extract
ME = methanol extract ACE = acetone extract
EAE = Ethyl acetate extract HE = hexane extract

The phytochemical composition analysis shows that alkaloids constituted 2.75 ± 0.15%, flavonoids constituted 3.92 ± 0.06 %, saponnins constituted 7.42 ± 0.05% and phenols constituted 1.16 ± 0.07 %.

The reducing power of the plants extracts tested shows a remarkable inhibition (68.11% - 78.67%) at 100µg/ml concentration as compared with standard ascorbic acid (Vitamin C) (82.64%), (Vitamin C is an electron donor, a potent water-soluble antioxidant in humans) (Padayatty, 2003) . the ethylacetate extract shows the highest antixodant property, 78.67%, followed by water extract (77.92%), acetone extract (71.32%), methanol extract (70.37%) and for hexane extract is 68.11%, at 50µg/ml concentration, the percentage inhibition for the extracts ranges from 38.67%, hexane extract to 73.77%, ethylacetate extract. While that of Vitamin C at same concentration was 80.56 % (Table 2).

This study shows that, the potency of the *Erythrina senegalensis* stem bark extracts as an antioxidant activity shows concentration dependant (table 2). The antioxidant property may be due the presence of phenolic and flavonoid (Vinay *et al.,* 2010). The phytochemicals like phenolic acid, polyphenols, flavonoids, flavonols, terpenoids, vitamin C and vitamin E scavenge the free radical activity thus inhibiting the oxidative mechanism that lead to emergence of various disease as the molecules are electron rich (Neeharika *et al*., 2012).

CONCLUSION

The erythrina senegalensis stem bark contain some phytochemical to signicant amount (2.75 ± 0.15% alkaloids, 3.92 ± 0.06 % flavonoids, 7.42 ± 0.05 % saponnin and 1.16 ± 0.07 % phenol) (table 1). The plant extracts is also observed to inhibit free radical activities as compared with standard ascorbic acid, this is attributed to the presence and concentration of the phytochemical available in the plant. This implies that the erythrina senegalensis stem bark has potential antioxidant ingredient therefore, it is may conclude that eryrithrina senegalensis stem bark may be used as a remedy against some chronic diseases as claimed by the traditional healer.

CONFLICT OF INTEREST
The authors have no conflict of interest.

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