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Cytotoxicity and Antioxidant Evaluation of *Khaya senegalensis* Stems Bark

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ABSTRACT
The use of medicinal herbs in traditional system of medicine is a common practice in many cultures around the world, especially in African society. Medicinal plants are a great source of bioactive molecules for treatment of different pathology of microbial and non-microbial origin. *Khaya senegalensis* A. Juss belongs to the family Meliaceae and is being used by the herbal practitioner in the treatment of various ailments including fever, headache, amenorrhea, smallpox, jaundice, lumbago and rheumatism. The present study aimed at evaluating the cytotoxicity and antioxidant assay of the *Khaya senegalensis* stem bark extracts. The plant sample was extracted using soxhlet extractor with petroleum ether, ethylacetate, ethanol and water in that order. The extracts were after extracted were concentrated and dried in a desiccator the subjected for cytotoxicity test. The water, ethanol and ethylacetate extracts of the *Khaya senegalensis* stem bark shows LC_{50} at 146 µg/cm^3, 225 µg/cm^3 and 238 µg/cm^3 respective against brine shrimp which shows that stem bark in non-toxic. The antioxidant activity reveals that water, ethanol, ethylacetate and petroleum ether gives 68.17%, 66.47%, 67.23% and 58.38% inhibition respectively as compared to ascorbic acid, 82.64% inhibition all at 100 µg/cm^3 extract concentration. The extract therefore can be said to have good antioxidant potency.
Keywords: Non-toxic, Antioxidant, Cytotoxicity, *Khaya senegalensis*

INTRODUCTION
Medicinal plants have been used for the ailment of several microbial and non-microbial originated diseases due to their valuable effects in health care delivery. The accessibility, reliability, and low toxicity of most of medicinal plants in therapeutic use have made them popular and acceptable by good number of religions for implementation in medical health care all over the world (Akharaiyi, 2011). Plants are indeed the first material used in alternative medicine type of remedy against many diseases. Several plants have therapeutic and pharmaceutical effects, for antibacterial, anti-fungal, antioxidant, anti-infectious and antitumor activities. Herbal medicine has been widely used as an integral part of primary health care in many countries (Anju, *et al.,* 2012). The therapeutic effects of medicinal plants are attributed to the phytochemicals in them including: flavonids, alkaloids, steroids, trepenoids, phenolic acids, tannins, saponins among others (Nyamai *et al.,* 2016). These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with proline-rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Herbs that have tannin as their main components are astringent in nature and hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). The biological function of flavonoid includes protection against

allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxins and tumors (Okwu, 2004).

K. senegalensis A. Juss belongs to the family Meliaceae (mahogany); it is a tall evergreen tree which grows in the Sahara savannah area from Senegal to Uganda. *Khaya senegalensis* is a popular medicinal plant among the Nupes, Yorubas and the Hausa Fulani's in Nigeria. The aqueous stem bark extract is traditionally used by these tribes in the treatment of malaria, jaundice, edema and headache (Makut *et al.,* 2008). The Hausa and Fulani tribes in Northern Nigeria also use *K. senegalensis* as a remedy for several human and animal ailments (Celestine, 2016). The extract from the bark of *K.senegalensis* is characterized by its bitter constituents; it is used extensively as a bitter tonic for the treatment of a variety of pro-inflammatory disease. *K.senegalensis* is commonly used in African traditional medicine for pain and inflammation (Marvit, *et al.,* 2018).

In view of the above mentioned characteristics and uses of *K. senegalensis*, the study seeks to determine the cytotoxicity and antioxidant potential of the plant stem bark extracts.

MATERIALS AND METHODS

Sample Preparation

Khaya senegalensis stem bark was collected from Potiskum, Yobe state and identified in the Department of Biological Science, Abubakar Tafawa Balewa University, Bauchi. The plant material was air dried in the laboratory then powdered using mortar and pistil.

Extraction procedure

Soxhlet extraction was employed using petroleum ether, ethyl acetate, acetone, methanol, and distilled water sequentially in the increasing order of solvent polarity throughout the extraction. The soxhlet extractor setup consists of a round bottom flask, siphon tube, distillation path, expansion adapter, condenser, cooling water inlet, cooling water outlet, heat source and thimble. The extraction was carried out following standard method as described by Gopalasatheeskumar (2018).

100 g of the solid sample was placed inside a thimble made from thick filter paper which was loaded into the main chamber of the Soxhlet extractor. Extraction solvent is taken in the round bottom flask and heated by using heating source like heating mantle. The heating temperature is built on the solvent employed to extraction. Due to heat the solvent in the bottom flask vaporizes into the condenser and then drip back to the sample thimble. When liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the end of the process was indicated the clear solution in the siphon tube. The extracts obtained were concentrated under vacuum by using a rotary evaporator and dried in a desiccator. The percentage recoveries of the extracts were the recorded and keep for further analysis. The percentage recoveries were calculated using the formula:

$$\%\ recovery = \frac{mass\ of\ extract}{mass\ of\ sample} \times 100$$

Cytotoxicity Test

Brine shrimp eggs were commercially available. For this experiment, brine shrimp egg without shells "Artemia Revolution" 120 g were obtained from NT labs (fry care) laboratories LTD UK, Serial No. 7//3380900038///3.Made in England.Eggs were stored in a refrigerator at 5^0c**.**

Preparation of artificial sea water

Artificial sea water was prepared by dissolving 35 g of sea salt in1litre of distilled water for hatching the brine shrimp eggs.

Hatching of brine shrimp

An artificial seawater was Prepared at full strength. To obtain an optimum result a solution of specific gravity of 1.022 at 24ºC was prepared by dissolving 35g/dm^3 of sea salt, (NaCl) in water. The seawater was added to the brine shrimp hatcher in a heated aquarium aerate from bottom of the unit so that all eggs are kept in suspension and moving. The brine shrimp bottle was shaken before dispensing into the aquarium (each drop gives from 1500 to 2000 nauplii, three drops (5000 nauplii)) and are hatched in approximately 250 cm^3 sea water. The hatcher is illuminated very well for a minimum of three hours preferably for 12 hrs. The hatching time depend on temperature at 24ºC (which is an average tropical

aquarium temperature) hatching takes place between 24-48 hrs (maximum hatch 44-48 hrs). The Nauplii is then used directly for the cytotoxicity test

Brine shrimp cytotoxicity

Brine shrimps eggs were hatched in the laboratory at room temperature as explained above. 2 cm^3 of brine shrimp stock solution (containing about 15-20 shrimps) was added to 5 cm^3 of sea water in a vial the extracts in various concentrations (25, 50, 100, 200, 400 ppm) were added to the vials. An incubation period of 24 hrs was given at room temperature. Visual counting of living brine shrimp was performed at 0 hr, 6 hrs, 12 hrs and 24 hrs. The number of dead shrimp at 24 hrs was determined with the percentage mortality and the LC_{50} was determined (Adoum, 2009; Moshi, *et al.,* 2010).

Statistical analysis

The percentage of deaths and (LC_{50}) were determined using statistical analysis. Percentage mortality (M %) was calculated by dividing the number of dead nauplii by the total number, and then multiply by 100%.

$$percentage\ motality\ (\%M) = \frac{Total\ nauplii - Alive\ nauplii}{Total\ nauplii} \text{ x } 100$$

LC_{50} values was obtained from the graph plotted the percentage mortality against concentration of the crude extracts by determining the concentration at which 50% of the brine shrimp were dead (Adoum, 2009; Moshi, *et al.,* 2010).

The brine shrimp results in this study was interpreted as follows: LC_{50} <1.0 µg/ cm^3 – highly toxic; LC_{50} 1.0 to 10.0 µg/ cm^3 – toxic; LC_{50} 10.0 to 30.0 µg/ cm^3 – moderately toxic; LC_{50} 30 -100 µg/ cm^3 – mildly toxic, and LC_{50} > 100µg/ cm^3 as non-toxic (Moshi, *et al.,* 2010).

In-vitro Antioxidant Assay (DPPH Radical Scavenging Assay)

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scarvenging activity was carried out according to the method reported by Deepka and Rajinder (2011), and Bivasubramanian and Brindha (2013).

The antioxidant activity of the plants extract was examined on the basis of scavenging effect on the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity. DPPH solution (0.004% w/v) was prepared in 95 % ethanol. Five different concentrations of extracts under study were taken in test tubes. 1 ml of freshly prepared DPPH reagent was added to the test tubes and incubated in dark. After 10 minutes of incubation, the absorbance was measured at 517 nm using spectrophotometer. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared incubated for 10 mins and then the absorbance was measured. All determinations 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.

RESULTS AND DISCUSSION

Cytotoxicity Assay

The study reveals that, the percentage mortality rate ranges from 32.50% to 75.00% for water extract, 29.16% to 72.00 % for ethanol extract, 25.00 % to 63.63 % for ethyl acetate extract and 22.22% to 47.05% for petroleum ether extract over the extracts concentration of 25 µg/cm^3 to 400 µg/cm^3. The water, ethanol and ethylacetate extracts of the *Khaya senegalensis* stem bark shows LC_{50} at 146 µg/cm^3, 225 µg/cm^3 and 238 µg/cm^3 respectively against brine shrimp. This implies that, the *Khaya senegalensis* stem bark is non-toxic (Moshi, *et al.,* 2010).

Figure 1 gives the summary of the result.

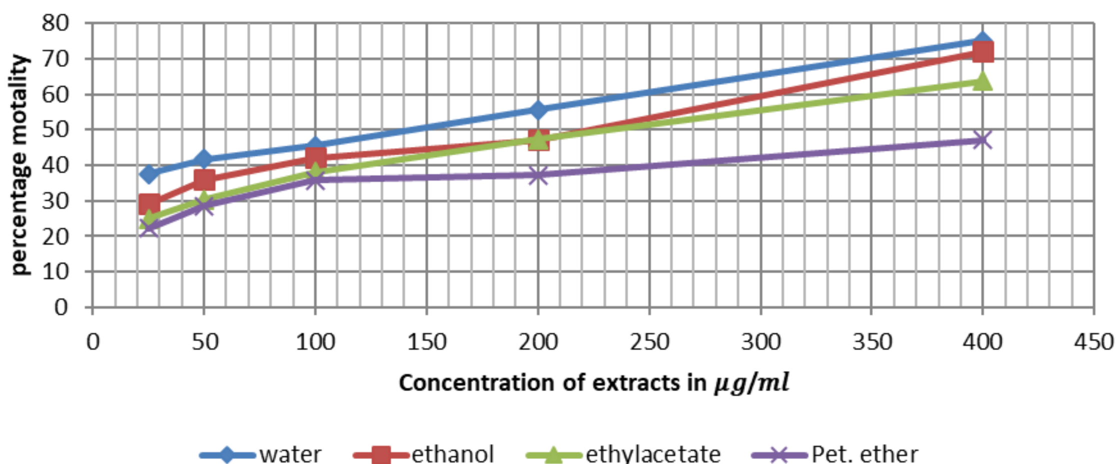

***Figure 1**: Brine Shrimp Assay of Khaya senegalensis Stem Bark Crude Extracts*

The cytotoxicity activity of the water, ethanol and ethylacetate extracts of the *Khaya senegalensis* stem bark in this study shows LC_{50} at 146 µg/cm^3, 225 µg/cm^3 and 238 µg/cm^3 respectively against brine shrimp. This is in line with the report from Omonike, peter and Adekunle (2017) who found the LC_{50} of *Acanthospermum hispidum, Alchornea laxiflora and Boerhavia* extracts at 183.70 µg/cm^3, 142.40 µg/cm^3, and 424 µg/cm^3 respectively.

Antioxidant activities of *Erythrina senegalsensis* Stems Bark Extracts

The reducing power of the plants extracts tested shows a remarkable inhibition (58.38% - 68.17%) at 100 µg/cm^3 concentration as compared with standard ascorbic acid (82.64%) and an inhibition range of 45.38% to54.99% at 50µg/cm^3 compared with the standard ascorbic acid (80.56%). The extract with the least reducing power is the petroleum ether extract, 58.38% inhibition at 100µg/cm^3 and at 50µg/ml concentration the percentage inhibition for the petroleum ether extract was 45.38%. Therefore *Khaya senegalensis* can be considered as having antioxidant potential. Table 1 gives the summary of the result.

Table 1: Antioxidant Activities of *Khaya senegalensis* Stem Bark Extracts

Sample	100µg/cm^3 Absorbance	% inhibition	50µg/cm^3 Absorbance	% inhibition
Water extract	0.169	68.17	0.239	54.99
Ethanol extract	0.178	66.47	0.262	50.65
Ethylacetate extract	0.174	67.23	0.252	52.54
Pet. ether extract	0.221	58.38	0.290	45.38
Control	0.092	82.64	0.103	80.56
Blank	0.530		0.530	

Key: pet. ether = petroleum ether, Control = Ascorbic acid

The antioxidant activity of *K. senegalensis* in this study shows an ihhibition of 68.17%, 66.47%, 67.23% and 58.38% for water, ethanol, ethylacetate and petroleum ether respectively. This study is in concurrence with Ukekpe *et al.,* (2019) who reported the scarvenging activity of acetone, ethanol and methanol extracts of stem bark of *K. senegalensis* as 66.282%, 84.838%, 85.921% scavenging ability respectively at 100µg/cm^3 compared with 97.437% of the standard, ascorbic acid. Atawodi et al., (2009) also reported that the leaves, stem bark and roots of *K. senegalensis* showed radical scavenging capacity with IC50 values of 178, 91 and 122 µl, respectively.

CONCLUSION

In conclusion, the findings of this study indicates that *Khaya senegalensis* stem bark is not toxic and can be said to have good antioxidant property compared with vitamin C, hence it can be applied in managing oxidative stress.

RECOMMENDATION

The researcher recommend that, further study should conducted to isolation, purify and the secondary metabolites responsible for the antioxidant activity.

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