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Research Article

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF STEM BARK CRUDE EXTRACT OF Danielliaoliveri.

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ABSTRACT
This research seeks to explore the phytochemical screening and antioxidant activity of stem bark crude extract of *Danielliaoliveri* stembark. The stem bark of the plant was extracted using various solvents with varying polarities. Standard methods were adopted to screen antioxidant and phytochemical nature of the plant. The results of extraction revealed the following order of percentage recovery: 3.82 <7.62 < 14.12 < 87.50 obtained from petroleum ether, chloroform, ethyl acetate and aqueous extracts respectively. Results of phytochemical screening of various solvent extracts revealed the presence of saponins, terpenoids, alkaloids, phenols, flavonoids, steroids and tannins in the stem bark of the plant. The antioxidant activity of the crude fractions of stem bark of the plant ranged from 21.19± 1.54 (IC_{50} = 47.74 µg/ml) to 47.01± 0.98(IC_{50}=21.29±0.44 µg/ml).The stem bark of the plant was found to contain good natural antioxidants. Hence it can be used for the treatment of inflammatory diseases, cardiovascular and diabetes.

Key words: phytochemical, extraction, *Danielliaoliveri*

INTRODUCTION

Medicinal plants are used by almost 80% of the world's population for their basic health care because of their low cost and ease in availability (Shazadi *et al*. Yakubu, *et al*., 2019) From the dawn of civilization, people have developed a great interest in plant-based drugs and pharmaceutical products(Yakubu, *et al*., 2019).Traditional medicine as defined by World Health Organization (WHO) may be summarized as the sum total of all the knowledge and practical, whether explicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing. Traditional medicine might also be considered as a solid amalgamation of dynamic medical know-how and ancestral experience (Mornier, 2016). Natural products are products from various natural sources – plants, microbes and animals. Natural products can be an entire organism (e.g. plant, an animal or a micro-organism), a part of an organism (e.g. leaves or flowers of a plant, an exudates), or pure compound (e.g. alkaloids, coumarins, flavonoids, lignin, steroids and terpenoids), isolated plants, animals or micro-organisms (Sarker and Nahar, 2007). From time immemorial, medicinal plants have been found to be important therapeutic aid for various ailments and diseases. Almost all cultures have depended partially or fully on herbal medicine because of its availability, efficacy, affordability, low toxicity and acceptability (Coker and Ogundele, 2002). Medicinal plants are the richest bio-resource of drugs in traditional systems of medicines, modern medicines, pharmaceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Tiwari, 2011).The therapeutic value of plants lies in some phytochemical constituents present in it that may be useful for healing of human diseases (Paradeepa *et al.,* 2016). Any part of plant may contain active components such as bark, leaves, flowers, roots, fruits and seeds (Santhi and Sengottuvel, 2016). In Nigeria, herbal therapies occupy a special position in health delivery especially among rural populace, because of easy acceptability and low cost of treatment enhance patronage (Adaku and Okwesili, 2008). *Danielliaoliveri* (Rolfe) Hutch & Dalziel (called Maje in Hausa) belongs to the family of fabaceae. It is commonly known as West African copal tree, African copaiba balsam, Ilorin balsam, Accra copal and Benin gum copal. The plant is found in both temperate and tropical regions of the world, Amazon region, South America and Africa. It is an indigenous plant of Africa found extensively in Benin, Cameroon, Gambia and Nigeria (Coker and Ogundele, 2002). *Danielliaoliveri*: (Rolfe) is a tall tree with conical crown whose shape is generally tapered. It can be recognized from the right

appearance of its trunk, light gray back. The leaves are peripinnately. Various parts of this plant such as bark, leaves and branches are medically important. Matured and young leaves are used to treat tuberculosis and headache respectively (Soro *et al.,* 2016). The plant is also used in Senegal to treat colic. The resin is used either externally or internally (or both) to treat vaginal discharge, bronchitis, renal pain, sore muscles and so on. The roots are used as aphrodisiac by some ethnic groups, as well as against tuberculosis. "Stipules", 10 – 15cm long, are used on wounds and ulcers. The roots are also used against mental diseases combined with leaves from *Ficus gnaphalocerpa*. The resin from this plant has traditionally been used to treat dental infections (Hope, 2005). In Nigeria, leaves of the plant are used in ethnomedicine to treat diabetes, diarrhea, and gastro intestinal disorder and as a diuretic (Augustine *et al.,* 2014). It is also used as herbal remedy for hyperglycaemia (Adaku and Okwesili, 2008). The aim of this research is to carry out phytochemical screening and antioxidant potential on stem bark crude extract of *Daniellia oliveri*

MATERIALS AND METHOD

Sample Collection and Preparation

The fresh stem barks of *Daniellia oliveri* were collected from Gudum na Hausawa, Bauchi Local Government Area of Bauchi state, and identified at the department of Biological Sciences, Abubakar Tafawa Balewa University (ATBU) Bauchi. Samples were then washed with running tap water 3 times, dried under shade, powdered using laboratory mortar and pestle, sieved and stored for further use.

Extraction of the Plant Materials

Dried powder (200g) was extracted on a shaker with 600ml of 50% ethanol at room temperature for 2 days. The extract was then filtered, concentrated using a rotary evaporator and dried in an oven at 40^0C to obtain a crude ethanol fraction (CF). The extraction yield was determined (29.50%). Partition of the CF was performed further by the method of Zhao *et al.,* with slight modification as described by Parasad *et al*, (2009). The dried CF was dissolved in 100ml water and then partitioned sequentially with 50ml each of petroleum ether, chloroform, ethyl acetate, and water respectively 3 times each. The fraction of each solvent was collected and concentrated using a rotary evaporator to remove the solvents. The various solvent extracts were stored in a refrigerator for further use.

Preparation of Reagents

Dragendoff's reagent

Dragendoff's reagent is a color reagent used to detect alkaloids in a test sample. Alkaloids, if present in the solution of sample, will react with Dragendoff's reagent and produce an orange or orange red precipitate. This reagent was invented by the German pharmacologist, John Georg Dragendroff (1836 – 1898) at the University of Dorpat. Dragendoff's reagent is a solution of potassium bismuth iodide prepared from basic bismuth nitrate ($Bi(NO_3)_3$), tartaric acid, and potassium iodide (KI) (Wikipedia, 2017). Dragendoff's reagent was prepared as follows: $Bi(NO_3)_3$ (0.88g) was dissolved in a mixture of distilled water (40ml) and acetic acid (10ml). KI (8.0g) was weighed separately and dissolved in 20ml of distilled water. The two solutions were mixed together in a 250ml volumetric flask and made up to volume with distilled water.

Ferric chloride solution

Preparation of 2% ferric chloride solution

Ferric chloride (2.0g) was weighed in a beaker containing small quantity of water and then transferred quantitatively into a 100ml volumetric flask and water was added to make up to volume.

Preparation of 0.1% ferric chloride solution

Ferric chloride (0.1g) was weighed and dissolved in a beaker containing small amount of water and then transferred quantitatively into a 100ml volumetric flask and distilled water was added to make up to volume.

Preparation of 10% ferric chloride solution

Ferric chloride (10g) was weighed and dissolved in a beaker containing small amount of water and then transferred quantitatively into a 100ml volumetric flask and made up to capacity with distilled water.

Phosphate buffer solution

Preparation of 1.00M phosphate buffer solution

Phosphate buffer (KH_2PO_4) (16.31g) was weighed and dissolved in a beaker containing small amount of water and then transferred quantitatively into a 100ml volumetric flask and made up to capacity with distilled water.

Preparation of 0.20 M phosphate buffer solution

Phosphate buffer (1.00M) (20ml) was measured into a 100ml volumetric flask and made up to volume with distilled water.

Preparation of Potassium ferricyanide (1g/100cm^3)

Potassium ferricyanide (1g) was weighed and dissolved in a beaker containing small amount of water and then quantitatively transferred into a 100ml volumetric flask and made up to volume with distilled water.

Preparation of Trichloroacetic acid (10g/100cm^3)

Trichloroacetic acid (10g) was weighed and dissolved in a beaker containing small quantity of water and then transferred quantitatively into a 100ml volumetric flask and made up to mark with distilled water.

Preparation of Extracts

Stock solution

The extracts (200mg) each was weighed and dissolved in a beaker containing small amount of water and then quantitatively transferred into 100ml volumetric flask and made up to volume with distilled water. The resulting solution has a concentration of 2mg/ml.

Serial dilution of the extracts

The stock solutions (1ml) each was measured and transferred quantitatively into a 100ml volumetric flask and made up to volume with distilled water. The resulting solutions each has a concentration of 0.02mg/ml (20 μg/ml).

Phytochemical Screening

The presence of bioactive compounds were tested in the extracts using standard methods as described by Santhi and Segottuvel, (2016), Yadav and Agarwala, (2011).

Detection of phenolic

Ferric chloride test

Extracts (10 ml) each was treated with few drops of ferric chloride solution (2%).Formation of bluish black color indicated the presence of phenols.

Detection of flavonoids

Sulfuric acid test

Extracts (0.5 ml) were treated with few drops of concentrated H_2SO_4. Formation of orange color indicated the presence of flavonoids.

Detection of terpenoids

Salkowski's test

The extracts (1ml) each were mixed with chloroform (2ml) and concentrated H_2SO_4 (3ml) was carefully added to form a layer. An appearance of reddish brown color in the inter face indicated the presence of terpenoids.

Detection of saponins

Crude extracts (0.5 mg) were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Detection of steroids

Crude extracts (2 ml) were mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.

Detection of alkaloids

The test solutions were mixed with little amount of HCl and Dragendoff's reagent. Formation of white precipitate indicated the presence of alkaloids.

Detection of tannins

The extracts (0.5ml) each were mixed with 1ml of distilled water and 3 drops of 10% ferric chloride solution were added. Formation of blue or green black coloration indicated the presence of tannins.

Antioxidant Activity by Reducing Power Assay

This method was described by Rajan *et al.*, (2010). The method is based on the following principle:
Substances which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm.

METHODOLOGY

A 1ml of the plant extract solutions (20μg/ml) were mixed with 2.5ml phosphate buffer (0.2m. pH 6.6) and 2.5ml potassium ferricyanide [$K_3Fe(CN)_6$] (1g/100cm^3), then the mixture was incubated at 50^{0}C for 20 minutes. To this, 2.5ml of trichloacetic acid (10g/100cm^3) was added and centrifuged at 3000rpm for 10 minutes. Finally, 2.5ml of the supernatant solution was mixed with 2.5ml of distilled water and 0.5ml $FeCl_3$ (0.1%) and the absorbance was measured at 700nm in UV-visible spectrophotometer and phosphate buffer was used as blank solution. The absorbance of the final reaction mixture was expressed as mean $\pm$ standard error of the mean of triplicate. Increased absorbance of the reaction mixture indicates stronger reducing power. The antioxidant activity of the stem bark extract was expressed as IC_{50} and compared with standard.

Assessment of % inhibition and IC_{50}

Radical scavenging activity of extract and standard were expressed in terms of % inhibition. It is calculated by using the formula:

$$IP = \left[\frac{A\ control - A\ sample}{A\ control}\right] \times 100$$

Where A control is the absorbance of the control and A sample is the absorbance in the presence of the sample of ethanol, petroleum ether, chloroform, ethyl acetate and aqueous extracts. The cIC_{50} value is defined as the concentration (in μg/ml) of extracts that produced 50% antioxidant effect.

$$IC_{50} = \frac{Concentration\ of\ extract}{\%\ inhibition} \times 50$$

Statistical analysis

All data were expressed as mean $\pm$ SEM. Statistical analysis was performed by one way ANOVA using manual method and confirmed by SPSS software version 20 and $P \leq 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Results

Extraction

The stem bark of *Daniellia oliveri*was extracted using various solvents, the nature and crude extract recoveries are expressed in table 1 below:

Table1: Nature and Recovery of the Crude Extracts of *Daniellia oliveri* Stem Bark

Extracts	Texture	Color	Wt of Sample (g)	Wt of Extracts (g)	% Recovery
Pet. Ether	oily, sticky Semi solid	Dark Brown	55	2.1	3.82
Chloroform	solid powder	Light Brown	52.5	4	7.62
Ethyl acetate	semi solid	Brown	47.5	6.5	14.12
Aqueous	semi solid	Pale Brown	40	35.0	87.5
Ethanol	oily sticky solid	Raddish Brown	200	59.0	29.5

Wt = weight

Phytochemical Screening of the Sample

The crude extracts were screened for the presence of secondary metabolites and the result obtained are tabulated in table 2 below

Table 2: Phytochemical Screening of Various Solvent Extracts of *Daniellia oliveri* Stem Bark

Phytochemicals	EE		PE		CE		EAE		AE	
saponin		+		-		+		+		+
Phenol	+		-		-		+		+	
Steroid	-		+		-		-		-	
Terpenoid		+		+		+		+		+
Alkaloid		+		+		-		+		+
Flavonoid		+		-		-		+		+
Tannin	+		-		-		+		+	

Keys: + =present, - = absent
EE = Ethanol extract, PE= Petroleum ether extract
EAE = Ethyl acetate extract, AE= Aqueous extract
CE =Chloroform extract

Antioxidant Activity

The antioxidant activity of the crude extracts was carried out by Reducing Power Assay Method and the results were presented in table 3 below

Table3: In Vitro Free Radical Scavenging Effect of Crude Extracts of *Danielliaoliveri* Stem Bark by Reducing Power Assay Method

Extracts (20 μg/ml)	I.P	IC_{50}
Ethanol	39.11 ± 1.16^{b}	25.46 ± 0.62^{bcde}
Pet. Ether	21.19 ± 1.54^{d}	47.74 ± 3.75^{a}
Chloroform	34.80 ± 1.15^{c}	28.79 ± 1.59^{bce}
Ethyl acetate	47.01 ± 0.98^{a}	21.29 ± 0.44^{bde}
Aqueous	42.63 ± 1.08^{b}	23.49 ± 0.58^{bcde}

Key: I.P= Percentage Inhibition $I.C_{50}$ = concentration (in μg/ml) of extracts that produced 50 % anti-oxidant effect

NOTE: The results are Mean ± Standard Error of the Mean (n = 3). Values on the same column with the same letters are significantly the same, while values on the same column with different letters are significantly different at $p \leq 0.05$.

Discussion of Results

Table 1 shows the nature and recovery of each fraction of *Daniellia oliveri*stem bark. The aqueous fraction gave the highest percentage recovery (87.50 %) while petroleum ether fraction gave the lowest percentage recovery (3.82 %).The highest percentage recovery of the aqueous extract might be due to the higher polarity index of water,]it has been noted that percentage recovery increases with increase in the polarity of the solvent, for this research, the order of percentage recovery of the various solvent extracts is 3.82 < 7.62 < 14.12 < 87.50 obtained from petroleum ether, chloroform, ethyl acetate & aqueous extracts respectively. The results of

phytochemical screening of various solvent extracts of *Daniellia oliveri*stem bark (Table 2) revealed the availability of many polar and non-polar chemical constituents. The results revealed the presence of saponins, terpeniods, alkaloids, phenols, flavonoids, steroids and tannins in the stem bark of the plant. All the tested phytochemicals except steroids were found to be present in ethanol, ethyl acetate and aqueous extracts; steroid, terpenoid and alkaloid were present in petroleumether extracts while chloroformextracted only saponin and terpenoind. The presence of some phytochemicalsin one extract and their absence in the other might be due to variation in solvent polarity which agrees with the Rule of Thumb which says 'like dissolves like'. Table 3 shows the results of antioxidant activity of various extracts of *Daniellia oliveri*stem bark with their corresponding IC_{50}. The results ranged from 21.19±1.54 ($IC_{50}$of 47.74 ±3.75 μg/ml) to 47.01 ± 0.98 (IC_{50} of 21.29 ±0.44 μg/ml). The highest value was obtained from ethyl acetate fraction and the lowest value was obtained from petroleum ether extract. The higher the value of IP (low IC_{50}value), the higher the antioxidant activity. The order of polarity index of the solvent is: EA <ETOH<water. Although, ethyl acetate has the lowest polarity index, it has highest molecular weight compared to ethanol and water. It has been noted (Kassim *et al.,* 2011),that the higher the molecular weight of the solvent the lower the polarity, which allow other substances of about the same molecular weight like catechins to be easily extracted, hence, ethyl acetate extract has a better antioxidant activity. All the extracts except Petroleum ether extracts how potent antioxidant activity when compared with ascorbic acid standard with IP of 32.91 ±1.15at 20 μg/ml (IC_{50} of 34.63±5.41 μg/ml at 100 μg/ml concentration of the standard)as determined by Rajan *et al.,* (2011). Based on the results of this study, the crude stem bark extracts of Daniellia *oliveri* were found to contain good natural antioxidants. The antioxidant activities shown by the crude fraction explain the traditional use of the plant in the treatment of inflammatory diseases, cardio vascular and diabetes. All the results of IP are significantly different with the exception of ethanol and aqueous extracts which are significantly the same at $P \leq 0.05$.The results of IC_{50} show that IC_{50} of petroleum ether extract is significantly different from that of the other extracts, while the IC_{50} of ethanol and aqueous extracts is significantly the same and that of the chloroform extract is significantly the same with all other extracts except petroleum ether extract at 95% confidence level, this show that values that are statistically the same have almost similar antioxidant activity.

CONCLUSION

Based on the results of this study, it is concluded that crude stem bark extracts of Daniellia *oliveri* contain good natural antioxidants. The antioxidant activities shown by the crude fraction explain the traditional use of the plant in the treatment of inflammatory diseases, cardio vascular and diabetes.

Recommendation

In the light of the results of antioxidant activity of stem bark of this plant, the following further studies on the stem bark of the plant are recommended: Determination of total phenol and flavonoids of the crude extracts should be carried out in order to establish relationship between antioxidant activity and total phenolic and flavonoids contents of *Daniellia oliveri*. Isolation and identification of the compounds responsible for the antioxidant activity of the stem bark crude extract of *Daniellia oliveri*.

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