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(RESEARCH ARTICLE)

Phytochemical analysis and free radical scavenging activity of *Isoberlinia doka* leaves

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Abstract

Isoberlinia doka is dominant species in the savannah of West Africa belonging to the family Fabaceae. It is used in traditional medicine for treatment of muscular – skeletal system disorders, Jaundice scorpion bites, Convulsion, diabetes, ulcer, wounds and cough. The objectives of the study are to determine the pharmacognostic and antioxidant activity of *Isoberlinia doka* leaves. Three extracts of *Isoberlinia doka* leaves were obtained by sequential maceration using n-hexane, ethyl acetate and methanol The extracts were screened qualitatively for the presence of saponins, carbohydrates, tannins, flavonoids, cardiac glycosides, anthraquinones and alkaloids using standard procedures. Thin layer chromatography was carried out to determine the separation profile of various extracts. Antioxidant activity of the methanol leaves extract of *Isoberlinia doka* was determined using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) Assay. The phytochemical screening of the methanol extract revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides and saponins. The methanol leaves extract of *Isoberlinia doka* demonstrated strong radical scavenging activity and reducing power ability with concentration dependent responses. The results of this study suggest that the plant can serve as a good source of antioxidant which can aid in the management of diseases associated with oxidative stress.

Keywords: *Isoberlinia doka*; Free radical scavenging; Phytochemical analysis; 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay

1. Introduction

Medicinal plants play a pivotal role in the health care since ancient times. Many cultures use mainly plant based drugs or formulations to treat various human ailments because they contain chemical constituents with therapeutic value [1] Free radicals play a major part in the development of chronic and degenerative ailments, the imbalance in the ROS generating and scavenging systems in the body causes damage of tissue and cellular organs [2]. Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, carcinoma and Parkinson's disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources [3]. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases [4]. Medicinal plants are important source of antioxidant compounds; these natural antioxidants reduce the risk of many chronic diseases. The secondary metabolites- like phenolic compounds and flavonoids- from plants have been reported to be the potent free radical scavengers, they are found in all parts of plants such as leaves, fruits, seeds, roots and barks [5].

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Isoberlinia doka (Fabaceae) is a tree 10-18m high or more. *Isoberlinia doka* is distributed in west and central Africa from Guinea in the west to Sudan, Uganda in the east. It is not known to occur in the South of the Equator [6]. The tree has been used by traditional medical practitioners for the treatment of diabetes, ulcer, wounds and cough [7]. The study was carried out to determine the phytochemicals present the free radical scavenging activity of the plant.

2. Material and methods

2.1. Plant collection and identification

The Plant was collected in Samaru, Zaria Local Government Area of Kaduna State, Nigeria in April, 2017. The plant was identified and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, where the voucher specimen is deposited.

2.2. Drying and storage

The leaves of *Isoberlinia doka* was shade dried for seven days, after which it was grounded using a pestle and mortar, weighed and packed in an airtight container for further use.

2.3. Method of extraction

The powdered leaves of *Isoberlinia doka* was extracted by maceration, using methanol as solvent. 500 g of the powdered sample was weighed and macerated in 2500 ml of methanol for 24 hours. The extract was filtered.

2.4. Phytochemical analysis

N-hexane, ethyl acetate and methanol extracts were analyzed for presence of phytochemicals using standard procedures [8].

2.4.1. Test for Flavonoids

Shinoda test

10 mg of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of pink color indicates the presence of Flavonoids.

Lead acetate test

10 mg of extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

2.4.2. Test for phenols and tannins

Lead acetate test

10 mg of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

Ferric chloride test

5 mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

Sodium hydroxide test

5 mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

2.4.3. Test for anthraquinones

Borntragers test

About 0.5 g of the extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinone.

2.4.4. Test for carbohydrates

Molisch's test

Small portion of the extract was put in a test tube; 10 ml of distilled water was added and shaken vigorously and gently. The mixture was then filters and divided into two portions. To the first portion, two drops of Molish's reagent was added followed by few drops of concentrated sulphuric acid by the wall of the test tube. Formation of brown or purple ring at the interphase indicated the presence of carbohydrates.

Fehling's test

To the second portion of the filtrate, few drops of Fehling's solution A & B was added and boiled for 5 minute on a water bath. Brick red coloration indicates the presence of reducing sugar.

2.4.5. Test for alkaloids

A small portion of the extract was stirred in 5 ml of 1% aqueous hydrochloric acid on a steam bath, allowed to cooled and filtered. 1 ml of the filtrate was treated with a few drops of Meyer's reagent and to another 1 ml of the filtrate, a few drops of Dragendorff reagent was added. To another 1 ml of the filtrate, a few drop of Wagner's reagent was added. Turbidity or precipitation with either of the reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated [9].

2.4.6. Test for saponins

Frothing test was used to detect the presence of saponins. Small quantity of the plant extract was mixed and shaken with water in a test tube. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins [10].

2.5. Radical scavenging activity DPPH assay

The effect of *Isoberlinia doka* on DPPH radical was assayed using the modified method of Mensor *et al* [11] Sample stock solution of each extract (0.1 mg/ml) will be diluted with methanol into various concentrations: 100, 80, 60, 40, 20 µg respectively. To each sample, 2.5 ml (20, 40, 60, 80, and 100 µg/ml in methanol) will be added to 1 ml of DPPH solution (0.2 mM in methanol). After 30 minutes of reaction at room temperature, the absorbance of the solution will then be measured at 518 nm. The free radical scavenging activity of the extract will be determined by comparing its absorbance with that of a blank solution (no sample). The ability to scavenge the DPPH radical will be calculated using the following equation;

$$Percentage\ inhibition\ of\ absorbance = \frac{A'control - A'sample}{A\ control} \times 100$$

Where A'control is the absorbance value of the control group, and A'sample is the absorbance of the sample.

3. Results and discussion

The phytochemical screening of the *Isoberlinia doka* leaves revealed the presence of alkaloids, flavonoids, tannins and saponins in the methanol extract. The methanol leaves extract of *Isoberlinia doka* demonstrated strong radical scavenging activity and reducing power ability with concentration dependent responses comparable to ascorbic acid.

The extracts revealed the presence of saponins, carbohydrates, tannins, flavonoids, cardiac glycosides and alkaloids. The medicinal values of plants are due to the presence of phytochemicals, which produce definite physiological actions [12]. Phenolic compounds such as tannins, flavonoids and saponins show free radical activity [13]. Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage

caused by free-radicals [14]. They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers [15].

Table 1 Preliminary phytochemical screening of *Isoberlinia doka* leaves

Sr. No.	Plant constituents	Test	N-hexane extract	Ethyl acetate extract	Methanol extract
1	Alkaloids	Wagner	Absent	Absent	Present
		Dragendorff	Absent	Present	Present
		Mayer	Absent	Present	Present
2	Flavonoids	Shinoda	Absent	Present	Present
		Lead sub-acetate	Absent	Present	Present
3	Saponins	Frothing test	Absent	Present	Present
4	Tannins & Phenols	Lead acetate	Absent	Present	Present
		Ferric chloride	Absent	Present	Present
5	Anthraquinones	Borntrager's	Absent	Absent	Absent
6	Carbohydrates	Molish	Present	Present	Present
		Fehling	Present	Present	Present
7	Steroids	Salkowski test	Present	Absent	Absent

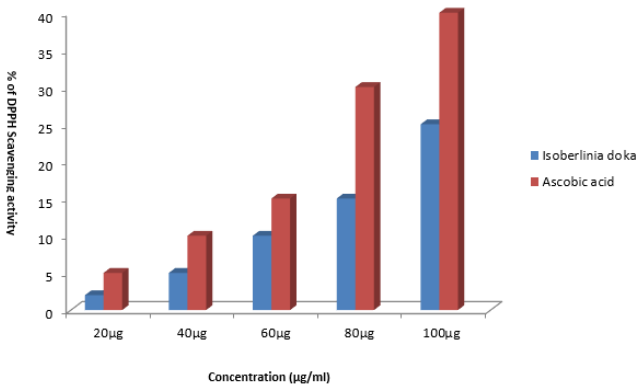

Figure 1 DPPH free radical scavenging activity of *Isoberlinia doka*

Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases [4]. DPPH is a stable radical commonly used to determine the antioxidant activity of various compound. It is a stable free radical because of its spare electron delocalization over the whole molecule. This method is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant, inducing a color change from purple to yellow at 517 nm. The degree of reduction in absorbance measurement indicates the radical scavenging (antioxidant) power of the extract [16]. The result of the antioxidant activity shows a dose dependent free radical scavenging activity comparable to ascorbic acid. Several studies have shown that the scavenging effect on the DPPH radical increases sharply with the increasing concentration of the samples and standards to a certain extent [17].

4. Conclusion

The results of this study suggest that the plant can serve as a good source of antioxidant which can aid in the management of diseases associated with oxidative stress.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of Interest

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