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**Phytochemical Constituents and Antifungal Studies of Methanol Leaf
 Extract and It's Fractions of *Isoberlinia Doka***

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ABSTRACT

Isoberlinia doka is a plant species 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 aim of the study was to conduct phytochemical and antifungal studies of methanol leaf extract and its fractions of *Isoberlinia doka*. The plant material was collected identified and air dried. Powdered plant material was subjected to maceration using methanol to obtained crude methanol leaf extract which was partitioned using *n*-hexane, chloroform ethyl acetate and *n*-butanol. The extract and fractions obtained were subjected to phytochemical screening using standard procedure, the antifungal studies of the methanol extract and its fractions against *Candida albican*, *Candida crusic*, *Trychophyтом rubrum* and *Trychophyтом mentagrophyte* were investigated using agar well diffusion method at different concentration (100 – 12.5 mg/mL). The phytochemical screening revealed the presence of various secondary metabolites which varies in the extract and fractions. The methanol extract and its fractions showed significant ($p < 0.05$) antifungal activity against all the fungal isolates with the ethylacetate fraction having the highest mean zone of inhibition ranging from 8-23 mm followed by the *n*-butanol fraction with the mean zone of inhibitions from 6-21 mm the methanol extract and the *n*-hexane fraction had the mean zone from 5-17 mm and 6-19 mm respectively while chloroform recorded lower mean zone of inhibition from 6-14. The standard drug fluconazole had the mean zone of inhibition from 6-20 mm. The most sensitive organism was *Candida albican*, while the least sensitive organisms were *T. mentagrophyte* and *T. rubrum* for the ethyl acetate fraction. The study has validated the ethnomedicinal claim for the use of this plant in treatment of the fungal infections.

Keywords: Phytochemical screening, Fungal isolates, *Isoberlinia doka*, Fractions and Fluconazole

1.0 INTRODUCTION

Natural products are chemical compounds or substances isolated from living organism. The chemistry of natural product includes their biosynthesis, extraction, identification, quantification, structural elucidation, chemical reactions, physical, and chemical properties. They are produced by the pathway of primary or secondary metabolism (Nwokeji *et al.*, 2016). Natural products compounds can be in the form of primary or secondary metabolites, the plant secondary metabolites are organic compounds or phytochemicals that are not directly involved in the normal growth, development or reproduction of the plant. Their biosyntheses are derived from primary metabolism pathways, which include tricarboxylic acid cycle (TCA), methyl erithrotol phosphate

(MEP) pathway, mevalonic and shikimic acid pathway. They can be extracted from plants using organic solvents and modern separation techniques to get the analyte of interest (Nwokeji *et al.*, 2016). *Isoberlinia doka* is a plant species 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 (Ahmed *et al.*, 2018). Traditionally, *Isoberlinia doka* presented in fig 2 is also used for posts, poles, handicrafts and agricultural implements. It is suitable for light construction, flooring, panelling, moulding, ship building, railway sleepers, boxes, crates, veneer and pulpwood. As there are no physical differences between the heartwood and the very wide sapwood,

the latter can be used just like the heartwood after treatment with preservatives. Wood ash is used in soap making. The inside of the fruits is used to scour earthenware pots. Several parts of the tree have traditional medicinal uses. In Benin a decoction of young leafy shoots enters into a potion or bath against convulsions; gum from branches is used to treat abscesses and purulent infections. In northern Ghana the leaves are used as fixative when dyeing nails with henna (Ahmed *et al.*, 2018). Previous phytochemical screening on other plants revealed the presence of alkaloid, tannins, saponins, flavonoids, carbohydrate glycosides and steroid (Mukhtar *et al.*, 2022). Bassey (2012) reported the isolation of flavone from *Selaginella tamariscina* exhibited potent antifungal activity against several pathogenic fungal strains and has a very low *hemolytic* effect on human *erythrocytes*. The three compounds eupomatenoid-3, eupomatenoid-5, and conocarpan isolated from *Piper solmsianum* exhibited antifungal action

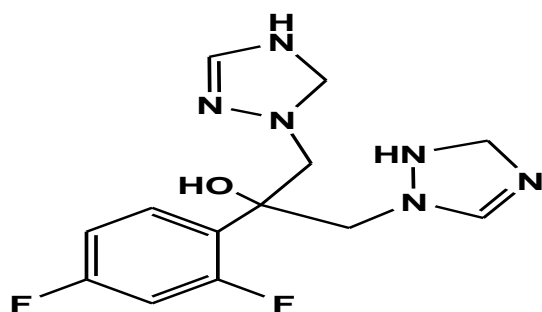


Fig: 1 Fluconazole

1.2 Objective of the Research

The objective of the research was to carry out preliminary phytochemical screening on the methanol leaf extract and fractions of *I. doka* using

2.0 MATERIALS AND METHODS

2.1 Sample Collection, Identification and Drying

The *Isobberlinia doka* was collected from Zuru L.G.A in Kebbi State of Nigeria. The sample was identified at the Herbarium Unit of Biological Sciences, Usmanu Danfodiyo University Sokoto by

against the tested *dermatophytes* tested. Ahmed *et al.*, (2018) reported the free radical scavenging activity of *Isobberlinia doka* leaves.

Despite the fact that extensive research dedicated to the development of new therapeutic strategies, there are only a limited number of available drugs to fight against fungal infections (Patrick *et al.*, 2012). Some of the antifungal agent currently used in clinical practice to treat essentially topical and systemic fungal infections. These include: Fluconazole(i), Voriconazole(ii), Butoconazole (iii), (Itraconazole I Ciclopirox(iv) and itraconzole (Mukhtar *et al.*, 2022).

Fluconazole presented in fig.1 is a tablet or suspension to treat yeast infections of the vagina, mouth, throat, esophagus, abdomen, lungs, blood and other organs. Fluconazole is also used to treat meningitis and can prevent yeast infections in patients who are likely to become infected due to chemotherapy or radiation therapy before a bone marrow transplant (Simonetal,2015).



Fig: 2 *Isobberlinia doka*

standard procedures and evaluate the effect of methanol leaf extract of *I. doka* and its fractions (*n*-hexane, chloroform, ethylacetate, and *n*-butanol) against some fungal isolates using agar well diffusion method.

comparing with specimen number (UDUS/ANS/0155). The plant material was shed dried, pulverized to powder and stored in a polythene bag three days before the extraction (merceration).

2.2 Extraction and Partitioning

1000 g of powdered sample was macerated with 6 L of methanol with occasional agitation for 3 days, the extract was filtered and the solvent was evaporated with the aid of rotary evaporator at 40

2.3 Phytochemical Screening

Various chemical tests were conducted on crude methanol leaf extract and fractions of *I. doka* to identify the presence phytochemical constituents such as alkaloids, flavonoids, terpenoids, tannins,

2.4 Antifungal Assay

2.4.1 Test organisms

The fungal isolates obtained from Department of Microbiology Sokoto State University, Sokoto were used for the study. They include *Candida albican*, *Candida crusie*, *Trychophyton rubrum* and *Trychophyton mentagrophyte*.

2.4.2 Preparation of test organisms

Test organisms were sub cultured and grown on 10 mL Sabouraud dextrose agar slants and was eventually kept in the refrigerator at 8 °C.

2.4.3 Preparation of reference antifungal agents

Stock solutions of fluconazole (5 mg/ mL) was prepared by dissolving 50 mg of the powder in 10 mL of distilled water from which 0.05 mg/mL (50 µg/ mL) working concentration was prepared.

2.4.4 Preparation of crude extract and fractions of *Isobberlinia doka*

Stock concentrations of 100 mg/mL was prepared with 10 % dimethyl sulfoxide (DMSO) by dissolving 0.5 g (500 mg) each of the crude extract and fractions (*n*-Hexane, chloroform ethylacetate, and *n*-butanol) in 5 mL of 10 % DMSO and two-fold serial dilution was carried out to obtain three more concentrations of 50, 25 and 12.5 mg/ mL

2.4.5 Preparation of the media

The Sabouraud Dextrose agar (SDA) as growth media were weighed and prepared with distilled

°C to obtain crude methanol leaf extract (110 g) which was then suspended in 1 L of distilled water which was filtered and partitioned with solvent of increasing polarity to obtain *n*-hexane (HF), chloroform (CF), ethylacetate (EF) and *n*-butanol (BF) fractions.

saponins, phenols, steroid cardiac glycoside according to the methods described by Evans (2002).

water according to the manufacturer's instructions. SDA was gently heated to aid its dissolution, it was dispensed into sterile petri dishes and allowed to cool and solidify.

2.4.6 Antifungal assay of *I. doka*

The antifungal activities methanol extract of *I. doka* and fractions (*n*-hexane, chloroform, ethyl acetate and *n*-butanol) was determined through susceptibility test using agar well diffusion method (Olowosulu *et al.*, 2005). Wells were bored into the solidified SDA plates using a sterile cork borer of 4 mm in diameter. 0.1 cm³ of the inoculum was seeded and spread evenly over the surface of the sterilized media using a sterile cotton swab stick. The wells were filled separately with 200 µL solution of the graded concentration of extract and fractions. 0.05 mg/ mL fluconazole (which served as positive control), 10 % (DMSO) used as negative control were dispensed into the wells. The plates were incubated at 72 hour, the zone of inhibition was measured using transparent ruler. The experiment was carried out in triplicates.

3.0 STATISTICAL ANALYSIS

The data obtained was subjected to the analysis of variance (ANOVA) using SPSS software followed by post hoc test, values were considered significant at $p < 0.05$ and data was expressed as mean \pm standard deviation

4.0 RESULT AND DISCUSSION

4.1 Extraction and fractionation

The extraction of 1000 g of *Isobberlinia doka* yield 110 g of the crude extract and the percentage yield from the partitioned fractions are presented in Table 1.

Table: 1: percentage (%) yield of partitioned fractions of *i. Doka*

Solvent	Weight(g)	Yield (%)	Colour
Water insoluble	64.015	6.402	Green
Methanol	110.0	11.00	Green
n-hexane	9.660	8.782	Green
Chloroform	0.882	0.802	Light brown
Ethylacetate	12.801	11.637	Brown
<i>n</i> -butanol	24.731	22.483	Reddish brown

4.2 Preliminary phytochemical screening of methanol extract and its fractions

Preliminary phytochemical screening of the methanol leaf extract and its fractions (*n*-hexane,

chloroform, ethylacetate and *n*-butanol) revealed the presence of flavonoids, tannins, saponins, cardiac glycosides, steroids/terpenes, phenol and alkaloid presented in table 2

Table:2: Phytochemical Constituents of Extract and it Fractions

Constituent	Test	Observation	ME	HF	CF	EF BF
Flavonoids	Shinoda	Orange color	+	-	+	+
	Sodium hydroxide	Yellow color	+	-	+	+
Alkaloids	Mayer's	A cream ppt	+	-	-	+
	Dragondoff's	Orange ppt	+	-	-	-
Saponin	Frothing	Formation froth	+	-	+	+
Tannins	Lead sub-acetate	Cream color ppt	+	-	+	+
Triterpenoids/steroids	Salkowki's	Red brown color	+	+	-	-
	Lieberman	Purple color	+	+	-	-
Phenols	Ferric chloride	A dark green color	+	-	+	+
Cardiac glycoside	Keller-Kiliani	A brown ring at interface	+	-	+	+

Key += present - = absent; ME=methanol extract; HF=*n*-hexane fraction; CF= Chloroform EF=ethylacetate fraction and BF=*n*-butanol fraction,

4.3 Antifungal Assay

The result of antifungal susceptibility test of methanol extract and its fractions (*n* hexane,

chloroform, ethylacetate, and *n* butanol) of *I. doka* against some selected fungal species are presented in Table 3, 4, 5, 6 and 7 respectively.

Table .3: Antifungal Studies of Methanol Leaf Extract

Organisms	Mean zone of inhibition (mm)				
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	0.05 mg/mL
<i>C. albican</i>	17.00±1.00*	12.60±1.12*	10.00±1.00*	10.00±1.00a	11.20±0.04
<i>C. crusie</i>	10.60±0.06*	11.00±1.00*	11.00±1.00*	5.00±1.00*	20.30±0.04

<i>T. mentagraphyte</i>	11.50±1.00*	9.00±1.00*	6.00±01.00*	11.00±1.00*	0.00±0.00
<i>T. reburum</i>	11.00±1.00a	9.00±1.00a	7.00±1.00a	7.00±1.00*	11.3±0.04

Key: The data = mean ± SD * (p< 0.05) compared with PC, (ANOVA): a Indicates no statistical significant difference compared with PC (Positive control)

Table .4: Antifungal Studies Of N-Hexane Fraction

Organisms	Mean zone of inhibition (mm)				
	100 mg/mL	50 mg/mL	25 mg/mL	Fluconazole (PC)	
				12.5 mg/mL	0.05 mg/mL
<i>C. albican</i>	16.00±1.00*	12.00±01.00*	12.00±1.00a	7.00±1.00a	11.20±0.04
<i>C. crusie</i>	15.60±1.00a	13.00±1.00*	14.00±1.00*	11.00±100*	20.30±0.04
<i>T. mentagraphyte</i>	10.60±0.60*	11.00±1.00*	8.00±1.00*	6.00±1.00*	6.00±0.00
<i>T. rubrum</i>	19.00±1.00*	14.00±1.00*	13.00±1.00*	12.00±1.00a	11.30±0.04

Key: = The data mean ± SD * (p< 0.05) compared with PC, (ANOVA): a indicates no statistical significant difference compared with PC (Positive control).

Table .5: Antifungal Studies Of Chloroform Fraction

Organisms	Mean zone of inhibition (mm)				
	100 mg/mL	50 mg/mL	25 mg/mL	Fluconazole (P.C)	
				12.5 mg/mL	0.05 mg/mL
<i>C. albican</i>	12.00±1.00*	8.60±1.00*	14.00±1.00a	7.00±1.00a	11.20±0.40
<i>C. crusie</i>	9.60±1.00a	10.00±1.00*	9.00±1.53*	9.00±1.00*	20.30±0.40
<i>T. mentagraphyte</i>	10.50±1.00*	9.00±1.00*	8.00±1.00*	6.00±1.00*	0.00±0.00
<i>T. rubrum</i>	14.00±1.00*	11.00±1.00*	11.00±1.00a	8.00±1.00a	11.3±0.40

Key: The data = mean ± SD * (p< 0.05) compared with PC, (ANOVA): a indicates no statistical significant difference compared with PC (Positive control).

Table .6: Antifungal Studies Of Ethylacetate Fraction

Organisms	Mean zone of inhibition (mm)				
	100 mg/mL	50 mg/mL	50 mg/mL	Fluconazole (PC)	
				12.5 mg/mL	0.05 mg/mL
<i>C. albican</i>	23.00±1.00*	19.60±1.00*	15.00±1.00*	11.00±1.00a	11.20±0.40
<i>C. crusie</i>	18.60±1.00a	15.00±1.00*	13.00±1.00*	12.00±1.00*	20.30±0.40
<i>T. mentagraphyte</i>	19.00±1.00*	14.00±1.00*	10.00±1.00*	8.00±1.00*	0.00±0.00
<i>T. rubrum</i>	12.00±1.00*	11.00±1.00a	9.00±1.00a	8.00±1.00a	11.30±0.40

Key: The data = mean ± SD * (p< 0.05) compared with PC, (ANOVA): a indicates no statistical significant difference compared with PC (Positive control).

Table .7: Antifungal Studies Of N-Butanol Fraction

Organisms	Mean zone of inhibition (mm)				
	100 mg/mL	50 mg/mL	25 mg/mL	Fluconazole (P.C)	
				12.5 mg/mL	0.05 mg/mL
<i>C. albican</i>	21.00±1.00*	13.60±1.00*	12.00±1.00a	9.00±1.00a	11.20±0.40
<i>C. crusie</i>	17.60±1.00a	13.00±1.00*	13.00±1.53*	12.00±1.00*	20.30±0.40
<i>T. mentagraphyte</i>	13.50±1.00*	10.00±1.00*	10.00±1.00*	6.00±1.00*	0.00±0.00
<i>T. rubrum</i>	15.00±1.00*	12.00±1.00*	11.00±1.00a	9.00±1.00a	11.30±0.40

Key: The data = mean \pm SD * ($p < 0.05$) compared with PC, (ANOVA): a indicates no statistical significant difference compared with PC (Positive control).

4.5 Extraction and Fractionation Yield

The extraction of 1000 g of the powdered sample of *Isoberlinia doka* using methanol as extracting solvent yielded 110 g of methanol extract. The fractionation of water soluble portion of methanol leaf extract revealed that *n*-butanol fraction has the highest percentage (%) yield followed by ethylacetate, *n*-hexane, and finally chloroform.

4.6 Phytochemical Screening of Methanol Leaf Extract and its Fractions

Preliminary phytochemical screening of methanol leaf extract and its fractions (*n*-Hexane, chloroform, and ethylacetate, and *n*-butanol) of *isoberlinia doka* leaves revealed the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and flavonoids, ethylacetate and *n*-butanol fractions indicated the presence of similar constituents including flavonoids, alkaloids, tannins, saponins, phenols, steroid and cardiac glycoside while *n*-hexane contained only steroid, and triterpenoids as presented in Table.2. The presence of these phytochemical constituents in the other plants have been reported (Emaikwu *et al.*, 2019). These secondary metabolites are thought to be responsible for the pharmacological activities of plant extract (Emaikwu *et al.*, 2019). Abubakar *et al.*, (2020) reported the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and flavonoids, ethylacetate and *n*-butanol from methanol leaf extract of the other plant.

4.7 Antifungal Studies

The susceptibility test of methanol extract and its fractions (*n*-hexane, chloroform, ethylacetate and *n*-butanol) exhibited varying antifungal activities against the test organisms and the activities was concentration dependent. The methanol leaf extract and its fractions exhibited significant ($p < 0.05$) antifungal activities at the graded concentration (100 – 12.5 mg/mL) with mean zone of inhibition ranging from 6.00–23.00 mm against the test organisms (*C. albican*, *C. crusie*, *T. mentagraphyte* and *T. rubrum*) presented in (tables 3,4,5,6,7).

The methanol leaf extract of *I. doka* showed significant ($p < 0.05$) antifungal activity against *C. albican* at 100 mg/mL when compared with fluconazole at 0.05 mg/mL as presented in (Table 3). Duncan *et al.*, (2008) reported the antifungal activity of *Fragaria virganiaa* extract against *Candida albican* and *Candida crusie* in which the fluconazole was used as a positive control. *n*-hexane fraction indicated a higher antifungal activity against *T. rubrum* when compared with fluconazole (Table 4), similarly, the standard drug, fluconazole exhibited higher effect against *C. crusie* when compared with the fraction at 100 mg/mL although the effect was not statistically significant (Table 6). Chloroform fraction demonstrated significant antifungal activity against *T. rubrum* at 100 mg/ mL and *C. albican* at 25 mg/ mL when compared with standard drugs 0.05 mg/mL and the difference is statistically significant (Table 5). Emma *et al.* (2001) reported the antifungal activities of some medicinal plants against some fungal isolate (*Candida albican*, *T. mentagraphyte*, *A. niger* and *A. flavus*).

Ethylacetate fraction exhibited the highest antifungal activity against *C. albican* (Table 5). The highest activity observed by the ethylacetate fraction might be due to the concentration of moderately polar compounds such as flavonoids and their derivatives that have been reported to possess antifungal activity (Harborne *et al.*, 1993). Ethylacetate fraction is a very good antifungal agent for the treatment of different fungal infections such as *Onychomycosis*, *oral candidiasis*, *oesophageal candidiasis* and *viginal thrush* (Harborne *et al.*, 1993).

n-butanol fraction showed antifungal activity against *C. albican* when compared to fluconazole, even though it recorded the mean zone of 17.60 mm against *C. crusie* which was lower than that of fluconazole 20.30 mm but the difference is not statistically significant (Table.7). Mukhtar *al.*, (2022) reported the moderate antifungal activity of *n*-butanol fraction against *A. fumugatus* when compared with ketoconazole. However of all the fungal isolates used *C. albican* was the most

susceptible to ethylacetate fraction and is the most dangerous of all the many common fungal isolate causing *tinea cruris*, *oral trush* and *balanoposthitis* (Xia *et al.*, 2019).

5.0 CONCLUSION

Preliminary phytochemical screening of methanol leaf extract and its fractions (*n*-Hexane, chloroform, ethylacetate, and *n*-butanol) of *I. doka* revealed the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and

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flavonoids. The result of antifungal studies of methanol extract and its fractions of (*n*- hexane, chloroform, ethylacetate, and *n*-butanol) *I. doka* has demonstrated significant antifungal activities validating the ethnomedicinal claim for the use of the plant in the treatment of fungal infections.

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