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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF METHANOLIC LEAF EXTRACT OF *RICINUS COMMUNIS* L. ON SOME PATHOGENIC MICROORGANISMS

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ABSTRACT

Antimicrobial resistance is gradually becoming a ravaging global problem; as such, there is a quest for new antimicrobial agents. These agents can largely be extracted from medicinal plants with various amounts of phytochemicals. This research investigated the phytochemical screening and antimicrobial activities of methanolic leaf extract of *Ricinus communis* on some pathogenic microorganisms. The test organisms include five bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae* and *Salmonella typhi*; and two fungal strains: *Candida albicans* and *Aspergillus niger*. Antimicrobial activity was tested using Agar Well Diffusion method. Results showed that the extract has antimicrobial activity due to the presence of the phytochemicals.

Key words: *Ricinus communis* L., methanolic leaf extract; minimum inhibitory concentration; minimum bactericidal concentration; minimum fungicidal concentration
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INTRODUCTION

Medicinal plants have a vital role to preserve the human healthy life (Manoj, 2017). There has been an increasing interest in medicinal plant researches owing to their organic richness and far less side effects. Alternative medicine, which is the use of medicinal plants in the treatment of many ailments, is currently employed by a large percentage of the world's population (Sofowora, 1993; Tiwara, 2008). The continual use of this traditional system has increased the knowledge hub on vital phytochemicals that numerous plants and their parts possess.

Bentley and Trimen (2007) referred to medicinal plants generally as plants in which one or more of its parts contain essential phytochemicals that may be exploited for therapeutic purposes, or such that may be used as precursors in chemo-pharmaceutical synthesis. The presence of these phytochemicals in plants has been found to be very beneficial to human systems as most foods consumed by humans often contain less quantity of these biomolecules. Moreover, their consumption results in far less side-effects when compared with pharmaceutical or synthetic drugs (Kennedy and Wightman, 2013). Thus, their supplements with human food cannot be over-emphasised. Plant phytochemicals are known to possess essential biological activities.

The family Euphorbiaceae contains nearly about 300 genera and 7,500 species. Generally, they are the flowering plants. Amongst them, *Ricinus communis* or castor plant has high traditional and medicinal value for maintaining a disease-free healthy life (Manoj, 2017). Traditionally, the plant is used as laxative, purgative, fertilizer and fungicide etc. It also possesses beneficial effects such as anti-oxidant, anti-histamic, anti-nociceptive, anti-asthmatic, anti-ulcer, immuno-modulatory, anti-diabetic, hepato-protective, anti-fertility, anti-

inflammatory, antimicrobial, central nervous system stimulant, lipolytic, wound healing, insecticidal, larvicidal and many other medicinal properties. Jitendra and Ashish (2012) reported that these activities are due to the important phytochemical constituents such as flavonoids, saponins, glycosides, alkaloids, steroids etc. The aim of this research was to determine the antimicrobial activity of the methanolic leaf extract of *Ricinus communis* L. on certain pathogenic microorganisms.

MATERIALS AND METHODS

Study Area

The research was carried out at the Department of Pharmacology and Drug Development, and the Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Zaria is a city located in the Northern Guinea Savannah Zone of Nigeria, with coordinates ($11^0$04'N, $7^0$42'E).

Collection and Identification of Plant Material

The plant material, *Ricinus communis* (ABU0900233), was obtained from the Institute for Agricultural Research, Ahmadu Bello University, Zaria. It was appropriately identified at the herbarium of the Department of Botany.

Extract Preparation

The collected plant materials were air-dried, then ground to powder. Eighty (80) g of the ground plant material was weighed and poured in a glass container, and 500 ml of methanol was added. The container was covered for three days to ensure adequate extraction of the bioactive compounds. The mixture was filtered and the filtrate was evaporated and concentrated to leave only the extract. The extract was thereafter packaged in an air-tight container and preserved for use.

Test Organisms

Five clinically important bacterial strains and two fungal strains were used. The test microorganisms were collected from Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, A. B. U., Zaria. The bacterial strains include: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumonia* and *Salmonella typhi*. The fungal strains were *Candida albicans* and *Aspergillus niger*.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the leaf extract of *Ricinus communis* was carried out using standard procedure by their specific qualitative chemical confirmatory tests.

Test for Tannins

Ferric chloride Test: about 0.5 g of the extract was stirred with 10 ml distilled water and filtered. Two drops of 1% ferric chloride solution was added to 2 ml of the filtrate. Formation of blue-black precipitate indicated the presence of tannins (Trease and Evans, 2007).

Test for Saponins

Frothing Test: About 0.5 g of the extract was dissolved with water in a test tube and shaken, followed by warming on a water bath. Persistent frothing on warming indicated the presence of saponins (Edeoga *et al*., 2005).

Test for Alkaloids
Wagner's Test: About 0.5 g of the extract was stirred with 10 ml of 1% aqueous hydrochloric acid on a water bath and filtered. To the filtrate, conc. NH_4OH was added and extracted with chloroform. The chloroform layer was extracted further with 10 ml of 1% HCl. The aqueous layer was filtered out and 1 ml of Wagner's reagent was added to it. A reddish-brown precipitate indicated the presence of alkaloids (Trease and Evans, 2007).

Test for Steroids
Salkowski's Test: A small quantity of extract was dissolved in 1 ml chloroform. It was filtered and to it 1 ml of conc. H_2SO_4 was added down the side of the test tube. Formation of a red ring at the interface indicated the presence of steroids (Edeoga *et al*., 2005).

Test for Triterpenes
Lieberman- Buchard Test: A small portion of the extract was dissolved in chloroform and filtered. An equal volume of acetic anhydride was added to the filtrate, followed by conc. H_2SO_4 down the side of the test tube. Brown ring at the interface indicated the presence of triterpenes (Trease and Evans, 2007).

Test for Cardiac glycosides
Keller- Killiani Test: About 0.5 g of the extract was treated with 2 ml glacial acetic acid in a test tube. Few drops of ferric chloride solution were added followed by 1 ml of conc. H_2SO_4. This gave a brown ring at the interface, indicating the presence of cardiac glycosides (Edeoga *et al*., 2005).

Test for Carbohydrates
Molisch's Test: A small amount of Molisch's reagent was added to a little portion of the extract in a test tube. After mixing, a small amount of conc. H_2SO_4 was added slowly to the sides of the sloping test tube. A purple-red ring at the interface indicated the presence of carbohydrates (Molisch, 2017).

Standardization of the Test Organisms
The test organisms were sub-cultured and incubated overnight, then standardized with normal saline (0.9% NaCl). The standardisation method used is the dilution method (CLSI, 2012). The standardisation factor used for Gram-positive organisms was 1:1000 dilutions and 1:5000 dilutions for Gram-negative organisms.

Antimicrobial Assay
The Agar Well Diffusion method as described by CLSI (2012) was used to test for the sensitivity of the test organisms to the extract with control as the standard drugs Ciprofloxacin (10µg/ml) for the bacterial strains and Terbinafin (30µ/ml) for the fungal strains. The growth media used were Nutrient Agar (NA) for the bacterial strains and Sabouraud Dextrose Agar (SDA) for the fungi.

Determination of Zone of Inhibition
Ten (10) ml of the growth media was poured into agar plates and allowed to solidify. Five wells were bored on each plate for every organism to represent 5 different concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and control). Each plate was inoculated with a particular test organism obtained from freshly prepared overnight cultures, using the flooding method. One hundred (100) µl of the various concentrations of the extract and the control were introduced into the diffusion wells of each plate. The bacterial plates were then incubated

for 24 hours at 37ºC, while the fungal plates were incubated for 72 hours at 30ºC. The diameters (mm) of the zones of inhibition were appropriately taken (Malgadi *et al*., 2004).

Determination of Minimum Inhibitory Concentration (MIC)

Ten (10) ml of different concentrations of the extract mixed with liquid media were prepared and poured into agar plates, then allowed to solidify. Diffusion disks inoculated with an organism each were placed at different points on the plates, and replicated for all 10 concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.50 mg/ml, 6.25 mg/ml, 3.13 mg/ml, 1.57 mg/ml, 0.78 mg/ml and 0.39 mg/ml). The plates were then incubated appropriately. Growth on the media indicated that there was no inhibition (CLSI, 2012).

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The disks from the plates were transferred to different bottles of freshly prepared liquid media and incubated appropriately. Turbidity in the media indicated growth, implying that there was no bactericidal or fungicidal effect on the organism at that concentration. Conversely, a clear broth showed bactericidal or fungicidal effect (CLSI, 2012).

Statistical Analysis

The results were expressed as mean ± standard error. The mean growth inhibition was tested by Analysis of Variance (ANOVA) and the means were compared using the Duncan's Multiple Range Test (DMRT).

RESULTS

The phytochemical screening of the methanolic leaf extract of *Ricinus communis* revealed the presence of tannins, saponins, alkaloids, steroids, cardiac glycosides, carbohydrates and triterpenes. Flavonoids and anthraquinones were absent as shown in Table 1.

Table 1: Result of the phytochemical screening of the methanolic leaf extract of *Ricinus communis*

Phytochemical	Test	Result
Tanins	Ferric chloride test	+
Saponin	Frothing test	+
Alkaloids	Wagner's test	+
Steroids	Salkowski's test	+
Flavonoids	Sodium hydroxide test	-
Anthraquinones	Borntrager's test	-
Cardiac glycosides	Keller- killiani test	+
Carbohydrates	Molisch test	+
Triterpenes	Lieberman-Buchard's test	+

Keys: (+)= Present
(-)=Absent

Table 2: Mean inhibition zones exhibited by the methanolic leaf extract of *Ricinus communis* (mm)

Test organism	Concentration (mg/ml)				
Bacteria	(200)	(100)	(50)	(25)	(control)
Bacillus subtilis	14.00 ± 0.00^{b}	12.00 ± 0.00^{c}	10.00 ± 0.00^{d}	0.00 ± 0.00^{e}	22.00 ± 0.00^{a}
Escherichia coli	16.00 ± 0.00^{b}	13.00 ± 0.00^{c}	11.00 ± 0.00^{d}	0.00 ± 0.00^{e}	36.00 ± 0.71^{a}
Salmonella typhi	15.00 ± 0.00^{b}	13.00 ± 0.00^{c}	11.00 ± 0.00^{d}	0.00 ± 0.00^{e}	26.00 ± 0.00^{a}
Staphylococcus aureus	18.00 ± 0.00^{ab}	16.00 ± 0.00^{ab}	13.00 ± 0.00^{bc}	05.00 ± 3.54^{c}	24.00 ± 0.00^{a}
Streptococcus pneumonia	28.00 ± 0.00^{a}	25.00 ± 0.00^{b}	20.00 ± 0.00^{c}	18.00 ± 0.00^{d}	0.00 ± 0.00^{e}
Fungi					
Aspergillus niger	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	45.00 ± 0.00^{a}
Candida albicans	14.00 ± 0.00^{b}	12.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	23.00 ± 0.00^{a}

Data expressed as Mean ± SE (Standard Error), n=2. One-way ANOVA was used with DMRT at 5% level of significance (p = 0.05).
Values with the same superscript across the rows are not significantly different.
Control (bacteria) = Ciprofloxacin (10μg/ml)
Control (fungi) = Terbinafin (30μg/ml)

Table 3: The Minimum Inhibitory Concentration (MIC) for the methanolic leaf extract of *Ricinus communis*

Test organism	Concentration (mg/ml)									
Bacteria	(200)	(100)	(50)	(25)	(12.50)	(6.25)	(3.13)	(1.56)	(0.78)	(0.39)
B. subtilis	-	-	+	+	+	+	+	+	+	+
E. coli	-	-	+	+	+	+	+	+	+	+
S. typhi	-	-	+	+	+	+	+	+	+	+
S. aureus	-	-	+	+	+	+	+	+	+	+
S. pneumoniae	-	-	-	+	+	+	+	+	+	+
Fungi										
A. niger	+	+	+	+	+	+	+	+	+	+
C. albicans	-	-	+	+	+	+	+	+	+	+

Key: (+) = Growth
(-) = No growth

Table 4: The Minimum Bactericidal Concentration (MBC) for the leaf extract of *Ricinus communis*

Test organism	Concentration (mg/ml)		
	(200)	(100)	(50)
B. subtilis	+	+	+
E. coli	+	+	+
S. typhi	+	+	+
S. aureus	+	+	+
S. pneumonia	+	+	+

Key: (+) = Growth
(-) = No growth

Table 5: The Minimum Fungicidal Concentration (MFC) for the leaf extract of *Ricinus communis*

Test organism	Concentration (mg/ml)		
	(200)	(100)	(50)
Candida albicans	+	+	+
Aspergillus niger	+	+	+

Key: (+) = Growth
(-) = No growth

DISCUSSION

The result of the phytochemical screening of the methanolic leaf extract of *Ricinus communis* showed the presence of tannins, saponins, alkaloids, steroids, cardiac glycosides, carbohydrates and triterpenes. The presence of these phytochemicals and absence of flavonoids and anthraquinones in the leaf extract of *Ricinus communis* has been reported by Shahid *et al*. (2016). Phytochemicals such as flavonoids, steroids, alkaloids, glycosides and saponins have been reported to show antimicrobial activities, thus conferring on the plants their medicinal properties (Jitendra and Ashish, 2012). Several authors including Lomash *et al*. (2010) and Naz and Bano (2012) have demonstrated the efficacy of polar solvents such as ethyl acetate and methanol in the extraction process of phytochemicals.

The zones of inhibition of the extract (at 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml) were compared with those of the standard drugs Ciprofloxacin (10 μg/ml) for antibacterial activity and Terbinafin (30 μg/ml) for antifungal activity. The extract exhibited significant antimicrobial activity ($p < 0.05$). This could be as a result of the presence of saponins and other phytochemicals that have been reported to show antimicrobial activity as reported by Barisi and Omodele (2014). The extract, however, showed the strongest antibacterial activity on *Streptococcus pneumoniae* with 28.00 mm as mean inhibition zone (IZ). Antifungal activity was stronger on *Candida albicans* (IZ=14.00 mm); *Aspergillus niger* was not inhibited by any of the concentrations of the extract. This could be because the bioactive compounds of the plant extract lacked sufficient potency to affect *A. niger* at all the concentrations, implying that it would require a higher concentration to that effect, as reported by Suurbaar *et al*. (2017).

The problem of drug resistance was clearly demonstrated in this study. *Streptococcus pneumoniae*, which was most susceptible to the extract, showed a high resistance to the standard drug Ciprofloxacin. Although this organism resists treatment by this drug, the extract can be exploited to produce a potent antibiotic against it. Castor plant has also been reported to have anti-ulcer, anti-diabetic, anti-asthmatic, hepato-protective and anti-fertility activities (Jitendra and Ashish, 2012). The wide spectrum of activities exhibited by this plant makes it highly medicinal; as such, it can be exploited and targeted at other pathogenic organisms or used for other therapeutic purposes.

The minimum inhibitory concentration (MIC) for *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis* and *Candida albicans* were observed to be 100 mg/ml; that of *Streptococcus pneumoniae* was 50 mg/ml; whereas that of *Aspergillus niger* was > 200 mg/ml. The extract was able to inhibit all the organisms except *A. niger*, but could not kill any of the organisms at any concentration. The MBC for all the bacterial strains could be at a concentration higher than 200 mg/ml. Similarly, the MFC for the fungal strains could be at a concentration higher than 200 mg/ml. This corresponds with the range of values for MBC and MFC reported by Suurbaar *et al*. (2017), which were 200-400 mg/ml. Higher concentrations of this extract (above 200 mg/ml) could exhibit greater antimicrobial activity.

CONCLUSION

Ricinus communis L. contained phytochemicals which conferred medicinal value on the plant. The methanolic leaf extract of *Ricinus communis* exhibited bactericidal and fungicidal activities. Therefore, the extract could be exploited for the development of drugs to treat infectious diseases caused by such organisms.

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