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# EVALUATION OF ANTIMICROBIAL ACTIVITY OF METHANOLIC LEAF EXTRACT OF RICINUS COMMUNIS AGAINST SOME CLINICAL ISOLATES

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**Abstract**

Medicinal herbs have contributed to disease prevention and management. The castor bean plant, *Ricinus communis,* offers great traditional and therapeutic values in some communities. This study was carried out to determine the antimicrobial activity and phytochemical constituents of leaf extract of *Ricinus communis*. The antimicrobial evaluation was performed on some clinical isolates (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Aspergillus flavus,* and *Fusarium species*). Agar well diffusion method and agar dilution techniques were used for bacteria and fungi respectively. Concentrations of 62.5mg/ml, 125mg/ml, 250mg/ml and 500mg/ml of the extracts were used for bacterial activity. Ciprofloxacin and Dimethyl sulfoxide (DMSO) was used as positive and negative control respectively. For the fungi study, the extract was incorporated into molten Sabouraud dextrose agar to make up for the concentration of 62.5mg/ml, 125mg/ml, and 250mg/ml respectively. Fluconazole (5mg/ml) and Dimethyl sulfoxide (DMSO) were used as the positive and negative control respectively. The result showed that the extract was effective on the clinical isolates; however, the control drugs had greater inhibition zones. The zone of inhibition of the extract on *Staphylococcus aureus* ranged from 7mm- 13mm, Escherichia coli 6mm-9mm, *Klebsiella pneumoniae* 14mm-22mm, *Pseudomonas aeruginosa* 9mm-15mm, and *Candida albicans* 9mm-14mm. The extract also exhibited antifungal activities on *Aspergillus flavus* and *Fusarium* species. Qualitative phytochemical studies revealed the presence of tannin, saponin, glycosides, flavonoids, phenol, alkaloids, and steroids in the leaves which may have contributed to this activity. This research has provided solid evidence for the use of this leaf in herbal medicines as well as a foundation for the development of novel antimicrobial medications.

**Keywords**: Antimicrobial activity, clinical isolates, *Ricinus communi,* leaf extract *(11)*

## Introduction

Medicinal plants are important natural resources that have been utilized to treat a wide range of illnesses around the world. Ancient literature also highlights the widespread use of medical plants, such as herbal medicine and healthcare preparations (1). Plants have a wide range of biologically active compounds, which is an indication that they are a rich source of a wide range of therapeutics. Antimicrobials are chemotherapeutic agents that kill or inhibit the growth of microorganisms in amounts that are appropriate for the host (2). Antibiotic resistance has become a major public health issue throughout the world (3). Medicinal herbs have played an important role in disease prevention and management. The majority of the world's population relies on herbal medications. In India's medical system, about 95% of the medications are derived from plants (4). Around 80% of medicinal drugs are of plant origin. Mankind has been blessed by nature with large medicinal plants, from which a broad range of contemporary medications have been derived (5). Such medications are based on the plants' natural medicinal knowledge. In recent years, there has been an increase in multiple resistances in human pathogenic microbes, owing to the misuse of conventional antimicrobial medications routinely used to treat infectious conditions. This has prompted scientists to look for new antibacterial compounds in unexpected places, such as medicinal and herbal plants (3). Herbal medication's importance in the treatment of human ailments cannot be overstated. It is known that the plant kingdom contains an endless supply of active chemicals that are essential in the treatment of many challenging disorders (6). Plants have been used to treat infectious diseases for ages and are regarded as a valuable source of novel antibacterial medicines. Several studies on the antibacterial activities of herbal plant extracts, including leaves, flowers, stems, and roots, have been conducted. Many nations in Africa and other regions of the world have continued to promote screening of plants used in traditional medicine to identify their antibacterial activity and potential inclusion in basic health care (7). Plants, as they have been since ancient times, remain a major source of medicine. Traditions all around the world have learned how to employ medicinal plants to combat disease and sustain good health over time. The importance of natural products in illness treatment has grown as a result of their natural source and fewer side effects when compared to the complexity of constructing chemical-based pharmaceuticals, as well as rising costs, which has prompted global researchers to concentrate on medicinal plant research (8). Plants are a rich source of organic chemicals, many of which have been utilized to treat various ailments. *Ricinus communis i*s one of many natural crude medications derived from plants that have the ability to treat a wide range of ailments and disorders (9). *Ricinus communis* is a flowering plant that belongs to the Euphorbiaceae family. It is planted all over the world due to the commercial value of its oil. It is mostly grown in very dry climates and semi-arid regions in many nations, the most prominent of which being India, China, and Brazil (10). *Ricinus communis* L. is a species of *Ricinus communis*. Castor oil plant (Euphorbiaceae) is a soft wooden small tree that is widely distributed throughout the tropics and warm and temperate parts of the globe. Different components of the plant are widely utilized for healing a variety of ailments by various groups and forest dwellers in many parts of the world. Secondary metabolites [possible drug sources] and therapeutically important essential oils are abundant in medicinal plants. *Ricinus communis* is a toxic, attractive shrub or tree that grows in both tropical and temperate climates and has medical and industrial use. It is often used as a disinfectant, eye inflammation treatment, in cosmetics, and other pharmaceutical preparations (11). *Ricinus communis* has been reported to possess good antimicrobial activities against some pathogenic bacterial strains. Castor also shows antimicrobial activity against some fungal pathogens (12). Castor leaves are used by lactating mothers externally to increase the milk flow. Castor oil is a natural emollient that can be used to soften the skin and hair. Rheumatism, headaches, dropsy (oedema), abscesses, ringworms, and warts are all treated using Castor plant juice obtained from the leaves. Castor oil is used to relieve transient constipation, but it is ineffective in the treatment of persistent constipation. Castor oil is used as a topical

treatment for ringworm and itching. Castor oil has been shown to help in labour and delivery (13).

Isolation of some antimicrobial and a variety of other chemical components from plants can have a significant impact on healthcare. Plants' medicinal properties are attributed to several key chemical components that have physiological effects on humans (1).

The importance of this scientific research on *Ricinus communis* Linn is based on various evidence of its efficacy in the treatment of a variety of ailments, as well as the need to evaluate the validity of earlier studies on *Ricinus communis* Linn. Because of the significant health threat posed by antibiotic resistance, more research on this plant is needful to appreciate its therapeutic value in the development of novel antimicrobial drugs.

## Methods

### Collection of *Ricinus communis* leaves.

Fresh leaves of *Ricinus communis* L. were collected from Ukehe town in Igbo-Etiti Local Government Area Enugu State. The leaves were identified in the Department of Plant Science and Biotechnology of the University of Nigeria Nsukka.

### Preparation of plant extract

The leaves were severally washed and allowed to air dry properly. They were then ground using an electric grinder and homogenous fine powder was obtained. The homogenous fine powder of *Ricinus communis* L. leaves was exhaustively extracted successively in soxhlet apparatus using methanol. The extracts were concentrated under reduced pressure using a rotatory evaporator to get the crude. A dark green semi-solid mass was obtained. The extract was kept in a refrigerator at 4°C until required.

### Test organisms

The bacterial isolates were obtained from the Microbiology Department University of Nigeria Teaching Hospital (UNTH) while the fungal isolates were obtained from the Research laboratory. Four bacterial strains; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae.*, and three fungi strains namely, *Aspergillus flavus, Fusarium spp*, and *Candida albicans* were used.

### Assay for antibacterial activity

The agar well diffusion method was used for the determination of the antibacterial activity of *Ricinus communis* methanolic leaf extract (11). The bacterial strains were uniformly spread on nutrient agar with help of a sterilized wire loop. Uniform holes were made using a sterile 6mm diameter cork borer. Thirty microlitre of the crude extract at 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml were added into the wells. All the Petri plates were kept at room temperature for 30 minutes to allow the extract to diffuse properly in the agar. The plates were incubated at 37°C for 24 hours and examined for zones of inhibition. Ciprofloxacin (5mg/ml) was used as positive control and DMSO was used as a negative control. The assessment of the antibacterial activity was based on the measurement of the diameter of the inhibition zone formed using a meter rule. The experiment was repeated thrice and results were taken as the mean of the readings.

### Assay for antifungal activity

The Agar dilution method was used for the determination of the antifungal activity of *Ricinus communis* methanolic leaf extract (1). About 1000mg/ml of extract was made by dissolving 10g of the extract in 10ml of DMSO. Dilution of the plant extract and the Sabouraud dextrose agar at 45°c containing chloramphenicol was made to get 10mls of agar for different concentration; 250mg/ml(2.5 ml of plant extract + 7.5ml of Sabouraud agar), 125mg/ml (1.25ml of plant extract + 8.75ml of Sabouraud agar) and 62.5mg/ml( 0.625ml of plant extract + 9.375ml of Sabouraud agar) respectively. The plates were allowed to be set and dried in the incubator and a 4mm size of agar blocks from the 3-day old culture of molds (*Aspergillus flavus, Fusarium* species) was seeded on the agar. Fluconazole (5mg/ml) was used as a positive control for fungi and DMSO was used as a negative control. It was incubated at room temperature and monitored daily for growth. The growth extension of the molds was measured using a meter rule for three consecutive days and the measurements were compared with that of the positive and negative control.

Antifungal activity of the extract for Candida was done the same way the antibacterial activity was carried out but Sabouraud agar was used instead. It was incubated for 24hrs and the assessment of the antifungal activity was based on the measurement of the diameter of the inhibition zone formed using a meter rule. The experiment was repeated thrice and results were taken as the mean of the readings.

**Phytochemical analysis (qualitative)**

Preliminary phytochemical analysis of the homogenous fine powder of *Ricinus communis* L. was done following the standard phytochemical procedure (14, 15). Qualitative analysis was carried out to identify the medicinal value of the plant. 20g of the sample was soaked in 100mls of different solvents such as n-hexane, ethanol, distilled water, methanol, and ethyl acetate for not less than 24hours. The extract was decanted and heated for 3 minutes to concentrate it. The presence of alkaloids, saponin, flavonoids, steroids, phenols, glycosides, and tannin was checked using standard methods.

**Statistical analysis**

All statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad, San Diego, CA, USA). Ordinary One-way Analysis of variance (ANOVA) and Student t-test was used for comparison of mean differences between and among groups respectively at 95% confidence interval. P-value ≤ 0.05 is considered statistically significant.

## Results

Table 1 shows the comparison of the ZIDs for the antimicrobial activities of the plant extract and control drugs against the test organisms. From the table, *Klebsiella pneumoniae* showed the highest ZID in all the extract concentrations while *Escherichia coli* showed the least ZID in all the extract concentrations. Ordinary one-way ANOVA showed a statistically significant difference ($p<0.0001$) in the mean ZIDs of the test organisms concerning different extract concentrations. There is also a statistically significant difference ($p<0.001$) in the mean ZIDs of each test organism concerning all the extract concentrations. Positive control (Ciprofloxacin) showed the highest ZID against *Staphylococcus aureus* and the least ZID against *Escherichia coli.*

Table 2 shows the percentage inhibition diameter of growth (PIDG) for *Aspergillus flavus* and *Fusarium spp* on plates containing different concentrations of the extract. From the table, both *Fusarium spp* and *Aspergillus flavus* showed lower PIDG in culture media containing 125mg/ml and 62.5mg/ml of extracts concentration when compared with the 250 mg/mL and positive control (Fluconazole) plate. From the table, *Aspergillus flavus* showed a higher growth zone diameter in culture media containing 125mg/ml and 62.5mg/ml of extracts. Student t-test showed no statistically significant difference ($p>0.0001$) in the percentage inhibition diameter of growth for the two tested organisms (*Aspergillus flavus* and *Fusarium spp).*

The Phytochemistry results in table 3 shows the presnce of alkaloids, tannins, glycosides, flavonoids, and saponins.

## Discussion

Text The discovery of antimicrobials led to a reduction in mortality and morbidity as a result infectious diseases, but their improper and indiscriminate usage has led to emergence of resistant strains. Pathogenic bacteria use a variety of mechanisms to develop intrinsic resistance to antibiotics, including changing target sites, active drug efflux, and enzymatic degradation. Since 25-50 percent of present medications are derived from plants, this has sparked greater interest in medicinal plants (16). Nature has bestowed many medical plants on humans, and a diverse range of pharmaceuticals have been developed from these naturally occurring medicinal plants. Medicinal plants constitute essential natural sources that have been used to cure a variety of diseases as they contain a large number of physiologically active chemicals, providing them a valuable supply of pharmaceuticals (17). Given the large diversity of secondary metabolites found in crude extracts of medicinal plants, they could be used as an

alternative source of resistance modifying agents .They have the ability to bind to protein domains, causing protein-protein interactions to be modified or inhibited (16).

The assessment of the methanol leaf extract of *Ricinus communis* revealed antimicrobial activity which has been recorded previously (1, 18, 3, 11). Our result from methanolic leaf extract of *Ricinus communis* against the bacterial isolates showed that *Klebsiella pneumoniae* was significantly inhibited followed by *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* using agar well diffusion. This does not agree with the work done by Naz and Bano (1) in Pakistan in which the methanol leaf extract of *Ricinus communis* showed higher antibacterial activity against *Staphylococcus aureus* and less activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* There could be two possible explanations for this observation. First, the *Staphylococcus aureus* used in this present study might have acquired some level of resistance leading to its less response to the plant extract. Secondly, it could be that the concentration of the active ingredients in the leaf extract they used was higher than that used in the present study. This might be due to the varying environmental elements found in the area where the *Ricinus communis* plant thrives. Our study also showed that Ricinus communis had higher antibacterial activity against Gram-negative organisms and this does not agree with the observation of some researchers that reported that plant extracts show higher activity against Gram-positive bacteria than Gram-negatives (19).

The leaf extract of *Ricinus communis* also exhibited antifungal activity against the three fungal isolates; *Candida albicans, Aspergillus flavus, and Fusarium spp*. The antifungal effect was exhibited more at the concentration of 250mg/ml and less in 125mg/ml and 62.5mg/ml against *Aspergillus flavus* and *Fusarium spp*. There was a little more antifungal activity of the leaf extract against *Fusarium spp* than *Aspergillus flavus* using the agar dilution method. There was complete inhibition at 250mg/ml for both *Aspergillus flavus* and *Fusarium spp*. The antifungal activity against *Candida albicans* was seen only at the concentration of 500mg/ml and 250mg/ml and no inhibition was seen at the concentration of 125mg/ml and 62.5mg/ml. The reason could be that leaf extract of *Ricinus communis* L can only exhibit antifungal activity against Candida albicans at higher concentrations. This study agrees with the work of some researchers that *Ricinus communis has* antifungal potential (1).

Preliminary qualitative phytochemical analysis carried out in the course of this study using different solvents like N-Hexane, Water, Methanol and Acetone revealed the presence of phytocompounds like tannins, saponins, glycoside, flavonoid, phenol, alkaloid, and steroid in the leaves which may have contributed to its activity. This work agrees with the works done in Bangladesh which reported that *Ricinus communis* leaves contain phytocompounds like alkaloids, saponins, tannins, glycosides, flavonoids, etc which are responsible for the inhibition of the organisms (20, 21). The antibacterial activity in this methanolic extract may be due to the presence of flavonoids and tannins. Flavonoids' antimicrobial activity against a variety of bacterial and fungal pathogens is due to their interaction with microbial cell membranes (22). Membrane permeability and disruption are seen as a result of their interaction with membrane proteins found on the bacterial cell wall (16). Tannins have been demonstrated to have a variety of biological actions, including antibacterial and antifungal properties, in previous research. The deactivation of cell membrane transport proteins and microbial adhesins could be the mechanism of tannins' antibacterial activity (16, 23).

Multiple drug resistance is a serious global challenge and the situation is exacerbated by synthetic medications' decreasing efficacy and growing toxicity. This has prompted researchers to look for a solution in plant-based antimicrobials, which are known to play an important role in the formulation of effective drugs. Antibiotics used with phytoactive components, either alone or simultaneously, may be a tool in combating worldwide antimicrobial resistance (16).

## CONCLUSION

Our study has shown that *Ricinus communis* Linn methanol leaf extract has significant potential to inhibit the growth of bacterial and fungal although the standard drug Ciprofloxacin gave more zone of inhibition diameter. Since the antimicrobial activity varies with the different concentrations of methanol

leaf extract of the plant, therefore an increase in its concentration might produce at least the same or better effect than most antibacterial and antifungal agents available. This study lends support to the traditional use of this medicinal plant in the treatment of bacterial and fungal infections. It has the potential to be exploited as a natural source of antifungal and antibacterial remedies in the future. The antimicrobial activity of *Ricinus communis* has justified its efficacy in the treatment and prevention of infection in man and has provided scientific support of medicinal plants in having the potential to be good therapeutics.

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## Conflict of Interest

There is none to declare.

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**Table 1: Comparison of the inhibitory effects of the plant extracts on the test organisms**

| | Mean zone of inhibition (mm) ± SEM | | | | | |
|---|---|---|---|---|---|---|
| **Extract Conc. (mg/ml)** | *Staph aureus* | *Escherichia coli* | *Klebsiella pneumonia* | *Pseudomonas aeruginosa* | *Candida albicans* | P-value |
| 500mg/ml | 12.67 ± 0.33 | 8.67 ± 0.33 | 20.33 ± 0.88 | 13.67 ± 0.88 | 13.33 ± 0.67 | <0.0001 |
| 250mg/ml | 9.67 ± 0.88 | 7.67 ± 0.33 | 18.67 ± 0.88 | 11.33 ± 0.67 | 10.00 ± 0.57 | <0.0001 |
| 125mg/ml | 8.33 ± 0.67 | 6.67 ± 0.33 | 17.33 ± 0.67 | 9.67 ± 0.67 | 0.00 ± 0.00 | <0.0001 |
| 62.5mg/ml | 0.00 ± 0.00 | 4.00 ± 2.00 | 15.67 ± 1.20 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.0083 |
| Positive Control | 40.33 ± 2.03 | 26.33 ± 0.88 | 35.67 ± 0.88 | 24.0 ± 5.57 | 20.0 ± 1.53 | 0.0022 |
| **P-value** | **<0.0001** | **<0.0001** | **<0.0001** | **0.0010** | **<0.0001** | |

**Table 2: Percentage inhibition diameter of growth for the moulds.**

| | Percentage inhibition | |
|---|---|---|
| **Extract Conc. (mg/ml)** | *Aspergillus flavus* | *Fusarium spp.* |
| 250 mg/ml | 100% | 100% |
| 125 mg/ml | 53.4% | 74.4% |
| 62.5 mg/ml | 15.2% | 45% |
| Positive control | 100% | 100% |

**Table 3: PHYTOCHEMICAL ANALYSIS (QUALITATIVE) OF VARIOUS EXTRACTS OF RICINUS COMMUNIS (CASTOR LEAF)**

| Parameters/Solvents | N-Hexane | Water | Methanol | Acetone |
|---|---|---|---|---|
| Alkaloids | + | + | + | + |
| Phenols | - | + | - | - |
| Saponins | - | - | + | - |
| Steroids | - | + | - | - |
| Tannins | + | + | + | + |
| Glycosides | + | + | + | + |
| Flavonoids | + | + | + | + |