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ANTIBACTERIAL ACTIVITY OF *BRYOPHYLLUM PINNATUM* AGAINST CLINICAL ISOLATES OF *ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIA* FROM URINARY TRACT INFECTION PATIENTS

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ABSTRACT

The emergence and spread of resistant strains of bacteria to routinely used antibiotics has made it imperative to continuously search for alternatives that can be used to cure infections, so as not to return to pre-antibiotic era. Plants are known to contain bioactive compounds that can be explored and used in the treatment of infection. The study investigated the antibacterial activity of ethanolic extracts of dried leaf extracts of *Bryophyllum pinnatum* against clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from urinary tract infection patients attending Shehu Muhammad Kangiwa Medical Centre. The photochemical screening of the crude extracts of *Bryophyllum pinnatum* (leaf of life) carried out using standard procedures described by Trens and Jens 2002, revealed the presence of Saponins, Flavonoid, Terpenoids, Alkaloids, Phenol and Tannins . The antibacterial activity of the extracts was carried out using agar cup diffusion method. Four concentrations (200,100, 50 and 25mg/ml) of the extract were tested against *Escherichia coli* and *Klebsiella pneumoniae*. The zones of inhibition measured at 200,100, 50 and 25mg/ml were: 00,00,00 and 00 mm respectively. The extracts therefore showed no antibacterial activity against the isolates at all the four concentrations tested. The results of this study proofs that the ethanolic extract of *B. pinnatum* leaves has no effect on *E. coli* and *K. pneumoniae* isolated from patients with Urinary Tract Infection, and hence cannot be used to treat infections caused by these bacteria.

Keywords: *Bryophyllum pinnatum, Escherichia coli, Klebsiella pneumoniae*, Antibacterial activity

INTRODUCTION

Urinary tract infections (UTIs) are considered to be the most common bacterial infection, affecting more than 150 million people annually worldwide (Smelov *et al*., 2016). Urinary tract infections are treatable and will usually not result in complications when diagnosis is made early and antibiotics are administered. In recent times however, the continuous emergence and spread of resistant strains of bacteria to routinely used antibiotics, the shy attitude of most people in discussing infections that affect genitourinary tract and the high cost of health care in developing countries has made the people to explore other alternatives such as herbs. Traditional medicine as defined by the World Health Organization is the total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, treatment of physical and mental illness (Moussaoui *et al*., 2016). The use of local plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America, USA, China, Japan and Africa (Dhama *et al*., 2014). Globally, there are evidence-based studies to verify the efficacy of medicinal plants, and some of this evidence have provided insights into the synthesis of plant-based compounds with therapeutics application (Dhama *et al*., 2014).

Escherichia coli and Klebsiella pneumoniae are normal flora of the gut that have been implicated in a number of urinary tract infections. The ability of *E coli* to alter its genetic makeup with rapidity contributes to the emergence and spread of resistant strains of *E coli*.

Bryophyllum pinnatum is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China and Australia, the divine herb contains a wide range of active compounds including alkaloids, titerpenes, glycoside, flavonoids, steroids bufadienolides, lipids and organic acids. In Nigeria, it is referred to as 'Shuka halinka' by the Hausas, 'Abamoda' by the Yorubas and 'oda-opue' by the Igbos. The plant is widely used in traditional medicine for the treatment of variety of ailments and well known for its haemostatic and wound healing properties. The pharmacological studies are reviewed and discussed focusing on different extracts from the plant and have been found to possess pharmacological activities such as Immunodulators, CNS depressant, analgesic antimicrobial, anti-inflammatory, anti-allergic, antianaphylactic, anti-lesishmanial, anti-tumorus, anti-ulcerous, anti-bacterial, anti-fungal, anti-histamine, antiviral, febrifuge, gastroprotective, immunosuppressive, insecticidal, muscle relaxant sedative (Balygina *et al*., 2002).

The aim of this study is to determine the antibacterial activities of *Bryophyllum pinnatum* Ethanol leave extract against clinical isolate of *Escherichia coli* and *Klebsiella pneumoniae* from urinary tract Infection patients attending Shehu Muhammed Kangiwa Medical Centre.

MATERIALS AND METHODS

Collection and Preparation of Plant Samples

Matured fresh-disease free leaves of *Bryophyllum pinnatum* were collected from a garden in Ugwan Rimi, Marafa Estate, Kaduna State. The plants were authenticated at the Department of Biological Sciences, Kaduna State University, Kaduna State, and authentication number KASU/BSH/2022 was issued. The leaves rinsed thoroughly with running tap water, dried at room temperature for 4 weeks and then pulverized using mortar and pestle.

Extraction of Plant Materials

The powder of the leaves were extracted using maceration method with ethanol as solvent. This was carried out by soaking 100g each of the finely grounded leaves in 300ml of ethanol for 72 hours, with vigorous agitation; the extracts were decanted and evaporated to dryness in a water bath. The

extracts were then kept in a sterile wide mouth container and stored in the refrigerator at 4^0C for further analysis.

Collection and Maintenance of Test Organisms
The test organisms used were clinical pathogens from urine samples. The test isolates were *Escherichia coli* and *Klebsiella pnemoniae obtained* from Shehu Muhammad Kangiwa Medical Centre. Three (3) isolates each of *Escherichia coli and Klebsiella pneumoniae* were collected from urine samples of urinary tract infection patients. The organisms were collected on sterile agar plates and then sub-cultured unto fresh media plates which were then incubated at 37^0C for 24 hours. These were kept as stock culture in the refrigerator at 4^0C. They were then subjected to Gram staining and biochemical tests for confirmation.

Media Preparation
All Media were prepared following standard manufacturer's instructions.

Phytochemical Screening of Crude Extract
Standard screening test were carried out on the powered leaves for various phytochemical constituents (Trease and Evans, 2002)

Test for Alkaloids
Meyer's test
About 2 drop of Meyer's reagent was added to 2ml of the acidified plant extract .Yelllow precipitate indicate the presence of alkaloids (Trease and Evans, 2002)

Test for Steroids
0.5g of plants extract was mixed in 2ml of chloroform and three drop of concentrated H2SO4 was added to form a lower layer. A reddish brown colour at the interface indicates a positive result (Trease and Evans, 2002).

Test for Tannins
0.5g of plant extract was boiled with water separately and filtered. Two drop of ferric chloride was added to the filtrate. A blue black, green precipitate indicate presence of tannins (Trease and Evans, 2002).

Test for Saponins
Fronthing test
About 0.5g of plant extract was shaken separately in a test tube, frothing which persisted for 15 minutes or when warm on water bath indicates the presence of saponins (Trease and Evans 2002).

Test for Flavonoids
0.5g of plant extract was boiled with water separately and filtered. To 2ml of the filtrate, 2 drop of ferric chloride (freshly prepared) solution was added. A green blue or violet colouration indicates the presence of phenolic hydroxyle group (Trease and Evans, 2002).

Test for Cardiac Glycosides
Plant extract was dissolved in glacial acetic acid containing traces of ferric chloride in a test tube, 1ml of concentrated sulphuric acid was added down the side to form a lower layer at the bottom. A purple ring colour at the interface indicate cardiac glycosides (Trease & Evans, 2002).

Test for Anthraquinones
Small portion of the plant extract was shaken with 10ml of benzene and filtered. 5ml of 10% ammonia solution was added to the filterate and stired the production of a pink-red or violet colour indicate the presence of free anthraquinoes (Trease & Evans, 2002).

Standardization of the Inoculum
The clinical isolates were sub-cultured in 5ml nutrient broth for 18-24 hours. A loopful of the overnight culture was used to prepare a tenfold serial dilution in three tubes each containing 9ml normal saline with a last tube containing 4ml normal saline which resulted in a concentration of 1:10, 1:1000 and 1:5000. The turbidity of the last tube containing the concentration of 1:5000 matched the 0.5 McaFarland standard (Cappuccino and Sherman, 2011).

Preparation of varied concentrations of crude extracts
The ethanol leaf extract of *Bryophyllum pinnatum* was prepared in accordance with the dilution method described by (Baker et al., 1993). The stock solution was prepared by dissolving 2g of the ethanol leaf extract of *Bryophyllum pinnatum* in 10ml of distilled water separately, to make a concentration of 200mg/ml. This concentration (200mg/ml) was then further diluted to make working concentrations of 100mg/ml, 50mg/ml and 25mg/ml.

Antibacterial Activity
The antibacterial activity of Bryophyllum *pinnatum* against the clinical isolates of *E coli and K. pneumoniae* were carried out using agar well diffusion method described by Ofokansi and Esinmone (2005).
Agar plates containing 20ml of Nutirent agar were seeded with 0.1ml of the standardized bacterial preparation in sterile Petri dishes. The Petri dishes were rotated slowly to ensure a uniform distribution of the organisms. Wells of 7.0mm diameter were bored on the agar plates using a sterile cork bore. 0.1ml of the extracts of different concentrations was used to fill each well. The Petri-dishes were allowed to stand for about 30minutes at room temperature to allow for proper diffusion of the extracts to take place. The plates were then incubated at 37^0C for 24 hours. The zones of inhibition if any were measured in millimetres and recorded**.**

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
The MIC of the extract was determined using the agar dilution method (Lorian, 2005). 0.1ml of the different concentrations of the extract was incorporated into nutrient agar medium, poured into Petri dishes and allowed to solidify. The standardized inoculum was inoculated with the solidified medium using point inoculation techniques on paper disc. The plates were then incubated at 37^0C for 24 hours. After the period of inoculation, the lowest concentration of the extract which showed no visible growth was recorded as the MIC. The plates which showed no visible growth was then sub cultured onto fresh medium with no extract, incubated at 37^0C for 24 hours. The concentration which showed no visible growth was then recorded as the MBC.

RESULTS
The results of the phytochemical screening of the ethanol leaf extracts of *Bryophyllum pinnatum* is shown in Table 1 Saponins, Flavonids, Phenol, Tannins, Terpeniods and Alkaloids were present in *Bryophyllum pinnatum*.

Table 1. Phytochemical analysis of the crude extracts

Constituents	*Bryophyllum pinnatum*
Saponins	+
Flavonoid	+
Terpeniods	+
Alkaloids	+
Tannins	+
Phenol	+

Key: + = present, - = absent

The antibacterial activity of the ethanol extracts of *Bryophyllum pinnatum* leaves against *E. coli* and *K. pneumoinae* is shown in Table 2.

The results shows that the extracts of *Bryophyllum pinnatum* had no activity on *E. coli* and *K. pneumoniae* clinical isolates at the four concentrations tested (200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml).

Table 2. Antibacterial activity of *Bryophyllum pinnatum* ethanol leaf extracts on *klebsiella pneumonia* and *Escherichia coli* using Agar well diffusion method

	Concentrations (mg/ml) of the zones of inhibition (mm) Ethanol extract (*B.pinnatum*)				
Test organisms	**200**	**100**	**20**	**25**	**control**
E. coli	-	-	-	-	40mm
E. coli	-	-	-	-	40mm
E. coli	-	-	-	-	50mm
K. pneumoniae	-	-	-	-	22mm
K. pneumoniae	-	-	-	-	30mm
K. pneumoniae	-	-	-	-	30mm

Key: - = no zone, Control = Gentamycine

The minimum inhibitory concentration of both extracts against the clinical isolates is shown in Table 3.

The results shows that there was visible growth at all the four concentrations (200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml) tested against *E. coli* and *K. pneumoniae*.

Table 4.2. Minimum inhibitory concentration (MIC) of the ethanol leaf extracts of *Bryophyllum pinnatum* against *E. coli and K. pneumoniae*

Concentration of extract Mg/ml	clinical isolates	
	Escherichia coli	*Klebsiella pneumoniae*
200	+	+
100	+	+
50	+	+
25	+	+

Key; + = presence of growth, - = absence of growth.

DISCUSSION

The phytochemical screening of the ethanolic crude extract of *Bryophyllum pinnatum* revealed the presence of Saponins, Flavonoid, Tannins, Alkaloid, Terpenoid and Phenol. This is similar to the findings reported by M.I. Izundu *et al.* (2021), Uchegbu *et al.* (2017).

In the present study, the ethanolic leave extract of *B. pinnatum* showed no antibacterial activity against isolates of *E. coli* and *K. pneumoniae*. This agrees with the findings reported by Umimbabazi *et al.* (2015) and Ibikunle *et al.* (2017). Nagaratna and Prakash (2015) reported antibacterial activity of *Bryophyllum pinnatum* leaf extract against *E. coli* using methanol as solvent with no activity against *K.* pneumoniae. Similarly Odunayo *et al.*(2007) reported antibacterial activity of methanol extract of *Bryophyllum pinnatum* against isolates of *E coli* and *Klebsiella pneumoniae* . In a study conducted by Azuonwu *et al.*,(2017) to test the antibacterial activity of ethanol extract of *Bryophyllum pinnatum* against *E coli* a zone of inhibition of 1mm was reported .The differences in antibacterial activity reported in different studies can be attributed to solvent used in the extraction process. A good solvent is characterized by its optimal extraction and its capacity in conserving the stability of the chemical structure of desired compounds (Harborne, 1999). Therefore, the type of extraction solvent and its polarity may have a significant impact on the level of extracted Polyphenols (Liux *et al.*, 2007). The absence of antibacterial activity against *E coli* can also be attributed to the fact that *E. coli* can alter their genetic makeup with astonishing rapidity resulting in unstable susceptibility patterns (Uwimbabazi *et al.*, 2015). Though plants have been used to treat infections since time immemorial, there has always been a general misconception by herbal medicine practitioners that a single herb can provide cure to most if not all infections, leading people to continuously use such herbs to treat infections believing that they will be cured even when the symptoms persist, resulting in complications that would have been avoided, if alternative drugs with scientifically proven activity was used.

The findings from this study shows that the ethanopharmacological claim on the use of herbal plants to cure most if not all infections should constantly be subjected to test in other to provide scientific evidence to either support or refute such claims.

CONCLUSION

The results of this study revealed the presence of phytochemicals in the ethanolic leave extracts of *Bryophyllum pinnatum*. The study also revealed that the ethanolic extract of *Bryophyllum pinnatum* leaves had no activities against clinical isolates of *E. coli* and *K. pneumoniae* from urinary tract infection patients. This provides scientific evidence to counter any claims by herbal practitioners of the use of *Bryophyllum pinnatum* leaves for the treatment of infections caused by *E coli and K pneumonia.*

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