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Microwave Assisted Extraction, Phytochemical and Antimicrobial Evaluation of Ethyl Acetate Extracts of Stem Bark of *Ficus exasperata* (Vahl)

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ABSTRACT: Stem bark of *Ficus exasperata* was extracted using ethyl acetate by microwave-assisted solvent extraction. Phytochemical screening and antimicrobial evaluation were carried out on the extract. Phytochemical screening revealed the presence of cardiac glycosides, tannins, flavonoids, triterpenes and steroids. Antimicrobial evaluation revealed that the extract is active against Methicillin – resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Candida stellatoidea* and *Candida tropicalis*. The zones of inhibition (mm) for the test organisms were (24 - 31) mm. Minimum inhibition concentrations (mg/L) of extract against MRSA, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Candida stellatoidea* and *Candida tropicalis* were 2.5, 1.25, 1.25, 2.5, 1.25, 1.25, 1.25 and 2.5 respectively. Minimum bactericidal concentrations (mg/L) of extract against Methicillin – resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Streptococcus pneumonia*, *Salmonella typhi*, *Proteus mirabilis Shigella dysenteriae Candida stellatoidea* and *Candida tropicalis* were 5, 2.5, 2.5, 5, 5, 2.5, 2.5 and 5 respectively. The extract showed high inhibition against MRSA and *Candida tropicalis.*

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One of the most outstanding successes in modern medicine is in the area of the development of Concentration, Microwave antimicrobial agents (Jamuna *et al.*, 2011). Moraceae is a family of flowering plants. The family contains over 500 species of the genus *Ficus* (Amponsah, 2012). They are mostly found in the tropics and semi-tropics. The genus *Ficus* consists of woody shrubs which are collectively referred to as fig trees. There are over 45 different species of *Ficus* in Nigeria (Adebayo *et al.*, 2009). In traditional medicine, *Ficus* species are used to cure various diseases. For instance, they have been used for the treatment of hypotension, dysentery and as astringents (El-Haway *et al.*, 2012). *Ficus exasperata* is a medium sized tree or a shrub of about 20 – 30-meter height (Uzama *et al*., 2018). In Nigeria, the plant is found in secondary rain forest and sometimes besides streams and rivers (Shagal *et al.*, 2011). It is known as forest sandpaper tree (Julius *et al.*, 2020). It is known by several vernacular names in Nigeria: *Ipin* (Yoruba), *Inwalinwa* (Igbo) (Ughachukwu *et al*., 2012). It is called *Hi-tur* in Tiv language and *Ijakpi* in Igala (Ugbenyo, 2017). *Ficus exasperata* is used in the treatment of several diseases in African traditional medicine (Nnamonu et. al. 2016). It is used in the treatment of fungal infection, dysentery, itching and rheumatism (Uzama *et al*., 2018). A decoction of the root of *F. exasperata* is used in the treatment of pneumonia in Tanzania. Its leaves are consumed as vegetable by Edo people of Nigeria (Nnamonu *et al*. 2016). The leaves are also used as abrasives for polishing furniture and utensils (Amponsa *et al*., 2013). Cold bark extract of *Ficus exasperata* is used to treat dizziness. Sap from the bark is used as a remedy for bleeding. The root is used in the treatment of cough, tuberculosis, urinary tract infection and gonorrhoea (Uzama *et al*., 2018). As a route in discovering antibacterial resistant drugs, microwave assisted extraction of stem bark of *Ficus exasperata* led to phytochemical screening and antimicrobial evaluation on the crude extracts.

MATERIALS AND METHOD

Collection and Preparation of Plant Material: Stem bark of *Ficus exasperata* was collected from Ibaji Local Government of Kogi State (North Central of Nigeria), identified and authenticated at the Herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria with voucher number 2733. The plant sample was collected, washed with distilled water and dried under shade for three weeks, after

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which it was pulverized and stored at room temperature until the time of extraction.

Microwave Assisted Solvent Extraction: Microwave assisted extraction (MAE) was carried out in line with the method described by Nnamonu *et al*. (2016). Ethyl acetate was used as solvent for extraction. Pulverized plant sample (500 g) was extracted with ethyl acetate (1.6 L) for 30 minutes (3 minutes at a time for ten times) using A domestic microwave oven (70 Watts/Defrost function). After extraction, extracts were allowed to cool to room temperature, carefully filtered and subjected to evaporation at room temperature (Muktar *et al*., 2018).

Phytochemical Screening: Phytochemical screening was carried out to test for presence of the following secondary metabolites: carbohydrates, cardiac glycosides, saponins, tannins, alkaloids, terpenoids, steroids and flavonoid using methods described by Muktar *et al*. (2018), Idris *et al*. (2019) and Anyam, (2011).

Test organisms: The bacteria used viz Methicillin – resistant *Staphylococcus aureus (*MRSA), *Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus, Salmonella typhi, Pseudomonas fluorescens, Proteus mirabilis, Shigella dysenteriae* and *Proteus vulgaris;* and the fungi *Candida stellatoidea*, *Candida tropicalis*, and *Candida pseudotropicalis* were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria.**:**

Antimicrobial Screening: Mueller Hinton Agar was the medium used as growth medium for the bacterial and Sabouraud Dextrose agar was used for fungi. The media were prepared according to manufacturer's instruction, sterilized at 121^0C for 15 minutes, poured into sterile Petri dishes and allowed to cool and solidify. Agar Diffusion method was used for antimicrobial screening of the plant extracts. The sterilized medium was seeded with 0.1 mL of the standard inoculum of the test microbe, the inoculum was evenly spread over the surface of the medium by use of sterile swab. Using a standard cork borer (6mm in diameter), a well was cut at the centre of each inoculated medium. The solution of the extract (0.1 mL) of concentration 10 mg/mL was introduced into each well on the medium.0.1g of extract was dissolved in 10 mL of DMSO (Dimethyl sulfoxide) to obtain a concentration of 10mg/ml. This was the initial concentration of the extract used to determine the antimicrobial activities from the plant. Incubation was carried out at 37^oC for 24 hours for bacteria and at 30^0C for 7 days for fungi. Each plate of the medium was observed after the stated time for zone of inhibition of growth. Zone of inhibition was measured using a transparent ruler (mm) (Idris *et al*. 2019).

Minimum Inhibition Concentration (MIC): The extract's minimum inhibitory concentration was determined using the broth dilution method. Mueller Hinton agar and Sabouraud dextrose broth were made as directed by the manufacturer, distributed into test tubes (10 ml), sterilised at 121℃ for 15 minutes, and allowed to cool. A turbid solution was made using Mc-turbidity Farland's standard (scale number 0.5). A sterile test tube was filled with 10 mL of normal saline solution. The test microorganisms were inoculated and cultured for 6 hours at 37 oC. A microbe broth was diluted in normal saline until the turbidity reached that of the Mc Farland standard, at which point the test microorganisms attained a concentration of around 1.5x 108 CFU/mL, as determined by visual comparison. Two times in sterile broth, the extract was serially diluted, providing concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.625 mg/mL. To obtain the starting concentration, the extract (0.1g) was dissolved in sterile broth (10 mL). The microbe suspension in normal saline (0.1 mL) was injected into various amounts of extract in nutrient broth. For bacteria, the broths were incubated at 37°C for 24 hours, and for fungus, at 30°C for 7 days. For bacteria, the findings were recorded after 24 hours, and for fungi, after 7 days (Idris et al., 2019).

Minimum Bactericidal Concentration (MBC). Mueller Hinton and Sabouraud dextrose agar were prepared as per manufacturer instruction; sterilised at 121°C for 15 minutes, placed into sterile petri dishes, and allowed to cool and harden. The contents of the serial dilution of MIC were then sub-cultured on Mueller Hinton agar for bacteria and Sabouraud dextrose agar for fungi. Bacteria were incubated at 37°C for 24 hours and fungi were incubated at 30°C for 7 days. The colony growth on each plate was then monitored. The plates with the lowest concentration of the extract without colony growth had the lowest bactericidal/fungicidal concentration (ldris et al., 2019).

RESULTS AND RESULTS

Extraction of stem bark of *Ficus exasperata* (500 g) with ethyl acetate using microwave gave a crude extract (8.9 g), percentage yield was 1.78 %. Phytochemical screening revealed the presence of anthraquinones, cardiac glycosides, steroids, triterpenes, and tannins (Table 1). The extracts were found to be effective against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Candida stellatoidea*, and *Candida tropicalis* in antimicrobial sensitivity

testing (Table 2). Minimum Inhibition Concentration of the extract against the test microbes showed the extracts to be effective against MRSA (2.5 mg/mL), Staphylococcus aureus (5 mg/mL), Streptococcus pneumoniae (1.25 mg/mL), Salmonella typhi (2.5 mg/mL), Proteus mirabilis (1.25 mg/mL), Shigella dysenteriae (1.25 mg/mL), Candida stellatoidea (1.25 mg/mL), and Candida tropicalis (2.5 mg/mL) (Table 3). Minimum Bactericidal/Fungicidal Concentration of the extract against the test microbes showed the extracts to be effective against MRSA (5 mg/mL), *Staphylococcus aureus* (2.5 mg/mL), *Streptococcus pneumoniae* (2.5 mg/mL), *Salmonella typhi* (5 mg/mL), *Proteus mirabilis* (5 mg/mL), *Shigella dysenteriae* (2.5 mg/mL), *Candida stellatoidea* (2.5 mg/mL), and *Candida tropicalis* (5 mg/mL) (Table 4).

Table 1: Phytochemical screening result

S/N	Phytochemical	Test	Observation	Conclusion
1	Carbohydrate	Fehling test	No change	–
2	Anthraquinone		Pink colouration	+
3	Cardiac Glycosides	Kella-Killiani Test	Brown ring	+
4	Steroid	Liberman- Burchard test	A colour change from violet to green	+
5	Triterpenes	Salkowski's Test	Redish-brown	+
6	Alkaloids	Dragendorff's test	No change	–
		Meyer' test	No change	–
		Wagner's test	No change	–
7	Tannins	$FeCl_3$ test	Green coloration	+

Keys: + = Present – = Absent

Table 2: Sensitivity/ Zone of inhibition (mm) of Extract Compared with Standards (Control Drugs).

Test Organism	Crude extract	Ciprofloxacin	Fluconazole
MRSA	S (27)	R (0)	R (0)
Staphylococcus aureus	S (30)	S (37)	R (0)
Streptococcus pneumoniae	S (31)	S (38)	R (0)
Bacillus cereus	R (0)	S (38)	R (0)
Salmonella typhi	S (26)	S (42)	R (0)
Pseudomonas fluorescens	R (0)	R (0)	R (0)
Proteus mirabilis	S (27)	S (32)	R (0)
Shigella dysenteriae	S (29)	S (41)	R (0)
Proteus vulgaris	R (0)	S (30)	R (0)
Candida stellatoidea	S (29)	R (0)	S (34)
Candida tropicalis	S (24)	R (0)	S (35)
Candida pseudotropicalis	R (0)	R (0)	S (32)

***R**= Resistant **S**= Sensitive. Figures in bracket represent average triplicate zone of inhibition (in mm).*

Table 3: Minimum Inhibition Concentration of the Extract against the test Organism

Test Organism/Concentration (mg/mL)	10.0	5.0	2.5	1.25	0.62
MRSA	-	-	0*	+	++
Staphylococcus aureus	-	-	-	0*	+
Streptococcus pneumoniae	-	-	-	0*	+
Bacillus cereus					
Salmonella typhi	-	-	0*	+	++
Pseudomonas fluorescens					
Proteus mirabilis	-	-	-	0*	+
Shigella dysenteriae	-	-	-	0*	+
Proteus vulgaris					
Candida stellatoidea	-	-	-	0*	+
Candida tropicalis	-	-	0*	+	++
Candida pseudotropicalis					

***Keys**: -= NO growth, 0* = MIC, + = Turbid (growth), ++ = moderate turbidity.*

Phytochemical Screening: Result of phytochemical screening showed presence of cardiac glycosides, tannins, flavonoids, anthraquinones, steroids and triterpenes. Lawal *et al*., (2012) reported the presence of saponins and cardiac glycosides with traces of anthraquinone in methanolic root bark extracts of the plant. Similarly, Shagal *et al*., (2011) reported the presence of tannins, flavonoids, saponins, glycosides, phenols and steroids from water and ethanolic extracts of leaves, stembark and roots of the plant. These phytochemicals have a wide range of therapeutic effects. Cardioactive substances, such as cardiac glycosides, affect the heart muscles and stimulate renal flow, which is a critical homeostatic mechanism that protects the kidney from arterial pressure spikes that would otherwise cause harm to the glomerular capillaries. They affect neurons, affecting the mechanical and electrical activity of the heart, as well as the vascular system's resistance and capacitance. (Lawal *et al*., 2012). Tannins are known for their use

in preventing urinary tract infections and other bacterial infections (Noce *et al*., 2021). The presence of flavonoids in a plant indicates that natural phenolic compounds which have beneficial effects in human diet as antioxidant and neutralizing agent for free radicals are contained in the plant (Suleman *et al*., 2019).

Table 4: Minimum Bactericidal/Fungicidal Concentration of the Extract against test Organisms

Test Organism/Concentration(mg/mL)	10.0	5 .0	2.5	1.25	0.62
MRSA	-	0*	+	++	+++
Staphylococcus aureus	-	-	0*	+	++
Streptococcus pneumoniae	-	-	0*	+	++
Bacillus aureus					
Salmonella typhi	-	0*	+	++	+++
Pseudomonas fluorescens					
Proteus mirabilis	-	0*	+	++	+++
Shigella dysenteriae	-	-	0*	+	++
Proteus vulgaris					
Candida stellatoidea	-	-	0*	+	++
Candida tropicalis	-	0*	+	++	+++
Candida pseudotropicalis					

Keys: - = NO growth 0 = MBC/MFC, + = Turbid (growth), ++ = Moderate turbidity, +++ = Heavy colonies growth.*

Antimicrobial Screening: Extract showed activity against three gram-positive bacteria (MRSA, *Staphylococcus aureus* and *Streptococcus pneumoniae*), three gram-negative bacteria (*Salmonella typhi, Proteus mirabilis* and *Shigella dysenteriae*) and two fungi (*Candida stellatoidea, Candida tropicalis). Bacillus cereus* (gram positive bacterium), *Proteus vulgaris and Pseudomonas fluorescens* (gram negative bacteria) and *Candida pseudotropicalis* (fungus) were resistant. Presence of tannins, flavonoids, triterpenoid, steroids and glycosides in plants is responsible for different curative properties of such plants (Hossain and Nagooru, 2011). Triterpenes and glycosides possess a wide range of biological activity including bactericidal, antiviral, fungicidal, cardiovascular and anticancer activity (Mayer *et al*., 2007). It is significant that the extract was sensitive against MRSA which is resistant to ciprofloxacin (a drug used for the treatment of bacterial infections). The result for zone of inhibition of plant extract against test organisms showed that it was significantly active. Any activity above 12 mm is taken as a strong activity, although the percentage extracted was low when compared to other reported MAE (Momtaz *et al*., 2008). Extracts showed a very competitive activity when compared with ciprofloxacin. This may be due to the extraction method used. Microwave assisted extraction has been shown to improve on the inhibition percentage of plant extracts. Kenmogne *et al.* (2014) reported that analgesic compounds in *Ximenia americana* obtained by microwave extraction had improved percentage of inhibition when compared with soxhlet and maceration methods. The MIC of extract against *Methicillin – resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococcus pneumonia, Salmonella typhi, Proteus mirabilis Shigella dysenteriae Candida stellatoidea* and *Candida tropicalis* was *(*1.25 - 2.5) mg/mL, whilst *MBC/MFC* was (2.5 – 5) mg/mL.

Conclusion: This study demonstrated that *Ficus exasperata* has the potential to be used as a source of antimicrobial compounds; for this reason, isolation and characterisation of the bioactive components from its extracts is ongoing.

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