Microwave Assisted Solvent Extraction Study of *Ficus Exasperata* (Vahl) Stem Bark Antimicrobial Potential Agaisnt Methicillin

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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 Staphylococus aureus (MRSA), Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Proteus mirabilis, Shigella dysenteriea, Candida stellatoidea and Candida tropicalis. The zones of inhibition (mm) for the test organisms were 27, 30, 31, 26, 27, 29, 27and 24 respectively. Minimum inhibition concentrations (mg/L) of extract against *Methicillin – resistant Staphylococus aureus (MRSA), Staphylococcus aureus, Streptococcus pneumonia, Salmonella typhi, Proteus mirabilis, Shigella dysenteriea, 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/fungicidal concentrations (mg/L) of extract against Methicillin – resistant Staphylococus aureus (MRSA), Staphylococcus aureus, Streptococcus pneumonia, Salmonella typhi, Proteus mirabilis Shigella dysenteriea Candida stellatoidea and Candida tropicalis were 5,2.5,2.5,5,5,2.5,2.5 and 5 respectively.*

Keywords: Antimicrobial evaluation, *Ficus exasperata*, Minimum Inhibition Concentration, Microwave, Phytochemical screening

Introduction

One of the most outstanding successes in modern medicine is in the area of the development of of antimicrobial agents (Jamuna *et al.*, 2011).

Moracae 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) (Ugachukwu *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 furnitures 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).

Materials and Method

Collection and Preparation of Plant Material

Stem bark of *Ficus exasperata* was collected from Tarauni Local Government of Kano State (North West of Nigeria), identified and authenticated at the Department of Biological Sciences, Bayero University Kano with voucher number 0133. The plant sample was collected, washed with distilled water and dried under shade for three weeks, after 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. The same procedure was repeated using ethyl acetate as solvent.

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 (Brain and Turner, 1975, Sofowora, 1984, Trease and Evans, 2002, Hassan *et al*, 2004, Edeoga et al., 2005 and Anyam, 2011).

Test organisms

The bacteria used viz Methicillin – resistant *Staphylococus aureus (*MRSA), *Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus, Salmonella typhi, Pseudomonas fluorescens, Proteus mirabilis, Shigella dysenteriae* and *Proteus vulgaris;* and the fungi *Candida stettoidea, Candida tropicalis,*and *Candida pseudotropicalis* were obtained from the Aminu Kano Teaching Hospital Zaria road Kano

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 ^{0}c 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 center 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 (Dimethylsulfoxide) 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 ^{o}C for 24 hours for bacteria and at 30^{0}C 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).

Minimum Inhibition Concentration

Minimum inhibition concentration of the extract was carried out using broth dilution method. Mueller Hinton agar and Sabouraud dextrose broth was prepared according to manufacturer's instruction and dispensed into test tubes (10 ml) and sterilized at 121^{0}C for 15minutes, and allowed to cool. Mc-Farland's turbidity standard was (scale number 0.5) was prepared to give a turbid solution. Normal saline (10 ml) was prepared and dispensed into sterile test tube. The test
microbe was inoculated and incubated at 37^{0}c for 6 hours). Dilution of the microbe was done in the normal saline until turbidity reached that of the Mc Farland standard by visual comparison. At this point, test microbes had a concentration of about 1.5x 10^{8} cfu/mL. Two-fold serial dilution of the extract in the sterile broth was made to obtain the concentration of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.625 mg/mL. The initial concentration was obtained by dissolving 0.1g of the extract in 10 mL of the sterile broth.

From suspension of the microbes in normal saline, 0.1 mL was inoculated into different concentrations of extract in nutrient broth. The broths were incubated at 37 ^{o}C for 24 hours for bacteria and at 30 ^{0}C for 7 days for fungi. The results were recorded after 24 hours.

Minimum Bactericidal/ Fungicidal concentration

Mueller Hinton and Sabouraud dextrose agar were prepared, sterilized at 121 ^{0}C for 15 minutes, poured into sterile petri dishes and allowed to cool and solidify. The contents of MIC in the serial dilution were then sub-cultured onto prepared media, bacteria on Mueller Hinton agar and fungi on Sabouraud dextrose agar. Incubation was made at 37 ^{0}C for 24 hours for bacteria and at 30 ^{0}C for 7 days for fungi. Each plate was then observed for colony growth. The Minimum bactericidal/fungicidal concentration was the plates with lowest concentration of the extract without colony growth.

Result

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 %.

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	Dragendoff's test	No change	–
		Meyer' test	No change	–
		Wagner's test	No change	–
7	Tannins	$FeCl_3$ test	Green coloration	+

+ = Present – = Absent

Table 3: Sensitivity/ Zone of inhibition (mm) of Extract Compared with Standards (Control Drugs).

Test Organism	Crude extract	Ciprofloxaxcin	Fluconazole
MRSA	S (27)	R (0)	R (0)
Staphylococcus aureus	S (30)	S (37)	R (0)
Streptococcus pneumonia	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 zone of inhibition (in mm).

Table 4: Minimum Inhibition Concentration of the Extract against the test Organism

Concentration/Test Organism	**10 mg/mL**	**5 mg/mL**	**2.5 mg/mL**	**1.25 mg/mL**	**0.62 mg/mL**
MRSA	-	-	0*	+	++
Staphylococcus aureus	-	-	-	0*	+
Streptococcus pneumoniae	-	-	-	0*	+
Bacillus cereus					
Salmonella typhi	-	-	0*	+	++
Pseudomonas fluorescens					
Proteus mirabilis	-	-	-	0*	+
Shigella dysenteriea	-	-	-	0*	+
Proteus vulgaris					
Candida stellatoidea	-	-	-	0*	+
Candida tropicalis	-	-	0*	+	++
Candida pseudotropicalis					

Keys: - ➡ NO growth 0* ➡MIC, + ➡Turbid (growth)
++ ➡ Moderate turbidity, +++ ➡Heavy colony growth

Table 5: Minimum Bactericidal/Fungicidal Concentration of the Extract against test Organisms

Test Organism/Concentration	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL
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

Discussion

Phytochemical Screening

Result of phytochemical screening shows 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 different medicinal significance. For example, cardiac glycosides are cardioactive compounds. They act on the heart muscles and increase renal flow. They exert some effects on neural tissues and thereby influence the mechanical and electrical activities of the heart and modify the resistance and capacitance of the vascular system (Lawal *et al*.,2012). Tannins are known for their use in preventing urinary tract infections and other bacterial infections (Ekeanyanwu *et al.,* 2010). 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 (Shagal *et al*., 2011).

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 dysenteria*) and two fungi (*Candida stellatoidea, Candida tropicalis). Bacillus cereus* (gram positive bacterium), *Proteus vulgaris and Psudomonas fluorescense* (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 (Alamgir *et al.,* 2013). Triterpenes and glycosides possess a wide range of biological activity including

bactericidal, antiviral, fungicidal, cardiovascular and anticancer activity (Patocka , 2003). 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 strongly active. Any activity above 12 mm is taken as a strong activity (Mudi *et al*, 2011). 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. Minimum inhibition concentration (in mg/mL) of extract against *Methicillin – resistant Staphylococus aureus (MRSA), Staphylococcus aureus, Streptococcus pneumonia, Salmonella typhi , Proteus mirabilis Shigella dysenteriea Candida stellatoidea and Candida tropicalis was* 2.5 1.25 1.25 2.5, 1.25, 1.25, 1.25 and 2.5 respectively,*whilst MBC/MFC* (in mg/mL) was respectively 5, 2.5, 2.5, 5, 5, 2.5, 2.5 and 5 .

Conclusion

This study showed that *Ficus exasperata* has potential as a useful antimicrobial agent. Further research to isolate and characterize the active principle from ethyl acetate etxtract of stem bark of *Ficus exasperate* is on-going in our laboratory.

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