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Comparative Antimicrobial Activity of Ethanol and Hexane Leaf Extracts of Ficus Exasperata on Five Microbial Isolates

Godwill Azeh Engwa

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Abstract

This study evaluated the antimicrobial activity of Ficus exasperata and compared the efficacy of ethanol and hexane extracts on five microbial isolates. To ascertain the set objective, after extraction with ethanol and hexane, the disk and well diffusion agar methods were employed to investigate the antimicrobial activity of the extracts and its minimum inhibitory and bactericidal concentrations. A phytochemical screening was done for the confirmation of the result and the data was statistically analysed.

Index terms— ficus exasperata, antimicrobial activity, ethanol, hexane, extract, phytochemicals, microbial isolates.

1 I. Introduction

nfectious diseases are the number one cause of death due to illnesses across the world and account for approximately one-half of all deaths in tropical countries. According to World Health Organisation (WHO) report, about 15 million (>25%) of 57 million annual deaths worldwide are the direct result of infectious diseases [1]. Of these infectious diseases, microorganisms are the commonest organisms responsible for morbidity and mortality [2,3]. As such, bacterial and fungal diseases continue to remain a major public health problem [4].

Efforts in the management of bacterial and fungal infections had been very effective for long with the use of antibiotics till the emergence of antimicrobial drug resistance in the past two decades [5,6]. Since then, the use of conventional drugs have been challenging in the treatment and management of these diseases and the quest for alternative solutions have been a major global concern to WHO and other public health institutions and organizations.

In recent time, there is so much concern on the use of plants and their constituents for treatment as have extensively been used in folk medicine for the treatment of many ailments [7,8]. So many plants have been shown to have medicinal properties against microbial and fungal infections [9,10]. One of such plant is Ficus exasperata, locally known as "sand paper plant" and "Ewe ipin" in the Yoruba language of Western Nigeria. Different parts of the plant are locally used for treating various infectious diseases such as eye-sores, ring worm, stomach pains and leprosy etc. [11][12][13]. The leaf extract of Ficus exasperata has been reported for the treatment of various diseases including coughs, intestinal pains, colics, bleeding, ulcer, wounds, bacterial, fungal infections etc. [13] ??14][15] ??16] ??17][18][19][20]. Various pharmacological actions such as anti-hypertensive, antioxidant, anti-inflammatory, anti-ulcer, anti-lipidic, anti-bacterial and anti-fungal activities have been described for Ficus Exasperata ??16] ??17][18][19][20][21][22].

These pharmacological activities are attributed to the presence of certain bioactive components in the plant [23] which have been identified and characterized and are now the basis for new therapies. Synergism is reported to be the most probably mechanism of action responsible for the overall pharmacological activity of medicinal plants [24,25]. Synergic effect depends on the photochemical load; that is, the number of various types of phytochemicals extracted which is determined by the extraction method employed. Polar solvent have shown to recover more bioactive components from plants than nonpolar solvents hence, a greater phytochemical load [26,27]. In this study, we compared the effect of ethanol (polar) and hexane (non-polar) extracts of Ficus exasperata on five microbial isolates. The crude extracts were further subjected to phytochemical screening to evaluate the phytochemical load.

2 II. Materials and Methods

3 a) Plant material

Fresh leaves of Ficus exasperata were collected from Enuguagu, Achi, Orjiriver local Government, Enugu and transported to the Chemistry Laboratory of Godfrey Okoye University, Enugu State of Nigeria.

4 b) Preparation of ethanol and hexane extracts

The leaves of Ficus exasperata were air dried for two weeks and ground to fine powder with a Binatone blender (Model BLG-401). Extraction was done as described by Adebayo and Ishola using a soxhlet extractor [28]. 70 g of each portion of the leaf powder were dissolved in Hexane and Ethanol and the mixture was transferred in the extractor separately. Hexane extraction was done at 69 o c adding 0.080g of antibulbing chips to speed up the boiling point while ethanol extraction was done at 50 0 C with 0.070g of anti-bulbing chips.

5 c) Specimen collection and culture

Clinical isolates of Salmonella typhi, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Cadida albicans were obtained from the Microbiology Laboratory of Godfrey Okoye University. The isolates were tested for viability by resuscitating them in buffered peptone water and only the viable isolates were sub-cultured. Plates containing 15ml of sterile nutrient agar (Oxoid, England) after autoclaving each were inoculated with the viable isolates by aseptic streaking and cultured at 37°C for 24 hours.

6 d) Evaluation of antimicrobial activity

In vitro antimicrobial activity was evaluated by the agar well and disc diffusion methods.

i.

7 Agar well Diffusion method

Agar well diffusion technique as described by Adeniyi et al. was adopted for the study [29]. Using sterile pasteur pipette, 5mm diameter wells were created at the centre of each plate and 1ml of the various concentrations of the plant extracts were dispensed into each well. The extracts were allowed to diffuse into the medium for 1hour pre-diffusion at room temperature after which the plates were incubated at 37 o c for 24 hours and the zones of growth inhibition measured in millimetre (mm). ii.

8 Disc diffusion method

In this method, 0.5mm sterile filter paper disc was soaked in extract solution for 2 hours then placed on the surface of the agar plate. The plates were kept at room temperature for 2 hours pre-diffusion at room temperature and incubated at 37 o c for 24 hours.

Also, a standard antibiotics, chloramphenicol of 250mg was dissolved in 2.5ml of distilled water to obtain a concentration of 100mg/ml. A twofold serial dilution was done trice to obtain the following concentrations; 50, 25, and 12.5 mg/ml. These four concentrations of chloramphenicol which served as positive control were impregnated on filter papers disc and placed alongside the ethanol plant extract (100mg/ml) filter paper disc on the surface of the agar plate. The plates were kept at room temperature for 2 hours pre-diffusion at room temperature and incubated at 37 o c for 24 hours.

9 e) Minimum inhibitory concentration (MIC)

The minimum inhibition concentration was determined using the dilution method as described by Robbers et al. [30] which made use of a twofold dilution assay. The extract was diluted with distilled water trice at a ratio of 1:2 to obtain concentrations of 200, 100, 50, and 25ug/ml. Nutrient agar broth was prepared according to manufacturer's instruction and dispensed into separate test tubes. 1ml each of the four extract dilutions was added in order of decreasing concentrations to the broth and incubated at 37°c for 24 hours.

10 f) Minimum Bactericidal Concentration (MBC)

The minimum bacterial concentration was determined as described by Robbers et al. [30]. The broths from the MIC assay were streaked on a solid nutrient agar plate and incubated at 37°c for 24 hours. Various dilutions of the plant extracts were impregnated on sterile filter papers and placed on the surface of the solid dry agar plate. After pre-diffusion of the plate at room temperature for 2 hours, they were incubated at 37 o c for 24 hours.

11 g) Phytochemical screening

Phytochemical analysis of both the hexane and ethanol extract was carried out for tannins, glycosides, saponin, flavonoids, alkaloids and steroids using standard methods of Sofowora, Trease and Evans, and Harbon [31,32,33].

12 III. Data Analysis

The zone of inhibition was considered as the distance of the clear zones that showed no growth on the surface of agar plate after culture and measured in millimetres (mm) using a ruler. The lowest concentration of extract which showed no turbidity in the broth culture was recorded as the MIC and the concentration that exhibited no bacterial growth after culture was considered as the MBC value.

The data was expressed as Mean ± SEM. The differences between the groups was compared using the analysis of variance method (ANOVA) followed by

13 IV. Results and Discussion

Extraction is a key step for the recovery and isolation of bioactive phytochemicals from plant materials. The pharmacological activities of plants greatly depend on the extraction method being employed [34]. Solvent extraction has widely been used to recover and isolate bioactive molecules as well as in the evaluation of their in vitro activity [35,36]. In this ethanol and hexane were used in the extraction investigate the activity of Ficus exasperata against microbial isolates. The percentage yield after extraction was higher in ethanol compared to hexane. Ethanol extraction recovered 10. 2g of extract with a percentage yield of 14.6% compared to 7.5g of hexane extract with a yield of 10.6% (Table 1). After extraction, the activity of Ficus exasperata was evaluated on five microbial isolates; Salmonella typhi, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Candida alblican. Antimicrobial screening using the disk and well diffusion methods showed Ficus exasperata extracts (ethanol and hexane) to possess antibacterial and antifungal activities as were able to inhibit the growth of all the microbial isolates.

In the disk diffusion method, the zone of inhibition (mm) ranged from 2.5 to 18.5 and the highest inhibition was recorded for the ethanol extract while the lowest was for the hexane extract. Inhibition was concentration dependent that is; the 200mg/ml extract concentration showed the highest inhibition while the 25mg/ml showed the least microbial growth inhibition. Comparing the inhibition profile for the various concentrations and microbial isolates, ethanol extract showed a greater inhibition than hexane extract and was significantly different ($p < 0.05$) for the 150 and 25mg/ml concentrations. The inhibition pattern varied in respect to the concentration for all the microbial isolates with the greatest inhibition recorded on Salmonella typhi followed by Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli in decreasing order while Candida alblican showed the least inhibition (Table 2). The well diffusion method also showed antimicrobial activity on all the microbial isolates but not as effective as the disk diffusion method. The zone of inhibition ranged from 2.0 to 15.5mm and the highest inhibition was recorded for the ethanol extract while the lowest was observed for the hexane extract. Similarly, the inhibition was concentration depedent as the highest extract concentration (200mg/ml) showed the greatest inhibition that reduced with decreasing concentration. Comparatively, ethanol extract showed a greater antimicrobial activity than hexane having a greater zone of inhibition for all the different concentrations and isolates and the difference was significant for the 100 and 25mg/ml concentrations ($p < 0.05$). Also, the microbial inhibition profile was similar to that of the disk diffusion method with Salmonella typhi having the highest inhibition followed by Klebsiella pneumoniae Staphylococcus aureus, Escherichia coli and Candida alblican in decreasing order (Table 3). The minimum inhibitory concentration (MIC) is the least concentartion of a plant extract that shows no growth of microbial isolates in broth. In this study, the MIC was the 100mg/ml concentration and was observed for both the ethanol and hexane extracts. The ethanol concentration inhibited the growth for all the isolates except Candida alblican while growth was observed for Klebsiella pneumoniae and Candida alblican with the hexane extract (Table 4).On the other hand, the minimum bactericidal concentration (MBC) which is the least concentration that will completely kill a particular microorganism was the 200mg/dl concentration. This concentration; both for ethanol and hexane extracts was effective in killing microbial isolates. For the ethanol extract, this concentration was sensitive in killing Salmonella typhi, Staphylococcus aureus, and Escherichia coli while only Salmonella typhi and Staphylococcus aureus isolates were killed by the hexane extract concentration (Table 5). Ficus exasperata extract was effective against all the different microbial isolates. When the activity of the plant extract was compared to various concentrations of the standard drug chloramphenicol, it was shown to be similar to that of the 100mg/ml exthanol plant extract concentration (Table 6). Previous studies have shown Ficus exasperata to possess antimicrobial activities against several microbial species [37][38][39]28]. However, the extracts had the least activity on Candida alblican suggesting that ficus exasperata is more effective on bacterial species especially gram negative bacteria such as Salmonella typhi and Escherichia coli which showed the highest inhibition pattern as well as the gram positive species than fungal species. This result confirms the local use of Ficus exasperata for medicinal purposes in treating infectious diseases caused by gram negative bacteria such as gastro intestinal infections, diarrhoea, typhoid etc [10][11][12]. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of Ficus exasperata which makes it a potential candidate for a prospective antimicrobial drug. In all, ethanol extract of Ficus exasperata showed an overall better inhibition pattern against microbial isolates than hexane though both were effective in inhibiting microbial growth (Figure 1). These results confirm the findings of previous studies which have also shown ethanol extract to possess the strongest antimicrobial activity and most effective in in vitro studies compared to other solvents used for extration [40]. The reason for a greater activity of ethanol over the hexane extract could be attributed to the polarity of the solvent which has earlier been reported to be responsible for the extraction of a wide range of phytochemicals that potentiates the

pharmacological activity of plant extracts [40][41][42]. The polarity of ethanol gives it the ability to penetrate cell membrane to extract intracellular ingredients from plant and also, since most phytochemicals are mostly aromatic or saturated compounds which are uncharge, they can easily be extracted by charge or polar solvents **??**43]. As such, ethanol; a polar solvent will yield more phytotochemicals which in synergy will generate a greater pharmacological activity than hexane which is non polar. Thus, the greater the phytochemical load, the greater the activity of a plant extract. and more effective solvent compared to hexane as it recovered a greater number (load) of phytochemicals. Out of the seven phytochemicals screened, five; tannin, saponin, alkaloid, flavanoid and glycosides were identified in the ethanol extract against three; tannin, alkaloid and flavanoid for hexane extract (Table 7). Phytochemicals are plant molecules that are not directly involve in plant's growth but for other secondary activities such as protection against pest, pigmentation, abiotic stress etc. [44]. These chemicals have been reported in several studies to be responsible for the healing potentials of medicinal plants **??**45]. In this study, a wide range of phytochemicals; tannin, saponin, alkaloid, flavanoid, glycosides were recovered which have been report for antimicrobial activities through different mechanisms of action **??**45].

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Hence, the collaborative or synergic action of these phytochemicals is responsible for the antimicrobial activity of Ficus exasperata.

15 V. Conclusion

Ficus exasperata possess a broad spectrum of antimicrobial activities and thus, a potential candidate for a prospective antimicrobial treatment whose activity will be at its best if ethanol is used for extraction to recover a wide range of phytochemicals. Due to its broad spectrum of activity, the local use of Ficus exasperata for various medicinal purposes is therefore encouraged.

16 VI. Acknowledgement

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17 VII.

Volume

Figure 1:

Figure 2: Figure 1 :

1

Extraction Method	Initial weight of plant (g)	Weight of plant extract (g)	Percentage Yield (%)
Ethanol	70	10.2	14.6
Hexane	70	7.5	10.6

Figure 3: Table 1 :

2

Concentration of Extract (mg/ml)	Extraction method	Zone of Inhibition (mm) ST	SA	EC	KP	CA	Mean±SEM	p-value
200	EE	18.5	12.2	18.3	11.5	10.3	14.16±1.76	
	HE	15.0	10.2	10.3	16.1	8.2	11.96±1.52	0.377
150	EE	14.5	10.0	8.3	12.4	9.2	10.88±1.13	
	HE	12.2	10.3	6.2	10.0	4.5	08.02±0.55	0.047
100	EE	10.0	8.0	7.0	8.1	7.0	06.60±1.12	
	HE	10.0	8.1	4.5	6.4	4.0	08.64±1.42	0.089
50	EE	6.0	5.5	5.2	5.5	5.0	05.44±0.17	
	HE	5.0	5.0	5.0	5.0	5.0	05.00±0.00	0.060
25	EE	4.0	3.8	3.7	3.8	2.8	03.62±0.21	
	HE	4.0	3.0	3.0	3.0	2.5	03.10±0.25	0.031

[Note: Legend: ST: Salmonella typhi, SA: Staphylococcus aureus, EC: Escherichia coli, KP: Klebsiella pneumoniae CA: Candida alblican, EE: Ethanol Extract, HE: Hexane Extract]

Figure 4: Table 2 :

3

Concentration of Extract (mg/m)	Extraction method	Zone of Inhibition (mm) ST	SA	EC	KP	CA	Mean±SEM	p-value
200	EE	15.3	10.5	7.5	12.2	9.0	10.90±1.35	
	HE	12.1	9.2	8.2	13.1	6.1	09.74±1.28	0.250
150	EE	10.2	8.3	7.5	8.0	7.5	08.30±0.49	
	HE	10.1	8.2	5.3	7.2	4.1	06.98±1.06	0.111
100	EE	8.4	6.4	5.0	6.2	5.2	06.24±0.21	
	HE	8.0	5.4	3.8	4.3	3.5	05.00±0.82	0.010
50	EE	3.9	3.5	3.1	3.0	3.1	03.32±0.17	
	HE	4.2	3.1	3.5	3.8	3.0	03.52±0.22	0.389
25	EE	3.5	3.0	3.0	2.8	2.0	02.86±0.24	
	HE	3.0	2.5	2.5	2.5	2.0	02.50±0.16	0.021

Figure 5: Table 3 :

4

Concentration of Extract (mg/ml)	Extraction method	Microbial growth profile ST	SA	EC	KP	CA
200	EE	?	?	?	?	?
	HE	?	?	?	?	?
100	EE	?	?	?	?	+
	HE	?	?	?	+	+
50	EE	+	+	+	+	+
	HE	+	+	+	+	+
25	EE	+	+	+	+	+
	HE	+	+	+	+	+

Legend: + Growth; ?No growth

Figure 6: Table 4 :

5

Concentration of Extract (mg/ml)	Extraction method	Microbial growth profile ST	SA	EC	KP	CA
200	EE	?	?	?	+	+
	HE	?	?	+	+	+
100	EE	+	+	+	+	+
	HE	+	+	+	+	+
50	EE	+	+	+	+	+
	HE	+	+	+	+	+
25	EE	+	+	+	+	+
	HE	+	+	+	+	+

Figure 7: Table 5 :

6

Chloramphenicol (mg/ml)	100	50	25	12.5	Ethanol plant extract (100mg/ml)
Zone of Inhibition (mm)	14.60	7.01	3.55	1.78	12

Figure 8: Table 6 :

7

Screened Phytochemicals	Tannins	Saponins	Alkaloids	Flavonoids	Steroids	Glycosides	Anthraquinones
Ethanol Extract	+	+	+ + _			+	_
Hexane extract	+	_	+ + _			_	_

Figure 9: Table 7 :

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Figure 10:

.1 Conflict of Interest

The authors declare no conflict of interest in conducting this research.

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