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Qualitative Phytochemical Screening and *In-vitro* Antimicrobial studies of extracts of *Dyschoriste pedicellata* (Acanthaceae)

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ABSTRACT

The plant *Dyschoriste pedicellata* (Acanthaceae) was investigated for its medicinal potential. The method of cold maceration was employed for extraction using petroleum ether, ethyl acetate and methanol sequentially. The preliminary phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponnins, glycosides and steroids. The crude extracts were evaluated for antimicrobial potential using the following clinical isolates; *Staphylococcus aureus*, *Salmonella typhi*, *Streptococcus pyogenes*, *Escherichia coli*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Candida albicans*, and *Aspergillus niger*. The zone of inhibition of test organisms ranged from 10 – 17 mm (pet ether), 13 – 23 mm (ethyl acetate), and 12 - 28 mm (methanol). These were compared with Ampiclox (26 mm), ciprofloxacin 20 – 24 mm, Amoxicillin 15 mm and Fluconazole (20 mm). The Minimum Inhibitory Concentration (MIC) showed that pet ether extract had an MIC of 12.5 mg/ml against *Streptococcus pneumonia* and *Klebsiella pneumonia*. The methanol and ethyl acetate fractions had MIC values of 6.25 mg/ml against *Aspergillus niger*. The presence of phytochemicals such as alkaloids, tannins, flavonoids, saponnins and glycosides and also the observed antimicrobial activity of the extracts are potentials present in *Dyschoriste pedicellata* worth investigating.

Key words*: Dyschoriste pedicelleta*, maceration, Plant extract, *Acanthaceae*, antimicrobial agents.

INTRODUCTION

Man since earliest times has used many plants as medicines. These plants have healing properties against human infections due to the presence of secondary metabolites, which have been found to act as antimicrobial agents against human pathogens (Iqbal *et al*., 2015; Amin *et al.,* 2017). Over the past decade, much attention has been placed on the study of phytochemicals for their antibacterial activity, especially against multidrug-resistant Gram-negative and Gram-positive bacteria (Jaradat *et al*., 2017; Guo, 2017). In recent years, many studies have shown that phytochemicals exert their antibacterial activity through different mechanisms of action, such as damage to the bacterial membrane and suppression of virulence factors, including inhibition of the activity of enzymes and toxins and bacterial biofilm formation (Barbieri, 2017). *Dyschoriste pedicellata* C. B. Cl. a small shrub found in Gambia, Mali, Northern Nigeria and in Ubangi-Chari (now the Central African Republic). It is known among the Fula-Pular people of Gambia as boru, where a leaf-infusion is given to children as a febrifuge. In Kenya the stems and leaves of *D. radicans* are used in the treatment of diarrhoea (Burkill,1985). The study was aimed at investigating the presence of phytochemicals and antimicrobial activity of the extracts, so as to ascertain the claims of its ethno-botanical use.

MATERIALS AND METHODS

Plant material

The whole plant *Dyschoriste pedicellata*, was collected from Kamuru town in Zango-Kataf local government area of Kaduna state, Nigeria. In the month of November, 2017 and identified at the Herbarium unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria. A specimen voucher number 498 was assigned and deposited in the Herbarium. The plant material was air-dried, pulverized and then stored in an air-tight container for further use.

Extraction of plant material

The air-dried powdered plant (972g) material was exhaustively extracted with petroleum ether (60-80^{o}C), ethyl acetate and methanol (general purpose grade) using cold maceration method. After filtration, the extracts were concentrated under vacuum and the weight of the extracts recorded.

Phytochemical screening

Qualitative phytochemical screening for alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponnins, Anthraquinones, steroids, terpenoids, and tannins were carried out according to the method as described by Trease and Evans, (1983) and Sofowora (1993).

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Antimicrobial screening

Test organisms

The following clinical isolates of *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes, Aspergillus niger* and *Candida albicans* were used.

Antimicrobial culture

Broth cultures of the test organisms were prepared by suspending a portion of the colonies of each organism in nutrient broth and incubated aerobically at 37^0C for about 18 h. The respective suspensions were adjusted to a turbidity of 10^8 colony forming units per ml (CFU/ml) using 0.5 McFarland standards for visual comparison.

Determination of Zone of Inhibition

The antimicrobial screening of the extracts was carried out using agar-in-well diffusion method. Briefly; sterile Mueller Hinton's agar plates were flooded with 0.1ml of the standardised bacterial and fungal suspensions. These were streaked uniformly on the surface of the culture media. Wells of 6 mm diameter were punched on each plate with sterile cork borer. The compound was dissolved in minimal quantity of Dimethyl sulfoxide (DMSO). About 0.1 ml of the test extracts at 200 mg/ml was added to each well and allowed to stay for about 1hr to enhance diffusion through the media. The plates were incubated (inverted) aerobically at 37 ^{o}C for about 18 – 24 h. At the end of the incubation period, the diameters of the zones of inhibition of growth were measured using a transparent rule and recorded. The extracts were tested in duplicates and the mean zones of inhibition were calculated (Akerele *et al.*, 2011).

Minimum Inhibitory Concentration (MIC)

The MIC was determined using broth dilution method as performed by Vellokobia *et al*. (2001). Briefly; two-fold serial dilution of the extracts were made to obtain 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5mg/ml and 6.25mg/ml. About 0.2 ml of the suspended standard inoculum of each organism was inoculated into the different concentrations of the test extracts. Then incubated at 37 ^{o}C for 24 h after which they were observed for inhibition of growth. Inhibition of growth was indicated by a clear solution, the least concentration of the extracts inhibiting the growth of each organism was recorded as the MIC.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The contents of the MIC tubes and the preceding tubes in the serial dilution, were sub-cultured into appropriately labelled nutrient agar plates by dipping a sterile wire loop into each tube and streaking on the surface of each agar plate respectively. The plates were then incubated at 37 ^{o}C for 24 h after which they were observed for colony growth. The lowest concentration of the subcultures with no growth was considered to be the minimum bactericidal/fungicidal concentration (MBC/MFC) of the extracts (Abdullahi *et al.*, 2011).

RESULTS

The findings of this research are presented below (tables 1-5). Table 1 gives the percentage yield of extracts, table 2, the phytochemicals present and tables 3-5, the results of antimicrobial assays.

Table 1: Weight and percentage yield of extracts

Sample	Weight (g)	% extracted
Dried sample	972	-
Pet ether extract	14.5	1.49
Ethyl acetate extract	13.2	1.36
Methanol extract	17.4	1.79

Table 1:Phytochemical screening of the crude extracts

Constituents	PE	EE	ME
Carbohydrates	+	+	+
Anthraquinones	-	-	-
Glycosides	+	+	+
Cardiac Glycosides	+	+	+
Saponins	-	-	+
Tannins	-	-	+
Flavonoids	-	-	+
Steroids & Triterpenes	+	+	+
Alkaloids	-	+	+

Key = + present, - absent, PE pet ether extract, EE ethyl acetate extract, ME methanol extract

Table 2: Zone of inhibition

Organisms	Zone Diameter (mm) of the Test Extracts at 200 mg/ml			Standard drugs used as positive control (µg)	Zone Diameter (mm) of the control
	EE	ME	PE		
Staphylococcus aureus	16	14	16	Ampiclox (30)	26
Salmonella typhi	16	15	12	Chloramphenicol (30)	17
Streptococcus pyogenes	14	12	10	Ciprofloxacin (10)	20
Escherichia coli	16	13	12	Ciprofloxacin (10)	24
Streptococcus pneumonia	20	19	17	Amoxicillin (20)	15
Klebsiella pneumonia	15	13	15	Pefloxacin (30)	27
Candida albicans	13	12	12	Fluconazole (25)	20
Aspergillus niger	23	28	15	Amphotericin B (10)	15

Key = EE ethyl acetate extract, ME methanol extract, PE pet ether extract

Table 3: Minimum Inhibitory Concentration (MIC)

Organisms	EE					ME					PE				
	Concentrations of two-fold serial dilution of extracts in mg/ml														
	100	50	25	12.5	6.5	100	50	25	12.5	6.5	100	50	25	12.5	6.5
S. aureus	-	-	-	-	+	-	-	+	++	++	+	++	++	++	++
S. typhi	-	-	+	++	++	+	++	++	++	++	+	++	++	++	++
S. pyogenes	-	-	-	+	++	-	-	+	++	++	+	+	++	++	++
E. coli	-	-	-	+	++	-	+	++	++	++	+	++	++	++	++
S. pneumonia	-	-	+	++	++	-	-	+	++	++	-	-	-	-	+
K. pneumonia	-	-	+	++	++	-	-	-	-	+	-	-	-	-	+
C. albicans	-	-	+	++	++	+	++	++	++	++	-	-	-	+	++
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++

Key = - no growth, + scanty growth, ++ moderate growth, EE ethyl acetate extract, ME methanol extract, PE pet ether extract. All concentrations in mg/ml

Table 4: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Organisms	EE					ME					PE				
	Concentrations of two-fold serial dilution of extracts in mg/ml														
	100	50	25	12.5	6.5	100	50	25	12.5	6.5	100	50	25	12.5	6.5
S. aureus	-	-	-	+	++	-	+	+	++	++	+	++	++	++	++
S. typhi	-	+	+	++	++	+	++	++	++	++	+	++	++	++	++
S. pyogenes	-	-	+	+	++	-	+	+	++	++	+	+	++	++	++
E. coli	-	-	+	+	++	+	+	++	++	++	+	++	++	++	++
S. pneumonia	-	+	+	++	++	-	+	+	++	++	+	++	++	++	+
K. pneumonia	-	+	++	++	++	-	-	-	+	++	-	-	-	+	+
C. albicans	-	+	++	++	++	+	++	++	++	++	-	-	+	++	++
A.niger	-	-	-	+	++	-	-	-	+	++	-	-	+	++	++

Key = - no growth, + scanty growth, ++ moderate growth, EE ethyl acetate extract, ME methanol extract, PE pet ether extract. All concentrations in mg/ml

DISCUSSION

The percentage yield of the extract as reported in table 1 showed that methanol extract had the highest yield (1.79%) followed by the pet ether extract (1.49%) and then the ethyl acetate extract (1.36%). The result of the phytochemical screening of all extracts revealed the presence of secondary metabolites namely; alkaloids, flavonoids, glycosides and triterpenes These substances may be responsible for the antimicrobial activity of the plant. Alkaloids were present in both methanol and ethyl acetate extracts. The methanol extract had the highest number of metabolites present. Tannins and flavonoids were only found in the methanol extract. Odoh *et al.,* (2011) reported the presence of alkaloids, flavonoids, tannins, glycosides, saponnins and sterols in the phytochemical screening of *Dyschoriste perrottetii* Nees. Yakubu et al., 2018 also reported the presence of hexadecanonic acid,2-methylnonane, linoleic acid from the GC-MS analysis of ethyl acetate fraction of the whole plant extract of *Dyschoriste perrotteii*. The phytochemical compounds detected are known to have medicinal importance and because of this they are very often used in medicines due to their well-known biological activities (Aba *et al.,* 2015). For example, alkaloids have been reported as powerful poison and many alkaloids derived from medicinal plants show biological activities like, anti-inflammatory, antimalarial, antimicrobial, cytotoxicity, antispasmodic and pharmacological effects (Iqbal *et al.,* 2015). Steroids derived from plants are known to have cardio tonic effect and also possess antibacterial and insecticidal properties. Tannins are also known to have antibacterial, antitumor and antiviral activities. Cardiac glycosides are known to treat congestive heart failure and cardiac arrhythmia (Ewansiha *et al.,* 2012). Other members of the Acanthaceae family such as *Acanthus ilicifolicus* have been found to have glycosides such as [2R]-2-o-β-D-gluco-pyranosyl-2H-1,4-benzoxazin-3(4H)-one present, flavonoids such as cinnamic acid, phenolic compounds such as α-tocopherol and triterpenoids such as β-amyrin have been isolated from this family (Awan *et al.,* 2014). Standard cultures of eight different microorganisms; *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes, Aspergillus niger* and *Candida albicans* were used to determine the antimicrobial activity of the extracts. The zones of inhibition (10-28mm), minimum inhibitory concentration (MIC) and minimum bactericidal

concentration (MBC) as shown in tables 3,4 and 5 respectively reports that all obtained extracts of the plant *Dyschoriste pedicellata* had substantive activity against the organisms used. Different extracts of other members of this family such as *Streptococcus pneumoniae* of the ethyl acetate (EE), methanol (ME) and pet ether (PE) extracts at 200mg/ml were 20,19 and 17 mm respectively as when compared with a standard drug; amoxicillin 20µg which gave a zone of 15mm. At 200mg/l when tested against *Aspergillus niger*, EE gave a zone of 23mm, ME 28mm and PE 15mm as against a standard drug 10µg Amphotericin B which gave 15mm. the MIC studies of PE inhibited the growth of *streptococcus pneumonia* and *Klebsiellla pneumonia* at 12.5mg/ml while *Candida albicans* and *Aspergillus nigger* at 25mg/ml. For the MBC analysis the best sensitivity was observed at 50mg/ml. The MIC studies for EE showed that at 50mg/ml the growth of the organisms where inhibited. At 6.5mg/ml the growth of *Aspergillus nigger* was inhibited. In the MBC analysis the best sensitivity against *Staphylococcus aureus* and *Aspergillus nigger* was observed at 25mg/ml. The MIC studies for ME showed that the growth of *Staphylococcus aureus*, *Streptococcus pyogenes* and *pnueumonia*, *Klebsiella pneumonia* and *Aspergillus nigger* was inhibited at 50mg/ml. At 6.25mg only *Aspergillus nigger* was inhibited. In the MBC studies all organisms were sensitive at 100mg/ml except *Salmonella typhi*, *E coli* and *Candida albicans*. At 25mg/ml, *Candida albicans* and *Aspergillus nigger* were sensitive. This implies that all the extracts exerted different levels of activity as compared with different standard test drugs used as positive controls and of which the ethyl acetate extract had the highest activity.

CONCLUSION

The result of the study revealed the presence of carbohydrates, flavonoids, anthraquinones, cardiac glycosides, alkaloids, steroids, triterpenes, saponnins, tannis and glycosides. The activity of the extracts was established by determining the zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration(MBC/MFC) on the following organisms; *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes, Aspergillus niger* and *Candida albicans* The average zone of inhibition for the ethyl acetate extract (EE) as noted was the highest. For the MIC studies, at 50mg/ml and 100mg/ml it inhibited the growth of all the organisms, *A. niger* was also inhibited at 6.5mg/ml and 12.5mg/ml and *S. aureus* was inhibited at 12.5mg/ml. In the MBC/MFC studies, the EE at 100mg/ml was able to kill the organisms, at 25mg/ml it was also able to kill *S. Rhinac anthusnasutus*, *Androgrphis paniculata* have been active against *Aspergillus niger*, *Caandida albicans*, *Cryptococcus neoformans* and so on (Awan *et al.*, 2014). The diameter for the zone of inhibition against *aureus* and *A. niger*, thus showing greater bactericidal/fungicidal activity. The research findings showed that the extracts were both bacteriostatic and bactericidal/fungicidal, thus confirming the ethno botanical use of the plant and in further contribution to knowledge, the plant can be worked on to isolate compounds of interest.

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