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PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITIES OF THE CRUDE EXTRACTS OF *CLEOME MONOPHYLLA* (LINN.)

*[1,2]E. Uwaiya, [2]G. I. Ndukwe, [2]H. Ibrahim and [2]A. B. Aliyu

[1]Department of Science Laboratory Technology, NILEST, Zaria, Nigeria

[2]Department of Chemistry, Ahmadu Bello University Zaria Nigeria

*Corresponding author: emmaiya2015@gmail.com

ABSTRACT

Preliminary phytochemical screening and antibacterial activities of the Chloroform and n-Hexane extracts from the whole plant parts of *Cleome monophylla* were investigated in order to evaluate its medicinal properties. Antibacterial activity was determined against ten bacteria using the Agar Well-diffusion method. The antimicrobial screening results of the Chloroform and n-Hexane extracts showed that they both had activity against *Methicillin-Resistance Staphylococcus aureus* (*MRSA*), *Escherichia coli*, *Salmonella typhi*, *Candida stellatoidea*. The n-Hexane extract also inhibited the growth of *Staphylococcus aureus* while the Chloroform extract inhibited the growth of *Helicobacter pylori* but no activity was observed against *Vancomycin Resist. enterococci*, *Candida albicans*, *Candida krusei* and *Proteus mirabilis*. The Chloroform extract of the whole part of the plant had minimum inhibitory concentrations at 5 mg/ml while the n-Hexane extract of the whole plant part showed minimum inhibitory concentration at 5 mg/ml on all the pathogens with sensitivity. Phytochemical screening of the whole plant parts gave positive results for glycosides, terpenes, cardiac glycoside and steroids in both extracts, while carbohydrates and saponins were only present in the chloroform extract. The result of this study justified the use of this plant in ethno-medicinal treatment of ailments.

Key words: Antimicrobial screening, *Cleome monophylla*, crude extract, inhibitory, phytochemical constituents.

INTRODUCTION

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants [1-2]. These medicinal plants are used for the treatment of all kinds of ailments such as skin infections, sores, intestinal and respiratory conditions etc. [3-5].

*Cleome monophylla (*Linn) belongs to the family, Cleomaceae (spindle flower family). It is a single leaf species in the compound leaf genera. It is commonly known as spindle pod and Tamil vernacular name is Naaikadugu and Ellukku sakkalathi. The synonym is *Cleome cordata*, *Cleome subcordata* and *Cleome massae*. It is an erect stocky annual herb up to 90 cm high, a weed of cultivated land, common around villages, and especially near to cattle-pounds. It is recorded from the Sahel areas of the Region from Senegal to Northern Nigeria, and occurring throughout tropical and subtropical Africa. In Nigeria crushed leaves are rubbed on the head for relief of headache, and the leaf, finely ground, is sometimes put in the eye to relief irritation [6, 7]. It is commonly used in traditional and folklore medicines for treating various diseases. *Cleome monophylla* cures ulcer, boils, wounds, cough, headache, swellings, hasten maturation, ear discharges, anthelmintic [8, 9], fever [10, 11], headache [12] and bile enlargement [13]. The leaf extract of *Cleome monophylla* had anti-HIV-1 reverse transcriptase activity [14]. The plant possesses anti-inflammatory, anthelmintic and antidermatosis activity [15].

MATERIAL AND METHOD

Plant collection and preparation

The plant, *Cleome monophylla*, was collected from Makurdi, Benue State in the month of August, 2019. They were properly identified and confirmed at the Herbarium, Department of Botany, Ahmadu Bello University, Zaria. The whole plant was washed and air-dried under shade for two weeks in readiness for the laboratory work. The dried plant was pounded into coarse powder using wooden mortar and pestle.

Extraction Procedure

The pulverized plant was weighed and extracted exhaustively with redistilled n-hexane (100 g in 300 ml) and chloroform (100 g in 300 ml) for 24 hours in a Soxhlet extractor. Concentration of the extracts was done *in vacuo* at 40 ^{o}C using rotary evaporator (*Rota vapor*).

Phytochemical analysis of the plant material

The chloroform and n-hexaneplant extracts were screenedfor plant metabolites.Standard techniques of Brain and Turner, Sofowora and Prajapati [16-18] were employed in the phytochemical screening. These metabolites include carbohydrates, cardiac glycosides, tannins, saponins glycoside, flavonoids, steroid and triterpenes.

Antimicrobial screening test

Pure clinical isolates of *Staphylococcus aureus, Methicillin-Resistant Staphylococcus aureus (MRSA), Escherichia coli, Helicobacter pylori, Proteus mirabilis, Salmonella typhi, Vancomycin-Resistant Enterococci* (*VRE*), *Candida albicans, Candida krusei and Candida stellatoidea,* obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, were grown on a nutrient agar slant in bijoubottles in an incubator at 37 ^{o}C for 24 h. Stock solutions of the respective plant extracts were prepared by initially dissolving 1 g ofeach methanol extract in 1 ml of DMSO to obtain stock solutions of each. From the stock solution, concentrations of 20.0, 10.0, 5.0, 2.5 and 1.25 mg/ml were prepared by serial dilution. The cork and bore diffusion method of Bauer *et al.* [19] and Barry and Thornsberry [20] were used in the anti-microbial screening. Inoculation of the prepared plates with the organism was done using a wire loop to transfer a strand of the organism into the plate followed by cross-streaking with the same wire loop to achieve uniform spread on the plate. A control was set up alongside using pure DMSO for each strain of organism. The plates were incubated ata temperature of 37 ^{o}C for 24 h after which they were examined for zones of inhibition of growth.

Determination of the minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration was carried out on the n-hexane and chloroform extracts because they showed sensitivity against the growth of the test organisms. The medium was nutrient agar solution which was prepared according to the manufacturers' standard of 28 g/1000 ml. In this case double strength was prepared by dissolving 28 g in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution. Then 5 ml of it was dispensed into 30 sets of universal bottles and sterilized in an autoclave at a temperature of 121 ^{o}C for 15 min. The agar was allowed to cool to at a temperature of 45 ^{o}C and each graded solution was then mixed gently with molten double strength nutrient agar in a Petri-dish and allowed to solidify for one hour. Extracts' concentrations of 20.0, 10.0, 5.0, 2.5 and 1.25 mg/ml were prepared by serial dilution. Each plate was divided into equal sections and labeled accordingly to correspond to the test organisms. Two 5 mm diameter paper discs (Whatman No.1) were placed aseptically into each labeled section of the plate using sterilized forceps. With an automatic micropipette, 0.1 ml of each bacterial

suspension was taken and transferred aseptically and carefully into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 h ata temperature of 37 ^{o}C after which they were observed for growths or death of the test organisms. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration.

Determination of the minimum bactericidal concentration (MBC)

This was carried out to know if the organisms could be killed completely or their growths could only be inhibited. Another 30 sets of plates of nutrient agar were prepared according to the manufacturers' standard and sterilized in an autoclave as earlier described. The paper discs in all the plates from the MIC tests were reactivated. Emphasis was paid to the MIC plates and the preceding plates. The re-activation was done in a mixture of 0.5% egg lecithin and 3% Tween 80 solution in a test tube. The reactivated organisms were sub-cultured into appropriately labeled quadrants of the sterilized nutrient agar plates using wire loop into each test tube and streaking uniformly on the labeled quadrants. This was then incubated for 24 h at a temperature of 37^{o}C after which they were observed for growths. The MBC was the quadrant with the lowest concentration of the extract without growth.

RESULTS AND DISCUSSION

The phytochemical screening of medicinal plants detected secondary metabolites which contribute significantly towards the biological activities of these plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, anti-leprosy activities etc. [21]. The phytochemical screening of the n-hexane extract of the whole plant parts of *Cleome monophylla* revealed the presence of glycosides, cardiac glycosides, steroids and triterpenes while the chloroform extracts of the whole plant parts of *Cleome monophylla* revealed the presence of carbohydrates, cardiac glycosides, glycosides, steroids and triterpenes and saponins, while tannins, flavonoid and alkaloids were absent in both as shown in Table 1. Phytochemicals like glycosides, steroids, saponins and terpenes have potentially significant applications against bacteria [22]. Saponins, terpenoids and steroids have been reported to have anti-inflammatory effects [23, 24]. Saponins are also important therapeutically as they are shown to have hypolipidemic and anticancer activity. Saponins are also necessary for activity of cardiac glycosides [25]. Studies have confirmed that steroids are responsible for cholesterol-reducing properties. Steroids also help in regulating the immune

response [26]. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties [27-29]. Plants containing carbohydrates, glycosides are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements [30]. Several authors have linked the presence of these compounds to the antimicrobial properties of plant extracts.

The antimicrobial screening of the n-hexane extracts of the whole plant parts showed that it was active on five microorganisms (Table 2). This could be due to the presence of the active bioactive substances in this fraction.

Table 3 shows the MIC results of the crude n-hexane extracts and chloroform extract of the plant. The n-hexane extracts inhibited the growth of *MRSA,* S. *aureus*, *E. coli*, *S. typhi*, and *C. stellatoidea* at a concentration of 5 mg/ml each while the chloroform extract inhibited the growth of *MRSA, E. coli, H. pylori,S. typhi and C. stellatoidea* at a concentration of 5 mg/ml each. The antimicrobial activities reported in this work confirms why this plant is used in traditional medicine to treat ulcer, boils, wounds, cough, headache, swellings, hasten maturation, ear discharges, anthelmintic, fever, headache and bile enlargement [8-13].

Table 1: Phytochemical constituents of the whole plant part of *Cleome monophylla*

		INFERENCE	
CONSTITUENTS	*TEST*	n-Hexane extract	Chloroform extract
Carbohydrate	a. Molisch's	-	+
	b. Fehling	-	+
Cardiac Glycosides	a. Keller-kiliani	+	+
	b. Kedde's	+	+
	c. Salkowski	+	+
Saponins glycoside	Frothing	-	+
Steroid & Triterpenes	Liebermann-Burchard's	+	+

Tannins	a. Iron (III) chloride	-	-
	b. Lead Subacetate	-	-
Alkaloids	a. Wagner	-	-
	b. Mayer	-	-
Flavonoid	a. Shinoida	-	-
	b. 10%NaOH & HCl	-	-
Steroidal Glycosides	Liebermann-Burchard's	+	+

Key:+ denotes Present, - denotes Absent

Table 2: Antimicrobial Activities and Zones of Inhibition of n-Hexane and Chloroform extract of the whole plant part of *Cleome monophylla* with control.

	ACTIVITY				ZONE OF INHIBITION (mm)			
TEST ORGANISMS	**n-Hexane Extract**	**CF Extract**	**CONTROL**		**n-Hexane Extract**	**CF Extract**	**CONTROL**	
			Cipro.	**Fluc.**			**Cipro.**	**Fluc.**
MRSA	S	S	R	R	26	21	ND	ND
VRE	R	R	S	R	ND	ND	32	ND
S. aureus	S	R	S	R	25	ND	34	ND
E. coli	S	S	S	R	23	23	38	ND
H. pylori	R	S	S	R	ND	22	30	ND
P. mirabilis	R	R	R	R	ND	ND	ND	ND
S. typhi	S	S	S	R	23	24	41	ND
C. albicans	R	R	R	S	ND	ND	ND	35
C. krusei	R	R	R	S	ND	ND	ND	31
C. stellatoidea	S	S	R	S	25	23	ND	34

KEY: R= Resistance. S= Sensitive. mm= Millimetre(s), Cipro. = Ciprofloxacin: Fluc. = Fluconazole, ND = Not determined, CF = Chloroform

Table 3: Minimum Inhibition Concentration (MIC) of Extract against Microorganisms.

	CONCENTRATION (mg/cm^3)									
TEST	**n-Hexane fraction**					**Chloroform fraction**				
ORGANISM	20	10	5	2.5	1.2	20	10	5	2.5	1.2
MRSA	-	-	o*	+	++	-	-	o*	+	++
VRE										
S. aureus	-	-	o*	+	++					
E. coli	-	-	o*	+	++	-	-	o*	+	++
H. pylori						-	-	o*	+	++
P. mirabilis										
S. typhi	-	-	o*	+	++	-	-	o*	+	++
C. albicans										
C. krusei										
C. stellatoidea	-	-	o*	+	++	-	-	o*	+	++

KEY: - = no growth (no turbidity), o*= MIC, + = light growth (Turbid), ++ = Dense growth (Moderate turbidity), mg = micro-gram.

Table 4: Result of Minimum Bactericidal (MB)/ Minimum Fungicidal (MF) Concentration of extract against Microbes

	CONCENTRATION (mg/cm^3)									
TEST	**n-Hexane extract**					**Chloroform extract**				
ORGANISM	20	10	5	2.5	1.2	20	10	5	2.5	1.2
MRSA	-	o*	+	++	+++	o*	+	++	+++	++++
VRE										
S. aureus	-	o*	+	++	+++					
E. coli	-	o*	+	++	+++	-	o*	+	++	+++
H. pylori						o*	+	++	+++	++++
P. mirabilis										
S. typhi	-	o*	+	++	+++	-	o*	+	++	+++
C. albicans										
C. krusei										
C. stellatoidea	-	o*	+	++	+++	-	o*	+	++	+++

KEY: - = no colony growth, + = Scanty colony growth, o*= MBC/MFC, ++ = moderate colony growth, +++ = Heavy colony growth, ++++ = Heavy colony growth.

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