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Phytochemicals screening and antimicrobial activities of Celosia laxa

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Abstract: *Medicinal plant Celosia laxa from Shika Zaria was collected. Shade dried and was extracted. The methanol extract was partitioned using petroleum spirit, chloroform and ethyl acetate. The phytochemical screening revealed the presence of carbohydrate, cardiac glycosides, saponins, steroids, triterpenes, flavonoids, tannins, combined reducing sugar. The microbial screening of the crude methanol and the chloroform extract were active against most of the microorganisms, however the chloroform extract showed more activity relative to the methanol extract. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the chloroform were carried out and the values determined respectively.*

Key words: *Medicinal plants, phytochemical analysis, antimicrobial activity, Celosia laxa*

I. Introduction

The world is fertile with natural and medicinal plants. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on the human body (Akinmoladun et al; 2007). Phytochemical, natural compound occur in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibbers to act against diseases or more specifically to protect against diseases.

The photochemical are grouped into two main categories (Krishnaiah et al, 2009)' namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc. and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compound etc.

(Krishnaiah et al, 2007; Edeoga et al, 2005). Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticidals (Kambu et al, 1982), antibacterial, antifungal (Lemos et al, 1990), anti constipative(Ferdous et al, 1992), spasmolytic (Santos et al, 1998), antiplasmodial (Benoituical et al, 2001) and antioxidant (Vardar –unlu et al, 2003) activities etc. The plants thus find their medicinal value due to respective phytochemical constituents they contains.

Infectious diseases are the leading causes of death throughout the world in the tropical countries, which are also becoming a serious problem in developed countries. It is calculate that infectious diseases are the main causes of death in 8% of the 9 deaths occurring in United States (Demissew and Dagne, 2001). In addition, antibiotics are sometime associated with adverse effects including hypersensitivity, immuno suppressant and allergic reactions. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plant. Celosia lexa is medicinally very important plant used by local practitioners for some aliment such as anthemintic, rheumatic, stomach ache, skin diseases and as embrocation for pans. Hence the aim of this study was to determine the phytochemical constituents and to investigate the antimicrobial properties so as to ascertain their uses in traditional medicines.

II. Materials and Method.

Plants collection: The work was carried out at the department of chemistry, Ahmadu Bello University, Zaria. The plant named celosialaxa was collected fromShikaSamaru, Zaria, Kaduna Sate. It was identified and authenticated at the Herbarium of the department of Biological sciences, Ahmadu Bello University Zaria. The leaves of celosialaxa was thoroughly washed with water and air-dried, it was then pulverized and was stored in an airtight polythene bags.

Phytochemical Analysis: The extract was analysed for the presence of Alkaloid steroid and Triterpeners, Terpenoids, Tannine, Saponin, flavoroid, Cardiac glycosides, carbohydrate, Anthraquinones,

Carhydrate (Molisch's Test): The plant material (1g) was extracted with distilled water (5mls) by heating in a water bath. It was filtered. Four drops of molisch' reagent were carefully added to the filtrate and concentrated sulphuric acid (3mls) added from the side of the test tube to form a lower layer. Reddish colouring was observed at the interphase.

Alkaloids: About 0.2g of the extracts was warmed with 2% H_2s0_4 for two minutes. It was filtered and few drop of the solution was treated with Dragendoff reagent. Residue did not show any colour change with any precipitation.

Flavonoids: Extracts of about 0.2g was dissolved in diluted Na0H and Hel was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

Cardiac glycosides: 5ml of the extract was treated with 2ml of glacial accetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrates H_2s0_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in acetic layer, a greenish ring may form just gradually throughout thin layer.

Anthraquinones (Borntrage's test): Small portion of the extract was shocked with 10ml of benzene and filtered. 5ml10% of ammonia solution was added to the filtrate and stirred. The production of a pink-red or violet colour indicates the presence of free antraguinones.

Saponins: About 0.2g of the extract was shaken with 5ml of distilled water and then heated to boil frothing (appearance of creamy miss of small bubbles) shows the presence of saponimes.

Tannine: Small quantity of the extracts was mixed with water and heated on water bath. The mixture was filtrate. A dark green solution indicate the presence of tannins.

Steroid and Triterpenes: Aqueous solution of acetic anhydride was added to the extract. 1ml of concentrated sulphuric acid was added down side the tube. The colour change was observed immediately and later Red, Pink or Purple colour indicates the pre3sence of triterpenes while blue or blue-green indicates steroids.

Antimicrobial screening of extracts: Pure clinical bacterial isolates of methcillia resistant staphylococcus aureus, salmonellatyphi, Pseddomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, streptococcus pyogenes, shigelladysecandida, Bacillus subtile, corynebaiterumulceran and Escherichia coli were collected from the medical microbiology laboratory of the Ahmadu Bello University Teaching Hospital, Zaria, they were sub-culture on sterile nutrient broth and incubated at 37oc for 24hours. The resulting cultures were diluted with sterile normal saline to obtain final dilution containing approximately 108 cfu/ml.

Preparation of solution of plant extract for antimicrobial screening test: The extract were made into solution of various concentrations using dimethyisulphoxide (DSMO) as solvent, i.e. 50, 25, 12.5, 6.25, 3.12 mg/ml., were prepared for the methanol and chloroform extracts. Ure DSMO (10%) was used as control.

Stock solution of the plant extract were equally prepared by initially dissolving each of methanol and chloroform extract (5g) of the plant extract I DMSO (10ml) to obtain a concentration of 50mg/ml. this was the initial concentration of the extract used to determine the antimicrobial activities of each of the extract.

Determination of the antimicrobial activities of the methanol and chloroformextract of celocialaxa , Mueller Hinton agar was the medium used as the growth for the microbs. The medium was prepared according to the manufacture's instruction. Sterilize at 121oc for 5minutes and the medium poured into sterile petri-dishes, the plates were covered and allowed to cool completely and to solidify. Diffusion method was the method used for screening the extracts. The sterilized medium was seeded with 0.1ml of the standardninoculums of the test microorganisms. By the use a standard corkborer of 6mm in diameter, a well was cut at the concentration of 50mg/ml was then introduced into each well in the medium. The inoculated plates were incubated at 37oc for 24 hours after which each plate was observed for possible zones of inhibition. The zones were measured with a transparent ruler and the result recorded in millimetres.

III. Results

Result of the photochemical screening of the plant of celosia laxa

Table 1

Property Tested	Inference
Carbohydrate	+
Cardiac glycosides	+
Anthraquinones	-
Saponins	+
Steriod and Triterpenes	+
Flavanoids	+
Tannins	+
Alkanoids	-

Key + = Positive, - = Negative

Antimicrobia activity screening of the methanol extract of Celosia laxa

Table 2

Microorganism	Methanol extract
Methicillin R.S.A	S
Staphylococcus aureus	S
Staphylococcus pyogenes	R
Bacillus subtile	S
Corynebacterumulceram	S
Escherichia coli	R
Proteus mirabilis	R
Proteus Vulgaris	R
Pseudomonas aeruginosa	S
Salmonella typhi	S
Shigelladysenteria	S
Candida albicans	S
Candida krusei	R
Candida tropical	S

Zone of inhibition of the methanol extract

Table 3

Microorganism	Zone of Inhibition (mm) of methanol extract
Methicillin R.S.A	19
Staphylococcus aureus	21
Staphylococcus pyogenes	0
Bacillus subtile	20
Corynebacterumulceram	17
Escherichia coli	0
Proteus mirabilis	0
Proteus Vulgaris	0
Pseudomonas aeruginosa	17
Salmonella typhi	0
Shigelladysenteria	22
Candida albicans	18
Candida krusei	0
Candida tropical	17

Minimum Inhibitory Concentration (MIC) of the methanol extract of the leaves of Celosia laxa

Table 4

Micro-organism		Concentration (mg/ml)				
		50	25	12.5	6.25	3.12
Methicillin R.S.A	-	MIC	+	++	+++	
Staphylococcus aureus				MIC	+	++
Staphylococcus pyogenes	_	_	_	_	_	
Bacillus subtile		_	_	MIC	+	++
Corynebacterunulceran	_	MIC	+	++	+++	
Eschrichia coli		_	_	_	_	_
Proteus mirabilis	_	_	_	_	_	
Proteus vulgaris	_	_	_	_	_	
Pseudomonas aeruginosa	_	MIC	+	++	+++	
Salmonella typhi	_	_	_	_	_	
Shigelladysenteria		_	_	MIC	+	++
Candida albicans	_	MIC	+	++	+++	
Candida Krusei		_	_	_	_	_
Candida tropical	_	MIC	+	++	+++	

Key: _ = No turbidity, MIC = Minimum Inhibitory Concentration, + = Turbid (Light Growth), ++ = Moderate turbidity, ++++ = High turbidity.

Minimum Bacterial Concentration of the methanol extract of the leaves of Celosia laxa
Table 5

Micro-organism	Concentration (mg/ml)				
	50	25	12.50	6.25	3.12
Methicillin R.S.A	MBC	+	++	+++	++++
Staphylococcus aureus	MBC	+	++	+++	++++
Staphylococcus pyogenes					
Bacillus subtile	MBC	+	++	+++	++++
Corynebacterunulceran	MBC	+	++	+++	++++
Eschrichia coli					
Proteus mirabilis					
Proteus vulgaris					
Pseudomonas aeruginosa	MBC	+	++	+++	++++
Salmonella typhi					
Shigelladysenteria		MBC	+	++	+++
Candida albicans	MBC	+	++	+++	++++
Candida Krusei					
Candida tropical	MBC	+	++	+++	++++

Key: _ = No turbidity, MBC = Minimum Bacterial Concentration, + = Turbid (Light Growth), ++ = Moderate turbidity, +++ = High turbidity.

The Antimicrobial of the chloroform extract of the leaves of Celosia laxa
Table 6

Test organism	Extract	Ciprofloxacin	Fluconazole
Methicillin R.S.A	S	S	S
Staphylococcus aureus	S	S	R
Staphylococcus pyogenes	R	S	R
Bacillus subtile	S	S	R
Corynebacterunulceran	S	S	R
Eschrichia coli	S	S	R
Proteus mirabilis	R	S	R
Proteus vulgaris	R	S	R
Pseudomonas aeruginosa	S	S	R
Salmonella typhi	S	S	R
Shigelladysenteria	S	S	R
Candida albicans	S	R	S
Candida Krusei	R	R	S
Candida tropical	S	R	S

Key: S = Sensitive, R = Resistance

The Antimicrobial of the chloroform extract of the leaves of Celosia laxa
Table 7

Test organism	Extract	Ciprofloxacin	Fluconazole
Methicillin R.S.A	26	0	0
Staphylococcus aureus	25	42	0
Staphylococcus pyogenes	0	40	0
Bacillus subtile	26	47	0
Corynebacterunulceran	24	37	0
Eschrichia coli	25	34	0
Proteus mirabilis	0	37	0
Proteus vulgaris	0	32	0
Pseudomonas aeruginosa	27	0	0
Salmonella typhi	26	30	0
Shigelladysenteria	22	42	0
Klebsiella pneumonia	21	37	0
Candida albicans	21	0	32
Candida Krusei	0	0	30
Candida tropicalis	23	0	35

Key: S = Sensitive, R = Resistance

The Antimicrobial Activity of the chloroform extract of the leaves of Celosia laxa
Table 8

Test organism	**Concentration in mg/ml**				
	30	15	7.50	3.25	1.625
Methicillin R.S.A	_	_	MIC	+	++
Staphylococcus aureus	_	_	MIC	+	++
Staphylococcus pyogenes					
Bacillus subtile	_	_	_	MIC	++
Corynebacterunulceran	_	_	MIC	+	++
Eschrichia coli	_	_	MIC	+	++
Proteus mirabilis					
Proteus vulgaris					
Pseudomonas aeruginosa	_	_	_	MIC	+
Salmonella typhi	_	_	MIC	+	++
Shigelladysenteria		_	_	MIC	+ ++
Klebsiella pneumonia		_	MIC	+	++ +++
Candida albicans	_	MIC	+	++	+++
Candida Krusei					
Candida tropicalis		_	_	MIC	+ ++

Key: _ = No turbidity, MIC = Minimum Inhibitory Concentration, + = Turbid (Light Growth), ++ = Moderate turbidity, +++ = High turbidity.

The Antimicrobial Activity of the chloroform extract of the leaves of Celosia laxa
Table 9

Test organism	30mg/ml	15mg/ml	7.5mg/ml	3.35mg/ml	1.625mg/ml	
Methicillin R.S.A	_	MBC	+	++	+++	
Staphylococcus aureus	_	MBC	+	++	+++	
Staphylococcus pyogenes						
Bacillus subtile	_	MBC	+	++	+++	
Corynebacterunulceran	_	MBC	+	++	+++	
Eschrichia coli	_	MBC	+	++	+++	
Proteus mirabilis						
Proteus vulgaris						
Pseudomonas aeruginosa	_	MBC	+	++	+++	
Salmonella typhi	_	MBC	+	++	+++	
Shigelladysenteria		MBC	+	++	+++	++++
Klebsiella pneumonia		MBC	+	++	+++	++++
Candida albicans	MBC	+	++	+++	++++	
Candida Krusei						
Candida tropicalis		MBC	+	++	+++	++++

Key: _ = No Colony growth, MBC = Minimum Bacterial Concentration, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth, ++++ = Very heavy colonies growth.

IV. Summary and Conclusion

The Preliminary phytochemical analysis was carried out on the plant Celosia laxa and the phytochemical screened. Plant metabolites were found in the plant material, they are carbohydrate, cardiac glycosides, saponins, sterioids, triterpenes, flavonoids, tannins, combined reducing sugar are present in the plant Celosia laxa.

The antimicrobial screening of the extracts were carried out in vitro on some microorganisms. The chloroform extract of celosia laxa has a zone of inhibition greater than that of the crude methanol from which the chloroform was partitioned. This show that the chloroform extracts was more active compare to the crude methanol extract which was also revealed in the minimum inhibitory concentration were 25mg/mi and 7.5mg/ml for the methanol and chloroform respectively. For the minimum bactericidal concentration, the MBC for the methanol extract starts from 50mg/ml for Methicillin Resistant Staphylococcus aureus, Staphylococcus aureus, Bacillus subtile, Corynebacterumulceran, Pseudomonas aeruginosa, Candida albicans and Candida tropicalis but was 15mg/ml for Methicillin Resistant Staphylococcus aureus, Staphylococcus aureus, Bacillus subtile, Corynebacterumulceran, Escherichia coli, Pseudomonas aeruginosa, Salmonella typi, while other test organism on chloroform extract had a MBC of 30mg/ml. These activities could be ascribe to the presence of secondary

metabolites in it, thereby justifying the usage of the plant in folk medicine in the treatment of sores, boils stomach ache, diarrhoea, urethral disorder.

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