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Phytochemical and antimicrobial studies on the aerial parts of *Synedrella nodiflora* Linn

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ABSTRACT

Extraction of the aerial parts of Synedrella nodiflora was done by continuous and exhaustive extraction of the powdered plant material using petroleum spirit (60-80°C), Chloroform, Ethyl acetate and methanol as solvents respectively. The extracts were obtained were concentrated in- vacuo at 40°C using rotary evaporator. General phytochemical screening of the aerial parts of Synedrella nodiflora Linn (Asterceae) revealed the presence of flavonoids, alkaloids, tannins steroids, glycoside and cardiac glycoside. The antimicrobial screening of the extracts showed significant zones of inhibition against 70% of the test organisms. The Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) against various organisms were conducted. The antimicrobial analysis of the isolated components was also conducted. These prove the folkloric usage of the plant and also indicated a broad spectrum activity of the plant extracts.

INTRODUCTION

Synedrella nodiflora Linn (Geartn) belongs to the family of Asteraceae. A straggling annual herb that grows nearly 2 m high a Fibrous woody root stock, a native of tropical America and now dispersed pan-tropically, which occurs throughout the region in waste places. It is an annual plant which grows in many parts of Nigeria and Africa in general and is given different names by different Communities and regions. In Ghana it is called kimkim, akara aje (Yoruba- Nigeria), chienhn waka (Ivory Coast), an-bal-kayan, ngulu-gbe (Sierra Leone) [1]. The leaf infusion is drank as laxative and leaf sap is used for treating dental infections in the Congo. [2]. A paste of the leaf is also used in topical embrocation for oedema and rheumatism in Ivory Coast [3]. When the leaves are mixed with other ingredients, they cure cough, heart troubles, stomach ache and ear ache. In Ghanaian traditional medicine, the aqueous extract of the whole plant is taken for the treatment of epilepsy .The plant is used extensively in Nigeria for cardiac troubles, Wounds healing and to stop bleeding [4]. In Malaysia and Indonesia, the plant is used for the treatment of headaches, earaches, stomachaches and in embrocation for rheumatism [1]. Alkaloids, sequiterpense lactones, tannins, flavoniod, steroids and terpeniod are the most abundant principles of this genus [1]. The compounds present in the genus represent several bioactivities ranging from anti-inflammatory, antioxidant, analgesic, astringent, antimicrobial among a host of many others [5].

MATERIALS AND METHODS

The aerial parts of the plants were collected from Samaru- Zaria; Kaduna State in August 2010.

It was identified by Mallam Umar Galla of the Herbarium of Biological Sciences Department, Faculty of Science, Ahmadu Bello University Zaria, Nigeria. A specimen was deposited in the herbarium with voucher number 359; the plant material was pulverized by the use of a wooden mortar and pestle and then stored in polythene bag until needed for use.

Extraction and Isolation
The classical procedure for obtaining organic constituents from dried plant was used [6]. Air-dried and pulverized plant material (100 g) was extracted successively using the soxhlet extractor with petroleum ether (60-80°C), chloroform, ethyl acetate and methanol in order of their polarities. The solvents were collected and concentrated *in-vacuo* using a rotary evaporator at 40^{0}C.

Phytochemical Screening:
The methods described by [7] were used to test for the presence of phytochemicals in the extracts.

Antimicrobial sensitivity test:
Antimicrobial sensitivity test was carried out *in vitro* to determine the extent to which an extract can inhibit the growth of pathogenic microorganisms. The sensitivity test was carried out using paper disc agar diffusion technique [8, 9].

Isolation of active compound
Chromatography was the major technique used in the purification of the extracts.

Thin layer chromatography (TLC).
This was used to determine the number of chemical components in the ethyl acetate fraction and the most suitable mobile phase for good resolution. The plant extract was dissolved in minimum amount of methanol, spotted at the base of the plate and developed using petroleum ether, chloroform, ethyl acetate and methanol in different ratios. The resulting chromatograms, after air-drying was viewed under short ultraviolet wave length of (254 nm) and long visible wavelength of (366 nm). Finally, a ratio of 7:3 of petroleum ether and diethyl ether was found to be the most suitable mobile phase that provided an excellent resolution. This culminated in the selection of this mixture as the best solvent system for column chromatography.

Column chromatography
The separation of the ethyl acetate extract into different chemical components was carried out using column chromatography technique with silica gel (60-120 mesh) as the stationary phases. The purified sample obtained in form of white needle-like crystals was labeled SEA.

Antimicrobial screening of the compound isolated.
Antimicrobial activity of the isolated component was determined using some pathogenic microorganisms. The microbes were obtained from the Department of Microbiology A.B.U Teaching Hospital, Zaria. All the microbes were checked for purity and maintained in slants of nutrient agar for the bacteria and slants of Sabaurand dextrose agar for the fungi. Disc diffusion method was the method used to determine the antimicrobial activities of the pure extract from the plant [6].

RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of alkaloids, tannins, saponin and cardiac glycosides as shown in Tables 1.

Table 1: Class of secondary metabolites of the aerial part of *Synedrell nodiflora* L

Class of compounds	PEE	CHE	EAE	ME
Alkaloids	+	+	+	+
Saponins	-	-	-	-
Glycosides	+	+	+	+
Phlobotannins	-	-	-	-
Tannins	+	+	+	+
Steroids	-	-	-	-
Flavonoids	-	+	+	+
Cardiac glycoside	+	+	+	+
Coumarins	-	-	-	-

PEE= Petroleum Ether Extract; CHE= Chloroform Extract; EAE = Ethyl Acetate Extract; ME= Methanol Extract

The antimicrobial sensitivity test of the four crude extracts of *Synedrella nodiflora* were carried out using ten pathogens namely:- *Staphylococcus aureus, Streptococcus pyogenes, Corynbacterium ulcerans, Klebsiella pneumonia, Salmonella typhi, Escherichia coli, Proteus rettgeri, Candida stellatoidea, Candida tropicalis and Pseudomonas aeruginosa.* The results obtained are shown in Table 2. *Streptococcus pyrogenes*, *Proteus rettgeri* and *Pseudomonas aeruginosa* were resistant to all the four extracts, *Candida stellatoidea* and *Candida tropicalis* were resistant to only the petroleum spirit extract but sensitive to chloroform, ethylacetate and methanol extracts. However, *S. aureus*, *C. ulcerans, K. pneumonia, S. typhi* and *E .coli* were sensitive to all the four extracts. This indicates that the extracts of *Synedrella nodiflora* had broad spectrum of activity. The four crude extracts had significant zones of inhibition against all the tested microorganisms except *Streptococcus pyrogenes*, *Proteus rettgeri* and *Pseudomonas aeuroginosa* which had no zones of inhibition with chloroform, ethyl acetate and methanol respectively. Petroleum spirit extract did not show zones of inhibition on *S. pyrogene, P. rettgeri*, *P. aeruginosa*, *C. stellatoidea* and *C.tropicalis*. The chloroform extract showed the highest zones of inhibition range of 20-29 mm, then methanol had 18-26 mm, ethylacetate had 14-24 mm, petroleum spirit had the lowest range of zones of inhibition of 12-19 mm. Minimum inhibitory concentration is important in diagnostic laboratory to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents [10]. The result of the minimum inhibitory concentration (MIC) for petroleum spirit extract shown in Table 2 revealed that the petroleum spirit extract inhibited the growth of *Staphylococcus aureus, Corynbacterium ulcerans, Klebsiella pneumonia, Salmonella typhi, Escherichia coli* at 7.5 mg/ml. Minimum inhibitory concentration (MIC) for chloroform extract revealed that the chloroform extract inhibits the growth of *Staphylococcus aureus, Corynbacterium ulcerans, Klebsiella pneumonia, Salmonella typhi, Escherichia coli, Candida stellatoidea, Candida tropicalis* at a concentration of 3.75 mg/ml .This shows that this extract is the most active followed by ethyl acetate and methanol and the least active is the petroleum spirit extract. The result of the minimum inhibitory concentration (MIC) for ethyl acetate extract revealed that the ethyl acetate extract inhibited the growth of *Staphylococcus aureus, Corynbacterium ulcerans, Salmonella typhi, Escherichia coli* at concentrations of 3.75 mg/ml and 7.5 mg/ml for *Candida stellatoidea, Candida tropicalis and Klebsiella pneumonia* respectively. The result of the minimum inhibitory concentration (MIC) for methanol extract revealed that the methanol extract inhibited the growth of *Staphyloccus aureus, Corynebacterium ulcerans, Klebsiella pneumonia, Salmonella typhi, Escherichia coli* at concentrations of 3.75 mg/ml and 7.5 mg/ml for *Candida stellatoidea and Candida tropicalis* respectively. The result of the minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC)) for petroleum spirit extract as shown in Table 2 showed concentration of 30 mg/ml for *Staphylococcus aureus, Corynebacterium ulcerans, Klebsiella pneumonia, Salmonella typhi, and Escherichia coli.* This shows that this extract does not have antifungal effect even at the highest concentration against the test fungi used in this research. The result of the minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) for chloroform extract as showed concentration of 7.5 mg/ml for *Staphylococcus aureus, Corynbacterium ulcerans, Salmonella typhi, Escherichia coli* and 15 mg/ml for *Candida stellatoidea, Candida tropicalis and Klebsiella pneumonia* respectively. The result of the minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) for ethyl acetate extract showed concentrations of 15 mg/ml for *Staphylococcus aureus, Corynebacterium ulcerans, Salmonella typhi, Escherichia coli* and 30 mg/ml for *Candida stellatoidea, Candida tropicalis and Klebsiella pneumonia* respectively. The result of the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) for methanol extract showed concentration of 7.5 mg/ml against *Staphylococcus aureus only,* 15 mg/ml for *Corynebacterium ulcerans, Salmonella typhi, Escherichia coli and Klebsiella pneumonia, lastly,* 30 mg/ml for *Candida stellatoidea and Candida tropicalis*. This also means that the chloroform, ethyl acetate and methanol extract had antifungal activity and so it can be used to cure diseases caused by these test fungi.

Antimicrobial screening of the isolated component:
The isolated compound coded SEA was also screened for biological activity using ten pathogenic microorganisms namely: - *Staphylococcus aureus, Streptococcus pyogenes, Corynbacterium ulcerans, Klebsiella pneumonia, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Candida stellatoidea, Candida tropicalis and Candida albicans.* The results obtained are as shown in Table 3. The results obtained showed that the isolated compound was active against *Staphylococus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli, Candida stellatoidea,* and *Candida albicans whereas C.ulcerans, P. aeruginosa and C. tropicalis* were resistant to the isolated compound. This shows that the isolated compound possibly exhibit broad spectrum activity. The result for zones of inhibition (Table 3) of the isolated compound on the test organisms. The result shows that the isolated compound had a good level of toxicity against the microorganisms when compared to the crude extract. The result of the minimum inhibitory concentration (MIC) for the isolated compound as shown in Table 3 revealed that the compound inhibited the growth *of Staphylococcus aureus, C. albican, and C. stellatoidea* at concentration of 0.005 mg/ml while *Streptococcus pyrogenes, Klebsiella pneumonia, Salmonella typhi, Escherichia coli* had MIC of 0.0025 mg/ml respectively. This implies that isolated compound is even more active and therefore, inhibited the growths of the organisms at much lower concentration than Sparfloxacin which is a standard drug use for the treatment of these infections. The result of the minimum bactericidal concentration (MBC/MFC) for the isolated compound as shown in Table 3 revealed that *Staphylococus aureus, Streptococcus pyrogenes. Escherichia coli C. albicans* had MBC at concentration of 0.01 mg/ml respectively. *Klebsiella pneumonia, Salmonella typhi* had concentration of 0.005 mg/ml *and Candida* stellatoidea had MBC at 0.02mg/ml. This implies that isolated compound has a high activity and would therefore kill the organisms; it also means that the isolated compounds would be very effective in curing diseases caused by any of these microorganisms. The results of the control analysis (Table 4) using DMSO as solvent and the test drugs Sparfloxacin and fluconazole at concentrations of 0.02 mg/ml and 0.05 mg/ml respectively. The test organisms were resistant to DMSO. The result also shows that the sparflaxocin had activity on all the organisms except *Pseudomonas aeruginosa, Candida albicans, Candida tropicalis and Candida stellatoidea* whereas, fluconazole had no activity on all the organisms except all the fungi used, *Candida albicans, Candida tropicalis and Candida stellatoidea.* The zones of inhibition Table 5 of the test drugs as compared to the isolated compound revealed that the isolated compound is more active than the test drug Sparfloxacin for *S.aureus, S.pyrogene, E. coli, S. typhi and K. pneumoneae.* Fluconazole had zones of inhibitions on *Candida albicans, Candida tropicalis and Candida stellatoidea that* are comparable to the isolated compound hence; the extract is as effective as the test drugs.

Table 3: Antimicrobial activity of the isolated compound SEA

Test organisms	A	ZI (mm)	MIC (mg/ml)	MBC (mg/ml)
S aureus	S	29	0.005	0.01
S.pyrogenes	S	32	0.0025	0.01
C. ulcerans	R	0	-	-
E.coli	S	32	0.0025	0.01
S.typhi	S	30	0.0025	0.005
K.pneumoniae	S	35	0.0025	0.005
P.aeruginosa	R	0	-	-
C.albicans	S	27	0.005	0.01
C.tropicalis	R	0	-	-
C.stellatoidea	S	25	0.005	0.02

A=Activity→ Sensitivity (S) or Resistance (R); ZI = Zone of inhibition; MIC = Minimum inhibitory concentration; MBC = Minimum inhibitory concentration

Table 4: Result of the control on the test microbes

Test organisms	DMSO	Sprafloacine	flouconazol
S.aureus	**R**	**S**	**R**
S.pyrogenes	**R**	**S**	**R**
C. ulcerans	**R**	**S**	**R**
E.coli	**R**	**S**	**R**
S.typhi	**R**	**S**	**R**
K.pneumoniae	**R**	**S**	**R**
P.aeruginosa	**R**	**R**	**R**
C.albicans	**R**	**R**	**S**
C.tropicalis	**R**	**R**	**S**
C.stellatoidea	**R**	**R**	**S**

DMSO= Dimethyl sulphoxide; S= Sensitivity; R = Resistance

Table 5: Zone of inhibition of the test drug against the microbes

Test organisms	DMSO	Sparfloxacin	flouconazol
S.aureus	**0**	**24**	**0**
S.pyrogenes	**0**	**30**	**0**
C. ulcerans	**0**	**30**	**0**
E.coli	**0**	**31**	**0**
S.typhi	**0**	**27**	**0**
K.pneumoniae	**0**	**31**	**0**
P.aeruginosa	**0**	**0**	**0**
C.albicans	**0**	**0**	**27**
C.tropicalis	**0**	**0**	**25**
C.stellatoidea	**0**	**0**	**27**

DMSO= Dimethyl sulphoxide

CONCLUSION

Synedrella nodiflora possesses high medicinal values as shown by activities of its crude extracts on various microorganisms used during the research work, the medicinal values of the isolated compounds was even more higher and so if synthesized would go a long way in improving the healthcare of the local users.

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