Analytical Chemistry: An Indian Journal

Research Article | Vol 21 Iss 5

Phytochemical Analysis And Anti-Microbial Activities Of The Extracts Of *Alternanthera spp.*

Edah O. Alexander[1], Okoye Maryann[2], Okoronkwo Ngozi[3], Terfa Jude Igba[4], Pofi A'aron Amos[4] and John Christopher Izang[4]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical sciences, University of Jos, Nigeria.

[2]Department of Medicinal Chemistry and quality control, National Institute of Pharmaceutical Research and Development, Industrial area, Abuja.

[3]Department of Microbiology and Biotechnology, National Institute of Pharmaceutical Research and Development, Industrial area, Abuja.

[4]Department of Chemistry, Faculty of Natural Sciences, University of Jos, Nigeria.

[*]**Corresponding author**: Edah O. Alexander, Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical sciences, University of Jos, Nigeria., E-Mail: jidimmaaugustine@ymail.com

Received: November 04, 2021; **Accepted:** November 10, 2021; **Published:** November 25, 2021

Abstract

This study was carried out in order to explore the phytochemical constituents and anti-microbial character in extracts of *Alternanthera pungens*, *Alternanthera sessilis*, and *Alternanthera brasiliana*. The plant material was extracted using water and acetone. Phytochemical screening of the crude plant extract was carried out using the methods outlined by Trease and Evans. The major classes of the phytochemicals were present in all samples evaluated except for Saponins which was present in *Alternanthera sessilis* and alkaloids were absent in the same *Alternanthera sessilis*. In vitro anti-microbial activity was assessed against three fungi specie which are *Aspergillus niger*, *Trichonphyton* and *Penicillum spp*. The anti-microbial test results revealed that the acetone extract has a more promising potential activity against fungi with a maximum zone of inhibition of 33mm against *Aspergillus niger* at a concentration of 400mg/ml. Nestatin was used as control because of its high anti-fungal potency at a standard concentration of 10mg/ml but showed a maximum zoning of 24mm against *Aspergillus niger*. Minimum Inhibitory Concentrations of the various plant extracts were determined and Minimum Bacterial Concentration was also determined. This research has shown that plant extracts of *Alternanthera pungens*, *Alternanthera sessilis* and *Alternanthera brasiliana* are rich in biologically active compounds and substances which are effective against fungi that can cause alterations to the normal functioning of the human skin.

Keywords: ***Antimicrobial; Phytochemicals; Alternanthera sessilis; Alternanthera brasiliana; Alternanthera pungen;, MIC; MBC***

Introduction

Plants were the first weapon used by man against different types of health ailments as they are the largest storehouse of the

Citation: Edah O. Alexander, Okoye Maryann, Okoronkwo Ngozi, Terfa Jude Igba, Pofi A'aron Amos and John Christopher Izang, Phytochemical Analysis And Anti-Microbial Activities Of The Extracts Of *Alternanthera Spp*. Anal Chem Ind J. 2021;21(5):171.

biochemical compounds. With the advancement of organic chemistry and modern technology man shifted from the traditional system towards synthetic drugs for healthcare needs. This was mainly because pure compounds were easily obtained and structurally modified to produce potentially more active and safer drugs. The synthetic drugs were preferred as there were many issues associated with the natural products like lack of authentication from legal authorities and there is also concern about the lack of validity of phytochemical products.

Alternanthera is a perennial herb and commonly noticed as a mat like structure in vacant lots, along roadside, railway tracks, lawns, etc. Its stem is hairy, 10-50 cm long, prostrate and occasionally develop roots the nodes. Leaves are green and ovate to obovate in shape and are generally 0.5 to 4.5 cm long and 0.3 to 2 cm wide [1]. Flowers are without stalk, sparsely velvety spikes with spiny bracts and bracteoles. In traditional medicine it was used as painkiller, for stomach ache, swelling and nasopharyngeal infections and also reported for lactation stimulus in veterinary. Alternanthera belongs to the family of Amaranthaceae, its common names are Joyweed, SenNdighis in mwaghavul language and in Shendam area of Plateau State it is called Zamfa. Other varieties of Alternanthera serves both as food and medicine in Asia and African countries. Species of Alternanthera have been used in the past and are still being used in present times for treatment of skin infections in rural areas due to ease of access and the high cost of pharmaceutical anti-fungals.

Taxonomical Classification

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Caryophyllales
Family: Amaranthaceae
Genus: *Alternanthera*
Species: *alternanthera*

Phytochemicals are biologically active substances that exist in plants in small amounts, which are not established nutrients but nevertheless; contribute significantly to protection against degenerative diseases. Some Phytochemicals are alkaloids, flavonoids, saponnins, tannins, steroids and terpenes, cardiac glycosides and anthraquinones. Fungi are micro-organisms that can be found everywhere both indoor and outdoor in all parts of the world. Some species have a wide range of importance but others are very toxic and can cause alterations to the normal functioning of the human body.
Trichonphyton is a genus of fungi (parasitic varieties) that causes skin diseases such as athlete' s foot, ring worm, eczema and other skin and scalp diseases.
Aspergillus niger is a common specie of the fungi genus Aspergillus. It causes black molds on fruits and vegetables and in humans it causes otomycosis (fungal ear infection), which causes pain in the ear and leads to temporary hearing loss.
Penicillum spp is a ubiquitous soil fungi present wherever organic material is found. In humans it causes urinary tract infections.
The aim of this study is to analyse the Phytochemicals and Anti-microbial activities of the extracts of Alternanthera pungens, Alternanthera sessilis, and Alternanthera brasiliana.

Materials And Methods

Sample collection

The Alternanthera plant species were collected from Jos environs in Plateau State, Nigeria. The samples were dried for two weeks at room temperature on a laboratory workbench and grinded into powder using laboratory piston and mortar.

Extraction of plant material

Acetone extraction was carried out by weighing 100g of *Alternanthera pungens* and soaking the powder in 800ml of Acetone with shaking and left to stand for 48 hours in a refrigerator (5-6˚C) to avoid fermentation with the lid covered using foil paper to avoid the acetone from evaporating. The mixture was then filtered by passing it through filter paper fitted in to a funnel and the filtrate was left to dry.

Aqueous extraction was carried out by soaking the plant samples in distilled water in well labelled conical flasks with shaking and was left to stand in a refrigerator to avoid fermentation for 24 hours and then the mixture was filtered. The filtrates were allowed to dry in an oven at a temperature of 45˚C. All extracts were collected in well labelled and air-tight containers.

Phytochemical Screening

Test for Alkaloids

One gram of powdered sample was boiled with water and 10 cm3 hydrochloric acid on a water bath and then filtered. Very small quantities of the following reagents were added separately to about 0.5 cm3 of the filtrate in a different test tube and were observed.

1. Few drops of Mayer's reagent were added to the filtrate in a test tube and a cream precipitation formed indicating the presence of alkaloids.
2. Dragendoff's reagent was added to extract forming rose – red precipitation.
3. Picric acid solution was added to extract the test tube was observed for turbidity indicating the presence of alkaloids.
4. About 10% tannic solutions were added to extract, a yellow precipitation was observed.

Test for Flavonoids

One gram of the powdered dried plant was boiled with 10ml of distilled water for 5minutes and filtered while hot. Few drops of 20% sodium hydroxide solution were added to 1ml of the cool filtrate. A change to yellow colour was observed which on addition of acid changed to colourless solution indicating the presence of flavonoids.

Test for Cardiac glycosides

Five cm3 of extract was treated with $2cm^3$ of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with about 1ml of concentrated sulphuric acid. A brown ring at the interface was observed indicating the presence of cardiac glycosides.

Test for Tannins

One gram of powdered sample was boiled with 20 cm3 distilled water for 5minutes in a water bath and was filtered while hot. One ml of cool filtrate was added to 5 cm3 distilled water and a few drops of 10% ferric chloride were added. It was observed for any formation of precipitates and any colour change; a bluish - black or brownish - green precipitate indicated the presence of tannins.

Test for Terpenes and Steroids

0.5g of plant extract was dissolved in chloroform. Two cm3 of acetic aldehyde was added to 0.5 cm3 methanol extract of sample with 2ml H_2SO_4. The colour changes from violet to blue or green indicating the presence of terpenes and steroids.

Test for Saponins

One gram of powdered sample was boiled with 10 cm3 distilled water in a water bath for 10 minutes. The mixture was then carried out to observe persistent froth (bubbles/foaming). A portion of the filtrate (2.5 cm^3) was diluted to 10ml with distilled water and shaken vigorously for 2 minutes, frothing indicated the presence of saponins. To the above solution 2 drops of olive oil was added and shaken vigorously for a few minutes. Formation of a fairly stable emulsion indicated the presence of saponins.

Test for Anthrquinones

Bortrager's test was used for the detection of Anthraquinones. 0.5g of each extract was taken into a dry test-tube and 5 cm3 of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with 100% ammonia solution, a pink-violet or red colouration in the ammonical layer (lower layer) indicates the presence of anthraquinones.

Anti-microbial Sensitivity analysis

The agar well diffusion method as described by Bauer et al., (1966) with slight modification was adopted in this assay. A loopful of the standardized (0.5 McFarland) fungal cell suspended was inoculated into well dried sterile Potato Dextrose Agar. The plant extract was reconstituted in Dimethylsulfoxide (DMSO) to obtain the working concentration of 400 mg/mL, 200 mg/mL, 100 mg/ml, 50 mg/mL and 25 mg/mL. A quantity (0.1 cm^3) of each extract was inoculated into wells earlier bored with a sterile borer in each plate to accommodate the different plant extract concentrations. The plates were allowed to stand for 30 minutes on the work bench for pre – diffusion of extracts. The Potato Dextrose Agar plates were incubated at 37 ^oC and room temperature for 168 hours (7 days). The antimicrobial activity of the extracts were determined after the incubation period by measurement of mean diameter zones of inhibition produced by the extracts against the test organisms (fungi) and the results were recorded in millimeter (mm) using a transparent ruler [2]. Nestatin was used as control with a standard concentration of 10mg/ml because of its potency against fungal infections.

Minimum Inhibitory Concentration (MIC)

This is a test usually done to ascertain the minimum concentration of the extract needed to stop the growth of a micro-organism to avoid wastage of the potent plant extract. This was done by suspending the fungi samples in solutions of the plant extract at concentrations of 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml respectively with shaking, if turbidity was noticed then it signifies that the fungi will not be affected by the plant extract at that given concentration.

Minimum Bactericidal Concentration (MBC)

The MBC values were determined following the modified method of El-Mahmood and Doughari (2009). This was done by removing a loopful of bacterial suspension from the MIC tubes that did not show any growth and sub-cultured into Nutrient agar plates. The plates were incubated at 37°C for 24 hours. After incubation, the concentration at which no visible growth was seen was recorded as the MBC.

Results

TABLE.1. **Phytochemical Constituents**

Phytochemicals	**Acetone extract**	**Water extract**

	A. pungens	A. Sessilis	A.brasiliana	A. pungens
Saponnins	-	+	-	-
Tannins	+	+	+	+ +
Terpenes/Steroids	+	+	+	+
Cardiac glycosides	+ +	+	+	+ +
Alkaloids	+	-	+	+ +
Flavonoids	+	+	+	+
Anthraquinones	+	+	+	+

Key + = phytochemical present, - = phytochemical absent ++ = phytochemical very present

TABLE.2. **Anti-microbial Activity of *Alternanthera spp***

Micro-organism	**Extract**	**Concentrations (mg/ml)**					
		400	200	100	50	25	C
Acetone extract	Aspergillus niger	33	30	26	-	-	24
(A.pungens)	Penicillumspp	30	27	20	11	-	23
	Trichonphyton	32	28	20	12	-	22
Water extract	Aspergillus niger	28	24	16	-	-	23
(A. brasiliana)	Penicillumspp	32	26	20	16	-	24
	Trichonphyton	26	24	20	12	-	23
Water extract	Aspergillus niger	19	12	10	-	-	23
(A. sessilis)	Penicillumspp	25	20	16	11	-	24
	Trichonphyton	24	22	18	10	-	22

Key: - = no zone formed

A. pungens = *Alternanthera pungens*

A. brasiliana = *Alternanthera brasiliana*

A. sessilis = *Alternanthera sessilis*

C = Control which is Nestatin because of its potent anti-fungal action at a concentration of10mg/ml. All zoning values are measured in millimetres (mm).

TABLE.3. **Minimum Inhibitory Concentration (MIC) of *Alternanthera spp*.**

Micro-organism	**Extract**	**Concentration (mg/ml)**				
		400	200	100	50	25
Aspergillus niger	Acetone(A.pungens)	-	-	+	+	+
Penicillum spp		-	-	+	+	+
Trichonphyton		-	-	+	+	+
Aspergillus niger	Water (A. pungens)	-	-	+	+	+
Penicillum spp		-	-	-	+	+
Trichonphyton		-	-	+	+	+
Aspergillus niger	Water (A. brasiliana)	-	+	+	+	+
Penicillum spp		-	-	+	+	+
Trichonphyton		-	+	+	+	+

KEY: - =No turbidity

+ =there is turbidity

A. pungens= *Alternanthera pungens*

A. brasiliana = *Alternanthera brasiliana*

TABLE 4. **Minimum Bacterial Concentration (MBC) of *Alternanthera spp*.**

Micro-organisms	**Extracts**	**Concentrations(mg/ml)**				
		400	200	100	50	25
Aspergillus niger	Acetone(A.pungens)	-	-	+	+	+
Penicillumspp		-	+	+	+	+
Trichonphyton		-	+	+	+	+
Aspergillus niger	Water (A. pungens)	-	+	+	+	+
Penicillumspp		-	+	+	+	+
Trichonphyton		-	+	+	+	+
Aspergillus niger	Water (A. brasiliana)	+	+	+	+	+
Penicillumspp		-	+	+	+	+
Trichonphyton		+	+	+	+	+

KEY: - =No growth

+ =there is growth

A. pungens = *Alternanthera pungens*

A. brasiliana = *Alternanthera brasiliana*

Discussion

From the results in **TABLE 1** it is observed that the phytochemical parameters are present in all samples except for Saponin which was observed only in *Alternantera sessilis* and Alkaloids were absent in the same sample. Also, the presence of Tannins was strongly noticed in the aqueous extract of *Alternanthera pungens*, Cardiac glycosoids were strongly noticed in both acetone and aqueous extracts of *Alternanthera pungens*, the same was observed with Alkaloids which were most present in aqueous extract of *Alternanthera pungens*.

TABLE 2 shows results of microbial activity and a maximum zone of 33mm of Acetone extract against the fungus *Aspergillus niger* at a concentration of 400mg/ml and the lowest value of zones observed was 10mm from the water extract of *Alternanthera sessilis* against *Aspergillus niger* and *Trichonphyton* at concentrations of 100mg/ml and 50mg/ml respectively. Nestatin which was the control showed a steady zone sizes with a mean value of 23mm.

TABLE 3 shows the MIC results and from that it is seen that the minimum concentration required for acetone extract is between the concentrations of 200mg/ml and 100mg/ml, the same goes for water extract of *Alternanthera pungens* except for *Penicillum spp* which is between concentrations of 100mg/ml and 50mg/ml. That of water extracts of *Alternanthera brasiliana* shows the MIC is between 400mg/ml and 200mg/ml but that of *Penicillum spp* is between 200mg/ml.

TABLE 4 results shows that for the acetone extract of *Alternanthera pungens* there will be bacterial growth at concentration of 200mg/ml and less except against *Aspergilus niger* were growth was observed from concentrations of 100mg/ml and less. Growth was observed also in water extracts of *Alternanthera pungens* at concentrations of 200mg/ml and less, also the same thing was observed in water extracts of *Alternanthera brasiliana* except against *Aspergillus niger* and *Trichonphyton* were growth was observed even at concentrations of 400mg/ml and less.

The results obtained show that *Alternanthera pungens* is a better antifungal than the control that was used (Nestatin) also *Alternanthera sessilis* and *Alternanthera brasiliana* showed better antifungal action against the fungi *Penicillum spp* and *Trichonphyton* than the control that was used. There are so many suggestions that aqueous and ethanolic extract from plants used in allopathic medicine and potential sources of antiviral, antitumoural and antimicrobial agents (Chung et al., 1995; Vlietinck et al., 1995).

Conclusion

The results of the phytochemical analysis of *Alternanthera* extracts show that the major classes of phytochemicals are present in all samples except for Saponin which is present in *Alternanthera sessilis* only and Alkaloid which is absent in the Alternanthera sessilis specie. The anti-microbial (anti-fungal) activity reveals that *Alternanthera* plant acetone extracts have promising potential activity against fungi such as *Aspergillus niger*, *Penicillum spp* and *Trichonphyton* which affects the human and animal skin tissue.

Recommendation

It is recommended that more studies should be carried out on other species of Alternanthera and fungi to further ascertain its potency and possibly isolate the active components for Pharmaceutical purposes. Toxicity studies should also be carried out ‘in vivo’ to evaluate the isolates.

REFERENCES

1. Jakhar S, Dahiya P. Antimicrobial, antioxidant and phytochemical potential of *Alternanthera pungens* HB&K. Int J Pharm Sci Res 2017; 9(8):1305.
2. Bauer, A. T. Antibiotic susceptibility testing by a standardized single disc method. Am J clinpathol 1966; 45:149-158.
3. Naidu, V. S. G. R. Hand book on weed identification 2012.
4. Vlietinck AJ, Van Hoof L, Totte J. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J. Ethnopharmacol.. 1995;46(1):31-47.
5. Chandran RP, Manju S, Vysakhi MV. In vitro antimicrobial activities of Hygrophila schulli (Buch.-Ham) leaf and root extracts against clinically important human pathogens. Biomed Pharmacol J. 2013; 6(2):421-8.
6. Fang JB, Duan HQ, Zhang YW. Antitumor constituent from Alternantherahiloxeroides. J Asian Nat Prod Res 2007.
7. Gordon Brown, The Conversation June eds 2018.
8. Guerra RN, Pereira HA, Silveira LM. Immunomodulatory properties of Alternanthera tenella Colla aqueous extracts in mice. Braz. J. Med. Biol. Res. 2003; 36:1215-9.
9. Jalalpure SS, Agrawal N, Patil MB, Chimkode R, Tripathi A. Antimicrobial and wound healing activities of leaves of Alternanthera sessilis Linn. Int. J. Green Pharm.2008;2(3).
10. Kannan, M., Chandran, R. P, Manju, S. Preliminary phytochemical and antibacterial studies on leaf extracts of Alternantherabrasiliana (L.) Kuntze. Int J Pharm Pharm Sci. 2014;626-628.
11. Kumar MS, Rani GS, Kumar SS. Screening of aqueous and ethanolic extracts of aerial parts of *Alternanthera sessilis* Linn. R. br. ex. dc for nootropic activity. Int J Pharm Sci Res 2011; 3(6):1294.

12. Manju S, Chandran RP, Shaji PK. In vitro free radical scavenging potential of Acorus Calamus L. rhizome from Kuttanad Wetlands, Kerala, India. Int J Pharm Pharm Sci. 2013;5:376-80.
13. Niño J, Narváez DM, Mosquera OM. Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two Rubiaceae plants from Colombian biodiversity. Braz. J. Microbiol. 2006;37:566-70.
14. Wayne PA. National Committee for Clinical Laboratory Standards 2000.
15. Wink M. Phytochemical diversity of secondary metabolites. J Encyclopedia of Plant and Crop Sci. 2004.
16. World Health Organization.Diethyl phthalate (ConciseInternational Chemical Assessment Document 52). Geneva,Switzerland: Author 2003.
17. Yadav NP, Dixit VK. Recent approaches in herbal drug standardization. Int J Integr Biol. 2008; 2(3):195-203.
18. Zhou BN, Blaskò G, Cordell GA. Alternanthin, a C-glycosylated flavonoid from Alternanthera philoxeroides. Phytochemistry. 1988;27(11):3633-6.
19. Huang Y, Ge Y, Wang Q. Allelopathic Effects of Aqueous Extracts of Alternantheraphiloxeroides on the Growth of Zoysiamatrella. Pol. J. Environ. Stud. 2017; 26(1).