EVALUATION OF ANTIMICROBIAL ACTIVITIES AND INHIBITORY EFFICACY OF LEAVE EXTRACTS OF *Agaratum conyzoides* and *Acacia alata* ON SOME PATHOGENIC BACTERIA SPECIES

Abstract

Aim: The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of extracts of leaves of *Ageratum conyzoides* and *Acacia alata* were evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. Methodology: The antimicrobial activity was determined in the extracts using agar disc diffusion method. The disc diffusion method was used to determine the antimicrobial activity of these plants on the test organisms. The fresh and ethanol extracts of *Ageratum conyzoides* and *Acacia alata* showed significant zones of inhibition greater than (5mm) on all the test organisms. Results: The fresh and ethanol extracts of *Ageratum conyzoides* and *Acacia alata* showed significant zones of inhibition on *Candida albicans* (7mm) and a low inhibition on other organisms which are bacterial species. Conclusion: The minimum inhibitory and microbial concentrations evaluated on both the fresh leaf and ethanol extracts of the plants were concentration dependent. The results of this research showed that the plants have a good antimicrobial activity and inhibitory potentials on the test organisms.

Key words: Antifungal activity, antibacterial activity, *Ageratum conyzoides, Acacia alata*

1. INTRODUCTION

"The use of herbal medicine for the treatment and prevention of diseases and infections is as old as mankind and is attracting attention by scientists worldwide" [1]. "This is corroborated by the World Health Organization in its quest to bring primary health care to the people" [2]. "In the developing countries, a vast number of people are in extreme poverty and some are suffering, and dying for want of safe water and medicine" [3].

"Traditional medicine is the oldest method of curing diseases and infections and various plants have been used in different parts of the world to treat human diseases and infections" [4]. "Different plant parts have also been used for various forms of diseases and infections. *Ageratum conyzoides* and *Acacia alata* are examples of medicinal plants and have been found to be useful in the treatment of some microbial infections. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body" [5].

"Medicinal plants are known to owe their curative potentials to certain biologically active substances, which exists in the parts of the plants. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoids, bioflavonoids, alkaloids, benzophenones, phenolic compounds as well as some metabolites such as tannins, saponins, cyanates, oxalate and terpenoids" [6].

"Medicinal plants are widespread in nature ranging from herbs and shrubs to trees in tropical and temperature regions all over the world" [7]. "The concentration of medicinal compounds in these plants and consequently their therapeutic efficacy varies and depends on source and handling, the part of the plant, the age of the plant and ecological factors such as neighbouring plant species, seasonality, and diurnal changes in light, climatic and soil conditions" [8].

"Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern" [9]. "The clinical efficiency of many antibiotics in existence is being treated by the emergence of multi-drug-resistant pathogens" [10]. "Throughout the history of mankind, many infectious diseases have been known to be treated with herbal remedies. The natural herb products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug lead because of the incomparable availability of diversity of chemicals. This results to a never-ending and urgent need to discover new antimicrobial compounds with different chemical structures and new mechanisms of action for re-emerging and new infectious diseases" [11]. "Therefore, researchers are increasingly turning their attention to folk medicine. Continuous search leads to developing better drugs against microbial infections" [12]. "Several medicinal plants are being screened for their potential microbial activity based on the increasing failure of chemotherapeutics and antibiotic and antifungal resistance exhibited by pathogenic agents" [13].

2. MATERIALS AND METHOD

2.1 METHODOLOGY

The antimicrobial activity of the extracts were determined using the agar disc diffusion method. The disc diffusion method was used to determine the antimicrobial activity of these plants on the test organism.

2.2 COLLECTION OF THE PLANT EXTRACTS

The plants used in this work was freshly harvested leaves of *Ageratum* conyzoides and *Acacia alata* obtained from uncultured gardens around the Academic Complex of Okpara University of Agriculture, Umudike, Abia State. The plants were taxonomically authenticated by Dr. Mathias Eka, a Plant Health Management Lecturer of the Department of Plant Health Management in the College of Crop and Soil Science, Okpara University of Agriculture, Umudike, Abia State.

The freshly harvested leaves meant for fresh leaf extract were washed, ground and extracted. The leaves were extracted using ethanol and collected while still fresh and. The drying period lasted for 7 days. The dried leaves were pulverised into powder using Thomas Wiley Mill Model E.D. 5 from Soil Laboratory, National Root Crops Research Institute (NRCRI), Umudike, Abia State.

2.3 Fresh leaf extracts preparation

The freshly harvested leaves were washed in water and grinded using a sterilized mechanical grinder from the Central Laboratory, National Root crops Research Institute, Umudike, Abia State. After grinding the leaf extract was squeezed with a sterilized muselein cloth into a Whatman No. 1 filter paper suspended into a clean sterile beaker. The extract was stored at 4°C in a refrigerator. 100 mls of the fresh extract was evaporated to dryness using a water bath regulated at 100°C to obtain a dry extract which will be used for the minimum inhibitory concentration determination.

2.4 Ethanol extracts preparation

"20.0 grams of the pulverized leaves of *Ageratum conyzoides* and *Acacia alata* were weighed using Satoric A. G. Gottingen electronic weighing balance. The weighed sample was soaked in 200 mls of ethanol contained in a conical flask. The mixture was swirled. After 48 hours elaption with interval stirring, the mixture was filtered using Whatman No. 1 filter paper into a clean sterile beaker and it was finally evaporated to dryness using a steam bath at 100°C" [14, 15].

The yield was recovered as a percentage of the quantity of initial plant material (20.Og) used and expressed as follows.

Percentage yield = weight of the powdered leaf used ÷ weight of the extract produced x 100

2.5 MEDIA PREPARATION

"Nutrient agar was prepared by weighing 28 grams of the powdered agar and introducing it into 1000 mls of distilled water in a clean sterilized conical flask. It was swirled until it became a mixture. It was then covered with foil and autoclaved at 121°C for 15 minutes. The medium was cooled at 47°C and 20 mls of the molten medium was poured into a sterile disposable Petri dish and allowed to solidify. The sterility of the medium was tested for by incubation for eight (8) hours and looking out for contaminants" [15].

2.6 STANDARDIZATION OF TEST ORGANISMS

A sterile wire loop was used to pick a colony of the test organism from a 24 hours agar plate and placed into a sterile test tube containing 2 mls of peptone water. "The broth culture was adjusted with the broth to obtain turbidity optically comparable to that of the 0.5 Macfarland's standard; The MacFarland's standard was prepared as described by Cheesbrough" [16]. A 0.6ml of 1% $^{w}/_{v}$ solution of Barium chloride (0.5g $BaCl_2.\ H_2O$ in 50ml of distilled water) was added to 99.4 ml of 1% $^{v}/_{v}$ solution of sulphuric acid (1ml concentration of sulphuric acid to 99 ml of water). A 10 ml volume is transferred to a capped tube and stored in the dark at room temperature (20 - 28°C).

2.7 BACTERIAL AND FUNGAL SPECIES CONFIRMATION

Clinical strains of microorganisms used are *Proteus mirabilis*, *Klebsiella pneumoniae*, *Candida albicans* and *Streptococcus pyogenes*. These organisms were obtained from the Microbiology Laboratory of Federal Medical Centre (FMC), Umuahia, Abia State.

"The test organisms were further identified and the biochemical and morphological characteristics were confirmed by standard methods" [16]. *Candida albicans* was sub-cultured onto Sabouraud dextrose agar and incubated at 37°C for 24 hours. After 24 hours, cream white pasty colonies were observed in the agar.

2.8 TESTS FOR ANTIMICROBIAL ACTIVITY

Evaluation of the sensitivity of test isolates to the plant extracts

The disc diffusion technique as described by Ogbulie et al. [17] was used to evaluate the antimicrobial activity of the extract. A 0.02ml aliquot of each extract was dropped on sterile filter paper discs of about 6mm diameter. These were allowed to dry in an oven at 40°C for 20 minutes after which they were introduced into already inoculated agar plates. This agar was inoculated by using a sterile wire loop to pick four (4) colonies of the test organism and emulsifying in a sterilized test tube containing 2 mls of normal saline. The suspension was agitated well and adjusted to the same turbidity as MacFarland standard tube No. 0.5. A sterile cotton swab was used to take the fluid from the suspension. The excess fluid was removed from the swab tip prior to inoculation. The agar surface was fully inoculated in three (3) different directions to cover the surface well. The moisture was allowed to disappear from the agar surface before dropping test discs onto the agar and it was ensured that the discs were firmly placed on the agar surface [16]. The plates were incubated at 37°C for 24 hours. After 24 hours, zones of inhibition were measured. Controls were also set up and seeded in the discs which were subsequently placed onto the already inoculated plates and incubated [17]. The tests were carried out in triplicates.

2.9 Control experiments using amoxycillin, ketoconazole and sterile water

"Amoxycillin was used as a positive control in order to compare the diameter of the zones of inhibition between the extracts and the already standardized antibiotic (amoxycillin) and antifungal drug (ketoconazole). The experiment was carried out aseptically" [18].

2.10 Determination of the Minimum Inhibitory Concentration (MIC)

The tube dilution method described by Akujobi et al, [19] was used in determining the MIC. One (1) gram of each ethanol extract was dissolved in 4mls nutrient broth and one gram of the dried extracts for each plant was dissolved in 4ml nutrient broth and this gave a stock solution with a concentration of 250mg/ml. Thereafter two- fold serial dilutions were made from the original stock of 4 ml (containing 250mg/ml) using nutrient broth to obtain the following concentrations: 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml, 7.82 mg/ml, 3.91 mg/ml, 1.96 mg/ml and 0.98 mg/ml for nine tubes consecutively.

2.11 Determination of Minimum Bactericidal Concentration and Minimum Fungicidal Concentration (MBC and MFC)

The minimum bactericidal and fungicidal concentrations were determined by first selecting tubes that showed no growth (mostly those at concentrations of 250 mg/ml, 125 mg/ml and 62.5 mg/ml) during the determination of MIC. One loopful from each of these tubes was sub-cultured over the surface of extract free nutrient agar in Petri dishes and incubated at 37°C for 24 hours. The lowest concentration at which no growth was observed on the agar was recorded as the Minimum Bactericidal and Fungicidal Concentration [20].

3. RESULTS

Table 1: Diameters of zones of inhibition of fresh leaf extracts and ethanol extracts of *Acacia alata* and *Ageratum conyzoides* as well as their controls in millimeter (mm).

	Acacia alata				
Organisms	Fresh leaf extract	Ethanol extract	Amoxicillin	Ketoconazole	Sterile water
Candida albicans	10.00	5.00	0.00	12.00	0.00
Proteus mirabilis	4.00	2.00	15.00	0.00	0.00
Streptococcus pyogenes	6.00	3.00	16.00	0.00	0.00
Klebsiella pneumoniae	0.00	0.00	14.00	0.00	0.00

	Ageratum conyzoides				
Organisms	Fresh leaf extract	Ethanol extract	Amoxicillin	Ketoconazole	Sterile water
Candida albicans	6.00	5.00	0.00	12.00	0.00
Proteus mirabilis	12.00	5.30	15.00		0.00
Streptococcus pyogenes	11.10	11.00	16.00		0.00
Klebsiella pneumoniae	9.30	9.00	14.00		0.00

Results is expressed as mean ± Standard Deviation (n=5).

Table 2: Expression of the diameter of zones of inhibition as highly sensitive, sensitive and resistant.

Acacia alata		
Organisms	Extracts	
	Fresh leaf extract	Ethanol extract
Candida albicans	+++	+
Proteus mirabilis	+	+
Streptococcus pyogenes	++	+
Klebsiella pneumoniae	_	_

Ageratum conyzoides		
Organisms	Extracts	
	Fresh leaf extract	Ethanol extract
Candida albicans	++	++
Proteus mirabilis	+++	++
Streptococcus pyogenes	+++	+++
Klebsiella pneumoniae	+++	+++

Results is expressed as mean ± Standard Deviation (n=5).

Key: +++ = Inhibition ≥ 7.00mm (highly sensitive)

++ = Inhibition ≥ 5.00mm (highly sensitive)

+ = Inhibition < 5mm (Trace)

- = Inhibition Nil (Resistant)

Table 3: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) values for the fresh leaf extracts of *Acacia alata* and *Ageratum conyzoides* using nutrient broth in mg/ml.

Test plants	**Test organisms**			
	Candida albicans	*Proteus mirabilis*	*Streptococcus pyogenes*	*Klebsiella pneumoniae*
Acacia alata				
MIC	31.25	15.625	31.25	62.5
MBC/MFC	62.5	31.25	62.5	125
Ageratum conyzoides				
MIC	3.91	7.82	3.91	7.28
MBC/MFC	7.82	15.625	7.82	15.625

Results is expressed as mean ± Standard Deviation (n=5).

Table 4: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) values for the ethanol extracts of *Acacia alata* and *Ageratum conyzoides*.

Test plants	**Test organisms**			
	Candida albicans	*Proteus mirabilis*	*Streptococcus pyogenes*	*Klebsiella pneumoniae*
Acacia alata				
MIC	92.5	31.25	62.5	125
MBC/MFC	125	62.5	125	250
Ageratum conyzoides				
MIC	3.91	7.82	3.91	3.91
MBC/MFC	7.82	15.625	7.82	7.28

Results is expressed as mean ± Standard Deviation (n=5).

4. DISCUSSION

According to Ibekwe *et al* [21], “several investigators have reported that plants contain antimicrobial substances”. The results of this study agree with the reports of these investigators. The results hereby obtained indicate the potential use of the extracts of *Ageratum conyzoides* and *Acacia alata* for further development.

The extracts of *Ageratum conyzoides* and *Acacia alata* showed varying degrees of antimicrobial activity. This variation is presumed to be due to the different active compounds present in the plants. According to Ogbulie *et al.,* [17]. this could also be attributed to the presence of these active compounds in different concentrations hence the different degrees of antimicrobial activity.

Generally, the level of inhibition exhibited by the fresh leaf extracts and the ethanol extract of both plants indicate that the fresh leaf extracts had higher diameters of inhibition than the ethanol extracts. This is supported by Scalbert [22] in his report that excessive heating affects the activities of active chemical compounds such as flavonoids, Alkaloids, terpenoids, and other heterogeneuous phytoconstituents present in the extract. Also according to Obi and Onuoha [23], ethanol is the best solvent for the extraction of most plant active principles for medicinal purposes.

This is not disproved by this study because for the purposes of solvent extraction, ethanol is highly active and more active than other solvents but when compared with the fresh leaf extracts, it is not as active as supposed. Hence this study reports that fresh leaf extracts are more act.ve than ethanol extract in inhibiting microbial growth.

Ogbulie *et al.* [17] report that when the active chemical components of plants are present in low concentrations in a given plant, there will be a low rate of inhibition of the plant. This study supports this report as is observed in the fresh leaf extracts and ethanol extracts of *Acacia alata* exhibiting a low trend in inhibition when compared with the fresh and ethanol extract of *Ageratum conyzoides.*

The fresh leaf extracts and ethanol extracts of *Acacia alata* showed high diameters of inhibition against *Candida albicans* when compared with its inhibition against other bacterial isolates. This corroborates the *report* of Makinde et al. [24] who reported that *Acacia alata* has a high rate of antifungal activity. They also reported that when *Acacia alata* is active against bacteria species, it exhibits its activity against Gram positive bacteria and other Gram positive organisms. This study supports the report. “This is observed in the high inhibition of the plant against *Candida albicans* and *Streptococcus pyogenes* which are Gram positive organisms This activity of *Acacia alata* against *Candida albicans* and other fungi supports the traditional use of the plant for the treatment of fungal skin diseases and vaginal itching caused by a fungus” [25]. This further proves that this plant has potentials to be exploited as a natural source of antifungal remedy in the future.

Kamboj *et al* [26] reports that high zones of inhibition of plants against organisms are due to the presence of alkaloids, flavonoids and other active compounds in high concentrations. This study supports this report and is exhibited in the high trend in the inhibition against the test isolates by fresh leaf extracts and ethanol extracts of *Ageratum cosnyzoides.*

In addition, the positive controls (Amoxycillin and ketoconazole) had the widest zone of inhibition on all the organisms while the negative control (sterile water) had no effect on all the test organisms.

5. CONCLUSION

Ageratum conyzoides and *Acacia alata* are plants of wide usage in traditional medicine. Following these traditional usages, many studies have been conducted in laboratories to confirm the efficacy of the plants for the treatment of some diseases. This research has now made it evident that the plants have a good antimicrobial activity as a result of their wide uses, ones of inhibition on the test organisms and the presence of several active principles such as alkaloids, terpenoids, cyanogenic glycosides, saponins, steroids and flavonoids. Many other compounds which are demonstrated to have interesting pharmacological activities and properties can also be isolated from the plant since the plant has not been tested for all the desired pharmacological activities.

With the appreciable level of inhibition recorded for the test plant extracts on the test organisms, it is obvious that these plants are potential sources of antimicrobial drugs one further studies towards their conclusive phytochemical analysis and characterization to unravel the identity of the active principles are recommended. Commercial antibiotic and antifungal drugs cause side effects such as liver, kidney and gastrointestinal tract toxicity. However, herbal remedies often do not produce any side effects. Therefore, alternative medicine has become a popular remedy to various types of ailments. The results obtained from these plant extracts continues the numerous searches for more effective drugs of plant origin which are less toxic and available for low socio-economic populations in the treatment of diseases caused by pathogenic bacteria and fungi.

COMPLETING INTEREST

Authors have declared that no competing interests exist.

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