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PHYTOCHEMICAL CONSTITUENTS OF AGERATUM CONYZOIDES AND ACACIA ALATA AND THEIR ANTIFUNGAL ACTIVITIES ON CANDIDA ALBICANS

Onuoha U.N.[1] and Alaebo P.O.[2]*

[1]Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[2]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

***Corresponding Author: Alaebo P.O.**
Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

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ABSTRACT

The leaves of *Acacia alata* used traditionally for the treatment of snake bites, ringworm, and throat infections and *Ageratum conyzoides*used traditionally for the treatment of wounds, pneumonia, diarrhea, and stomach ailments were tested for their antimicrobial activity on *Candida albicans*, *Streptococcus pyogenes*, *Klebsiella pneumonia* and *Proteus mirabilis* using fresh leaf extracts and ethanol extracts. 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* showed significant zones of inhibition greater than 5mm on all the test organisms. The fresh and ethanol extracts of *Acacia alata* showed significant zones of inhibition on *Candida albicans* (7mm) and a low inhibition on other organisms which are bacterial species. The minimum inhibitory and microbial concentrations evaluated on both the fresh leaf and ethanol extracts of the plants were concentration dependent. Preliminary phytochemical screening carried out on the plants showed the presence of different antimicrobial compounds and this determined their degree of activity. The results obtained in this study are of significance in the health care delivery system and apparently justifies the use of the est plants in the treatment of snake bites, ringworm and pneumonia among others.

KEYWORDS: Phytochemical, *Ageratum conyzoides*, *Acacia alata*, *Candida albicans*, Antifungal activities, MIC.

1.0 INTRODUCTION

Candida albicans is a normal flora in about 50% individual and colonizes the oropharyngeal cavity, gastrointestinal, vaginal tract and skin of healthy individuals (Westh *et al.*, 2004). However, factors like systemic, local, hereditary and environmental changes leads to disturbances in homeostasis. Subsequently, the transition of the normal flora to the pathogen and an opportunistic infection occurs (Talapko *et al.*, 2021). In immune-compromised persons (people living with HIV/AIDS, hematological malignancies, and transplant patients), there is an increased risk of *candida* infections. Also, infection risks exist in patients on antibiotic therapy, alcohol consumption and smoking and old age (Robertson *et al.*, 2013; Hoversten *et al.*, 2019). The effects associated with the misuse and abuse of antimicrobials and its cost have resulted to research on low cost alternatives which include phytochemicals.

African traditional medicine is the oldest and perhaps the most assorted of all therapeutic systems. Africans considered being the cradle of mankind with a rich biological and cultural diversity marked by regional differences in healing practices (Gurib-Fakim, 2006). Plants do not just provide food and shelter, oxygen and beautification to animals but are also sources of phytomedicines (Njoku *et al.*, 2022). A single plant may contain many phytochemicals. For instance, bitter substances that stimulate digestion in humans and animals, possess anti-inflammatory compounds that reduce swellings and pain, phenolic compounds that can act as an antioxidant and venotonics, antibacterial and antifungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste products and toxins, alkaloids that enhance mood and give a sense of well-being (Shohawon and Mahomoodally, 2013). Hence, the importance of the African traditional medicine in healthcare systems cannot be overemphasized.

Ageratum conyzoides commonly known as goat weed and *Acacia alata* commonly called bush candle are examples of medicinal plants and have helped treat some

microbial infections. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Cowan and Steel, 1965). 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 (Damodaran, 2006). Throughout the history of humankind, many infectious diseases have been known to be treated with herbal remedies. Natural herb products, either pure compounds or standardized plant extracts, provide unlimited opportunities for novel drug discovery because of the incomparable availability of a diversity of chemicals. This results in a never-ending and urgent need to discover new antimicrobial compounds with different chemical structures and mechanisms for re-emerging new infectious diseases (Edeoga *et al*., 2005). Therefore, researchers are increasingly turning their attention to folk medicine and continuous search leads to the development of better drugs against microbial infections (Harbourne, 2003). This present study aims to investigate the phytochemical components of *Ageratum conyzoides* and *Acacia alata* and their antifungal effects on *Candida albicans.*

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plants

For this study, freshly harvested leaves of Ageratum conyzoides and Acacia alata were obtained from uncultured gardens around the Academic Complex of Michael Okpara University of Agriculture, Umudike, Abia State. The plants were taxonomically authenticated in the Department of Plant Health Management in the College of Crop and Soil Science, Michael Okpara University of Agriculture, Umudike, Abia State.

2.2 Preparation of the plant extracts

Fresh leaf extract and ethanol leaf extract were prepared for this study. The freshly harvested leaves meant for fresh leaf extract were washed and kept aside for subsequent grinding and extraction whereas the leaves meant for ethanol extract were collected while still fresh and sun-dried for 7 days. The dried leaves were pulverized 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 sterilized muslin 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 ml of the fresh extract was evaporated to dryness using a water bath regulated at 100°C to obtain a dry extract used for the minimum inhibitory concentration determination.

2.4 Ethanol extracts preparation

20.0 grams each of the pulverized leaves of *Ageratum conyzoides* and *Acacia alata* were weighed using Satori A. G. Gottingen's electronic weighing balance. The weighed samples were soaked in 200mls of ethanol in different labeled conical flasks and the mixture was swirled. After 48 hours of elation 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 (Ogbulie *et al.,* 2004). The colours of the extracts were recorded and the dried extracts were stored in bijou bottles at room temperature. The yield was recovered as a percentage of the quantity of initial plant material (20.0g) used and expressed as follows.

$$\textbf{Percentage yield} = \frac{Yield}{20.0g} * \frac{100}{1}$$

2.5 Collection and confirmation of isolate

The organism used for this study was collected from the Microbiology Laboratory of Federal Medical Centre (FMC), Umuahia, Abia State. Afterwards, biochemical tests were carried out on the test organism for confirmation. The test organism (*Candida albicans*) were sub-cultured onto Sabouraud dextrose agar (prepared according to manufacturer's instruction) and incubated at 37°C for 24 hours. After 24 hours, cream white pasty colonies were observed in the agar. Further confirmatory test was carried out using a simple wet preparation and germ tube test. For the wet preparation, a colony of the organism was collected from the sub-cultured agar using a sterilized wire loop and emulsified in a drop of normal saline on a clean, grease-free dry slide. A cover slip was placed on the slide, and the slide was observed under the microscope using the 10x objective. Budding yeast cells were observed. To further confirm the organism, the germ tube test was carried out by centrifuging 2mls of blood at 100 rpm for 10 minutes and using a Pasteur pipette to collect the serum. The serum was transferred into a sterilized test tube and a sterile wire loop was used to collect two colonies of the organism and emulsified in the serum. The preparation was corked with cotton wool and incubated for 2 hours at 37°C. After incubation, a Pasteur pipette was used to transfer a drop of the serum yeast culture to a glass slide and covered with a cover slip. The preparation was examined microscopically using the 10x and 40x objectives with the condenser iris diaphragm closed sufficiently to give a good contrast. Sprouting yeast cells that are a tube-like outgrowth from the cells were seen. The sub-cultured plate also had a distinctive yeast smell (Cowan and Steel, 1965).

Gram stain was also carried out on *Candida albicans*. After staining, the slide was allowed to air dry. A drop of oil immersion was placed on the slide and the slide was examined microscopically under the 100x objective.

2.6 Evaluation of the sensitivity of test isolates to the plant extracts

The disc diffusion technique as described by Ogbulie *et al*., (2004), was used to evaluate the antimicrobial activity of the extracts. The disc embedded with the plant extracts were placed on labeled agar plates inoculated with the fungi. This agar was inoculated using a sterile wire loop to pick four (4) colonies of the test organism and emulsify them in a sterilized test tube containing 2mls of normal saline. The suspension was agitated well and adjusted to the same turbidity as Mac Farland 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 (Ogbulie *et al.*, 2004). The plates were incubated at 37°C for 24 hours. After 24 hours, zones of inhibition were measured. Controls were also set up using ketoconazole (250mg) and seeded in the discs, subsequently placed onto the already inoculated plates and incubated (Ogbulie *et al.*, 2004). The tests were carried out in triplicates on plates labeled A1, A2, A3 and C1, C2 and C3 for *Acacia alata* and *Ageratum conyzoides* respectively.

2.7 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MBC and MFC)

In this study, the tube dilution method described by Akujobi *et al.*, (2004) 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 4ml (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. The ninth (9th) tube served as a control with only nutrient broth. Having obtained the different dilutions and concentrations, three drops of overnight broth cultures of the test organisms were inoculated into the dilutions. The organisms were sub-cultured on broth using a dilution of the organisms in 2mls of normal saline and adjusting to the standardized barium sulphate suspension (McFarland standard) according to Vandepitte *et al.*, (1991). The MIC tubes were plugged with cotton wool and incubated at 37°C for 24 hours. After 24 hours, the tubes were examined for microbial growth. The minimum inhibitory concentration of a drug/crude extract is the smallest concentration of such a drug/crude extract that can inhibit the growth of the test organism (Kamboj and Saluja, 2008). Afterwards, theminimum fungicidal concentrations were determined by first selecting tubes that showed no growth (primarily those at concentrations of 250 mg/ml, 125 mg/ml and 62.5 mg/ml) during the determination of MIC. One loop from each of these tubes was sub-cultured over the extract free nutrient agar surface 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 Fungicidal Concentration (Kamboj and Saluja, 2008).

2.8 PRELIMINARY PHYTOCHEMICAL SCREENING OF *Ageratum conyzoides* AND *Acacia alata*

Chemical tests were carried out on the crude powder of the specimens using standard procedures to identify the constituents.

2.8.1 Test for tannins

0.5 grams of the dried powdered samples were boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride (FeCl) were added and observed for a brownish-green or a blue-black colouration (Cowan and Steel, 1965).

2.8.2 Test for alkaloids

20 mg of the pulverized powdered material was dissolved in 1 ml of ethanol and was filtered, and a filtrate was obtained. 2 ml of the filtrate was added to 1 ml of the Hydrochloric acid (HCL) and heated. After heating, 1 g of the extract was added to 6 drops of Mayor's reagent and observed for a cream coloured precipitate (Cowan and Steel, 1965).

2.8.3 Test for saponins

20 mg of the powdered sample was boiled in 20mls of distilled water in a water bath and filtered. The filtrate was measured, and 10mls of the filtrate was mixed with 5mls of distilled water and shaken vigorously for a stable, persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. It was then observed for emulsion formation, indicating saponin's presence (Cowan and Steel, 1965).

2.8.4 Test for flavonoids

A portion of the powdered plant sample was heated with 10mls of ethyl acetate over a steam bath for three (3) minutes. The mixture was filtered, and 4mls of the filtrate was shaken with 1 ml of dilute ammonia solution. It was observed for a yellow colouration (Cowan and Steel, 1965).

2.8.5 Test for steroids

Two (2) ml of acetic anhydride were added to 0.5g ethanol extract of each sample with 2 ml of sulphuric acid. The colour change from violet to blue or green was watched out for (Cowan and Steel, 1965).

2.8.6 Test for cyanogenic glycosides (Keller Killani test)

Five (5) ml of each extract was treated with two (2) ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-laid with one (1) ml of

concentrated sulphuric acid. A brown ring of interface indicating the presence of cyanogenic glycosides was watched out for (Cowan and Steel, 1965).

2.8.7 Test for terpenoids (Salkowski test)

Five (5) ml of each extract was mixed in two (2) ml of chloroform and concentrated with 3 ml of H_2SO_4 (sulphuric acid), which was carefully added to form a layer. A reddish-brown colouration indicating the presence of terpenoids was looked out for (Cowan and Steel, 1965).

3.0 RESULTS AND DISCUSSION

3.1 Results of Confirmatory Tests on the Test Organisms

The test organism obtained was further confirmed, and the test results are summarized in the table below.

Table 3.1: Results of confirmatory tests on test organisms

Colonial characteristics	Germ tube test	Gram Reaction	Confirmed organism
Cream coloured pasty colonies with a distinctive yeast smell on Sabouraud dextrose agar	+	+	*Candida albicans*

3.2 Colours of the Filtrates after Filtration And Extraction

The filtrates had the following colours after filtering and extracting. *Acacia alata*.

Table 3.2: Percentage yield of the leaf extract and ethanol extracts of *Acacia alata* and *Ageratum conyzoides.*

Plant species	Extract type	Weight of pulverized sample used	Weight of dried extract/volume of fresh leaf extract	Percentage yield of extracts (%)
Acacia alata	Dried leaf extract	20.0g	3.0g	30%
	Fresh leaf extract		9.6ml	96%
	Ethanol extract		6.6g	66%
	Dried leaf extract		2.6g	26%
Ageratum conyzoides	Fresh leaf extract	20.0g	8.30ml	83%
	Ethanol extract		4.0g	40%

Table 3.3: Diameters of zones of inhibition of fresh leaf extracts and ethanol extracts of *Acacia alata* and *Ageratum conyzoides* and their controls in millimetres (mm).

	Acacia alata				
Samples	**Fresh leaf extract**	**Ethanol extract**		**Ketoconazole**	**Sterile water**
A1	10.30	5.00		12.00	0.00
A2	9.90	4.98		12.00	0.00
A3	9.80	5.02		12.00	0.00
	Ageratum conyzoides				
Samples	**Fresh leaf extract**	**Ethanol extract**		**Ketoconazole**	**Sterile water**
C1	6.00	5.00		12.00	0.00
C2	6.04	4.48		12.00	0.00
C3	4.49	5.01		12.00	0.00

3.4 ANTIMICROBIAL ACTIVITIES OF PLANT EXTRACTS

3.4.1 Diameters of zones of inhibition of organisms

The diameters of the zones of inhibition of the test organisms using the fresh leaf extracts and ethanol extracts of the two plants are presented in Table 3.3. The high inhibitions are graded as greater than 7.00 mm with values less than 5.00 mm regarded as trace. This is represented on Table 3.4. The antimicrobial activities of Acacia alata and Ageralum conyzoides were assayed in vitro by the agar disc diffusion method against four (4) microorganisms.

The fresh leaf extracts of both plants had conspicuous zones of inhibition. The fresh leaf of *Acacia alata* had conspicuous zones of inhibition on *Candida albicans.* *Streptococcus pyogenes* and *Proteus mirabilis* are 10.00 mm, 6.00 mm and 4.00mm, respectively, while on *Klebsiella pneumoniae*, the plant had no zone of inhibition. The ethanol extract of *Acacia alata* showed inhibition not greater than 3.00 mm on *Proteus mirabilis* and *Streptococcus pyogenes*, no inhibition on *Klebsiella pneumonias* and significant inhibition of 5.00 mm on Candida albicans.

The fresh leaf extracts and ethanol extracts of *Ageratum conyzoides* showed conspicuous inhibition zones greater than 5.00mm. On the contrary, 0.2ml of Amoxycillin and ketoconazole showed wide inhibition zones on the test organisms, which is incomparable to the plant extracts. Sterile water, which was the negative control, showed no

inhibition zones, and it was utterly resistant to all the test organisms.

The various inhibitions of fresh and ethanol extracts of both plants on all test organisms are represented on a histogram in Figures 3.1 and 3.2.

Table 3.5: Minimum Inhibitory Concentration (MIC) 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		
	A1	A2	A3
Acacia alata			
MIC	31.25	15.625	31.25
MFC	62.5	31.25	62.5
Ageratum conyzoides	C1	C2	C3
MIC	3.91	7.82	3.91
MFC	7.82	15.625	7.82

3.5.1 Minimum Inhibitory, Minimum Bactericidal and Minimum Fungicidal Concentrations Test Result.

The 9 tubes used for the MIC test contain a mixture of 5 ml nutrient broth, extracts and organisms at concentrations of 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml, 7.82mg/ml, 3.91 mg/ml, 1.96 mg/ml and 0.98 mg/ml. the ninth (9th) tube is the control containing only nutrient broth and test organisms. This is to be used to compare tubes in which there was no inhibition. The MIC, MBC and MFC test result values are shown in Tables 3.5 and 3.6.

The fresh leaf extracts of *Acacia alata* showed activities against the four test organisms with the highest activity against Klebsiella pneumonias (MIC = 62.5 mg/ml, 125 mg/ml) followed by Streptococcus pyogenes and Candida albicans (MIC = 31.25 mg/ml, MBC / MFC = 62.5 mg/ml). The least activity was shown against Proteus mirabilis (MIC - 15.625 mg/ml, MBC = 31.25 mg/ml).

The ethanol extracts of *Acacia alata* showed activity against the four test organisms, with the highest activity against *Klebsiella pneumonia* (MIC - 125 mg/ml, MBC = 62.5 mg/ml).

The fresh leaf extract of *Ageratum conyzoides* showed activities against the four test organisms with the highest activity against Proteus mirabilis and *Klebsiella pneumonia* which had the same values (MIC = 2.28 mg/ml, MBC = 15.625 mg/ml). The minor activity was shown on *Candida albicans* and *Streptococcus pyogenes*, and they had the same values, i.e. their lowest inhibition at the same concentration (MIC = 3.9 mg/ml, MFC/MBC = 7.28mg/ml).

The ethanol extracts of *Ageratum conyzoides* showed activities against the four test organisms, with the highest activity against Proteus mirabilis (MIC = 7.28 mg/ml, MBC = 15.625 mg/ml). The least activities were against Candida albicans, *Streptococcus pyogenes*and *Klebsiella pneumoniae*. They were inhibited and their lowest inhibition occurred in the same concentrations of the extracts (MIC = 3.91 mg/ml, MFC/MBC = 7.82 mg/ml).

The Minimum Bactericidal and Fungicidal (MBC and MFC) pattern of activity was similar to that of the Minimum Inhibitory Concentration for all the organisms used in this study. The histogram in Figure 3.2 clearly illustrates the different activities of the two test plants on the four test organisms. The results for the MIC, MBC and MFC for the dry extracts and ethanol extracts of both plants are shown in Tables 3.5 and 3.6.

Table 3.6: 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

3.6 RESULTS OF PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of *Ageratum conyzoides* showed the presence of flavonoids, steroids, terpenoids and alkaloids and the absence of tannins, saponins and cyanogenic glycosides. Preliminary phytochemical screening of *Acacia alata* showed the presence of flavonoids, saponins, terpenoids, cyanogenic glycosides and alkaloids and the absence of steroids and tannins. These results are represented in Table 3.7.

Table 3.7: Preliminary phytochemical analysis of *Acacia alata* and *Ageratum conyzoides.*

Test plants	Active compounds						
	Tannins	Saponins	Flavonoids	Steroids	Cyanogenic glycosides	Alkaloids	Terpernoids
Acacia alata	-	+	++	-	++	++	++
Ageratum conyzoides	-	-	+++	+++	-	+++	+

Key: + = present but in low concentration.
_ = absent.
++ = present in high concentration.
+++ = present in very high concentration.

4: DISCUSSION

The findings of Zaria *et al*, (1995) and Ibekwe *et al*, (2001) 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 *Acacia alata* and *Ageralum conyzoies* for medicinal purposes.

The plant extracts of *Acacia alata* and *Ageratum conyzoides* 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.*, (2004), 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 (1991) 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. This is said to support the high activity of the fresh extracts because a plant for ethanol extract collection is subjected to tedious heating processes such as drying the fresh leaves in the sun for days and collecting the extracts by heating at high temperatures in a steam bath. These processes could result in a reduction in the activity of active chemical constituents that enable antimicrobial activity (Scalbert, 1991). Ethanol is the best solvent for the extraction of most plant active principles for medicinal purposes according to Obi and Onuoha (2000). 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 active than ethanol extract in inhibiting microbial growth.

As reported by Ogbulie *et al.*, (2004) 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 agrees with the report as 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.*

This also support the high activity of 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 and Igoli (2007) 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 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 (Makinde *et al.*, 2007; Omar *et al.*, 2002). This further proves that this plant has potentials to be exploited as a natural source of antifungal remedy in future (Omar *et al.*, 2002).

Kamboj *et al.*, (2008) reports that high zones of inhibition of plants against organisms is 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 conyzoides*. This is due to the presence in high concentration of steroids, terpenoids, flavonoids and alkaloids as shown in the preliminary phytochemical screening. This high activity of *Ageratum conyzoides* is not surprising because the plant has been consistently listed among plants used in native medicine in Nigeria that their medicinal potentials have been established (Idu *et al.*, 2007). Also this study has confirmed the high activity *of Ageratum conyzoides* on both Gram positive and Gram negative bacteria and fungi. The diameter of its inhibition zones compared with standard antibacterial and antifungal drugs do not differ significantly. This confirms their high antimicrobial activity even at low concentrations. This further proves that the plant has the potential to be exploited as a natural source of antibacterial and antifungal remedies in the future (Kamboj *et al.*, 2008).

Preliminary phytochemical screening of the plants revealed the presence of saponins, flavonoids, steroids, alkaloids, terpenoids and cyanogenic glycosides in *Acacia alata* and the presence of flavonoids, steroids, alkaloids and terpenoids in *Ageratum conyzoides*. All these components are present in high concentrations except terpenoids which is present in a very low concentration in *Ageratum conyzoides.* This is supported by the report of Omar *et al.,* (2002) who carried out preliminary phylochemical screening for *Acacia alala* and confirmed the presence of saponins, flavonoids, alkaloids, terpenoids, cyanogenic glycosides among others. Also, Kamboj *et al.,* (2008) carried out phytochemical screening of the leaves of *Ageratum convzoides* and reports the presence of flavonoids, terpenoids, alkaloids and steroids with saponin and cyanogenic glycoside absent. However, there is a very high concentration of the compounds present hence their high activity. The report of Kamboj *et al.,* (2008) corroborates with this study.

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

Acacia alata and *Ageratum conyzoides* 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 made it evident that the plants have a good antimicrobial activity as a result of their wide 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.

Plant based antimicrobial have enormous therapeutic potentials hence there is need for further exploration in this direction.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

Author declared that no conflict of interest existed in this paper.

Ethical approval and consent to participate

The study was conducted by following the guideline set by National Institute of Health,USA as approved by the College of Veterinary Medicine,Mcheal Okpara University of Agriculture, Umudike. THE ETHICAL COMMITTEES REFERENCE NUMBER IS: MOUAU/CVM/REC/202015

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