

Preliminary Phytochemical , Chromatographic And Antimicrobial Evaluation Of Ethanol Leaves Extract And Fractions Of *Andrographis Paniculata* (Burm.F.) Nees Family Acanthaceae

T. U Onyekaba¹, Nwoye David Ifeanyi², Anowi Chinedu Fredrick³

1. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Delta State University. Abraka, Nigeria.

2. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Delta State University. Abraka, Nigeria

3. Department of Pharmacognosy and Traditional Medicine, Faculty of Pharma. Science, Nnamdi Azikiwe University. Awka, Nigeria.

Corresponding author: T. U Onyekaba

Abstract: This study is designed to examine the ethanol leaves extract of *Andrographis paniculata* (Burm.f.) Nees family acanthaceae for its in vitro antimicrobial potential against strains of *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* at five concentrations; 200, 100, 50, 25 and 12.5mg/ml respectively by agar well diffusion method to ascertain its folkloric claim to treat various infectious diseases. The extracts showed significant broad spectrum antibacterial activity. The minimum inhibitory concentration of the bacteria ranged between 7mg/mL and 12.5mg/mL while that of *Candida albicans* was 1.42mg/mL. Phytochemical analysis revealed the presence of terpenoids, tannins, flavonoids, saponins, alkaloids, reducing sugar, steroids, and cardiac glycoside. TLC analysis of the hydrolysed fractions obtained by solvent extraction technique resolved fraction A into three components ($R_f = 0.41, 0.46$ and 0.82), fraction B into two components ($R_f = 0.27$ and 0.58), fraction C into two components ($R_f = 0.42$ and 0.65), fraction D into one component ($R_f = 0.87$) and fraction E into two components ($R_f = 0.48$ and 0.76). Distillation of the volatile oil gave oil with the refractive index of 1.3685 and boiling point of 220 – 221°C. These findings explicitly support its traditional claims in treating skin infections, pneumonia, respiratory tract infection, diarrhoea and dysentery.

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I. Introduction

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted.

Plant-based antimicrobials have enormous therapeutic potential as they can serve purpose with lesser side effects that are often associated with synthetic antimicrobials (Purohit and Vyas, 2007). Many of these plants are readily available in rural areas, thus making traditional system of medicine relatively cheaper than the orthodox medicine. The medicinal value of plants depends on their chemical constituents that provide a definite physiological action on the human body. These bioactive constituents are tannins, flavonoids, alkaloids, phenol compounds, saponins, glycosides, anthraquinones etc.

1.1 General Description Of *Andrographis Paniculata*

Andrographis paniculata (Burm.f.) Nees, a member of the family of Acanthaceae, is an herb used in traditional medicine in China, India and Southeast Asia. It is known as king of bitters in English and Chuan Xin Lian in Chinese, but is more commonly referred to simply as andrographis.

Andrographis paniculata grows erect to a height of 30–110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance-shaped leaves have hairless blades measuring up to 8 centimetres long by 2.5 wide. Small white flowers 1-2 cm long, set along a thin upright stem. Tubular flowers can have purple coloured flecks on the inside of the lip-like formation. A fine oblong seed capsule 1.5 cm long contains several very small round seeds. Seeds will readily disperse if not picked. Leaves have no aroma. Flavour of the whole plant is extremely bitter. Propagation is by seeds, cuttings and layering stems. Plant seeds, spring and summer, germination may be in 10- 30 days. Plants will grow in a wide range of soils, in full sun or shade and thrives in moist conditions.



Fig1. Picture of *Andrographis paniculata*

1.2 Traditional Uses / Antimicrobial Properties of *Andrographis paniculata*.

Andrographis paniculata prominent 26 Ayurvedic formulations as evidenced from Indian Pharmacopoeia; while, in Traditional Chinese Medicine, it is an important “cold property” herb used to relieve body heat in fever. It is also effectively used as immunostimulant, and for asthma, gonorrhoea, piles, dysentery and dyspepsia, blood purification, influenza, diabetes, myocardial ischemia, jaundice, anti-typhoid, anti-snake venom, antimalaria, anti-HIV, anti-fertility, anti-inflammatory, and anti-glycaemic properties (Datta et al., 2012).

Andrographis paniculata has been used to treat upper respiratory tract infections (Poolsup et al., 2004; Coon et al., 2004 and Saxena et al., 2010). It is used to overcome sannipata type of fever, difficulty in breathing, homeopathy burning sensation, cough, skin diseases, fever, ulcer and worms.

As medicinal plants are gaining more importance in Pharmaceutical industries for the preparation of new phytomedicines, this study was undertaken to check its properties as a drug.

II. Method

2.0 Preparation Of Plant Extract

The fresh and healthy leaves of *A. paniculata* were collected from Abraka, Delta State of Nigeria, cleaned with distilled water, shade-dried at room temperature for seven days, after which, the dried leaves were pulverized using an electric blender. The resulting powder was kept in air-tight container until extraction.

2.1 Extraction

200g of the powdered plant leaves were subjected to soxhlet extraction using 1.4L of 70% ethanol as solvent. The extract was then concentrated using a rotary evaporator (Buchi Rotary Evaporator, R-210) at 40°C. The final concentrated extracts were stored at -18°C in labelled sterile bottles and kept as aliquots until further evaluation.

2.2 Preliminary Phytochemical Analysis

2g of the crude extract was dissolved in 70% ethanol and the presence of common secondary plants metabolites were tested for using the various methods below:

2.2.1 Alkaloids: 5mls of dilute Hydrochloric acid (HCl) was added to 2ml of the plant extract. This was followed by 2ml of Dragendorff’s reagent and the appearance of orange colour precipitate indicates the presence of alkaloid.

2.2.2 Glycosides: To a test tube containing 2ml of the plant extract, 2ml of 10% alcohol solution of α -naphthol was added and 2ml of concentrated sulphuric acid was finally added to the content of the test tube and the appearance of a deep violet ring at the interface indicates the presence of glycoside.

2.2.3 Cardiac Glycosides: 2ml of glacial acetic acid and 1ml of 0.1% Ferric chloride solution were added to 2ml of the plant extract in a test tube respectively. 2ml of concentrated Sulphuric acid was gradually added along the side of the test tube, a greenish ring at the interface indicates the presence of cardiac glycoside.

2.2.4 Phenols: 2ml of distilled water was added to 2ml of the plant extract, this was followed by two drops of 10% ferric chloride. A blue colour indicates the presence of phenol.

2.2.5 Reducing Sugar: 2ml each of Fehling’s reagent A and B was added to 2ml of the extract solution in a test tube. A light green colour indicates the presence of reducing sugar.

2.2.6 Terpenoids: To 5ml of the extract, 2ml of Chloroform and 3ml of concentrated Sulphuric acid was carefully added to form a layer. The presence of Terpenoids is indicated by a reddish-brown colouration at the interface.

2.2.7 Flavonoids: 5ml of dilute Ammonia solution was added to 5ml of the plant extract followed by the addition of 2ml of concentrated Sulphuric acid. A yellowish colouration indicates the presence of flavonoids

2.2.8 Steroids: To 2ml of the plant extract in the test tube, 3ml of Chloroform and 2ml of acetic anhydride added. 2ml of concentrated Sulphuric acid was carefully added along the slant side of the test tube. A colour change of violet to green indicates the presence of steroid.

2.2.9 Tannins: Three drops of 0.1% Ferric chloride was added to 2ml of the plant extract, a brownish green colour indicates the presence of tannin.

2.2.10 Saponin: (a).To 2ml of the plant extract in the test tube, 2ml of distilled water was added and shaken vigorously. A foamy lather formation indicates the presence of saponin. (b). 2ml of olive oil was added to 2ml of the plant extract in the test tube. Formation of an emulsion indicates the presence of saponin.

2.3 Solvent Extraction Of The Crude Extract

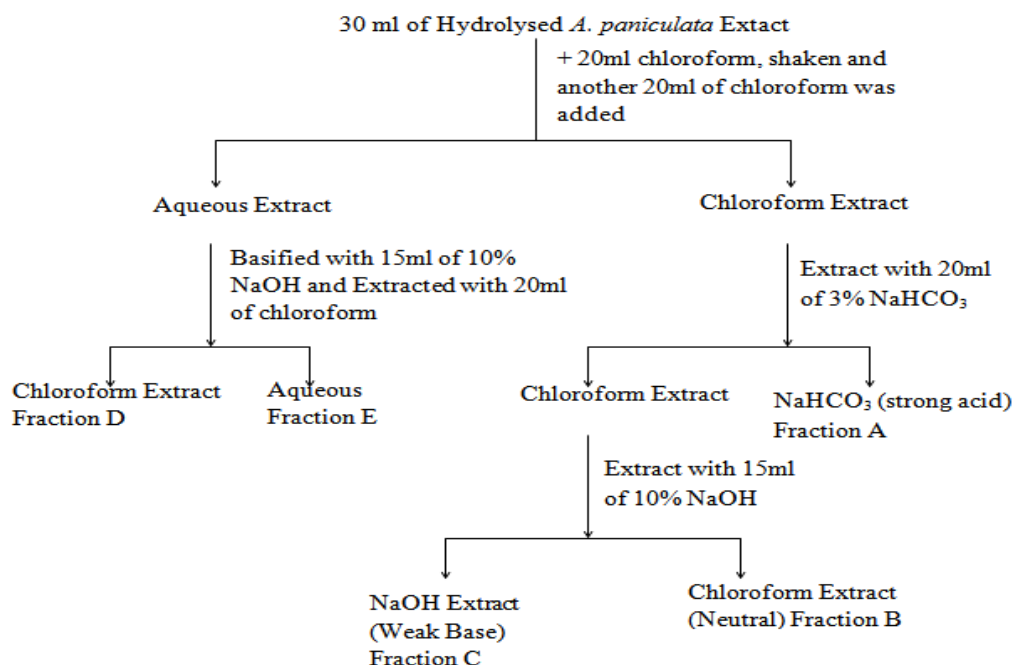
4g of the crude extract was heated with 50ml of 3N Hydrochloric acid solution under reflux for 3 hours. 30mls of the hydrolysed extract was measured into a clean separating funnel, 20ml of Chloroform was added and the mixture was shaken to facilitate the partitioning of the phytochemicals into the solvents. Then, another 20ml of chloroform was added without shaking the funnel and the elution of the chloroform phase was done. This was labelled the chloroform phase.

To the aqueous phase in the separating funnel, was added 3N (12%) sodium hydroxide solution to basify it. 40ml of chloroform was added to the basified extract and the elution of the chloroform extract was done, this was labelled fraction D and the sodium hydroxide extract was labelled fraction E.

To the chloroform phase was added 20ml of 3% sodium hydrogencarbonate solution to extract the strongly acidic compounds. The chloroform extract was eluted into a beaker. The sodium bicarbonate extract was labelled fraction A. 15ml of 10% sodium hydroxide solution was added to the chloroform phase and the separating funnel was shaken. The chloroform extract was eluted and it was labelled Fraction B while the sodium hydroxide portion was labelled fraction C

The diagrammatic representation of the solvent extraction procedure as described by Olaniyi and Agumbamila, (1998) is as shown below:

30 ml of Hydrolysed *A. paniculata* Extract



Phytochemical screening of the different fractions A, B, C, D, and E were carried out using the aforementioned methods.

2.4 Thin Layer Chromatographic Analysis

2.4.1 Preparation of the solvent system

150ml of ethyl acetate, 26ml of methanol and 19ml of distilled water were measured into a clean container and shaken vigorously to enable proper mixing. This ethyl acetate-methanol-water solvent system was transferred into a clean chromatographic tank and then covered for 15 minutes to allow for saturation of the tank.

2.4.2 Preparation of TLC plates for development

2cm was measured from one end of the thin layer chromatographic plate. This was taken as the point of origin. Each fraction of the extract was spotted along the 1 cm mark, 2cm apart from one to another using a narrow capillary tube. The spots were allowed to dry before the plate was placed into the developing tank. The developing tank was covered and left undisturbed until the solvent front got to a predetermined mark on the TLC plate. The plate was dried viewed using a UV-lamp and the various retention factors (R_f) were computed using the formula stated below:

$$R_f = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent (solvent front)}}$$

2.5 The Determination Of Refractive Index And Boiling Point of The Terpenoids Oil in Adrographis Paniculata Extract

4g of Adrographis paniculata extract was weighed and transferred into 250ml flat bottom flask. To this was added 50ml of 3M HCl acid and heated under reflux for 3hours. The refluxing was discontinued and the mixture was distilled. The volatile oil came at a boiling point of 220 – 221°C.

The refractive index of the received volatile oil was determined with Abbe's refractometer at 26.1°C.

2.6 Anti Bacterial Susceptibility Tests

2.6.1 Test microorganisms

Four bacterial cultures namely Escherichia coli, Streptococcus pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus were used in this investigation. All the cultures were procured from Pharmaceutical Microbiology Department, Delta state University, Abraka, Nigeria.

2.6.2 Media and Inoculums preparation

The media used for antibacterial test were Nutrient Agar/Broth and Muller Hinton agar. Mueller-Hinton agar was prepared by dissolving 7.6g of the Agar in 200ml of distilled water (according to the manufacturer instruction, 38g of the Mueller-Hinton agar powder dissolved in one litre of distilled water). The mixture was properly dispersed enabling dissolution, thereafter it was covered using cotton wool plug in foil paper strip and sterilized using the autoclave at 15lb pressure and 121°C for 15 minutes.

The test bacterial strains were inoculated into nutrient broth and incubated at 37°C for 24hrs. After the incubation period, the culture tubes were compared with the turbidity (opacity) of the standard.

2.7 Antibacterial assay

The crude extract of the plant was examined for antibacterial activity in accordance with the "Agar-well diffusion method". 19ml of the sterilized Mueller-Hinton Agar at 50°C was poured into test tubes containing 1ml of different strains of the organisms. This was mixed properly before pouring into a sterile Petri-dish, and allowed to set. Thereafter, wells were bored into the set agar using a cork borer and two (2) drops of the different concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of the extract introduced into the wells. 70% ethanol was used as a negative control while the standard antibiotic ciprofloxacin was used as a positive control. After allowing the plates to stand for 30 minutes to enable proper diffusion of the extract at room temperature, it was finally incubated at 37°C for 24 hours. The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone (mm) formed around the wells. The study was performed in triplicate and the results were expressed as means of the three measurements.

2.7.1 Minimum Inhibitory Concentration (MIC) of the Plant Extract

The minimum inhibitory concentration (MIC) of the crude extracts of A. paniculata was determined by agar well dilution method (EUCAST, 2000). The growth media, Mueller Hinton agar (MHA) was first prepared and sterilized by autoclaving as explained in 2.7 above. The sterilized MHA was allowed to cool to 50°C and 18 ml each of the molten Agars was added to test tubes which contained 2 ml of different concentrations of the test crude extracts (7 to 12.5 mg/ml). The mixture of the media and the crude extract were thoroughly mixed and poured onto pre-labelled sterile Petri-dishes on a level surface. Additional Petri-dishes containing only the

growth media were prepared in the same way so as to serve for comparison of growth of the respective bacteria. The plates were then set at room temperature and dried. The suspensions of the respective bacteria (corresponding to 10^8 CFU/ml) were inoculated onto the series of agar plates. The plates were then incubated at 37°C for 24 hours. Experiments were performed in duplicate and MIC values expressed as the lowest concentration of the plant extracts that produced complete suppression of colony of respective bacteria.

2.8 Antifungal Activity

2.8.1 Test microorganism

Candida albicans was used in this investigation. The culture was procured from Pharmaceutical Microbiology Department, Delta state University, Abraka, Nigeria.

2.8.2 Media and Inoculums Preparation

The media used for antifungal test was Sabouraud dextrose agar. The agar was prepared by dissolving 6.5g of the Agar in 100ml of distilled water (according to the manufacturer instruction; 65g of the Sabouraud agar powder dissolved in one litre of distilled water). The mixture was properly dispersed enabling dissolution, thereafter it was covered using cotton wool plug in foil paper strip and sterilized using the autoclave at 15lb pressure and 121°C for 15 minutes.

The test fungus was inoculated into nutrient broth and incubated at 37°C for 24hrs. After the incubation period, the culture tubes were compared with the turbidity (opacity) of the standard.

2.8.3 Antifungal assay

This was carried out in accordance with the ‘‘Agar–well diffusion method’’. 19ml of the sterilized Sabouraud dextrose agar at 50°C was poured into test tubes containing 1ml of different strains of the organisms. This was mixed properly before pouring into a sterile Petri-dish, and allowed to set. Thereafter, wells were bored into the set agar using a cork borer and two (2) drops of the different concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of the extract introduced into the wells. 70% ethanol was used as a negative control while the standard antibiotic ketoconazole was used as a positive control. After allowing the plates to stand for 30 minutes to enable proper diffusion of the extract at room temperature, it was finally incubated at 37°C for 24 hours. The assessment of antifungal activity was based on the measurement of diameter of inhibition zone (mm) formed around the wells. The study was performed in triplicate and the results were expressed as means of the three measurements.

2.8.4 Minimum Inhibitory Concentration (MIC) of the Plant Extract

A graph of the square of the diameter of inhibition zones of the plant extract and the antibiotics was plotted against the log of the concentration. The MIC of the extract was then calculated as the antilog of the x-intercept from the equation of the line obtained (Akanwari Wiak W G et al; 2012 and Chinedu F. A. et al., 2012).

III. Results

3.1 Result On Phytochemical screening

Tables 3a and 3b show the results of phytochemical screening of the crude plant extract and the hydrolyzed extracts of *Andrographis paniculata* respectively. The results show the presence of alkaloids, flavonoids, terpenoids, saponin, reducing sugar, phenols, steroids, cardiac glycoside and tannin.

After hydrolysis with 3N HCl for 3hours and subsequent fractionation, fraction A contained cardiac glycoside, reducing sugar and tannin; fraction B contained alkaloid and terpenoid; fraction C contained reducing sugar and saponin; fraction D contained alkaloid; and finally, fraction E contained cardiac glycoside and steroid.

Table 3a:Phytochemical Screening Results of the Crude Extract from *Andrographis paniculata* Leaves

S/N	Secondary plant metabolites	Inference
1	Alkaloids	++
2	Cardiac Glycoside	+
3	Phenols	++
4	Reducing sugar	++
5	Terpenoid	+++
6	Flavonoid	+
7	Steroids	++
8	Saponin	+++
9	Tannins	+++

KEY: - = Absent; + = Scanty present; ++ Moderately present; +++ = Highly Present

Table 3b: Phytochemical screening results of fractionated extract obtained by solvent extraction.

Phytochemicals	A	B	C	D	E
Alkaloids	-	+	-	++	-
Cardiac Glycoside	-	+++	-	-	++
Reducing sugar	++	-	++	-	-
Terpenoid	-	+++	-	-	-
Flavonoid	-	-	+	-	-
Steroids	-	-	-	++	-
Saponin	-	-	+++	-	-
Tannins	+++	-	+	-	-

KEY: - = absent; + = Scanty present; ++ = moderately present; +++ = Highly Present.

3.2 Results On Thin Layer Chromatography Analysis

The solvent system ethyl acetate: methanol: water (150:26:19) resolved fraction A into three components, fraction B into two components, fraction C into two components, fraction D into one component and fraction E into two components. The table below shows the result TLC analysis:

Table 3c: Result of thin layer chromatography of the extract and the various fractions.

Samples	Retention factor values of components				
Crude extract	0.31	0.47	0.64	0.58	0.76
Fraction A	0.41	0.46			0.82
Fraction B	0.27			0.58	
Fraction C		0.42		0.65	
Fraction D				0.87	
Fraction E		0.4		0.76	

3.3 The Result Of The Refractive Index And Boiling Point Of The Terpenoid Oil Extract.

Table 3d:Result of the Refractive Index and Boiling point of the Terpenoid oil extract.

Volatile oil	Refractive Index	Boiling Point
Terpenoid	1.3696	220 – 221°C
	1.3673	
Average =	$\frac{1.3696 + 1.3673}{2}$	
	= 1.3685 _{T=26.1°C}	

3.4 Antibacterial Effects Of Ethanol Leaves Extract Of A. Paniculata

Table 3.5a shows the result of the antibacterial activity of A. paniculata leaves extract on the test microorganisms. The positive control shows highest zone of inhibition (27mm) on S. aureus and P. aeruginosa while the negative control (70% ethanol) did not cause any inhibition.

Table 3.5b shows the result of the minimum inhibitory concentration (MIC) of the ethanol leaves extract of A. paniculata on the test microorganisms. The result revealed the MIC ranged from 7mg/ml to 12.5mg/ml for all the organisms.

Table 3e: Antibacterial activity of ethanol leaves extract of A. paniculata

	Concentrations of Extract (mg/ml)						+ve Control	-ve Control
	200	100	50	25	12.5	10 μ g	70% Ethanol	
<i>E. coli</i>	12	10	8	7	3	11	-	
<i>P. aeruginosa</i>	6.3	5.3	5.3	3	1	27	-	
<i>S. aureus</i>	13	12	10	9	7	27	-	
<i>S. pneumoniae</i>	6	5.4	5	3	3	21	-	

Positive control: Ciprofloxacin

Negative control: 70% ethanol

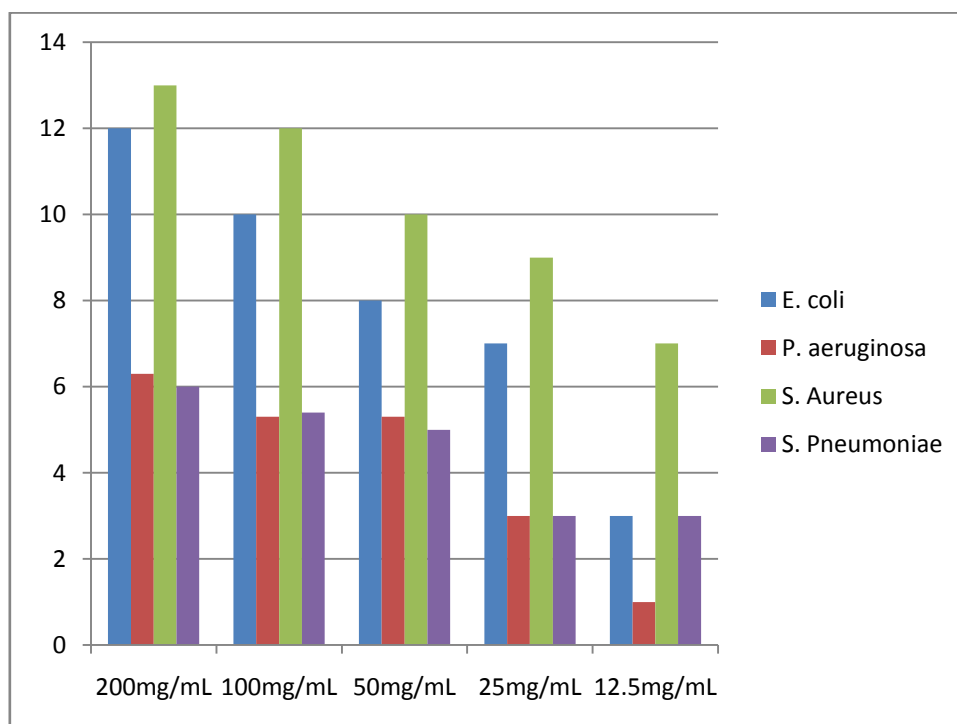


Fig 3a: Bar chart showing inhibition zone (mm) of the extract against different bacterial strains at different concentrations.

Table 3f: The Minimum Inhibitory Concentration (MIC) of the Ethanol Extract of *A. paniculata*

Conc. mg/ml	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pneumoniae</i>
12.5mg	–	–	–	–
11.5mg	–	+	–	–
10.5mg	–	+	–	–
9.5mg	–	+	+	–
7mg	+	+	+	–
3.5mg	+	+	+	+
1.75mg	+	+	+	+

3.5 Results of Antifungal Activity of Ethanol Leaves Extract of *A. Paniculata*.

3.5.1 Inhibition Zone Diameter

Table 3g is a table showing the inhibition zone diameter (mm) at different concentrations (mg/mL) of plant extract and reference drugs (Ketoconazole) against *Candida albicans*.

Table 3g: Antifungal activity of ethanol extract of *A. paniculata*

Concentration (mg/mL)	Diameter zone of inhibition (mm)	
	Extract	Ketoconazole
200	10	11
100	8.5	6.5
50	8	8
25	7	7
12.5	7	7

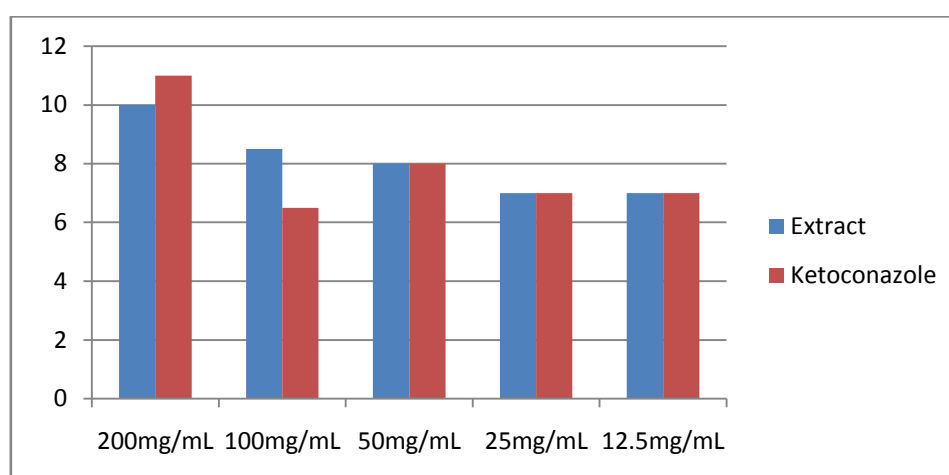


Fig 3b: Bar chart showing inhibition zone (mm) of the extract and ketoconazole against *Candida albicans*



Figure 3c: Culture plate showing Inhibition zones of Extract (2 and 5) and Reference drug (2c and 5c) against *Candida albicans*.

3.2 Minimum Inhibitory Concentration (MIC) of the Ethanol Extract of *A. paniculata* and Ketoconazole.

Table h: Log concentration and square of Inhibition zone Diameter (IZD)² of Extract

Conc.(mg/ml)	Inhibition zone Diameter (IZD)	Log conc.	IZD ²
200	10	2.301	100
100	8.5	2	72.25
50	8	1.697	64
25	7	1.3979	49
12.5	7	1.0969	49

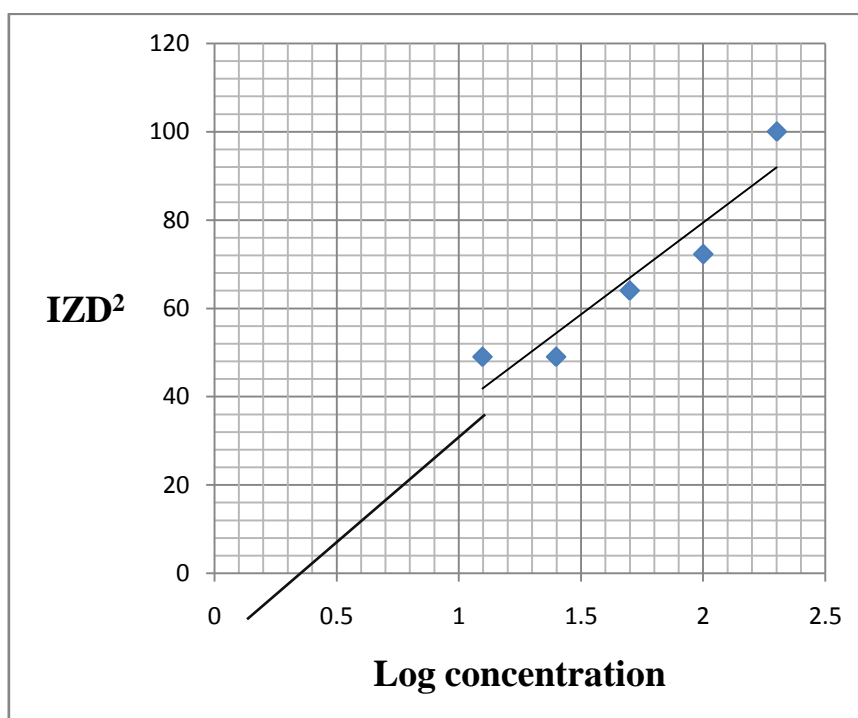


Figure 3d: A graph of the square of the diameter of inhibition zones of the plant extract against the log of the concentration.

Intercept on the x-axis (log conc.) = 0.15mg/ml

MIC = antilog (0.15) = 1.42mg/ml

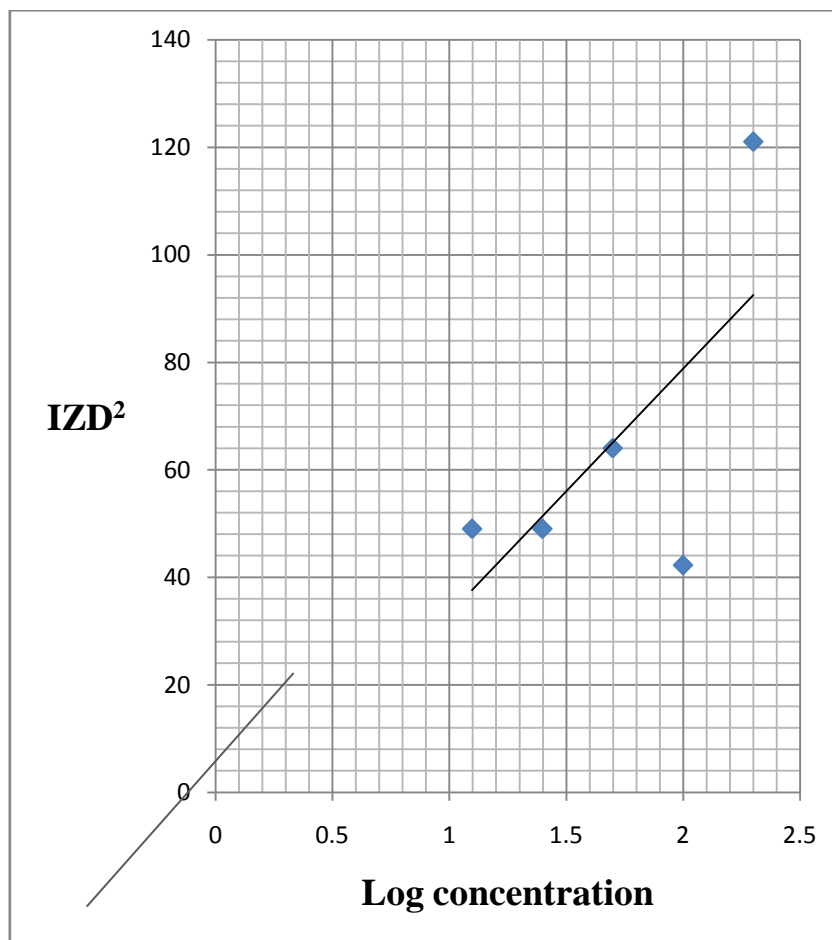


Figure 3c: A graph of the square of the diameter of inhibition zones of ketoconazole against the log of the concentration

Intercept on the x-axis (log conc.) = 0.22mg/ml

MIC = antilog (0.22) = 1.66mg/ml

IV. Discussion

Antimicrobial agents have been used in clinical practice for over 40 years (Abubacker et al. 2010) and resistant bacteria are emerging world wide as a threat to the outcome of common infections in community and hospital settings (Radhika et al 2008).

In this study, in vitro antibacterial and antifungal activities of antimicrobial activity of ethanol leaves extract of *A. paniculata* were determined using agar well diffusion test. In this experiment, the ethanol leaves extract of *A. paniculata* demonstrated antibacterial activities against *S. aureus*, *P. aeruginosa*, *Streptococcus pneumoniae* and *E.coli* as well as antifungal activity against *Candida albicans*. The diameter zone of inhibition was highest with *S. aureus* (13mm) and lowest with *P. aeruginosa* (1mm). This difference in the diameter zone of inhibition could have resulted from differences in the composition of the cell wall of Gram positive bacteria and Gram negative bacteria. The greater susceptibility of gram-positive bacteria to plant extracts has been previously reported in South American (Paz et al., 1995), African (Kudi et al., 1999; Vlietinck et al., 1995) and Australian (Palombo and Semple, 2001) medicinal plant extracts. The results obtained in this study confirm the antibacterial potential of the plant investigated (*A. paniculata*), and its usefulness in the treatment of skin infections. This in vitro study corroborates the antibacterial activity of *A. paniculata* used in folkloric medicine to treat pneumonia, respiratory infections, dysentery and skin infections (Kanokwan . et al., 2008; Poolsup . et al., 2004; Coon et al., 2004 and Saxena et al., 2010). The result for minimum inhibitory concentration shows that it ranges from 7mg/ml to 12.5mg/ml, with *P. aeruginosa* having the highest MIC (12.5mg/ml) and *Streptococcus pneumoniae* with the lowest (7mg/ml). This shows that *S. pneumoniae* is more sensitive than *P. aeruginosa* to the plant extract (Chinedu et al 2012). The plant extract showed a remarkable activity against *Candida albicans* which was comparable with the control (ketoconazole). MIC obtained on the fungus, *Candida albicans* for the extract and the control drug were 1.42mg/ml and 1.66mg /ml respectively. This plant's activity against *Candida albicans* also supports the work done by Radha et al, (2011).

The phytochemical analysis carried out on the plant extract revealed the presence of alkaloids, flavonoids, terpenoids, saponins, reducing sugar, phenols, steroids, cardiac glycoside and tannins. This result also supports the findings reported by Radha et al,(2011) and Salna et al. (2011). Some of these phytochemicals have been implicated in the antibacterial properties of *A. paniculata*. It has been shown that alkaloid protects against chronic disease (Akindele and Adeyemi , 2007).

Thin layer chromatography (TLC) analysis of the various fractions obtained using solvent extraction and the crude extract resolved the crude extract into five components, fraction A into three components, fraction B into two components, fraction C into two components, fraction D into one component and fraction E into two components. Resolving the fractions into various components verified the presence of different phytochemicals in the plant which could be responsible for its antimicrobial properties.

Distillation of the volatile oil present in the plant was carried out and the determination of the refractive index of the oil was done using Abbe's refractometer at 26.1°C. Result of this determination gave the refractive index of 1.3685.

V. Conclusion

The results obtained in this study explicitly showed that the ethanol leaves extract of *Andrographis paniculata* exhibited antibacterial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Escherichia coli* as well as antifungal action against *Candida albicans*. This result has therefore validated the traditional use of the plant in the treatment of laryngitis, pharyngitis, tonsillitis, dysentery, diarrhoea, pneumonia, respiratory infections, wounds and skin infections among other diseases caused by these microorganisms. Being sensitive to *Candida albicans*, the plant extract can be used in the treatment of candidiasis. However further study is required to identify the main active principles of medicinal value, its toxicological profile in human and to subsequently formulate it into conventional dosage form.

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