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Original Research Article

Antibacterial Activity of Ethanolic Leaves Extract of the Plant *Andographis Paniculata* on Clinical Isolates of *Staphylococcus aureus* and *Escherichia coli*

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Abstract: The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in rate of infection by antibiotic-resistant microorganisms. Antibacterial activity of the ethanolic leaf extracts of *Andographis paniculata* against *Staphylococcus aureus* and *Escherichia coli* was studied. The fresh leaves of *Andographis paniculata* were collected randomly from a local farm in Alulu Enugu East L.G.A Enugu State Nigeria. The leaf samples were morphologically identified, washed, air dried at room temperature and milled into powder. Thirty-nine grams of the powder total weight of the extract, was macerated using ethanol as solvent. Phytochemical analysis of the leaves extract was carried out and the results showed that Saponins (++), Tannins (++), Flavonoids (+++), Phenols (+++), Steroids (++) were present while Terpenoids (-), Alkaloids (-) and Glycosides (-) were absent. The extract was concentrated by evaporation at various concentrations (100mg/ml, 300mg/ml, and 500mg/ml) prepared using 5% DMSO. Antibacterial activity was determined using agar well diffusion method, and results showed that there was no antibacterial activity of ethanolic leaf extracts of *A. paniculata* against *E. coli* while there was inhibition zones of 20 mm, 17 mm and15 mm against *Staphylococcus aureus* respectively. The Minimum inhibitory concentration was determined and the results showed that at 12.5mg/ml concentration, the extract was able to inhibit the growth of *Staphylococcus aureus.* The Minimum inhibitory concentration was determined and the results showed that at 12.5mg/ml concentration, the extract was able to inhibit the growth of *Staphylococcus aureus.* This study therefore showed that ethanolic extracts of *Andographis paniculata* has activity against the *Staphylococcus aureus* since they exhibited comparable activity with the positive control ciprofloxacin.

Keywords: *Andographis paniculata,* Antibacterial activity, Ciprofloxacin, *Escherichia coli,* and *Staphylococcus aureus*.

INTRODUCTION

Microbial diseases has become a major global problem almost since the advent of mankind on earth and today more microbial diseases are still being discovered and treatments still being invented (Ali and Mir, 2020). The threat of bacterial diseases and viral diseases in the society is even more serious than other microbial diseases due to the recent

ability of bacteria and virus to form resistance to antimicrobial drugs and its ability to be dormant in cells for a long period of time and also its ability to reproduce quickly and massively within a short period of time (Bloom and Cadarette 2019). The introduction of antibacterial materials into general clinical use is one of the most successful approaches in chemotherapy, considerably contributing to the control of infections and diseases. The effects of bacterial diseases have continued to create an uprising alarm in the society and efforts are being made to minimize and reduce the number of mortality caused by bacteria (Bloom and Cadarette 2019).

Due to the increased use of wide antimicrobial drugs and antibiotics there is greater awareness on the importance of antimicrobial drugs and its effect on our daily life and also on the use of antimicrobial drugs in the society. Many of these bacteria have become resistant to the drugs that were previously bactericidal to them and this has also created more alarming question in the society as to whether the human race can ever eradicate bacterial diseases once and for all (Dogan *et al.,* 2020). Bacteria over the years has shown to be both helpful and harmful to human life, we cannot rule out the tremendous breakthrough we have had in trying to curtail bacterial spread and infection. Over the years' herbal treatment of diseases has been modified and scientist have been able to extract medicinal compounds from different medicinal leaves and plan (Dogan *et al.,* 2020). The existence of herbal plants has been since the advent of mankind on earth, and herbal plants has shown to be effective and reliable. In Nigeria *Andographis paniculata* has been reported by local herbalists to be effective against a variety of diseases such as *Necrotizing fasciitis, cellulitis* (Geetha *et al.,* 2017). *Andographis paniculata* also known as bitter weed or Green chiretta is located at different parts of the country and is been greatly used by local herbalists residing in villages to treat patients suffering from *Necrotizing fasciitis* popularly known as flesh eating disease. Its major photochemical components include Flavaniods, tepernoids, xanthones, polyphenols and noriridioides extracts which have shown to have medicinal properties (Geetha *et al.,* 2017). However, in this study ethanol extracts of *Andographis paniculata* leaves was used as an antibacterial agent against clinical isolates of *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Collection and Transportation of Plant Samples

Fresh leaves of *Andographis paniculata* from a local farm in Alulu Nike Enugu East L.G.A Enugu State were collected using stainless clipper that was sterilized in the hot air oven and were transferred into a sterilized stainless cylinder plate with lids. The lids were sealed immediately the plants had been placed in the plate. The samples were transported to Microbiology laboratory of Enugu State University of Science and Technology Enugu State Nigeria immediately after collection and the leaf samples was identified by a plant taxonomist Prof. J.C Okafor of Enugu State University of Science and Technology.

Collection of the Clinical Stock Culture

The stock clinical culture of *Staphylococcus aureus and Escherichia coli* was collected from Microbiology Laboratory Enugu State University of Science and Technology Teaching Hospital Enugu.

Isolation of the Organisms from the Clinical Stock Culture

This was carried out as described by (Turner, *et al.,* 2019). The organisms from clinical stock culture were serial diluted in 10-fold and cultured on Nutrient agar and incubated for 24 hours at 37^{o}C. After incubation, discrete colonies were sub cultured on Nutrient ager, MacConkey agar, Mannitol salt agar and Eosin Methylene Blue agar (EMB) to obtain pure culture. The sub-culturing of the isolates was done by placing each colony in the middle of the agar plate and streaked up and down and across the plate and incubated for 24 hours at 37^{o}C.

Identification of the Isolates from the Clinical Stock Culture

The identification was done using a method described by (Cheesbrough, 2000). The isolates were identified and characterized based on the morphological and biochemical test which include. Sub-culturing on selective and differential media (Mannitol Salt Agar), Gram staining, Indole test, Catalase, test and Oxidase test, Urease test, Citrate Utilization test, Methyl red test, Glucose fermentation test, Lactose fermentation test and Voges-proskaur test.

Ethanol Extraction of *Andographis paniculata* Leaves

The extraction was carried out as done by (Hossain *et al.,* 2019). Fresh leaf of *Andographis paniculata* weighing 39.7 g was washed in running water to remove unwanted materials. The leaves were air-dried for two weeks and ground into fine powder. 36.9g of ground sample was weighed and soaked in 270 milliliters of 90% ethanol solution (BDH, England), stirred and allowed to stand for 24 hours. The suspension was filtered using Muslin bag followed by Whatman No. 42 filter paper. The filtrate was evaporated under reduced pressure and

dried using a rotary evaporator at 55°C. The concentrated extract was stored in a labeled sterile screw capped bottle at 2°C.

Phytochemical Analysis of the Plant Extracts

The phytochemical analysis of the plant *Andoraphis paniculata* was carried out as illustrated by (Hossain, *et al.,* 2014). The presence of tannins, flavonoids, terpenoids, steroids, saponins, alkaloids, glycosides and phenols was determined.

Test for Tannins

0.1g of the extracts was stirred with 10ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to the filtrate. The presence of a blue-green precipitate indicated the presence of tannins.

Lead Ethanoate Test for Flavonoids

0.1g of the extracts was dissolved in water and filtered. To 5ml of the filtrate, 3ml of lead ethanoate was added. Appearance of a buff-pale yellow precipitate indicated the presence of flavonoids.

Test for Terpenoids

0.1g of the extracts was dissolved in ethanol. 1ml of acetic anhydride was then added, followed by the addition of conc H_2SO_4. Change in colour from pink to violet was not observed hence indicating the absence of terpenoids.

Liebermann-Buchard Test for Steroids

2ml of acetic acid was added to 0.1g of the plant extract. The solution was cooled well in ice after which conc. H_2SO_4 was added carefully. A colour change from violet to bluish-green indicated the presence of steroidal ring.

Test for Saponins

0.1g of the extracts was boiled with 5ml of distilled water and filtered. About 3ml of distilled water was added further and shaken vigorously for about for 5 minutes. Frothing which persisted on warming was observed which indicated the presence of saponins.

Test for Alkaloids

0.1g of the plant extracts was dissolved in dilute Hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). There was no formation of yellow coloured precipitate hence indicating the absence of alkaloids.

Test for Glycosides

0.1g of the plant extracts was mixed with 30ml of distilled water and heated on a water bath for 5 minutes. To 5ml each of the filtrates 0.2ml of Fehling's solution A and B was added until it turns alkaline. The solutions were heated on a water bath for 2minutes. No brick-red precipitate was formed indicating the absence of glycosides.

Ferric Chloride Test for Phenols

0.1g of the extracts was boiled with distilled water and then filtered. Few drops of 10% ferric chloride solution were added to 2ml of the filtrate. A blue colouration indicated the presence of a phenolic hydroxyl group.

Preparation of Different Concentrations of the Extract

The preparation of different concentration for the extract was carried out using the method described by (Geetha *et al.*, 2017). 500mg/ml, 300mg/ml and 100mg/ml concentrations of the crude extract of ethanol was prepared by dissolving 5g, 3g and 1g of the plant extract in 10mlof 5% DMSO respectively.

Preparation of Inoculums

This was done according to (Hossain, *et al.,* 2014). Inoculums were standardized to give a density of 10^6 colony-forming units (CFU)/ml. A loopful of the test organisms was inoculated into 5.0 ml of nutrient broth and incubated at 3 °C for 24 h. 0.2 ml from the 24-h culture of the organisms were dispensed into 20 ml sterile nutrient broth and incubated for 3–5 h to standardize the culture to 10^6 CFU/ml (corresponding to 0.5 McFarland standards). Plates were inoculated within 15 minutes of standardizing the inoculum, to avoid changes in inoculum density.

Antimicrobial Activity of Ethanolic Leaf Extracts of *Andrographis paniculata* on the Isolates

This was carried out by agar well diffusion method as described by (Bahtter *et al.,* 2015). The culture media used for the assay was the Mueller Hinton Agar. One hundred microlitres (10^6 CFU /ml) of fresh microbial culture of the isolates was spread on plates containing Muller Hilton agar using a sterile glass spreader. Six plates in total were used to carry out this assay. Three agar wells of 6mm diameter each was punched off into each of the plates using a sterile cork borer. The wells on each plate contained different concentrations of the plant extract, a negative control (DMSO) and 10mg of the antibiotic (Ciprofloxacin) which served as positive control. The plates were incubated at 37°C for 24 hours and were observed for inhibition.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of the active extracts was determined using tube dilution method as described by (Bahtter *et al*., 2015). Decreasing concentrations of the plant extract used in the antimicrobial assay that exhibited inhibition (100mg/ml) was prepared using two-fold dilution method using Mueller Hinton broth. Six sterile test tubes were used with each containing 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml concentrations of the ethanol extract respectively. Two tubes served as control: nutrient broth inoculated with bacteria was used as a positive control and nutrient broth containing the plant extract was used as a negative control. After incubating for 18 hours at 37°C, the tubes were examined for turbidity indicating the growth of the microorganisms. The lowest solution of the extract that inhibited the growth of the microorganism as detected by the lack of visual turbidity (matching the negative growth control) was designated the minimum inhibitory concentration.

Determination of Minimum Bactericidal Concentration (MBC)

According to (Bahtter *et al.,* 2015) the minimum bactericidal concentration was determined by assaying the test tubes resulting from MIC determination. A loopful of the content of each test tube was inoculated by streaking on a solidified Mueller Hinton agar plate and then incubated at 37 °C for 24 hours and observed for bacterial growth.

RESULTS

The morphological characteristics of the *Staphylococcus aureus* and *Escherichia coli* from clinical isolates are shown in Table 1.

Table 1: Morphological Characteristics of the *Staphylococcus aureus* and *Escherichia coli*

Bacterial Isolates	Macroscopic Characteristics	Microscopic Characteristics
Staphylococcus aureus	Consisted of smooth, golden yellow colonies on nutrient agar and also appeared yellow on Manitol Salt agar medium.	Consisted of Gram positive cocci in clusters which appeared purple.
Escherichia coli	Consisted of thick, metallic green sheen on Eosin Methylene Blue Agar and also appeared mucoid bright pink colonies on MacConkey Agar medium.	Consisted of Gram negative single short rods which appeared pink.

The biochemical characteristics of *Staphylococcus aureus* and *Escherichia coli* from clinical isolates are shown in Table 2.

Table 2: Biochemical Characteristics of *Staphylococcus aureus* and *Escherichia coli*

Bacterial isolate	Gram Stain	Indole test	Catalase test	Oxidase test	Urease test	Citrate Utilization	Methyl red test	Glucose fermentatio	Lactose fermentatio	Voges-p roskaur test
Staphylococcus aureus	+	-	+	-	+	+	+	+	+	+
Escherichia coli	-	+	+	-	-	-	+	+	+	-

KEYS; –: Negative, +: Postive

The phytochemical analysis of ethanol extracts of *Andographis paniculata* leaves was shown in Table 3.

Table 3: Phytochemical of *Andographis Paniculata* Leaves

Phytochemicals	Estimated Concentration
Alkaloids	-
Phenols	+++
Glycosides	-
Tannins	++
Saponins	++
Terpenoids	-
Steroids	++
Flavonoids	+++

KEYS; -: Absent, +: Scanty, ++: Moderate, +++: Abundant

The antibacterial activities of *Andrographis paniculata* leaves extract on *Staphylococcus aureus, and E. coli* are shown in Table 4.

Table 4: Antimicrobial Activity of Ethanolic Leaf Extracts of *Andrographis paniculata* on the Isolates

S/N	Plant Extract Ethanol (90%) Concentration (mg/ml)	Zones of Inhibition (mm)	
		Escherichia Coli	***Staphylococcus aureus***
1	500	-	20
2	300	-	17
3	100	-	15
4	Ciprofloxacin (10g/ml)	20	41
5	Control sterile disc	-	-

Controls: Negative – Sterile sensitivity disc, Positive – 10g/ml Ciprofloxacin

The minimum inhibition concentration and minimum bactericidal concentration of Ethanolic leaf Extracts of *Andographis paniculata* on *Staphylococcus aureus* are shown in Table 5.

Table 5: MIC and MBC of Ethanolic leaf Extracts of *Andographis paniculata* on *Staphylococcus aureus*

Bacterial isolate	**Different concentrations of ethanol extract of *Andographis paniculata* tested against *Staphylococcus aureus* with reference to MIC and MBC**					**MIC**	**MBC**
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml		
Staphylococcus aureus	-	-	-	-	+	12.5mg/ml	25mg/ml

KEYS; MIC: minimum inhibition concentration, MBC: minimum bactericidal concentration, (+): showing growth ie (Showed turbidity), (−): no growth ie. (There was no turbidity)

DISCUSSION

Studies were conducted on antibacterial activity of ethanol leaf extracts of *Andographis paniculata* against isolates from the clinical stock culture of Enugu State University of Science and Technology Teaching Hospital Enugu State Nigeria. The isolates were morphologically and biochemically identified as *Staphylococcus aureus* and *Escherichia coli* using standard identification techniques (Tables 1, 2). These are in line with the work of Cheesbrough, (2000).

Experiments on phytochemical analysis of ethanol extracts of *Andographis paniculata* leaves were conducted and result showed that Saponins, Tanins, Flavanoids, Phenols, Steriods were present with Phenols and Flavanoids being in higher concentration, while Terpernoids, Alkaloids and Glycosides were absent (Table 3) These contradicts the work of (Husain *et al.,* 2021). It may be due to geographical origin of the plant or other factors such as solvent used for the plant extraction process, or parts of the plant used. Antibacterial activity of ethanol leaf extracts of *Andographis paniculata* against *Staphylococcus aureus* and *Escherichia coli* was carried out using the agar well diffusion method with the ethanolic extract at 500mg/ml, 300mg/ml and 100mg/ml exhibiting inhibition zones of 20 mm, 17 mm and15 mm against *Staphylococcus aureus* respectively while ethanol extract showed no inhibition against *Escherichia coli* (Table 4). This is somewhat corresponds to the work of Shalini and Narayanan (2015). Studies were also carried out to determine the Minimum inhibitory concentration and Minimum bactericidal concentration of ethanol leaf extracts of *Andographis paniculata* on *Staphylococcus aureus* Results were obtained for the minimum inhibitory concentration which showed that at a concentration 12.5mg/ml the extract was able to inhibit the growth of *Staphylococcus aureus.* The Minimum bactericidal concentration was observed at a concentration of 25mg/ml.

As previously stated, the ethanolic leaves extracts of the plant *A. paniculata* used in this study recorded activity against the clinical isolates of *Staphylococcus aureus*, but there was no antibacterial activity of ethanol leaf extracts of *A. paniculata* against *Escherichia coli* (Table 5). The findings partly agree with (Polash *et al.,* 2017), as the ethanolic (70%) and aqueous (30%) leaf and stem extracts of *A. paniculata* showed activity against *E. coli* and *Staphylococcus aureus* in their study. It has also been reported that the crude aqueous as well as ethanolic extracts of *A. paniculata* do not show any antibacterial effect against gram-negative *E. coli* (Mazurek-Popczyk *et al.,* 2020) hence agreeing with this study.

CONCLUSION

This study showed that there was antibacterial activity of ethanol leaf extracts of *A.*

paniculata against *Staphylococcus aureus* probably due to the presence of phytochemicals such as Flavonoids, Phenols and Saponins. The ethanolic leaf extracts of *A. paniculata* showed no antibacterial activity against *Escherichia coli* which is probably as a result of the absence of vital active phytochemicals such as Alkaloids, Terpernoids and Glycosides as shown in the result of this research work. Further research should be done using other solvents for extraction as it plays a vital role in the activity of the leaf extracts.

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