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(RESEARCH ARTICLE)

Antimicrobial properties of *Andrographis paniculata* (vinegar) leaf on selected human pathogens

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Abstract

The study was conducted to investigate the antibacterial effect of *Andrographis paniculata* against selected human pathogens using microbiological techniques. Different solvents were used to extract the active components from the leaves. The antibacterial activity was studied against selected human pathogens viz, *Escherichia coli*, *Salmonella* sp, *Bacillus* sp and *Enterococccus* sp. For *E. coli*, the range of minimum inhibition zone at 2.5 mg/ml, 5.0 mg/ml, 7.5 mg/ml and 10.0 mg/ml ethanol extract was 15 mm-25 mm while that of aqueous extract was 13 mm-17 mm with a non-detectable zone at 2.5 mg/ml concentration. *Salmonella* sp had inhibition zone range of 13 mm-22 mm for ethanol extract while the same organism showed minimum inhibitory zone range of 11 mm-21 mm for aqueous extract. The inhibition zone range of ethanol extract against *Bacillus* sp was 16.5 mm- 25.5 mm while aqueous extract had 9.5 mm-19 mm. Finally for *Enterococcus* sp, the inhibition zone range of ethanol extract was 15 mm-25.5 mm while aqueous extract had 9 mm-25 mm. Among the two solvents, ethanol extract showed greater antibacterial activity against the test organisms when compared with the aqueous extract at 10.0 mg/ml concentration of the extracts. Preliminary screening of phytochemicals in the leaf extract of *Andrographis paniculata* revealed the presence of alkaloids (6.266±0.376 mg/100g), saponins (11.055±0.134 mg/100g), flavonoids (1.175±0.067 mg/100g), tannins (2.230±0.099 mg/100g) and cyannogenic glycosides (0.870±0.057 mg/100g). The results revealed that 10.0mg/ml concentration had effect on all the test bacteria.

Keywords: Antimicrobial; *Andrographis paniculata*; Human pathogens; Plant extract

1. Introduction

Over the past two decades, interest in traditional medicinal plants has grown greatly from the use of herbal products as natural cosmetics and for self-medication by the people to the research -based investigations of plants of their antimicrobial effects against human pathogens in human beings. Beyond this pharmaceutical view point to plants, there is a large disposition to utilize plants products to supplement the diet, mainly with the purpose of improving on the quality of life and preventing the elderly people from diseases. Globally, India has been recognized as a major resourceful area for the traditional medicines [1].

Andrographis paniculata popularly known as king of bitters belongs to the family Acanthaceae, it is an annual herbaceous plant and is extensively cultivated in Southern Asia, China and some part of Europe. In traditional medicine, *Andrographis paniculata* is widely used to get rid of body heat, dispel toxins from the body, prevent common cold, upper respiratory tract infections including sinusitis and fever and as antidote against poisons of snakes and insects [1].

The aerial parts and whole plant of *Andrographis paniculata* have been used for centuries in Asian countries as traditional medicine for the treatment of different ailments. It has been used by traditional medicine practitioners for

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stomach aches, inflammation, pyrexia and intermittent fever [2].The leaf extract of this plant is a traditional remedy for the treatment of infectious diseases, fever-causing, colic pain, loss of appetite, irregular stool and diarrhea [3].

The plant has been reported to exhibit different mode of antimicrobial activities *in vivo* as well as *in vitro* viz, antibacterial, antiviral, anti-inflammatory and anticancer [4]. The plant *Andrographis paniculata* demonstrates potential therapeutic action in curing, common cough, liver disorders and colds in human. The characteristic phytochemical substance encountered in this plant has considerably intensified its significance in the area of medicinal plant. This study will therefore examine the antimicrobial properties of *Andrographis paniculata* and its effect on selected human pathogens.

2. Material and methods

2.1. Sample collection

Fresh leaves of *Andrographis paniculata* plant were collected from a vegetable garden. The sample was stored in a sterile polythene bag and was transported to the chemistry laboratory in Akwa Ibom State polytechnic, Ikot Osurua for analysis. Fresh leaves of *A. paniculata* plant were washed thoroughly with distilled water and kept for the water to dry. Then the leaves were sliced into smaller sizes and air-dried at room temperature to remove the moisture content.

The dried sample were crushed into powdery form using a sterile blender, a fine powder was obtained and stored in an air-tight bottle for the extraction of ethanolic and aqueous extract

100 g of the grounded powder leaves were weighed into flask containing 200 ml of 75 % ethanol and soaked instantly at an interval of 20 minutes for three days for proper extraction. The setup was filtered using Whatman filter paper and the filtrate was transferred into a beaker and placed in a water bath regulated at temperature of 50 ^{0}C for dryness through evaporation method

The same procedure was carried out for aqueous extract; it was soaked for 24 hours before filtering with Whatman filter paper. The dried extract (paste) was carefully transferred into a sample bottle sealed immediately and stored in the refrigerator at 4 °C for further analysis of antimicrobial activity.

2.2. Morphological and Biochemical identification of bacterial isolates

Adequate inspections were carried out for all the isolates, the cultural characteristics were noted. The biochemical characterization as described by Cheesbrough [5] were carried out which included; catalase, coagulase, oxidase, indole, citrate, urease, motility, spores, capsule test, methyl red- voges proskaur test and carbohydrate fermentation.

2.3. Preparation of Antibacterial Sensitivity disc and inoculation of test bacteria

The residue obtained from the evaporated extract was dissolved with ethanols for ethanolic extract and distilled water for aqueous extract respectively and was subjected to different concentrations ranging from 10 mg/ml, 7.5 mg/ml, 5 mg/ml and 2.5 mg/ml and perforated Whatman paper No 1 of about 6 mm in diameter sterilized in hot air oven on cooling, it was soaked in different concentrations of the extract, removed and was allowed for few minutes to dry. According to NCCLS, [6] molten agar was poured into sterile petri dishes and allowed to solidify. A sterile wire loop was used to pick a loopful of the test isolates inoculated uniformly on the labeled solidified agar plate. Each of the prepared discs was placed equidistant in all the plates based on the concentrations. The conventional disc which included both Gram positive and Gram negative antibiotics were also inoculated at equidistant on separate plates. The plates were wrapped with aluminum foil and invertedly incubated at 37 ^{o}C for 18-24 hours for detection of clear zones of inhibition.

The inhibitory zones were interpreted as sensitive, intermediary or resistant, using the interpretation chart of zone size of Kirby- Bauer sensitivity test method described by Prescott et al., [7]. Interpretations of the result was done using inhibiting zone sizes, 18 mm and above were considered sensitive, 13-17 mm intermediate and 13 mm below as resistant according to NCCLS [6].

2.4. Phytochemical Screening of the plant sample

The powdered form of the leave sample was then subjected to phytochemical analysis to determine the bioactive components present in the leave sample which made it suitable for treatment of various ailments. The bioactive component includes Alkaloids, flavonoids, saponins, Tanins and Cyanogenic glycosides.

3. Results

3.1. Morphological and biochemical characteristics of bacterial isolates

The morphological (phenotypic) and biochemical characteristics of bacterial isolates were carried out for identification as shown in tables 1 and 2.

Table 1 Biochemical characteristics of bacterial isolates

Isolates	Isolate code	Gram stain	Spore	Catalase	Coagulase	Motility	Citrate	Urease	Indole	Oxidase	MR	VP	Glucose	Lactose	Maltose	Sucrose
1		-	-	+	-	+	-	-	+	-	+	-	A	A	A	A
2		-	-	+	-	+	-	-	-	-	+	-	A	A	A	A
3		+	+	+	-	+	+	-	-	+	-	+	A	A	A	A
4		+	-	-	-	-	-	-	-	-	-	+	A	O	A	A

Key;
A- Acid produced
O- No acid produced

Table 2 Phenotypic characteristics of bacterial isolates

Isolates	Cultural and morphological characteristics	Cell shape	Probable Bacteria
1	Metallic green, Dry, Flat, Entire, opaque, Aerobic	Short Rods	*Escherichia coli*
2	Creamy, Circular, Moist, Flat, Transparent, Aerobic	Rods	*Salmonella* sp
3	Milky, Circular, Dry, Raised, Dentate, Opaque	Rod single	*Bacillus* sp
4	Pale yellow, Circular, Mucoid, Raised, Entire, Aerobic	Cocci in pairs	*Enterococcus* sp

3.2. Antibiogram of Bacterial Isolates on different concentrations of plant extract

Antibacterial effect of *Andrographis paniculata* (vinegar) leaves extract on the four tests isolates was carried out using agar disc diffusion method (Table 3).

Table 3 Antibiogram of Bacterial Isolates on different concentrations of plant extract

	Extract Conc.(mg/ml)	Zone of Inhibition (mm)	
Isolated Bacteria		**Ethanol**	**Aqueous**
Escherichia coli	10.0	25.0	17.5
	7.5	20.6	17.0
	5.0	19.5	13.0
	2.5	15.0	No zone
Salmonella sp	10	22.0	21.0
	7.5	18.0	18.0
	5.0	15.0	13.5
	2.5	13.0	11.0
Bacillus sp	10.0	25.5	19.0
	7.5	23.0	15.5
	5.0	18.0	13.0
	2.5	16.5	9.5
Enterococcus sp	10.0	25.5	25.0
	7.5	19.0	18.0
	5.0	17.5	9.5
	2.5	15.0	No zone

Keys: Sensitivity: ≥18 mm; Moderate: 13 mm-17 mm; Resistance: ≤13 mm

3.3. Antibacterial susceptibility pattern of isolate to selected antibiotics

Antibacterial susceptibility pattern of bacterial isolates to selected gram positive and negative antibiotics was carried out as shown in Table 4.

Table 4 Susceptibility of bacterial isolates on antibiotics using disc diffusion method (Gram negative and positive disc)

Isolates	**SXT**	**S**	**PN**	**OFX**	**AU**	**CPX**	**NA**	**CN**	**PEF**	**CEP**
Escherichia coli	18.5	17.0	15.0	23.0	31.0	21.0	17.0	27.0	16.0	-
Salmonella sp	12.5	-	-	25.0	21.0	28.0	30.0	29.0	28.0	12.0
	NB	**AMX**	**APX**	**RD**	**S**	**CH**	**LEV**	**CPX**	**CN**	**E**
Bacillus sp	22.0	23.0	18.0	28.0	19.0	18.5	33.0	34.0	25.0	23.0
Enterococcus sp	14.5	11.5	12.0	20.0	14.0	22.0	11.5	15.5	20.0	16.0

Keys: Sensitivity: ≥18 mm; Moderate: 13 mm-17 mm; Resistance: ≤13 mm

SXT- Septrin, S- Streptomycin, PN- Ampicillin, CEP-Ceporex, OFX- Taravid, AU- Augmentin, CPX- CCiprofloxacin, CN- Gentamycin, NA- Nalidixic Acid, PEF- Reflacine, CH- Chloramphenicol, AMX- Amoxil, APX- Ampiclox, RD- Rifampicin, LEV- Levofloxacin, NB- Norfloxacin, E- Erythromycin, S- Streptomycin, CPX- Ciprofloxacin, CN- Gentamycin

Antibiotics	**Sensitivity**	**Intermediate**	**Resistance**
Ciproflaxacin [CPX] 10mcg	≥ 17	15-16	≤14
Amoxicillin (AMX) 20 mcg	≥18	16-17	≤ 15
Ampiclox[APX] 30mcg	≥21	15-20	≤ 15
Rifampicin [RD] 20 mcg	≥19	15-18	≤ 14
Septrin [SXT] 30mcg	≥ 16	11-15	≤ 10
Streptomycin [S] 30mcg	≥ 21	15-20	≤14
Ampicillin [PN] 30mcg	≥14	12-13	≤11
Ceporex [CEP] 10mcg	≥18	15-17	≤14
Taravid [OFX] 10mcg	≥ 15	12-14	≤11
Augmentin [AU] 30mcg	≥18	13-17	≤ 12
Ciprofloxacin [CPX] 10mcg	≥21	16-20	≤ 15
Gentamycin [CN] 10mcg	≥ 15	13-14	≤12

3.4. Phytochemical analysis of *Andrographis paniculata*

The phytochemical screening of *Andrographis paniculata* leaves showed bioactive components which include; alkaloids, flavonoids, saponins, tannins and cyanogenic glycosides (Table 5).

Table 5 Phytochemical screening of *Andrographis paniculata* leaves

S/N	Parameters	Concentration [Mg/100g]
1.	Akaloids	6.266±0.376
2.	Saponins	11.055±0.134
3.	Flavonoids	1.175±0.064
4.	Tannins	2.230±0.099
5.	Cyanogenic glycosides	0.870±0.057

4. Discussions

Infectious diseases are a major cause of mortality and morbidity worldwide, currently the ongoing battle against bacteria prevail certainty of evolving resistance. Also, advancement in medical research in more patients being in evaluative and immune suppressed states further creates a perpetual need for new antibiotics. As a result, it is the right time to discover new plant –based antimicrobial agent without the tendency to be resisted by pathogens [8]. *A. paniculata* is known to possess several water soluble lactone andrographoloidic antibiotics properties. Medicinal plants are more important in field of pharmaceutical industries for new drug preparation [9].

The present study was therefore out to assess the antibacterial activity of *Andrographis paniculata* of ethanolic and equeous leave extracts on some pathogenic bacteria. Four bacterial isolates which includes *Escherichia coli*, *Salmonella* sp, *Bacillus* sp and *Enterococcus* sp were used for the study. Maximum inhibition zone of 25 mm was recorded with ethanol extract against *E.coli*, while 17.5 mm maximium inhibition zone was recorded with extract against *E.coli*. This result is comparable with previous studies which reported that 75 µL methanol is better than other solvent used for antibacterial activity [10]. The maximum inhibition zones (22 mm and 21 mm) for *Salmonella* sp was obtained with 10.0 mg/ml of both ethanol and equoeous extracts. These results is in line with the work of Pushpendra et al., [10] who reported high efficacy of methanolic extract of *Andrographis paniculata* on *Salmonella* sp, than the equous extract. The ethanolic extract showed a high inhibition zone of 25.5 mm than 19mm recorded for equous extract at 10.0 mg/ml concentration for *Bacillus* sp which indicate better efficacy of the plant extract against *Bacillus* sp compared to the aquoes extract. Similar result was obtained by previous studies [10].

The efficacy of ethanolic and aqoeus extract against *Enterococcus* sp at 10.0 mg/ml concentrations were almost the same (25.5 mm and 25 mm) inhibition zone. Also, 19 mm and 18 mm zone of inhibition were obtained at 7.5 mg/ml concentration. This implies that both extracts can be effective against *Enterococcus* sp infections. The phytochemicals present in plants have been studied extensively to establish their efficacy and to understand the underlying mechanism of action. Phytochemicals are responsible for preventing diseases and promoting health. Preliminary screening of the *A. paniculata* leave extract revealed the presence of alkaloids, saponins, flavonoids, tannins and cyanogenic glycosides in varying concentrations. Sapronins was present in the plant in higher concentrations (11.055 ±0.13 mg/100g) followed by alkaloid (6.266±0.376 mg/100 g), tannins (2.230±0.099 mg/100 g), flavoids (1.175±0.067 mg/100 g) and the least concentration for cyanogenic glycosides (0.870±0.057 mg/100 g). These results is in line with the work of Hostettmann et al [11], who reported ethanolic extract of *A. paniculata* was rich in phytochemical constituents such as alkaloids, saponins, flavonoids and tannins. Alkaloids are natural products that contain heterocyclic nitrogen atoms and are basic in characters.

In assessing the general antibacterial activity of the ethanolic and aqeouos extracts of *A. paniculata* on the test bacteria, it could be deduced that the ethanolic extract exhibited high efficacy to all the test bacteria at concentrations ranging from 5.0-10.0 mg/ml while aqueous extract showed sensitivity to the test bacteria only at 10.0 mg/ml and 7.5 mg/ml for most bacteria. Some antibiotics used as control like Augmentin, ciprofloxacin, levofloxacin also demonstrated high efficacy against the pathogens.

5. Conclusion

This study reveals that the ethanolic and aqueous extracts of *Andrographis paniculata* posses and exhibit antibacterial effect against some pathogenic bacteria. However, the most efficacy of the antibacterial activity was obtained for the ethanolic extract at concentrations ranging between 7.5 mg/ml and 10.0 mg/ml. Thus it can be concluded that

antibacterial activities of the plant extracts is dependent on concentration and is comparable to the antibacterial results obtained for the conventional antibiotics.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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