ORIGINAL ARTICLES

ANTIBACTERIAL ACTIVITY OF FRACTIONS OF *UAPACA HEUDELOTTI* BAIL. SYNERGISTIC EFFECT WITH CIPROFLOXACIN

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ABSTRACT

AIM: The aim of the research was to evaluate the antibacterial activity of extracts of *Uapaca heudelotti* when used alone, and in combination with ciprofloxacin, on selected microorganisms.

METHODOLOGY: Fractions of *Uapaca heudelotti* were utilized alone and consolidated with ciprofloxacin to assess their antibacterial properties against some selected bacteria; *Escherichia coli, Salmonella typhi, Streptococcus pyogenes, Klebsiella pneumonia, Staphylococcus aureus, Proteus mirabilis and Bacillus subtilis*.

RESULTS: Results demonstrated that the extracts of Uapacaheudelotti showed significant antibacterial activity against the tried microorganisms with a zone of inhibition ranging from 11 – 27 mm. Joint use of the hexane, chloroform, ethyl acetate and methanol fraction of this plant with ciprofloxacin prompted better synergistic zone which extended in the vicinity of 21 and 41 mm. The results of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the standard drug (3.125 and 12.5 mg/ml respectively) were significantly decreased to 0.75 and 1.5 mg/ml respectively, when the fractions were in combination with standard drug ciprofloxacin.

CONCLUSION: This study shows that the combination of fractions of Uapacaheudelotti with antibiotics increased the antibacterial activity of the antibiotic drug. This may be useful in the use against emerging microbial drug resistance.

Keywords: ciprofloxacin combination, multi-drug, resistance, *Uapaca heudelotti*

INTRODUCTION

Antibacterial agents are a standout amongst the most critical weapons in battling bacterial diseases and have extraordinarily benefited the health-related nature of human life since their introduction. However, these antibacterial agents are under risk. The same number of regularly utilized anti-microbial drugs have turned out to be less and less potent against specific sicknesses not just on the grounds that a significant number of them produce harmful chemicals, but because of the development of resistance to a drug by certain microscopic organisms [1]. Treating bacterial contaminations with antimicrobial agents is helpful, yet their indiscriminate use has prompted resistance among microorganisms and additionally prompted the reoccurrence of old infectious diseases. Development of resistance among microorganisms to antimicrobial agents is credited to the overuse of current antimicrobial medications [2]. Today, clinically important microbes are described by single medication resistance, as well as by various antimicrobial resistance. This follows as a result of past many years of antimicrobial use and misuse [3]. The consistently expanding bacterial resistance to existing medications is a difficult issue, and along these lines, there is a critical need to scan for new classes of antibacterial substances, particularly from plant sources. Unlike synthetic medications, antimicrobials of plant sources are devoid of symptoms and have an awesome restorative potential to mend numerous infectious diseases [4,5]. At times, the utilization of single antitoxin does not deliver the desired inhibitory impacts and to defeat this, a blend of medications produce significant synergistic impact which outperforms their individual antimicrobial activity. The synergistic impact might be because of certain complex arrangement which turns out to be more viable in the hindrance of specific types of microorganisms either by restraining the cell wall or by bringing about its lyses or death [1]. The wide utilization of antimicrobials in the treatment of bacterial infections has prompted the rise and spread of resistance strains. An example is, vancomycin resistance S. aureus (VRSA) which has been accounted for [6,7,8,]. Along these lines, it is necessary to discover new antimicrobial medications or new ways that are powerful for the administration of irresistible infectious disease brought about by drug resistance microscopic organisms including MRSA [9]. There are different approaches to cure and control infections caused by multidrug resistant strain of bacteria one of which is by isolation of phytochemicals that can help to prevent the spread of infection and another method is to formulate new synergistic combination using different commercially available antibiotics with plant fractions with an active phytochemical that have antimicrobial properties [9]. The synergistic impact has been accounted for against gram-negative microscopic organisms [10,11,12] and the ability of plant extract to improve antibiotics has not been well explained [13]. With the increase in bacterial resistance to antibiotics, antimicrobial plant products have gained attention in the scientific research. The use of natural antimicrobial compounds is important not only in food preservation, but also in the control of human diseases of plant microbial origin [14]. Plants contain an extensive variety of substances that can be utilized to treat chronic diseases and infectious diseases. For instance, flavonoids are an extremely fascinating group of secondary metabolites that are possibly valuable wellsprings of antimicrobial formulations. Many reviews have been reported on the antimicrobial usefulness of plant metabolite [15,16,17]. Novel antibacterial activities of plant fractions or Phyto compounds have been reported [18,19,20,]. Also, few plant extracts and phytocompounds displayed synergistic association with antimicrobials against Gram-positive microscopic organisms [21].

MATERIALS AND METHODS

Collection and identification of plant

The fresh stem bark of *U. heudelotti* was collected from the Benue state, in Nigeria in January 2015. The plant was identified by Mal. S. Namadi of the Department of Botany, Faculty of Life Science, Ahmadu Bello University, Samaru, Kaduna State Nigeria.

Extraction of plant materials

The plant materials (the stem bark of *U. heudelotti*) were air-dried at room temperature (27°C) for 2 weeks, after which it was ground to a uniform powder. The crude methanol extract was prepared by soaking 500 g of the dry powdered plant materials in 1 L of methanol at room temperature for 48 h. The extract was filtered after 48h and was concentrated using a rotary evaporator with the water bath set at 40°C. A portion of this extract was reconstituted in water to yield a water-soluble fraction and water insoluble fraction. The two fractions were subsequently partitioned successively and exhaustively using hexane and ethyl acetate, which were then concentrated using a rotary evaporator with a water bath set at 40°C.

Microorganisms

The antibacterial activity was evaluated using *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Bacillus subtilis, Streptococcus pyogenes*, and *Salmonella* strain. All bacterial strains were taken from the microbiology laboratory Ahmadu Bello University Teaching Hospital. Bacterial strains were maintained by subculture on nutrient agar favorable to their growth for 24h in the dark at 37 °C.

Antibiotics

500 mg of standard ciprofloxacin was used.

Antimicrobial activity

The antimicrobial activity of the fractions was determined by the disk diffusion method which is based on the spread of antimicrobial compound in solid medium [22]. The Mueller–Hinton agar was poured in sterile petri dishes (90 mm diameter). The paper discs (6 mm diameter) that were impregnated with 2 mL of each plant extract and antibiotic, were tested. Standard discs were placed on the inoculated agar surface. Petri dishes were allowed to stand for 30 min at room temperature before incubation at 37 °C for 24 h. The effect on the plant fraction was reflected by the appearance around disc of a transparent circular zone corresponding to the absence of growth. The diameter of inhibition zone was measured in mm. The larger the diameter of the area, the more susceptible the strain [23]. To determine the synergistic effect of the combination of the plant fractions and antibiotics which are in the form of ready to use discs, 2 ml of each plant fraction was applied on the antibiotic disc to determine the zones of inhibition [24]. The obtained synergistic results were compared with those of the antibiotics and fractions tested on the same strains alone and by the same method with a standard deviation of ±0.2 mm.

RESULTS

The hexane fraction of *U. heudelotti* showed significant antibacterial characteristic against the tested microorganisms with the exceptions of *S. aureus* and *S. pyogenes* at a concentration of 50 mg/ml (Table: 1). The chloroform fraction was active against *B. subtilis,S. aureus* and *E. coli* with a zone of inhibition of 12, 18 and 15 mm at 50 mg/ml (Table: 1). The ethyl acetate fraction showed activity against *B. subtilis, S. aureus, E coli, S. pyogenes* with a zone of inhibition of 20, 22, 23, 23 and 24 mm respectively at a concentration of 50 mg/ml (Table: 1). At the highest concentration, the methanol fraction of *U. heudoletii* inhibited the activity is *S. aureus*, *S. pyogenes*, *E. coli* and *P. mirabilis* with a zone of inhibition of 15,17, 23, 24 mm respectively (Table:1).

Table 1. Zone of Inhibition of hexane, chloroform, ethyl acetate and methanol fraction of *U.heudelotti* (mm).

Micro organisms	Zone of Inhibition hexane fraction				Zone of Inhibition of chloroform				Zone of Inhibition of ethyl acetate fraction				Zone of Inhibition of methanol				ciprofloxacin
	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	10µg/ml
B. subtilis					12	12	10	12	-	-	-	-	12	12	10	12	37
P. mirabilis	-	-	-	-	-	-	-	-	24	23	32	19	-	-	-	-	17
S. aureus	-	-	-	-	18	13	-	-	15	13	11	-	18	13	-	-	28
K. pneumonia	12	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25
S.pyogenes	-		-	-	-	-	-	-	17	15	13	-	-	-	-	-	30
S. typhi	15	14	12	10	-	-	-	-	-	-	-	-	-	-	-	-	35
E.coli	-	-	-	-	15	15	12	-	23	20	-	-	15	15	12	-	32

Table 2. Zone of Inhibition of hexane, chloroform, ethyl acetae and methanol fraction of U. heudelotti in combination with ciprofloxacin.

Micro organisms	Hexane + Ciprofloxacin				Chloroform + Ciprofloxacin				Ethyl acetate + Ciprofloxacin				Methanol + Ciprofloxacin				ciprofloxacin
	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	10µg/ml
B. subtilis	39	40	42	45	33	25	35	36	30	35	37	39	28	30	33	35	17
P. mirabilis	35	36	38	40	35	30	38	40	27	30	33	35	30	31	35	37	25
S. aureus	30	34	36	40	25	35	32	34	26	32	34	36	25	29	30	33	30
K. pneumonia	34	35	36	38	30	35	34	36	25	32	34	36	25	26	30	34	35
S.pyogenes	32	34	36	40	30	32	35	37	30	31	35	36	30	32	35	36	35
S. typhi	25	26	28	30		37											35
E.coli	37	39	40	43	36	17	40	41	32	35	36	38	30	35	37	40	32

The hexane fraction showed strong synergistic activity against all tested microorganism. The combination of the antibiotic with chloroform, ethyl acetate, and methanol extract is shown in Table:2. The chloroform fraction enhanced the antibiotic activity against all microorganisms. The zone of inhibition of the standard drug ciprofloxacin against *B. subtilis, P. mirabilis, S. aureus, K pneumonia, S. pyogenes, S. typhi* and *E. coli* was 37, 17, 25, 30, 35, 35, 32 but was increased to 45, 40, 40, 38, 40, 30 and 43 respectively when combined with concentration of 0.625 mg/ml of the chloroform fraction. The highest degree of synergistic effects was observed against *B. subtilis* and *P. mirabilis* as the zone of inhibition of the drug above 37 and 17 mm increased to 45 and 40 mm in combination with 6.25 mg/ml of the fraction. The zone of inhibition of the chloroform fraction was in the range of 10 to 18 mm. The ethyl acetate and methanol plant reactions showed significant inhibition, as there was an increase in the zone of inhibition.

Table 3. Minimum Inhibition Concentration of fractions of U.heudelotti in combination with ciprofloxacin (mg/ml).

Micro organisms	Hex + Cip	Chl+ Cip	Ethy + Cip	Meth +Cip	Cip
B. subtilis	0.75±0.2	1.5±0.2	1.5±0.2	1.5±0.2	3.125±0.2
P. mirabilis	0.75±0.2	0.375±0.2	3.125±0.2	1.5±0.2	6.25±0.2
S. aureus	0.75±0.2	3.125±0.2	3.125±0.2	3.125±0.2	6.25±0.2
K. pneumonia	0.75±0.2	3.125±0.2	3.125±0.2	1.5±0.2	6.25±0.2
S.pyogenes	0.75±0.2	3.125±0.2	1.5±0.2	1.5±0.2	6.25±0.2
S. typhi	3.125±0.2	6.25±0.2	1.5±0.2	6.25±0.2	12.5±0.2
E.coli	0.75±0.2	0.75±0.2	0.375±0.2	1.5±0.2	6.125±0.2

Key: Hex + Cip= hexane fraction in combination with ciprofloxacin, Chl + Cip = chloroform fraction in combination with ciprofloxacin, Ethy + Cip = ethyl acetate fraction in combination with ciprofloxacin, Meth + Cip = methanol fraction in combination with ciprofloxacin, Cip=ciprofloxacin.

Table 4. Minimum Bactericidal Concentration of fractions of U.heudelotti in combination with ciprofloxacin (mg/ml).

Micro organisms	Hex + Cip	Chl+ Cip	Ethy + Cip	Meth +Cip	Cip
B. subtilis	1.5±0.2	3.125±0.2	6.25±0.2	6.25±0.2	12.5±0.2
P. mirabilis	3.125±0.2	3.125±0.2	3.125±0.2	3.125±0.2	6.25±0.2
S. aureus	3.125±0.2	6.25±0.2	6.25±0.2	6.25±0.2	12.5±0.2
K. pneumonia	3.135±0.2	3.125±0.2	3.125±0.2	3.12±0.2	6.25±0.2
S.pyogenes	3.125±0.2	1.5±0.2	3.125±0.2	3.125±0.2	6.25±0.2
S. typhi	6.25±0.2				12.5±0.2
E.coli	1.5±0.2	3.125±0.2	1.5±0.2	3.125±0.2	6.25±0.2

Key: Hex + Cip= hexane fraction in combination with ciprofloxacin, Chl + Cip = chloroform fraction in combination with ciprofloxacin, Ethy + Cip = ethyl acetate fraction in combination with ciprofloxacin, Meth + Cip = methanol fraction in combination with ciprofloxacin, Cip=ciprofloxacin.

The result of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the standard drug (3.125 and 12.5 mg/ml respectively) was significantly decreased (to 0.75 and 1.5 mg/ml respectively) when the fractions were in combination with standard drug ciprofloxacin Table 3 and 4.

DISCUSSION

All zones of inhibition of the fractions were more noteworthy at higher concentrations. This suggests that the action of the fractions increased with the increase of concentration. According to the obtained results, the combination of the fractions of *U. heudelotti* with ciprofloxacin antibiotic showed significant effects against the seven tested bacteria. Combined treatment of plant fractions with modern antibiotic agents have been accounted for bringing about a delay in the rise of microbe resistance, and furthermore in delivering synergistic impacts in the treatment of bacterial disease [25]. Drug synergism between known conventional antibiotics and bioactive plant fractions is a novel idea and could be gainful (synergistic) or pernicious (antagonistic) [26]. As the concentration of the fractions increased, synergistic activity though significant, decreased with the increase in the concentration of the fractions (Table 4). This could be attributed to the inability of higher concentration of plant fractions to diffuse through the nutrient agar medium.

This limitation in drug dissemination is a noteworthy challenge in the assessment of the antimicrobial impacts of plant concentrates when utilizing the agar diffusion method [27]. In this study, synergism impact, resulting from the blend of antimicrobial agent with fractions of this plant demonstrated a comparative pattern for all plant fractions. The result of the study is consistent with previous in vitro studies which reported synergistic effects with a significant reduction in MICs of the antibiotic drug due to the combination of the antibiotic drug with plant fractions [28]. Synergistic interaction between the drug and chloroform fraction means that their joint effect is stronger than the sum of effects of the individual agents. The result of this study shows that there is a decrease in the MIC when there is a combination of the fractions of *U. heudelotti* and standard drug against the tested microorganisms (Table: 9). This implies that the fractions increased the antibacterial activity of the antibiotics against the tested microorganism through synergistic interaction. Fractions of *U. heudelotti* tested, especially the chloroform fraction, showed powerful antibacterial activity against three tested organisms and combination with antibiotic showed synergistic activity.

This result may indicate that the chloroform fraction of *U. heudelotti* contains natural inhibitors working by different mechanisms or inhibitions. In conclusion, the result of this study was encouraging, although clinical studies are needed to define the real efficacy and possible toxic effect *in vivo*. The result of this experiment reveals that the combined use of plant fractions with antibiotics may be used to fight emerging drug-resistance problems.

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