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ANTIBACTERIAL ACTIVITY OF *Solenostemon rotundifolius* LEAF EXTRACT AGAINST SOME PATHOGENS ASSOCIATED WITH FOOD

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ABSTRACT
Food-borne diseases by bacterial contamination are major public health problems in Nigeria, especially by multidrug-resistant bacteria which could result in an increasingly growing problem of antimicrobial resistance. Therefore, there is need to find potentially effective, safer and natural antimicrobial alternatives against food poisoning bacteria. The antimicrobial activity of *Solenostemon rotundifolius* leaf extract was investigated against three Gram-negative pathogenic bacteria; Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa, two Gram-positive bacteria strains: *Enterococus fecalis* and *Staphylococcus* aureus. The antibacterial activity was evaluated by agar diffusion, minimum inhibition and bactericidal concentration methods. Results showed that *S. rotundifolius* leaf extract exhibited antimicrobial activity against both the gram-negative and gram-positive bacteria. The minimum inhibitory concentration of microbial growth ranged from 12.5 mg/ml to 50 mg/ml against the bacteria.

Keywords: Pathogenic Bacteria, Antimocrobial agent, Solenostemon rotundifolius and Food poisoning

Introduction

Bacterial pathogens are responsible for food borne diseases and cause other illnesses in man. These include: *Escherichia coli*, *Salmonella typhi*, *Enterococus fecalis, and Staphylococcus aureus*. Consumption of food contaminated with these pathogens results in gastrointestinal symptoms such as diarrhoea, nausea, abdominal cramps and vomiting. Bacterial diseases are major public health problems both in developing and developed countries, although, the global incidence of the disease is difficult to estimate. WHO (2007) reported that 1.8 million people died of diarrhoea in 2005, attributing a great proportion of the cases to consumption of contaminated food and drinking water. *Pseudomonas aeruginosa*, *S.typhi*and *E. coli are* common Gram-negative bacteria, while *E. fecalis* and *S. aureus* are the common Gram-positive bacteria.*P. aeruginosa* is found in the environment, mainly in soil and water. *S. typhi* and *E. coli* are rod-shaped, facultative anaerobic bacteria (Pui*et al.,* 2011; Lim *et al*., 2010). *Salmonella* is responsible for typhoid fever disease; common food-borne pathogens associated with fresh fruits and vegetables (Pui*et al.,* 2011). *Salmonella* was responsible for 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide (Pui*et al.,* 2011). *E. coli* are commonly found in the gut of warm-blooded animals, although most strains are harmless, but some strains can cause serious food-borne diseases. Cattle are said to be natural reservoir of pathogenic *E coli*, with an estimate of between 1% and 50% of healthy cattle carrying and shedding *E. coli* in their faeces, while other contaminated food vehicles of *E. coli* such as, vegetables are thought to have been contaminated through bovine faecal materials (Lim *et al* 2010). *P. aeruginosa* is an opportunistic microorganism, establishing itself in vulnerable patients, usually with compromised immunity. It affects various parts of the body, respiratory tract, central nervous system, urinary tract and the skin. *Enterococci* are part of the normal flora of humans and animals but some strains are known to cause serious diseases in humans. *Staphylococcal* food-borne disease is one of the most common, and results from contamination of food by preformed *Staphelococcus aureus* enterotoxins.

In livestock, antibiotics are used regularly for prevention of microbial infection and growth promotion. This prophylactic use of antibiotics in livestock has been associated with antibiotic- resistant organisms in the gut of farm animals and this resistance is thought to be transmitted to humans when such animals are consumed either through direct

infection with the resistant bacteria, or transfer of resistant gene to human pathogen (Chang *et al*., 2015). Hence, in 2006 the European Union ban on the feeding of non-therapeutic antibiotics of human importance to farm animals took effect (European Commission, 2005). Antimicrobial resistance is a big challenge facing mankind, and development of new and effective antimicrobials will help reduce the burden of antimicrobial resistance.

Herbal plants have rich phytochemicals with a long history of their antimicrobial properties in folklore medicine. *Solenostemon rotundifolius*(Common names: *Hausa potato*, *Frafra potato*, *Sudan potato*, *Zulu round potato*,*Coleus potato*, *Chinesepotato*) is a small herbaceous annual crop with a succulent stem (15-30 cm), thick leaves and pale violet small flowers. It has aromatic smell and small dark-brown tubers which cluster at the base of the stem. The tubers are believed to be the source of staple energy in the past but it is now being replaced by more starchy food, and only being consumed as a supplementary energy food, especially when income is lean (Enyiukwu *et al.*, 2014; Sugri *et al.*, 2013). Boiled leaves of *S. rotundifolius* is eaten as a pot herb and used in ethno-medicine for the treatment of dysentery, blood in urine and eye disorders including glaucoma (Enyiukwu *et al.*, 2014). The aim of this study therefore, is to determine the *in vitro* antibacterial activities against Gram-negative bacteria, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa;* Gram-positive bacteria, *E. fecalis* and *S. aureus* and their susceptibility to *S. rotundifolius* which is used traditionally for the treatment of dysentery. This study will contribute to on-going research efforts in search of effective antimicrobial agents with antibacterial activity,

Materials and Methods

Collection of plant materials

The leaves of *Solenostemon rotundifolius* were collected from the demonstration farm of National Root Crops Research Institute, Umudike, and identified by the Genetic Resource Unit of the Institute.

Preparation of plant extract

Dried pulverised plant materials were soaked in 90% ethanol for 72hrs and filtered through whatmann filter paper No 1. The filtrates were evaporated at temperature of 40ºC in a water bath and the extract was stored at room temperature until used.

Bioassay-guided fractionation of the ethanol extract

The method of Abbot and Andrew (1970) was used in the fractionation of the extract by column chromatography. Silica gel, 100-200 mesh, was used as the stationary phase, while gradient solvent system of combination of petroleum ether, chloroform and methanol was used as the mobile phase to elute the entire compounds from the sample. Thirty-nine fractions of 50ml each were collected. These were subjected to analytical thin-layer chromatography (TLC), and fractions with difference in retention factor (R_F), value of not more than 0.1 - 0.15 were pooled together. Five fractions were obtained after the pooling and were labeled: F1, F2, F3, F4 and F5.

Bacterial test strains

Bacteria strains of *Esherichia coli* (ATCC 25922 Gram negative), *P. aeruginosa* (ATCC 27853 gram negative), *S. aureus* (ATCC 25923 gram positive), *E.fecalis* (ATCC 7080 gram positive) and *S.typhi* (clinical isolate gram negative) were obtained from the Centre for Molecular Biosciences and Biotechnology, Michael Okpara University of Agriculture, Umudike. The bacteria test strains from nutrient agar slants were inoculated onto freshly prepared nutrient agar plates and incubated for 24 hours at 37ºC. A cell suspension of each microorganism was prepared by transferring 3-5 colonies from the nutrient agar plates to a sterile bottle containing physiological saline. The turbidity of the suspension was adjusted to 0.5 McFarland turbidity standard with sterile physiological saline.

Determination of antibacterial activity

The Kirby-Bauer disc diffusion technique (Biemer, 1973), was employed for antimicrobial testing. It determines the sensitivity of microorganisms to specific antimicrobial drugs; greater drug efficacy yields larger microbe-free zones surrounding drug-containing disks after overnight growth on solid media. Mueller-Hinton agar (MHA) was autoclaved for 15min at a temperature of 121ºC. For each test microorganism, 0.1ml of bacterial suspension was inoculated into the medium, mixed gently and poured into petri dish. Paper discs of 6mm in size obtained by perforating filter paper (Whatmann No.1) were sterilized in hot air oven at 140ºC for 1hr. Each paper disc was impregnated with 20μl of various concentrations of 1mg/ml, 10mg/ml and 100mg/ml of the respective plant extracts. The discs were dried in an incubator at 50ºC. Discs impregnated with Gentamicin served as the positive control while discs impregnated with dimethyl sulfoxide(DMSO) served as the negative control. The discs containing the extracts and the control were placed on the MHA plates, the plates incubated at 37ºC for 24hrs and the diameter of any clear zone obtained around the discs measured with a metre rule. For each concentration of an extract, three replicates were assayed.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an anti-microbial drug that prevents visible bacteria growth of a microorganism after overnight incubation in a media. MIC was determined using the broth dilution method as described by Andrews (2001). Briefly for each test microorganism to be studied, 126 test tubes containing 1ml of Mueller Hinton broth was set up in 7 rolls of 18 test tubes per roll. Exactly 1ml of each

plant extract, and fractions were added in triplicate to the test tubes in the 1st row and serially diluted row by row by transferring 1ml from each test tubes in the first row to each test tubes in the 2nd row. From each test tubes in the second row, 1ml was taken and serially transferred to the test tubes in the 3rd row, down to the last test tube of the 7th row from where 1ml was taken and discarded. The 1stto the 7th row contained concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.13mg/ml, 1.51mg/ml and 0.76mg/ml respectively. Similarly, the same procedure was carried out for gentamicin (positive control) during which 1ml of 0.005mg/ml of dissolved gentamicin was taken in triplicate into test tubes labelled antibiotic control containing 1ml of Mueller Hinton broth, 1ml was taken out and discarded. Two sets of negative control were prepared; the first set of three test tubes containing 1ml of Mueller Hinton broth and no plant extract was labelled as organism control and another three sets containing 1ml of Mueller Hinton broth and 1ml of DMSO labelled DMSO control. Each of the test tubes was inoculated with 50µl of the test bacterial suspension except the sterile control (containing only Mueller Hinton broth). All test tubes were incubated at 37ºC for 18hrs. MIC was recorded as the lowest concentration of each plant extract that inhibited the bacterial growth as detected by the absence of visual turbidity. The MIC values were confirmed by streaking the incubated culture tubes on freshly prepared nutrient agar plates. Scanty growth on the plate around the point of streak suggested MIC while complete absence of growth was indicative of MBC (Minimum bactericidal concentration).

Statistical Analysis

The software package used for data analyses was SPSS Version 20.0 (IBM SPSS Inc, Chicago, IL) and level of significance was estimated by One Way Analysis of Variance (ANOVA). Data were analysed using Duncan Multiple Range Test, and complemented with Student's t test for post-hoc test for comparisons of the means of the various doses and fractions. The probability level of less than 5% (($p<0.05$) was considered statistically significantly different between the test and control groups as well as among test groups for measured values.

Results and Discussion

Antibacterial activity of plants extract

The results of the anti-microbial activities of *S. rotundifolius* ethanol crude extract and sub-fractions obtained by the disc diffusion method are presented in Table 1. The antibacterial activity of the plants studied demonstrated clear zones of inhibition, followed by MIC and MBC values shown in Table 2and 3 respectively. The data obtained for the antibacterial activity of *S. rotundifolius* leaf is first reported to the best of our knowledge. The extract and the fractions showed different zones of inhibition at concentrations of 10 and 100 mg/disc against the tested 5 bacteria strains: *S. aureus*, *E. coli*, *E. fecalis*, *S. typhi* and*P. aeruginosa*. Their effects were comparable with that of gentamicin at 0.005mg/disc. The lowest concentration of *S. rotundifolius* leaf extract and sub-fractions capable of causing any visible inhibition of bacteria growth was 12.5 mg/ml given by F3 and F4 against *E. coli*, and by the crude extract, and F4against *S. aureus*. The MIC for *E. faecalis* growth was 25mg/ml produced by F3, F4 and F5, while *S. typhi* was at 25mg/ml given by F4. *P. aeruginosa* growth could only be inhibited by F5 at a minimum concentration of 50 mg/ml. However, the lowest concentration of the extract and fractions that could kill a microorganism was 25mg/ml and was caused by the crude extract, F3 and F4. The obtained antimicrobial result agrees with the results reported for other botanicals of lamiaceae family (Ilhan *et al.*, 2008; Sharma *et al*., 2013). The results of MIC and MBC of the plant extracts suggest that *S. rotundifolius* has the potential to control bacteria growth in food and gastroenteritis thereby validating the ethno-medicinal use of the plant in control of infectious diarrhoea. Therefore, they can be used to control diseases caused by bacterial food poisoning and can also be used as natural preservatives of food.

The gram positive bacteria were generally more sensitive to the extract than the gram negative bacteria. This observation agrees with the report of Kozlowska *et al*., (2015), on antimicrobial activity of some plants from Lamiaceae family. The antibacterial activity of *S. Rotundifolius* leaf extracts could be because of the high phenolic content of the extract. Harbor *et al.,* (2016), reported relatively high content of tannins and flavonoids. However, the antibacterial activity of phenols could be due to the interactions of the phenolic compounds with the bacterial cell surface (Bouarab-Chibane *et al*., 2019). Both tannins and flavonoids have been associated with antimicrobial activity. Plants synthesize flavonoids in response to microbial attack (Dixon *et al*., 1983), and these flavonoids are reported to be effective anti-microbial substances against a wide array of microorganisms (*in vitro*) studies (Xie, 2014). Flavonoids mechanism of antibacterial activity includes the alteration of bacterial cell membrane permeability and the inhibition of nucleic acid synthesis, cytoplasmic membrane and biofilm formation (Xie, *ibid*). Tannins ability to form complexes with proteins could be the basis for their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins. Tannins also form complexes with polysaccharides (Cowan, 1999).

Conclusion

The study analyzed the antibacterial activity of *Solenostemon rotundifolius* leaf extract against some pathogens associated with food. Results show that crude extract and sub-fractions of *S. rotundifolius* leaves has potentials as antimicrobial agents to control

growth of food borne and spoilage bacteria. However, in traditional use, medicinal plants are not used as single therapies, rather are used in combination with other herbal remedies, thereby acting in synergistic manner. We also recommend further assessment of the antimicrobial activity in combination with other herbal remedies as used in ethno-medicine.

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Table 1: Diameter zone of inhibition of *S. rotundifolius* against the tested pathogenic organisms

Conc. mg/disc	*S. aureus*	*E. coli*	*P. aeruginosa*	*E. fecalis*	*S.typhi*
1	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00
10	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00
100	10±0.20	10±0.54	0.00±00	8±0.55	7±0.5
Gentamicin (0.005mg/disc	20±0.50	19±0.02	21±0.025	20±0.03	19±0.02
DMSO	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00

Data are mean of three replicates (n=3)±standard error

Table 2: Results of minimum inhibitory concentration (MIC) of crude extract and fractions

Test Organisms	Crude (mg/ml)	F2 (mg/ml)	F3 (mg/ml)	F4 (mg/ml)	F5 (mg/ml)	F6 (mg/ml)
S. aureus	12.5	50	50	12.5	25	25
E. faecalis	50	50	25	25	25	50
E. coli	NI	50	12.5	12.5	25	NI
P. aeruginosa	NI	NI	NI	NI	50	NI
S. typhi	50	50	50	25	NI	NI

NI = no inhibition

Table 3: Minimum bactericidal concentration (MBC) of crude extract and fractions

Test Organisms	Crude (mg/ml)	F2 (mg/ml)	F3 (mg/ml)	F4 (mg/ml)	F5 (mg/ml)	F6 (mg/ml)	Genta-micin (mg/ml)	DMSO (10%)
S. aureus	25	50	50	25	50	50	10	NI
E. faecalis	50	50	50	50	50	50	10	NI
E. coli	NI	50	25	25	50	NI	10	NI
P. aeruginosa	NI	NI	NI	NI	50	NI	10	NI
S. typhi	50	50	50	50	NI	NI	10	NI

NI = no inhibition

Fig. 1: *Solenostemon rotundifoliusplant*
Source: *https://en.wikipedia.org/wiki/Plectranthus _rotundifolius*

Fig. 2: Growth inhibition of some tested food-borne bacterial strains caused by *S. rotundifolius leaf extract* (dark disc) and gentamicin control (white disc)