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COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *SOLANUM MELONGENA* (GARDEN EGG) AND *IPOMOEA BATATAS* SWEET (POTATO)

[1]ABIYE E., [2]RAPLONG H. H., [2]UDOKAINYANG A. D., [2]ABDULLATEEF H., [2]GUSHE D., [2]MAIMADU A., [2]OVYE M., [2]OKEZIE C. M., [2]DAKUP P. G., [2]MALI M. B., [2]IBRAHIM D., [2]ISA S. AND *[2]BATA S. I.,

[1]National Veterinary Research Institute Vom, Plateau State Nigeria.
[2]Federal College of Animal Health and Production Technology, Vom, Plateau State Nigeria

*Corresponding Author: Bata, S. I drshalangs2014@gmail.com +234 803 413 2995

ABSTRACT

An in-vitro antibacterial activity of aqueous and ethanolic extracts of *Ipomoea batatas* (sweet potato) and *Solanum melongena* (garden egg) leaf against some pathogenic bacteria was evaluated using agar well diffusion assay. Commercial ciprofloxacin (10µg/ml) was used as the positive control. The sweet potato leaf extracts even at high concentrations did not show any inhibitory activity on any of the test bacteria. On the other hand, the garden egg aqueous leaf extract showed antibacterial activity (mean zone of inhibition of 24.0±0.58) against *Escherichia coli*, *Salmonella gallinarum* and *S. aureus* and was not significantly different in activity when compared with ciprofloxacin (33.0±6.9) at concentration of 400mg/ml (p=0.076). The zone of inhibition produced by garden egg leaf extract decreased as the concentration of the extract decreased. The garden egg aqueous extract showed activity against all the test pathogens with average minimum inhibitory concentrations (MICs) ranging from 75mg/ml-150mg/ml across the bacteria with the highest concentration being against *S. aureus*. The result showed that the MIC for the control ranged between 0.156 and 5µg/ml. Below concentration ranges of 0.156 and 5 µg/ml the control did not show activity against *E. coli* and *Salmonella gallinarum* respectively. Even at 3000mg/ml there was no bactericidal activity against *E. coli and S. gallinarum* by the *Solanum melongena* leaf extract. The control however showed bactericidal activity at lower concentration of 1.25µg/ml for *E. coli* and 0.0391 for *S. gallinarum*. The activities of aqueous leaf extract of *Solanum melongena* on some bacterial pathogens as shown in this study support the local use of the plant in traditional therapy for diseases caused by the pathogens.

Keys: Antibacterial, concentration, inhibitory, *Solanum melongena*, *Ipomoea batatas*

INTRODUCTION

The use of medicinal plants in the treatment of ailments is a common practice in Africa and is usually the first health recourse for about 80% of the world population (Sofowora, 1993; Ordu *et al*., 2018). Plants that are medicinal are rich in compound that may potentially be natural drugs which may serve as sources of alternative, affordable and safe antibacterial treatment for common diseases. Secondary metabolites such as tannins, flavonoids, alkaloids, terpenoids, quinines, coumarins and saponins present in plants have been found to act on pathogens (Das *et al*., 2010; Bansode and Chavan, 2012). The concentration of bioactive substances or active principle of plants used for therapeutic purposes varies with the different parts of the plants. The concentrations of most bioactive metabolites are often high in some parts such as leaves and root (Nasir *et al.,* 2005).

Bacteria acquire and transmit resistance against some therapeutic agents. This has resulted in production of many new antibiotics by pharmaceutical companies in the last three decades (Ordu *et al*., 2018). The development of antibiotic resistant bacteria stems from a number of factors, including inappropriate use of antibiotics in human and animal health and their prolonged use as growth promoters at sub-clinical doses in poultry and livestock production (Elisha *et al*., 2017). Herbal drugs have been used since ancient times as remedies for various diseases across the world (Darsanaki and Parsa-Lisa, 2014). There has been an inadequate primary health care and veterinary service delivery in many rural parts of the world (Elisha *et al*., 2017) accounting for the sustained use of traditional medicine to treat both humans and animals. Even where the orthodox medicines are readily available, a large percentage of the population still use herbal remedies along with or in preference to conventional medicines (McGaw and Eloff, 2008).

Emergence and reemergence of pathogens with increasing rate of antibiotic resistance and the failure of chemotherapy has led to efforts being directed at the use of new natural antibacterial agents for treating infectious disease (Fankam *et al.,* 2014). *Solanum melongena* (Garden egg) belongs to the family solananceace, it is a perennial crop which contains alkaloids which are usually bitter in taste and is characterized by powerful physiologic activity. The plant has no known side effect except for those allergic to it. *Ipomoea batatas* commonly known as potatoes is a dicotyledonous plant that belongs to the morning glory family (Mohanraj and Sivasankar, 2014). It is to a great degree adaptable and delicious vegetable that possesses high nutritional value. The plant is also considered valuable for its medicinal use having anti-cancer, antidiabetic, and anti-inflammatory activities (Mohanraj and Sivasankar, 2014).

Antibacterial activity of some medicinal plants such as *S. melongena* and *Ipomoea batata* needs to be investigated against some bacteria (*Salmonella, Escherichia coli* and *Staphylococcus aureus*) to validate the traditional use as antibacterial agents. This study was therefore carried out to evaluate the antibacterial activity of Ethanolic and aqueous extracts of sweet potato *(Ipomoea batatas)* leaf and the aqueous extracts of garden egg (*Salanum melongena*) on some pathogenic bacteria.

Methodology

Ethical considerations

The study was approved by the Federal College of Animal Health and Production Technology, Vom, Project committee. The test procedure was not carried out on animals or humans and so did not require ethical permit.

Collection and preparation of the plant materials

The leaves of *S. melongena* and *I. batatas* were collected between the month of March and April from Chaha Fadama, Jos-South Local Government Area of Plateau State, Nigeria. The leaves were examined and those attacked by insects or microbes were removed. The harvested leaves were washed and dried at room temperature indoors. After drying, the samples were pulverized using mortar and pestle and sieved using gauze cloth as described by Aremo *et al.,* 2006. The powder was stored in tightly closed glass containers in the dark at room temperature.

Plant Extraction

Extraction was carried out as described by the Association of Official Analytical Chemists (AOAC). Briefly, 50g of the pounded leaves of sweet potatoes and garden egg were dissolved into 500ml distilled water. The process was repeated using absolute ethanol and the mixtures were allowed to stand on the bench for 24hours. Cotton wool was used to filter the mixtures through a funnel. The filtrates were then dried in hot air oven at 45^{o}C for 72hours.

Phytochemical Screening

The phytochemical constituents of the ethanolic and aqueous extracts (alkaloid saponin, tannin, anthraqiunones, flavonoids, cardiac glycoside, resin and steroidal rings) were determined as described by Sofowora, 1993.

Test Bacteria.

The test organisms consisted of the standard strains of Gram-positive bacteria-*Staphyloccus aureus* and Gram-negative bacteria -*Escherichia coli* and *Salamonella gallinarum*. The test organisms were collected from the Central diagnostic division of the National Veterinary Research Institute, Vom. All the bacterial strains were sub-cultured from the original culture, stored at −70°C and maintained on Müller-Hinton (MH) agar plates at 4°C, and grown at 37°C when required.

Growth of test bacteria

The bacteria were grown at 37^{o}C for 24 hours in nutrient both, 10µg/ml Ciprofloxacin was used as a standard antibiotic for in-vitro activity.

Determination of antibacterial activity

Ten micrograms per milliliter (10µg/ml) ciprofloxacin was used as a standard antibiotic for in-vitro antimicrobial activity using Agar well diffusion method.

Preparation of plant extracts concentrations

Different concentrations of the plant extract, 4000 mg/ml, 3000mg/ml and 1000mg/ml were prepared by dissolution in sterile distilled water.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the leaf extract of sweet potato was determined using the broth micro dilution method as described by Eloff, 1998. Serial dilution of the extracts was obtained thus; 10mg/ml, 5mg/ml, 2.5mg/ml, 1.5mg/ml, 0.623mg/ml i.e. 0.313mg/ml, 0.156mg/ml, 0.0782mg/ml, 0.0391mg/ml and 0.095mg/ml respectively. The extracts were added unto sterile 20-well micro-titre plates containing 100 µl nutrient broth medium inoculated with 10^{5}cfu/ml bacterial cells. Distilled water and 10µg/ml ciprofloxacin served as the negative and positive control respectively. After 24hours it was incubated at 37^{o}C, the minimum inhibitory concentration was determined as the lowest concentration that completely inhibited the growth of the bacteria. The assay was repeated twice with two replicates for the extract against the individual bacterial species of all the test concentrations. Iodonitrotetrazolium Chloride (20 µl, 0.2mg/ml, INT, Sigma-Aldrich) was added to the test wells at the

completion of the incubation period, and was further incubated at 37°C for 3hours. The presence of viable bacteria was determined based on the dye changing color from yellow to pink.

Minimum Bactericidal Concentration of the Extract.

The minimum bactericidal concentration (MBC) of the leaf extracts of sweet potato and garden egg were determined by withdrawing 20µl of the bacterial broth suspension that showed no color change, and it was spread onto nutrient agar plates, this was followed by incubation at 37°C for 24-48hours. The lowest concentration of the extract at which no bacterial growth was observed, was considered as the MBC.

Data analysis

The differences between the mean of the zone of inhibition of the plant extract and the control was determined using analysis of variance (ANOVA). P-values less than 0.05 were considered statistically significant. Results were presented in tables and charts.

RESULTS

Phytochemical composition of the ethanolic and aqueous extracts of sweet potatoes leaves and garden egg

The result showed presence of saponins, tannins, cardiac glycosides, steroids /terpenes, in the chemical composition of the ethanolic and aqueous leaf extracts of sweet potatoes and garden egg. Alkaloid, anthraquinone and resins were not present in the aqueous extract, while anthraquinone and resins were not present in the ethanolic extract of sweet potatoes. Only resins were absent in the aqueous leaf extract of garden egg while alkaloid, anthraquinone and resins were absent in the ethanolic extract (Table 1 and 2 respectively).

Activity of sweet potatoes leaf extracts on test organisms

The sweet potatoes leaf extracts (aqueous and ethanolic) did not inhibit bacteria growth. This therefore suggests that the sweet potato leaf extract does not have antibacterial activity on the test organism (Table 3).

Activity of garden egg leaf extract on test organism (zone of inhibition)

All the pathogens namely *E. coli*, *S. gallinarum*, *S. aureus* showed susceptibility to ciprofloxacin with zone of inhibition of 44, 32, and 20mm respectively. Only the garden egg aqueous leaf extract showed antibacterial activity against *Escherichia coli*, *S. gallinarum* and *S. aureus* similar with activity observed with ciprofloxacin at concentration of 400mg/ml based on the zones of inhibition that classified antibiotics into susceptible, intermediate and resistant (p=0.076) (Table 4).

The garden egg aqueous extract was active against all the pathogens with average MICs ranging from 75mg/ml-150mg/ml across the bacteria with the highest concentration against *S. aureus* (Table 5). The result showed that the MIC for the control ranges between 0.156 to 5µg/ml. Below 0.156 to 5 µg/ml the control did not show activity against *E. coli* and *Salmonella gallinarum* respectively (Table 6)

Even at 300mg/ml there was no bactericidal activity against *E. coli and S. gallinarum* by the *Solanum melogena* leaf extract (Table 7). The control however showed bactericidal activity at lower concentration of 1.25µg/ml for *E. coli* and 0.0391µg/ml for *S. gallinarum* (Table 8)

Table 1: Phytochemical constituents of aqueous and ethanolic extracts of sweet potato leaf

Constituent	Aqueous	Ethanolic
Saponins	+	+
Tannins	+	+
Cardiac glycoside	+	+
Flavonoids	+	+
Alkaloid	-	+
Steroids/Terpenes	+	+
Anthraquinone	-	-
Resins	-	-

Key: +ve = Present, -ve = Absent

Table 2: Phytochemical constituents of aqueous and ethanolic extracts of garden egg

Constituent	Aqueous	Ethanolic
Saponins	+	+
Tannins	+	+
Cardiac glycoside	+	+
Flavonoids	+	+
Alkaloid	+	-
Steroids/Terpenes	+	+
Anthraquinone	+	-
Resins	-	-

Key: +ve = Present -ve = Absent

Table 3: Activity of sweet potato (*Ipomoea batata*) ethanolic and aqueous extracts on test organisms

	Concentration (mg/ml)				
	Zone of Inhibition(mm)				
Organism	400	300	200	100	Control
E. coli	0.00	0.00	0.00	0.00	44
S. gallinarum	0.00	0.00	0.00	0.00	32
S. aureus	0.00	0.00	0.00	0.00	20

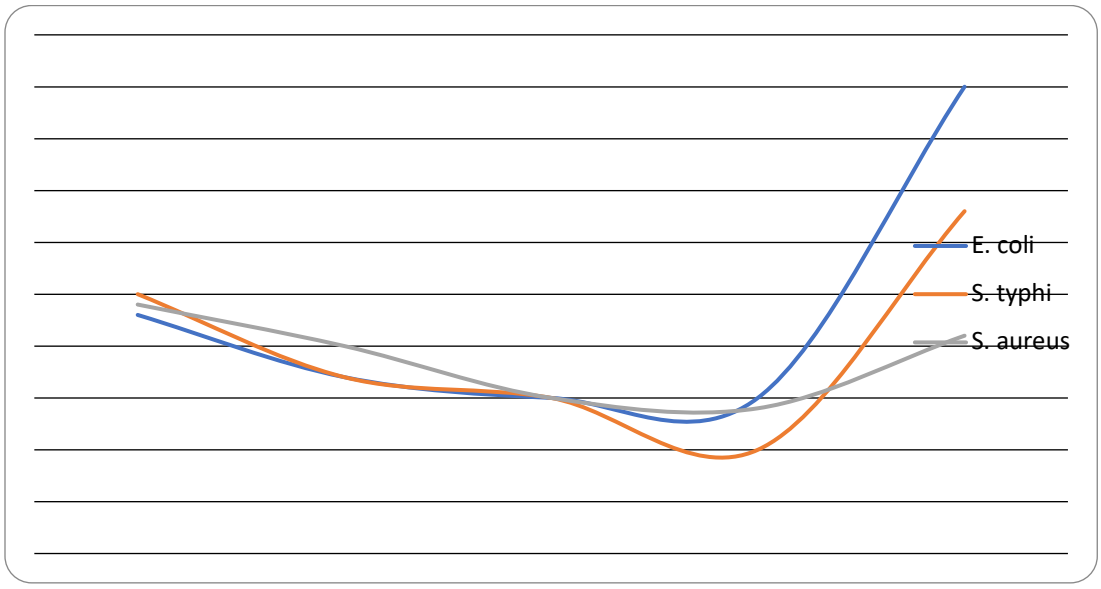

Fig 2: Activity of *Solanum melongena* aqueous leaf extract against the test bacteria based on the diameter of the zone of inhibition versus the control (ciprofloxacin)

Table 4: Activity of *Solanum melongena* (garden egg) aqueous extract on test organisms

	Concentration (mg/ml)				
	Zone of Inhibition(mm)				
Organism	400	300	200	100	Control
E. coli	23	17	15	15	45
S. gallinarum	25	17	15	10	33
S. aureus	24	20	15	14	21

Key: Control (Ciprofloxacin 10µg/ml) = or > 21mm (Susceptible) 16-20mm (Intermediate) < or = 15 (Resistant)

Table 5: Minimum inhibitory concentration of *S. melongena* aqueous leaf extract

	Concentration (mg/ml)									
	Zone of Inhibition(mm)									
Organism	300	200	150	100	75	50	25	12.5	6.25	3.125
E. coli	-	-	-	-	µ	+	+	+	+	+
S. gallinarum	-	-	-	µ	+	+	+	+	+	+
S. aureus	-	-	µ	+	+	+				

Key: + = Growth, - = no growth, -µ = MIC

Table 6: Minimum Inhibitory concentration of the control (ciprofloxacin)

~~Conc mg/ml~~ **Organism**	~~10~~	~~5~~	~~2.5~~	~~1.25~~	~~0.623~~	~~0.313~~	~~0.156~~	~~0.0782~~	~~0.0391~~	~~0.0195~~
Escherichia coli	-	-	-	-	-	-	µ	+	+	+
S. gallinarum	-	µ	+	+	+	+	+	+	+	+

Key: + = Growth, - = no growth, -µ = MIC, MIC = < 0.0198

Table 7: Minimum bactericidal concentration of *S. melongena* aqueous leaf extract on some bacteria

Conc mg/ml Organism	300	200	150	100	75	50	25	12.5	6.25	3.125
E. coli	+	+	+	+	+	+	+	+	+	+
S. galinarum	+	+	+	+	+	+	+	+	+	+
S. aureus	-	-	+	+	+	+	+	+	+	+

Key: + = Growth, - = no growth, Control (Ciprofloxacin 10µg/ml)

Table 8: Minimum bactericidal concentration of the control (Ciprofloxacin)

Conc mg/ml Organism	10	5	2.5	1.25	0.623	0.313	0.156	0.0782	0.0391	0.0195
E. coli	-	-	-	+	+	+	+	+	+	+
S. gallinarum	-	-	-	-	-	-	-	-	-	+

Keys: + = Growth, - = no growth

DISCUSSION

This study was carried out to investigate the antibacterial activity of ethanolic and aqueous extracts of *Ipomoea batatas* leaf and *S. melongena* on some pathogenic bacteria. The study revealed that sweet potatoes, both the ethanolic and aqueous leaf extracts had no activity against the test bacteria while the garden egg leaf showed activity at different concentrations. The lack of activity of leaf extracts of sweet potatoes may be attributed to the absence of some of the phytochemicals that play a role and will require further investigation. The garden egg aqueous leaf extract has shown a diameter of zone of inhibition against the pathogens comparatively similar in sensitivity with ciprofloxacin at higher concentration. The low values suggesting intermediate or resistance may be due to the fact that the plant extract is in its crude form therefore having small amount of the bioactive compounds. Studies by Ogbeche *et al*., 1997; Akinyemi *et al*., 2005 reported similar range of values confirming the antibacterial potentials of the plant.

All the pathogens namely *E. coli*, *S. gallinarum*, *S. aureus* showed susceptibility to ciprofloxacin with zone of inhibition of 44, 32, and 20mm respectively. The acceptable standard diameter zone of inhibition for sensitive organism for the ciprofloxacin used as control is >21mm (NCCLS, 1993). The MBC values obtained for the garden egg leaf extract against the pathogens are higher than MIC. Even at 300mg/ml the garden egg leaf extract did not sho w bactericidal activity suggesting that the leaf extract is bacteriostatic at lower concentrations and bactericidal at much more higher concentrations. Because concentrations of the traditional preparations are not specific or determined this may account for the large quantity of the extracts being recommended by the medical traditional practitioners

Conclusion

Garden egg aqueous leaf extract has showed antibacterial activity while sweet potatoes

aqueous and ethanolic leaf extracts did not show any antibacterial activity in this study. The activities of aqueous leaf extract of *Solanum melongena* on some bacterial pathogens as shown in this study support the local use of the plant in traditional therapy.

RECOMMENDATIONS

Further study that will investigate the in-vivo activity, safety margin of *S. melongena* leaf is recommended

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Authors' contribution

BSI, AE and HR conceived and designed the research. BSI, AE, OMC, DPG, MMB, DI and GD carried out the field and laboratory analysis. UAD, AH, BSI, OMC, DPG, MMB, DI and IS produced the results in tables and figures. BSI produced the first draft of the manuscript. HR and MA read the review of the first draft. The final draft was read and approved by all authors.

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