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In-Vitro Antibacterial Activity of Leaf and Stem Extract of *Passiflora edulis* (Passion Fruit) Planted in Federal University of Agriculture Makurdi, Central Nigeria

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Abstract: ***The organic extracts of Passsiflora edulis were tested for antibacterial activity against clinically important Gram positive and Gram negative bacteria viz: Staphylococcus aureus, Bacillus spp, Streptococcus spp, Escherichia coli, Salmonella spp and Shigella spp. The in vitro antibacterial activity was performed by disc diffusion method. The ethyl acetate leaf extract inhibited the growth of S. aureus, Bacillus spp,, Salmonella spp and Shigella spp, excellently. The ethanolic stem extract also showed antibacterial activity against S. aureus, E. coli and Shigella spp while the ethyl acetate leaf extract showed higher spectrum of antibacterial activity as compared to the ethanolic stem extract both at higher concentrations. Passiflora edulis plant could serve as a better source of antibacterial agent.***

Keywords: Antibacterial activity, Leaf and Stem extract, *Passiflora edulis,* Central Nigeria

1. Introduction

Passion flower is also known as maypop, apricot vine, passion vine and granadilla. It grows as much as 30 feet (10cm) tall, with a thick woody stem, vines (passiflora) have a genus of about 400 species of flowering plants and the largest in the family of *Passifloraceae* (Benica, 2007). They are mostly vines with some being shrubs and a few species being herbaceous. The species of this genus are distributed in the warm temperate and tropical regions of the world, but they are most rare in Asia, Australia and tropical Africa (Ingale and Hivrale, 2010). Species of *Passiflora* have been naturalized beyond their native ranges. Some of the species are noted for their showy flowers which includes; *P. incarnate, P edulis, P alata, P. laurifolia* and *P. quadrangularis* (Patil and Paikrao, 2012).

The passion flower was discovered in 1569 by Spanish explorers in Peru, who saw the flowers as symbolic of the passion of Christ and therefore a sign of Christ's approval of their efforts (Tyler *et al*., 1987). This is the origin of the scientific and common name. The folklore surrounding this plant possibly dates further into the past (Tyler, 1987). The floral parts are thought to represent the elements of the crucifixion (3 styles represent 3 nails, 5 stamens for the 5 wounds, the corona as the crown of thorns, the petals representing the 10 true apostles, with the white and bluish-purple colours those of purity and heaven) (Chevallier, 1996; Tyler, 1987). In Europe passion flower has been used in homeopathies medicine to treat pain, insomnia related to neurasthenia or hysteria, nervous exhaustion, epilepsy, tetanus, muscle spasms and leaves presented anxiolytic activity (Yuldasheva *et al*., 2005).

The genus *Passiflora* belongs to *Passifloraceae* family and includes passion fruit (Patil and Paikrao, 2012). It is the largest and the most widespread genus of tropical flora, about 21 subgenera (Cronquist, 1981). More than 350 species have been found in tropical regions and rain forests of South America and 60 of them are edible. Passion fruit is an important fruit in many exotic and subtropical countries due to its edible fruits, ornamental use and medicinal properties (Patil and Paikrao, 2012). Some species of *P. edulis, P. quadrangularis* and *P. lugularis,* are chiefly cultivated for the production of fruit juice (Patil, 2012). *P. incarnate* is reputed for its sedative properties and several other species are known for their ethno botanical use (Abascal *et al*., 2004).

Several species have edible fruits and attractive flowers about 40 species have been cultivated, but fewer than 6 are fruit crops in the neotropics and only *P. edulis* is economically important. A few species such as *P. foetida* and *P. tripartite* are recorded as weeds in distinctive parts of the world. Both *P. foetida* and *P. tripartita* are closely related taxonomically, whereas *P. edulis* belongs to a different subgenus (Waage *et al*., 1981).

The aim of this study is to determine the in vitro antibacterial activity of leaf and stem extract of *Passiflora edulis.*

2. Materials and Methods

Collection of Plant Materials

Fresh leaves and stem of *Passiflora edulis* were collected from the nursery bed from the Federal University of Agriculture Makurdi. Benue State. Central Nigeria.

Preparation of the Extract

The fresh leaves and stem of the plant were dried in the Biological Sciences laboratory of the University for a total of ten (10) days, ground into fine powder and stored in a glass container at 4^{o}C until ready for use.

Ethanolic Extract

Fifty grammes (50g) of the powdered leaves and 30 grammes of powdered stem was then weighed and poured

into a round bottom conical flask. 300mls of ethanol a solvent was measured and poured into the conical flask containing the sample as well. This was allowed to stay for 72 hours and was thoroughly shaken intermittently. After 72 hours the mixture was filtered into a beaker. The filtrate in the beaker was concentrated to dryness on a water bath at a temperature of 100°C (steam temperature). The beaker containing the dried dark brown extract was then covered with non-adsorbent cotton wool and aluminum foil and was then stored in the refrigerator at 4°C until required for use.

Ethyl Acetate Extract

Also 50g of the powdered leaves and 30g of powdered stem was weighed and poured into a conical flask and 300ml ethyl acetate was measured using measuring cylinder and poured into the conical flask containing the sample. It was left to stay for 72 hours and then thoroughly shaken intermittently. The mixture was filtered after 72 hours and the filtrate concentrated to dryness at a temperature of 100°C in a water bath. The extract was covered with non-adsorbent cotton wool and stored in refrigerator at 4°C until needed.

Extraction with Water

Fifty grammes (50g) of the powdered leaves and 30g of powdered stem was soaked in 300mls of sterile distilled water in flat bottom flask and allowed to stand for 24 hours covered with cotton wool to prevent contamination and to obtain higher yield of concentration. The mixture was filtered after shaken continuously for some time and filtrate dried on a water bath at a temperature of 100°C to water extract (dark brown in colour). The extract was stored in the refrigerator at 4°C until required for use.

Source of Test Organism

Bacterial isolates of pure cultures of Gram-positive bacteria (*Staphylococcus aureus, Streptococcus* spp. and *Bacillus* spp.) and Gram-negative bacteria (*Eschericha coli, Salmonella* spp. and *Shigella* spp.) were obtained from Tosema Specialist Diagnostic Laboratory, High Level Makurdi. Benue State.

Preparation of Media

Twenty eight grams (28g) of the powder was suspended and added to 1 liter of deionised water and was allowed to soak for 10 minutes. It was swirled to mix and then sterilized by autoclaving at 121°C for 15 minutes (equivalent heat process), cooled to 47°C and mixed well before pouring plates.

Sterilization of Glass Wares

All glass wares (test tubes, pipettes, petri dishes universal bottles and conical flask) were sterilized in an autoclave at 121°C for 15 minutes. Inoculating wire loop was sterilized under hot flame from a Bunsen burner and then cooled before each use.

Preparation of Concentration of Extract

Stock solution of the extracts (stem and leaf) was prepared by weighing 1g and 3g respectively of each extract using electronic weighing machine. The known weight of each extract was dissolved in 10ml of dimethylsulfoxide in a sterile sample beaker to obtain a stock solution of concentration 100 and 300mg/ml. The stock solution was labeled appropriately and stored in the refrigerator at 4^0C until required for use.

A standard antibacterial agent ciprofloxacin at a concentration 10mg/ml was used as positive control on the entire bacterial organisms and their zones of inhibition, if any was compared with those of the extract.

Determination of Antibacterial Activity

The antibacterial activity of the various extract was determined using disc diffusion method described by the National Committee for Clinical Laboratory Standard (NCCLS, 1993). The disc was prepared with sterilized filter papers (Whatman No.1 6mm in diameter) soaked in different concentration 100 and 300mg/ml of the extracts and then dried at 50°C for 30 mins.

Overnight cultures of each bacterial isolates were diluted using sterile normal saline to give an inoculum concentration size of about 10cfu/ml. The inocula were spread on the surface of the dried nutrients agar plates with cotton wool swabs, which had been dipped in the diluted suspension of the organism. The plates were then incubated at 35°C for 24 hours to allow the growth of the organism before the disc was further placed aseptically on the culture. The treated plates were again incubated at 37°C for another 24 hours. The same procedure was repeated without using 10mg/ml of ciprofloxacin. The plates without antibiotic or extract disc were set up as the negative control. The zones of inhibition above 6mm diameter of each isolate were used as a measure of susceptibility (positive reading) to the extracts that was compared to that of standard antibiotic (positive control).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of both extract was determined by broth dilution method described by Ingraham *et al.* (1995) and Chessbrough (2000) with slight modification.

In this method seven (7) test tubes labeled 1 to 7 were used for test organism on each extract. The seven (7) sterile test tubes were arranged in six rows in a test tube rack, each row for one of the six microorganisms that were positive to the antibacterial sensitivity test. 5ml of the sterile nutrient broth was pipette into all the test tubes. Thereafter, 5ml of the stock solution 100mg/ml of stem extract and 300mg/ml of leaf extract was transferred aseptically using sterile pipette into the first tubes of the six rows and mixed thoroughly to obtain concentrations of 50 and 150mg/ml. A serial dilution of the solution was then made in each row from test tube 1 to 5 to obtain concentrations of 25, 12.5 and 6.25mg/ml for stem extract and 75, 32.5 and 16.25mg/ml respectively. Test tube 6 and 7 served as control. Test tube 6 contained 2ml of ethanol while test tube 7 contained only 0.1ml of the test organism. The test tubes were covered with non-absorbent cotton wool and incubated at 37°C for 18 to 24 hours. After incubation, the Minimum Inhibitory Concentration (MIC) of the extracts on the test organisms was read by observing the tubes for turbidity.

Minimum Bactericidal Concentration (MBC)

The contents of the Minimum Inhibitory Concentration (MIC) tubes in the serial dilution were sub-cultured into

approximately labeled nutrients agar plates by dipping a sterile wire loop into each test tube and streaking the surface of the nutrient agar plates. The plates were then incubated at 27°C for 24 hours after which they were observed for colony growth. The lowest concentration of the sub-cultured plates with no growth was considered as minimum bactericidal concentration.

3. Results

Antibacterial Studies

The result of the antibacterial activity of the ethanolic, ethyl acetate and water extracts of *P. edulis* stem is presented in table 1. The water extract did not inhibit the growth of any of the bacteria tested. Also there was no antibacterial activity with the ethyl acetate of the extract used. However, ethanolic extract showed antibacterial activity on some of the tested organism. It inhibited the growth of *Staphylococcus aureus, Escherichia coli* and *Shigella* spp. with highest susceptibility recorded at highest concentration of 300mg/ml.

Table 2 presents the antibacterial activity of water, ethyl acetate and ethanolic leaf extract of *P. edulis.* The result showed that some extracts inhibited the growth of some microorganisms while others did not. The ethyl acetate extract of *P. edulis* showed considerable activity on *Staphylococcus aureus, Bacillus spp. Salmonella* spp. and *Shigella* spp. with the highest antibacterial activity exhibited by *Salmonella* spp. at 300mg/ml concentration. There was no antibacterial activity with water and ethanolic extracts.

Table 1: Antibacterial activity of Organic (ethanolic and ethyl acetate) and water extracts of stem of *Passiflora edulis.*

Plant parts	Solvent used	Concentration (mg/ml)	Diameter of inhibition zone					
			Staphylococcus aureus	*Bacillus spp.*	*Streptococcus spp.*	*Escherichia coli*	*Salmonella spp.*	*Shigella spp.*
Stem	Ethanol	100	9	-	-	7	-	6
		300	11	-	-	9	-	7
	Ethyl acetate	100	-	-	-	-	-	-
		300	-	-	-	-	-	-
	Water	100	-	-	-	-	-	-
		300	-	-	-	-	-	-
Standard antibiotic	Ciprofloxacin	10	35	25	-	32	37	28

Key: - = Resistant to test organism
Figures represent zones of inhibition = sensitive to test organism at ≥ 6mm

Table 2: Antibacterial Activity of Organic (ethanolic and ethyl acetate) and water extracts of leaves of *Passiflora edulis.*

Plant parts	Solvent used	Concentration (mg/ml)	Diameter of inhibition zone					
			Staphylococcus aureus	*Bacillus spp.*	*Streptococcus spp.*	*Escherichia coli*	*Salmonella spp.*	*Shigella spp.*
Leaves	Ethanol	100	-	-	-	-	-	-
		300	-	-	-	-	-	-
	Ethyl acetate	100	7	7	-	-	9	4
		300	9	11	-	-	12	6
	Water	100	-	-	-	-	-	-
		300	-	-	-	-	-	-
Standard antibiotic	Ciprofloxacin	10	35	25	-	32	37	28

Key: - = Resistant to test organism
Figures represent zones of inhibition = sensitive to test organism at ≥ 6mm

The Minimum Inhibitory Concentration (MIC) of ethyl acetate leaf extracts is presented in table 3. The MIC for *Staphylococcus aureus and Bacillus* spp. was found to be 75mg/ml while that of *Salmonella* spp. *and Shigalla* spp. was 32.5 respectively after turbidity test was carried out. *Salmonella* spp. and *Shigella* spp. were found to show the least MIC at a relatively lower concentration of 32.5mg/ml when compared to that of *S. aureus* and *Bacillus* spp.

The MIC of ethanolic stem extract of *P. edulis* presented on table 4 contains *S. aureus, Escherichia coli* and *Shigella spp.* representing 25mg/ml for each.

The minimum bactericidal concentration (MBC) of ethyl acetate leaf extract of *P. edulis* is presented in Table 5. The MBC of *S. aureus, Bacillus* spp, *Salmonella* spp. and *Shigella* spp. was found to be 150, 150, 75 and 75mg/ml respectively. Whereas the MBC of ethanolic stem extract shown on Table 6 was found to be 50mg/ml for each of *S. aureus, E. coli,* and *Shigella* spp.

Table 3: The minimum inhibitory concentration of ethyl acetate leaf extracts of *Passiflora edulis*

Test organisms	Concentration of extract (mg/ml)					Control		MIC
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	
	300	150	75	32.5	16.25	Negative	Positive	
Staphylococcus aureus	-	-	-	+	+	-	+	75
Bacillus spp.	-	-	-	+	+	-	+	75
Salmonella spp.	-	-	-	-	+	-	+	32.5
Shigella spp.	-	-	-	-	+	-	+	32.5

Key: + = Turbidity (growth of organism)
- = Inhibition

Table 4: The minimum inhibitory concentration of Ethanolic stem extracts of *Passiflora edulis*

Test organisms	Concentration of extract (mg/ml)					Control		MIC
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	
	100	50	25	12.5	6.5	Negative	Positive	
Staphylococcus aureus	-	-	-	+	+	-	+	25
Escherichia coli.	-	-	-	+	+	-	+	25
Shigella spp.	-	-	-	+	+	-	+	25

Key: + = Turbidity (growth of organism)
- = Inhibition

Table 5: Minimum bactericidal concentration (MBC) of ethyl acetate leaf extract of *Passiflora edulis*

Test organisms	Concentration of extract (mg/ml)					Control		MBC
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	
	300	150	75	32.5	16.25	Negative	Positive	
Staphylococcus aureus	*	*	-	+	+	-	+	150
Bacillus spp.	*	*	-	+	+	-	+	150
Salmonella spp.	*	*	*	-	+	-	+	75
Shigella spp.	*	*	*	-	+	-	+	75

Key: + = Turbidity (growth of organism)
- = Inhibition
* Lysed (Killed organism)

Table 6: Minimum bactericidal concentration (MBC) of ethanolic stem extract of *Passiflora edulis*

Test organisms	Concentration of extract (mg/ml)					Control		MBC
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	
	100	50	25	12.5	6.5	Negative	Positive	
Staphylococcus aureus	*	*	-	+	+	-	+	50
Escherichia coli.	*	*	-	+	+	-	+	50
Shigella spp.	*	*	-	+	+	-	+	50

Key:
+ = Turbidity (growth of organism)
- =Inhibition
* = lysed (killed organism)

4. Discussion

From the study, the ethyl acetate and ethanolic organic solvent extracts of *P. edulis* has some effects on some Gram-positive and Gram-negative bacteria. The ethyl acetate leaf extract was found to inhibit the growth of *S. aureus, Bacillus spp., Salmonella spp. and Shigella* spp. excellently. The ethanolic stem extract also showed antibacterial activity against *S. aureus, E. coli* and *Shigella* spp. The ethyl acetate leaf extract showed higher spectrum of antibacterial activity as compared to the ethanolic stem extract both at higher concentrations.

The result of this study showed that *P. edulis* and its extracts (solvent) have broad spectrum and magnitude of activity at higher concentration of the extract. Similar study carried out by Mohanasundari *et al.* (2007) on antibacterial properties of *Passiflora foetida* L. found that the ethanolic leaf extract exhibited variable degrees of antibacterial activity against *P. putida, V. cholerae,* and moderate activity was noted in *S. flexneri* and *S. pyogenes* respectively. The result indicated that all the test organisms were found to be more susceptible to higher concetnrations of the extract. Also in Mohanasundari *et al.* (2007) work, the ethanolic fruit extract showed moderate activity against the bacterial pathogens namely *V. cholerae, P. putida, S. pyogenes* and *S. flexneri*. Similarly, acetone extract too exhibited moderate to mild activity against *V. cholerae* and *P. putida* according to the varying concentration of the extracts. Others exhibited very poor activity even at higher concentration of the extract as the case with the present study.

This present study on *P. edulis* also agrees with the conclusion drawn by Afolayan and Meyer (1997), who

proved that antimicrobial activity of acetone extract from the aerial parts of *Helichrysum aureonitens,* had significant activity against Gram-positive bacteria and considerable result in fungal species depending on the concentration of the extract. Likewise, Balakrishna *et al.* (2000) worked on the antibacterial and antifungal activities of alcoholic extract of the aerial parts of *Solanum trilobatum* and concluded that the higher concentration of the extract exhibited better activity.

The extract was more active on Gram-positive bacterial than Gram-negative bacteria as revealed by zones of inhibition at varying concentrations, although there are some variations. This does not totally agree with the work of Lambert (2002) and Russell (2002), who in their study explained that the reason is as a result of the differences in the cell wall permeability of the two organisms to antimicrobial agents. The cell wall of Gram-negative organisms makes them less permeable to antimicrobials as a result of its high lipid content and that the extract was inactive on the other bacterial species probably because of innate resistance (Lambert, 2002; Russell, 2002).

The study has also shown that the phytochemical compounds such as alkaloids, phenol, tannins, and flavonoids was found to have very potent antibacterial activities. These phytochemical constituents were also reported by Ogundare *et al.* (2006) who showed that a similar plant species containing such compounds is responsible for many antimicrobial activities.

5. Conclusion

From the study, the extract (stem and leaf) of *Passiflora edulis* was found to be effective against both Gram-positive and Gram-negative bacteria except *Streptococcus spp.* which could be as a result of innate resistance. This suggests that the plant could serve as a better source of antibacterial agent.

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