Phytochemical Screening and Antibacterial Activity Of Passiflora edulis

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Abstract: This study examined the phytochemical constituents and antimicrobial activity of *Passiflora edulis* (Sims) (leaf, stem and fruits). Phytochemical analysis revealed the presence of carbohydrates, glycosides, flavonoids, resins and balsams, alkaloids, and phenolic compounds in all the plant parts investigated. Tannins were present in the leaf and fruit extracts but absent in the stem whereas saponins were present in the leaf and stem but not detected in the fruit sample. Terpenes were not detected in any part of the plant. The antimicrobial activities of the leaf, stem and fruit (hexane, water, ethyl- acetate and methanolic) extracts were screened against two gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, and four gram negative bacteria *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Klebsiella pneumoniae* and *Escherichia coli*, using the well- in- agar method. All the extracts (hexane, water, ethyl- acetate and methanolic extract) showed antimicrobial activity against the pathogenic bacteria tested. Amongst the extracts examined, hexane extracts exhibited the best antimicrobial activity against all the bacteria used in this study and the effect was significant (p < 0.05). Statistical analysis also showed that the antimicrobial activity was dependent on the type of solvent used for extraction as well as the part of the plant used (p < 0.05). This preliminary study indicated potential broad spectrum activity of the plant extracts and the presence of bioactive substances that can be of value in combating infections.

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1. Introduction

Infectious diseases account for approximately one -half of all deaths in tropical countries (Iwu et al., 1999). Multiple drug resistance in human pathogenic micro-organism has been observed increasingly in recent years due to indiscriminate use of commercial anti-microbial drugs commonly used in the treatment of such diseases. Over the last three centuries, intensive efforts have been made to discover clinically useful antimicrobial drugs (Ahmad et al., 1998). It is believed that by using new compounds which are not based on the existing synthetic antimicrobial agents, antibiotic resistance can be considerably minimized (Shah, 2005). These has led to the increasing interest in traditional ethno medicine which is believed will lead to discovery novel therapeutic agents (WHO, 2000). of Phytochemicals from medicinal plants showing antimicrobial activities will likely have different structures from microbial derived antibiotics and these are also likely to have different modes of action (Fabricant and Fansworth 2001).

Passiflora edulis with common names of passion fruit and purple granadilla is a vine species of passion flower that is native to Paraguay, Brazil and Northeastern Argentina (USDA, 2007). It has been introduced to warmer parts of the world for the cultivation of its fruit and is present across the West African region including Nigeria (Burkill, 1997). All parts of the plant have been ascribed to have medicinal properties. The leaf in decoction is considered in Congo to have anti diarrhoetic properties, the stem is pounded in cold water in Uganda and taken to treat tuberculosis and the edible fruit is a digestive stimulant used as a remedy for gastric tumours (Burkill, 1997). The flower extract of *P. edulis* has sedative and hypnotic effect (Capasso and Sorrentino, 2005).

There are a few existing studies on this plant from other parts of the world but this study is unique in assaying the extract of the fruits as well as using four different solvents for extraction and testing on gram positive and gram negative bacteria.

2. Materials and Methods

2.1 Collection and Identification of Plant Materials.

The fresh leaves, stems and fruits of *Passiflora edulis* were collected from the Law Faculty of University of Abuja in the month of December, 2009. The identification of the plant was done by qualified Plant Taxonomists. The voucher specimens of the plant materials were deposited with the herbarium section of the University of Abuja and at the National Institute for Pharmaceutical and Research Development (NIPRD), Abuja.

2.2 Extraction Methods and Procedures

Each plant material (leaf, stem, and fruit) was air dried for one to three weeks and pulverized. Fifty grams of each coarse powdered plant material was extracted with 250 ml each of the solvents namely hexane, methanol, ethyl- acetate and water in a beaker for 24 hours After this ,the different mixtures were separately filtered and the filtrates concentrated. The resultant extracts were evaporated to dryness on a water bath and the yield obtained was recorded and stored in a clean and dry container until used.

2.3 Phytochemical Screening

The coarse powdered of the plant parts (leaf, stem, and fruit) were chemically tested for the presence of carbohydrates, saponins, tannins, phenols, volatile oil, phlobatannins, anthraquinones derivatives, terpenes and sterols, resin and balsams, flavonoids and alkaloids using standard procedures as described by Sofowora (1982).

2.4 Test Organisms.

Five of the bacterial strains used in this study were clinical isolates obtained from the Microbiology Laboratory, University of Abuja Teaching Hospital -University of Abuja, Nigeria. The clinical isolates were strains of *Staphylococcus aureus, Escherichia coli, Salmonella paratyphi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The sixth test organism is a strain of *Bacillus subtilis* isolated from the environment.

2.5 Preparation of Stock and Working Solutions of the Plant Extracts.

The stock and working solution of the plant extracts were prepared by using the standard method of the National Committee for Clinical Laboratory Standard (NCCLS, 1993). Each extract was weighed and prepared in stock solution by adding known volume of sterile distilled water to obtain a concentration of 20mg/0.2ml.

2.6 Screening for Antimicrobial Activity of the Plant Extracts on Bacteria

Antimicrobial activity of the plant extracts were tested by well- in –agar method. The inoculum size of each clinical isolate was standardized matching a turbidity equivalent to a 0.5McFarland standard. For each organism, a sterile cotton swab was dipped into the suspension, rotated several times on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swab was drawn over the entire surface of already prepared plates of Mueller Hinton Agar to get uniform distribution of bacteria.

The plates were bored with 5mm cork borer with a pre-sterilized cork borer. Five of these were made in each plate at a distance of 2cm from the periphery of the plates. For each plate seeded with a test organism, four different extracts namely hexane, methanol, ethylacetate and water were used. Sterile distilled water was used for the fifth hole as a control.

To each plate, 0.2ml of each plant extract was added as eptically into the well. The plates were allowed to stand until extracts have been completely absorbed by the medium. The plates were later incubated at 37^{0} C for 24hrs. The effectiveness of these extracts was recorded by measuring the diameter of inhibition zone. Each experiment was performed in triplicate.

3. Results

3.1 Phytochemical Screening

The phytochemical screening results of the coarse dried powdered plant materials of the leaf, stem and fruit of *Passiflora edulis* (Sims) are presented in (Table 1).

The result showed that the leaf, stem and fruit examined contain carbohydrates, glycosides, flavonoids, alkaloids, phenols and resin and balsams. Saponin and volatile oil are present only in the leaf and stem and absent in the fruit sample part, and tannin present only in the leaf and fruit parts of the plant. The test for terpenes gave negative result for all the plant parts examined.

Part of plant investigated	Active components										
	Carbohydrate	Tannin	Glycoside	Flavonoid	Saponin	Resins and balsam	alkaloids	Volatile oil	Phenol	Terpenes	
Leaf	+	+	+	+	+	+	+	+	+	-	
Stem	+		+	+	+	+	+	+	-	-	
Fruit	+	+	+	+	-	+	+	-	+	-	
Key											

Table 1: phytochemical screening result of the leaf, stem and fruit of the plant material.

Key:

- Confirmed

Not Confirmed

3.2 Result of the Antimicrobial Activity of the Plant Extracts.

The results of the antimicrobial screening of the hexane, methanol, ethyl- acetate and water of the leaf, stem and fruit extracts of *Passiflora edulis* (Sims) are presented in Table 2.

For the leaf extract, the antibacterial activity revealed that the hexane and water extracts showed greater and significant activity. The hexane extract showed mean diameter of inhibition zone of 16mm for *S. paratyphi*, 15mm for *S. aureus*, 14mm for *P. aeruginosa* and 13mm for *K. pneumoniae*. The water extract showed degree of growth inhibitions of 15mm for *S. paratyphi* and 13mm for *S. aureus*.

The stem extracts showed greater and significant activities for the hexane and methanol extracts with degree of growth of inhibitions of 16mm and 14mm respectively for *K. pneumoniae*. While no activity was observed against all tested bacteria for the ethyl-acetate and water stem extracts.

The fruit extracts showed significant activities for the hexane and water extracts. The hexane extract showed mean diameter of inhibition zone of 16mm for *K. pneumoniae*, 14mm for *S. aureus* and *P. aeruginosa* and the water extract with mean diameter of inhibition zone of 16mm for *S. paratyphi*. We observed that the antimicrobial activities were dependent on the part of the plant used as well as the type of extract used and statistical analysis showed that these were significant (p < 0.05).

Table 2: Antimicrobial activity of hexane, methanol, ethyl- acetate and water extracts of the leaf, stem and fruits of *Passiflora edulis* (Sims) by well-in agar method.

Plant Part	Solvent	Concentration	Mean Diameter of Inhibition Zone (mm)						
		of Extract (mg/mm)	Staphylococcus aureus	Bacillus subtilis	Kleibsiella pneumoniae	Pseudomonas aeruginosa	Escherichia coli	Salmonella paratyphi	
Leaf	Hexane	20	15	13	13	14	-	16	
	Methanol	20	12	-	10	11	-	13	
	Ethyl-acetate	20	-	8	7	8	10	-	
	Water	20	13	10	9	-		15	
Stem	Hexane	20	13	10	16	13	14	10	
	Methanol	20	10	9	14	7	12	13	
	Ethyl-acetate	20	-	-	-	-	-	-	
	Water	20	-	-	-	-	-	-	
Fruit	Hexane	20	14	12	16	14	10	10	
	Methanol	20	-	-	-	10	-	15	
	Ethyl-acetate	20	12	10	15	11	13	12	
	Water	20	13	8	-	6	-	16	

4. Discussion

The preliminary analysis of the crude extract revealed the presence of the following biologically active compounds of therapeutic values: carbohydrates, glycosides, tannins, alkaloids, saponins, resin and balsam, volatile oil, flavonoids, and phenolic compounds. Similar observations were made by Johnson et al. (2008) on the antimicrobial properties of the leaf and callus of Passiflora edulis and found that the antibacterial efficacies of chloroform, ethanol, methanol, isopropanol and petroleum ether extracts varied in effectiveness and the solvent chloroform showed maximum extraction value and its extract showed the maximum bio-efficacy when compared with other solvents due to the presence of variety of compounds such as saponins, steroids, tannins, phenolics, triterpenoids, alkaloids and flavonoids. In a study of antibacterial effects of *P. edulis* leaf and stem extracts Ripa et al. (2009) observed differences in antimicrobial effects of extracts depending on the solvent used and the part of the plant used in agreement with our present study. Ripa et al. (2009) observed that the chloroform leaf extracts were much more active than the petroleum ether extract having average zones of inhibition of 7-10 mm by disc diffusion method.

In their study the gram negative bacteria Shigella dysenteriae and S. boydii (10 mm) were moderately inhibited and the stem chloroform extract showed the highest activity against the growth of *Vibrio mimicus* having the zone of inhibition of 17mm. Besides this, the extract showed good activity against the growth of V.parahemolyticus (16mm), S. dysenteriae (15mm) and S. boydii (14 mm). However, in contrast to their findings, our study showed no significant differences in susceptibility of gram positives and gram negative organisms except for the methanolic extract of the fruit. The observed difference could be due to the type of solvent used for extraction namely; hexane, methanol, ethyl- acetate and water this study as opposed to petroleum ether and chloroform used in the previous study. It could also be due to the

differences in the type of organisms used which were also different in this case as well as subtle but important differences in geographical location as well as variety.

This preliminary present study provides valuable information on the potentials of *Passiflora edulis* to yield bioactive compounds that could be potentially used to combat diseases caused by pathogenic bacteria.

Further work is required to find out the active compounds from the crude extracts of *Passiflora edulis* as well as the chemical structures and mode of action and toxicity studies of the isolated compound(s).

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