Phytochemical Screening and Antimicrobial Activity of Ethanoic Extract of *Passiflora edulis* var. *flavicarpa* Seed on Selected Pathogens

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Abstract The present situation of drug resistance in the world necessitates the need to intensify researches on the use of alternative strategies for effective control of infections using products obtained from plant extracts. Hence this study was undertaken to evaluate the phytochemical and antimicrobial properties of ethanoic extracts of *Passiflora edulis* var. *flavicarpa* seed by testing them against three clinically important pathogens namely: *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Agar diffusion method was used in determining the susceptibility pattern of the different seed extracts concentrations (25%, 50%, 75% and 100%) on the test organisms (*Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*). The *Passiflora edulis* var. *flavicarpa* seed extract was subjected to phytochemical screening for presence of bioactive compounds using standard procedures. From the results, the zone of inhibition of the ethanoic extract at varying concentrations (100%, 75%, 50% and 25%) on *Staphylococcus aureus* ranged from 6mm to 18mm, *Escherichia coli* 5mm to 11mm and *Candida albicans* 5mm to 18mm. The growth of all the organisms was inhibited, through to varying degrees with *Staphylococcus aureus* and *Candida albicans* being more susceptible than *Escherichia coli*. The phytochemical screening revealed the presence of tannins, flavonoids, saponins, alkaloids, steroid, glycosides, saponin glycoside, terpenes and cyanogenic glycosides. The antimicrobial activity of this extract is possibly linked to the presence of flavonoids, alkaloids, steroid, saponins and/or tannins. The result obtained in the study is an indication that passion seed has the potential to be used as a source for new broad spectrum antimicrobials.

Keywords Antimicrobial Activity, *Passiflora edulis* var. *flavicarpa*, Seed, Extract, Phytochemical Constituents

morbidity and mortality in man. In recent years drug resistance in human pathogenic microorganisms has developed and the use of several antibiotics has been ineffective in solving the global problem. Great efforts are being made to reverse this trend and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer and more effective agents that can be used to fight infectious diseases [1].

Plants are known to produce phytochemicals, which are potential sources of anticarcinogenic, anticancer, antimicrobial, and antioxidant activity. The antimicrobial activities of medicinal plants extracts have been linked to the presence of bioactive compounds such as tannins, flavonoids and alkaloids which sometimes serve to protect the plants themselves against bacteria, fungi and viral infections as well as exhibiting their antimicrobial properties on these organisms [2].

The passion fruit tree (*Passiflora edulis*) belongs to family *Passifloraceae* [3]. There are two recognized forms of passion fruit: purple (*P. edulis* Sims) and yellow (*P. edulis* var. *flavicarpa*) [4]. The passion flower is a fast growing perennial vine with a thick woody stem. 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 [5]. The leaves, stems, roots, and fruits of *Passiflora* species have long been used in folk medicine and are finding an increasingly important place in modern medicine [6].

The *P. edulis* var. *flavicarpa* fruits are round in shape, with a diameter between 8 and 10 cm and a green peel at maturity which later turns yellow. The edible part of the passion fruit (40%) consists of pulp with seeds, and 60% of the peel consists of mesocarp and epicarp. They contain many seeds (as do the other *Passifloraceae* species) surrounded by a gelatinous yellow pulp that has an intense aroma and a sweet-acid taste [7].

1. Introduction

Infectious disease still remains an important cause of

The most economically importance of passion fruit is in the form of concentrated juice [8]; however, for industrialization, passion fruit bark and seeds are usually discarded, and these represent over 60% of the fruit and are almost always treated as organic waste. An alternative to by-products from passion fruit industrialization would be its use in production of antimicrobials.

The present situation of drug resistance in the world necessitates the need to intensify researches on the use of alternative strategies for effective control of infection using products obtained from plant extracts. Hence, this study was undertaken to determine the antimicrobial activity of ethanolic extracts of passion seed by testing them against three clinically important pathogens namely; *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

2. Materials and Methods

Sample Collection

The passion fruits were obtained locally from Ofeme in Umuahia North Local Government Area of Abia State in South Eastern Nigeria. The plants were botanically identified in the herbarium section of Department of Biology/Microbiology, Abia State Polytechnic, Aba.

Figure 1. *Passiflora edulis* var. *flavicarpa*

Processing of Plant Materials

The fruits were prepared for use by washing with distilled water, the fruits were cut with a sterile knife and the seeds were obtained. The seeds were dried at room temperature and later dried in an electric oven at 90°C for 2 hours after which the seeds were grinded to fine powder using an electric blender.

Preparation of Extracts

About 25 grams of the powdered seed of passion fruit was extracted with 100ml of ethanol using soxhlet apparatus for 48hrs. After 48 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 extract was then stored in a well -sealed dark brown colored glass bottle under refrigerated condition at 4°C until use.

Test Organisms

Escherichia coli, *Staphylococcus aureus* and *Candida albicans* were used as the test organisms. The cultures were obtained from a high level private laboratory in Aba, Abia State. The bacteria were subcultured on to Nutrient Agar and Mac Conkey Agar plates while fungi was sub cultured on to Sabouraud dextrose agar and kept at 37°C for 24 hours. They were confirmed by Gram staining, Motility, IMViC, Sugar Fermentation (Glucose, Sucrose, Lactose and Maltose), Oxidation-Fermentation, Urease, Nitrate, Mannitol Motility, Triple Sugar Agar, Oxidase, Catalase and Coagulase Tests for both Gram positive and Gram negative bacteria. The cultures from solid media were further inoculated to peptone broth, incubated for 24 hours at 37°C and used as the inoculum for the determination of antimicrobial activity.

Antimicrobial Test

Pure cultures of the 24 hours test organisms were each seeded onto different freshly prepared nutrient agar plates by means of a sterile glass spreader to get uniform distribution of the microorganisms. With the help of a sterile borer, wells of 5mm diameter were made in the media at a distance of 1-2cm from the periphery of the plates. These plates were labeled and 0.2ml of each plant extract (at different concentrations of extracts i.e. 100%, 75%, 50%, and 25%) was added aseptically into the well. Then the plates were incubated for 24 hours at 37°C. The effectiveness of the extract was recorded by measuring the diameter of inhibition zone. The zones of inhibition above 6mm diameter of each isolate were used as a measure of susceptibility (positive reading) to the extracts [9].

Phytochemical Screening

The ethanol extract of *Passiflora edulis* var. *flavicarpa*

seeds were screened for various bioactive compounds including flavonoids, saponins, alkaloids, tannins, steroid, anthraquinones, volatile oil, glycosides, cardiac glycoside, saponin glycoside, balsam, terpenes and cyanogenic glycoside using methods described by Harborne, [10] and Kokate, [11].

Statistical Analysis

The IBM SPSS version 21 was used for data analysis. Means were compared using one way analysis of variance followed by a post hoc Tukey's test ($p < 0.05$) for mean comparison.

3. Results

The result of antibacterial screening of ethanolic extracts of passion seed against test organisms are represented in table 1. The extract showed variable degrees of antibacterial activity against *S. aureus*, *E. coli* and *Candida albicans*. The result indicates that all organisms were found to be more susceptible to higher concentration of the extract. There was

no statistical difference between the specified range of concentration of the zones of inhibitions among tested organisms as determined by the one – way Anova, $F(2,9) = 2.081$, $p = 0.180$.

A Tukey's post hoc test also revealed that there was no statistical significance between the mean differences at the concentrations of various zones of inhibitions of the tested organisms as that of *S. aureus* (12.75 ± 5.12 , $p = 0.172$), *E. coli* (5.75 ± 4.57 , $p = 0.373$) and *C. albicans* (10.75 ± 5.25 , $p = 0.841$).

Table 1. Antimicrobial activity of ethanolic extracts of passion seed against test organisms.

Organisms	Zones of inhibition of various concentrations				mean $\pm$ SD
	100%	75%	50%	25%	
<i>S. aureus</i>	18mm	15mm	12mm	6mm	12.75 ± 5.12^a
<i>E. coli</i>	11mm	7mm	5mm	Nil	5.75 ± 4.57^a
<i>C. albicans</i>	18mm	11mm	8mm	6mm	10.75 ± 5.25^a

N.B: Values followed by the same super script under the same row are not significantly different

Figure 2. Inhibition Zone of Diameter of Different Concentrations of Ethanolic Extract of Passion Seed

Table 2. Phytochemical analysis of seed extracts of *Passiflora edulis*

Compound groups	Ethanoic extract
Flavonoids	+
Alkaloids	+
Tannins	+
Anthraquinones	–
Glycosides	+
Balsam	+
Saponins	+
Terpenes	+
Steroid	+
Volatile oil	+
Saponin glycoside	+
Cardiac glycoside	-
Cyanogenic glycoside	+

Key: + = present; – = absent

4. Discussion

The antimicrobial activity of ethanoic extract of passion seed was tested on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The extract showed strong antimicrobial activity against *S. aureus* and *C. albicans* followed by moderate activity against *E. coli* as shown in the various zones of inhibition observed on agar plates. The result of this study showed that ethanoic extract of passion seed have broad spectrum and magnitude of activity at higher concentration although there was no statistical significance between the mean difference at the concentrations of various zones of inhibition of the tested organisms.

A similar study carried out by Mohanasundari *et al.* [12] on antibacterial properties of *Passiflora foetida* found that the ethanolic leaf extract exhibited variable degrees of antibacterial activity against *P. putida*, *V. cholera* 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 concentrations of the extract. Also in Mohanasundari *et al.* [12] work, the ethanolic fruit extract showed moderate activity against the bacterial pathogens namely; *V. cholera*, *P. putida*, *S. pyogenes* and *S. flexneri*. Wasagu *et al.* [13] reported the comparative chemical analysis, phytochemical screening and antimicrobial activities of the rinds, seeds and juice of (*Passiflora edulis* var. *flavicarpa*) passion fruit on *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus niger* by well-in agar method. The rind and seed showed significant antimicrobial activity against the test organisms. The finding of their study is similar to the present study.

Razia *et al.* [14] reported that all the extracts of *Passiflora edulis* showed antimicrobial activity against the pathogenic bacteria tested namely; *Escherichia coli*, *Bacillus subtilis*,

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*. Similar conclusions were drawn by Aernan *et al.* [9], who proved that extract of leaf and stem of *Passiflora edulis* had antibacterial activity against gram negative and gram positive bacteria namely; *S. aureus*, *Bacillus* spp., *Streptococcus* spp., *Escherichia coli*, *Salmonella* spp. and *Shigella* spp.

The extract was more active on Gram positive bacteria (*S. aureus*) than Gram –negative bacteria (*E. coli*) as revealed by zones of inhibition at varying concentrations, although there are some variations. This is agreement with the work of Aernan *et al.* [9]; Agu *et al.* [15] and may be attributed to 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.

Phytochemical analysis is responsible for the identification of components which are responsible for antimicrobial activity of plants. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. The phytochemical analysis of passion seed extracts revealed the presence of alkaloids, flavonoids, tannins, saponins and several other aromatic compounds. These phytochemical constituents were also reported by Wasagu *et al.* [13]. The presence of detected phytochemical constituents in seeds of *Passiflora edulis* var. *flavicarpa* might be responsible for the observed antimicrobial activity.

This work justifies the therapeutic use of *Passiflora* in traditional medicine to treat various infectious diseases caused by the microbes. Further research is required to study the active principle, chemical structures and mechanism of action of isolated compounds.

5. Conclusions

The findings of the study revealed that the ethanoic extract of passion seed was found to be effective against *S. aureus*, *C. albicans* and *E. coli*. This suggests that the seed extracts from passion fruit have potentials to serve as a better source of antimicrobial agent.

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