Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.19, 2016

Comparative Chemical Analysis, Phytochemical Screening and Antimicrobial Activities of the Rinds, Seeds and Juice of (Passiflora edulis var. flavicarpa) Passion Fruit

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Abstract
In this study, an attempt had been made to determine the proximate composition and anti-nutritional factors of the juice, rind and seed of *passiflora edulis var. flavicarpa*. Crude lipid was determined by Soxhlet extraction, crude protein by microkjeldahl method and crude carbohydrate by difference. The result of proximate composition showed that there was a significant difference ($p<0.05$) in fibre, protein, lipid, and moisture content between the rind, juice and seed. The oxalate, phytate, tannin and cyanide content of the rind, seed and juice were low but differed significantly. The methanol extract of the rind, seed and juice of *Passiflora edulis var. flavicarpa* were screened for the presence of secondary plant metabolites and tested for antimicrobial activity. Flavonoids, alkaloids, volatile oils and balsam were detected in all the extracts. Saponins and steroids were detected in seed and juice extracts. Glycosides and saponin glycosides were detected in seed and peel extracts. The antibacterial activity was tested against *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* by well-in agar method. The rind and seed showed significant antibacterial activity against the test organisms at all the concentrations tested (30, 60 and 90 mg/ml). The juice extract showed moderate activity. The rind, seed and juice showed antifungal activity. The fungus isolate used was *Aspergillus niger* using agar incorporation method. The methanol extracts of seed and rind showed extremely significant differences ($p<0.05$) against the *Aspergillus niger,* while juice extract considered not quite significant ($p>0.05$). The result showed a significant increase in the activity of the extracts at all the concentrations tested (10, 20, 40, 70 and 100 mg/ml). The observed result may be attributed to the presence of detected phytochemical constituents. It can be concluded that *Passiflora edulis var. flavicarpa* possess antimicrobial activity and is also nutritionally relevant and could serve as a rich source of nutrients.
Keywords: *Passiflora edulis var. flavicarpa,* Comparative, Chemical, Antinutritional. Phytochemical, antimicrobial.

Introduction

Whether a man eats for living or lives for eating, food is his major concern [14]. People do not eat nutrients but food and the food provide necessary nutrients [11]. Fruits are one of the food sources that provide nutrient to the body for effective metabolic activity.

Anti-nutrients are chemicals synthesized by plants and they interfere with the absorption of nutrients. Some of these plant chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed at appropriate amounts [17].

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves, but recent research demonstrates that they can also protect human against diseases. Plants that are rich in a wide variety of secondary metabolites such as tannins, flavonoids and alkaloids have been found to have in vitro antimicrobial properties [6].

Passiflora edulis is a vine species of passion flower that is native to Brazil, Paraguay and northern Argentina. It's cultivated commercially in tropical and sub-tropical areas for its sweet, seedy fruit and is widely grown in several countries of South America, Central America, the Caribbean, and Africa, southern Asia and United States [13]. Two types of *P. edulis* are grown commercially, the purple form (*P. edulis* Sims) and a yellow form (*P. edulis* var. *flavicarpa*) [3, 10]. Although the seed is edible, during juice production, the seeds and the rinds of the fruit serve as waste. A research on the comparative chemical analysis, phytochemical and antimicrobial activities of the rinds, seeds and juice of the fruit will be of great benefit to humans.

Materials and Methods

Collection of Sample and Authentication

Fresh *Passiflora edulis var. flavicarpa* fruits were collected from Farmers' market, Maitama, Abuja, Nigeria and were identified in the herbarium section of the Biological Science Department, Usmanu Danfodiyo University, Sokoto and was given the voucher specimen number (UDUH/ANS/0059).

Plant Sample Preparation

The fruits were washed with water and residual moisture evaporated. The juice of passion fruits were extracted from the fruits while the seeds and the rinds were air dried to a constant weight. The seeds and the rinds of the fruits were pulverized into coarse powdered form using pestle and mortar.

Plant Sample Extraction

Fifty grams (50g) of each sample were soaked in 500mls of 80% methanol for forty eight (48) hours under room temperature. The resulting mixtures were filtered using Whatman filter paper No 1. The filtrates were collected and concentrated to half of their original volume in a water bath and the residues were discarded.

Proximate analysis

The moisture content, ash content, crude protein, crude lipids, crude fiber, and total solid of the passion fruit were determined using standard methods described by association of official analytical chemical [2]. Available carbohydrate was calculated by subtracting the total of the percentages of crude protein, crude lipid, crude fibre and ash content from 100% moisture free sample.

Anti-nutritional analysis

Phytate was determined by the method of Ola and Obah [12], Oxalate was determined by the method of Day and Underwood [4], Cyanide was determined by the method of AOAC [2]. Tannins was determined by the method of van-Burden and Robinson [18].

Phytochemical screening

The methanol extract of the different parts of *Passiflora edulis var. flavicarpa* fruits (Rinds, Seeds and Juice) were screened for various bioactive compounds including flavonoids, saponins, alkaloids and tannins [7, 9, 15 and 16].

Test organisms

The test organisms used for antibacterial activities were *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella spp.* obtained from Microbiology post graduate research laboratory, Microbiology Department, UDUS. While *Aspergillus niger* is the organism for antifungal activity obtained from Mycology laboratory, Biological Science Department of the same institution.

Preparation of Culture Media for antibacterial activity

Twenty eight grams (28g) of the nutrient agar was weighed and transferred into a conical flask containing 1000ml of distilled water and mixed. The mixture was heated to dissolve the powder and then dispensed into sterilized Petri dishes or plates which were then sterilized by autoclaving at 121˚C for 15 minutes then allowed to cool at 45˚C.

Test for antibacterial activity

The bacteria activity was done using hole-in-plates bioassay procedure. Thirty test tubes each containing 5ml of sterilized distilled water were used for the dilution of the methanol extracts of the various parts of the fruits into various concentrations (30mg/ml, 60mg/ml and 90mg/ml) i.e. 0.03g, 0.06g and 0.09g respectively.

Plate of Nutrient agar was prepared. Three wells were made on each plate agar using sterilized 6mm cork borer. Each plate agar was inoculated with different isolates respectively using sterilized wire loop. After inoculation, the different concentration of the methanol extract was mixed with little plain agar and poured into the designated well/hole which carries the specific concentration (30, 60 and 90mg/ml respectively) of the extract. The inoculated plates were allowed to stand for 15 minutes before incubating for 24 hours at 37˚C, followed by observation of inhibition zone around wells. Streptomycin (0.10g) was used as a control for comparison with the previous test of the extract using the same procedure.

Preparation of Culture Media for antifungal activity.

Fourteen grams (14g) of Sabouraud Dextrose Agar (SDA) was homogenized in 500ml of sterilized distilled water. It was then heated on a hot plate to dissolve completely, and then poured into conical flask; cotton wool was plugged on the mouth of each conical flask containing the media and wrapped with aluminium foil. They were then autoclaved for 15 minutes. The agar was allowed to cool at 45˚C and then poured into media, allowed to solidify.

Antifungal Susceptibility Test

SDA was used for the inoculation of *Aspergillus niger*. Active cultures for the experiments was prepared by seeding a loopful of *Aspergillus niger* and incubated without agitation for 4 days at 27˚C. The inoculated plates were allowed to stand for 15 minutes before incubation, followed by observation of inhibition zone. Antifungal tablet (0.10g) was used as a control for comparison with the previous test of the extract using the same procedure.

Data analysis

The results were expressed as mean $\pm$ standard error of mean of three measurements followed by one-way Analysis of Variance (ANOVA) using the statistical package SPSS version 20. Differences in mean ($\pm$SEM) were considered at $p<0.05$ significant level.

Results

The results of proximate composition of juice, rind and seed extract of *Passiflora edulis var flavicarpa* is presented in table 1. The protein, carbohydrate, fat, fibre, moisture, ash and total solids of the juice, rind and seed differ significantly ($p<0.05$).

Table 1: Results of Proximate Compositions of the juice, rinds and seeds of *Passiflora edulis var. flavicarpa.*

Antinutritional factors

Table 2 reveals the concentration of antinutritional factors in the seed, rind and juice extract of *Passiflora edulis var. flavicarpa.* The phytate, oxalate, cyanide and tannin concentration of the seed, rind and juice differ significantly ($p<0.05$).

Table 2: Antinutritional factors present in seed, rind, and juice of *Passiflora edulis var. flavicarpa.*

Phytochemical Screening

Table 3 presents the result of the phytochemical screening of *Passiflora edulis var. flavicarpa* fruits (rinds, seeds and juice). The result revealed the presence of flavonoids, alkaloids, volatile oils and balsams in the rinds, seeds and juice of the fruit. Tannins, glycosides and saponin glycosides were present in the seeds and rinds only while saponins and steroids were present in the seeds and juice only. Terpenes and cyanogenic glycosides are present in the seeds alone, cardiac glycosides was present in the juice alone.

Table 3: Qualitative phytochemical analysis of seed, rinds, and juice extracts of *passiflora edulis var. flavicarpa.*

Antimicrobial Activity of the methanol extract of the fruit.

Table 4 presents the antibacterial activity of seed, rinds and juice of methanol extract of *Passiflora edulis var. flavicarpa.* The methanol extracts of the seed and rind of the fruits exhibited mild antibacterial activity against *S. aureus, Salmonella spp.* and *E.*coli, although the extracts had more effect on the organisms with higher concentration. The juice on the other hand showed no antibacterial activity against the three test organisms used.

Table 4: Antibacterial activity of seed, rind and juice methanol extracts of *Passiflora edulis var. flavicarpa.*

The results of antifungal activity of the methanol extract of *Passiflora edulis var. flavicarpa* is shown in table 5. Extracts of the rinds, seeds and juice exhibited antifungal activity against the fungal strain used. The extract from the rind had the highest activity against *A.niger* while the juice showed the lowest activity against it.

Table 5: Antifungal activity of methanol extract of seed, rind, and juice of *Passiflora edulis var. flavicarpa.*

Discussion

The results of the proximate analysis reveals that the percentage crude protein, carbohydrate, crude fat, crude fiber, moisture, ash for the rinds, seeds and juice were significantly different with the seeds having the highest value for crude protein (8.90 ± 0.88), crude fat (5.90 ± 0.07) and ash content 17.00± 0.29. This suggests that the seeds could serve as a rich source of protein, lipid and minerals. The juice had the highest carbohydrate content (75.30 ± 0.06) compared with the rind (53.60 ± 0.15) and the seeds ($20.70 \pm$0.06). The juice had a total solid content of (13.80 ± 0.06) indicating that the juice is a rich source of soluble sugars. The rinds had the highest moisture content (8.20 ± 0.44) compared with the seeds. Also the rinds had the highest crude fiber content (29.60 ± 0.33) compared with the seeds (27.40 ± 0.44) and the juice (0.80 ± 0.17). Dietary fibre have been credited to reduce cholesterol level in the body, thus minimizing the risks of cardiovascular disease caused by high plasma cholesterol level.

The oxalate contents of rind, juice and seed differed significantly ($p<0.05$). The cyanide, tannins and phytate concentrations were low in all the three samples. Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption [5]. Phytate works in a broad pH-region as a highly negatively charged ion, and therefore its presence in the diet has a negative impact on the bioavailability of divalent, and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} [8]. Dietary oxalate has been known to complex with calcium, magnesium and iron leading to the formation of insoluble oxalate salts and resulting in oxalate stone [19]. Hydrogen cyanide inactivates the enzyme cytochrome oxidase in the mitochondria of cells by binding to the Fe^{3+}/Fe^{2+} contained in the enzyme which causes a decrease in the utilization of oxygen in the tissues [8].Tissues that depend highly on aerobic respiration, such as central nervous system and heart are particularly affected.

The phytochemical screening of the various parts of *Passiflora edulis var. flavicarpa* fruits revealed the presence of flavonoids, alkaloids, volatile oils and balsams in the rinds, seeds and juice of the fruit. Tannins, glycosides and saponin glycosides were present in the seeds and rinds only while saponins and steroids were present in the seeds and juice only. Terpenes and cyanogenic glycosides are present in the seeds alone, cardiac glycosides was present in the juice alone. However, resins and anthraquinones were absent in the various parts of the fruits. Phytochemical constituents such as alkaloids, flavonoids, phenols and several other aromatic

compounds are secondary metabolites of plants that serve a defense mechanism against many micro-organism [1].

The result of antimicrobial study showed that the various part of the fruits possess variable degree of antibacterial and antifungal activity. The seed extract showed activity against *Staphylococcus aureus*, *Salmonella spp* and *Escherichia coli* while the rind extract exhibited activity against *Staphylococcus aureus* and *Salmonella spp.* However it showed no activity against *Escherichia coli.* The juice showed no antibacterial activity. This reveals that the seeds showed a better antibacterial activity than the rind. The rind, seed and juice of the fruit showed strong antifungal activity against *Aspergillus niger* with the rind showing the highest activity followed by the seed then the juice. These findings may be attributed to the presence of secondary metabolites.

Our findings showed that there is a significant difference in the nutritional content of the rind, seed and juice of *Passiflora edulis var. flavicarpa*, although they are all rich sources of nutrients. The anti-nutritional content for oxalate, phyatate, tannins and cyanides were low in the rind, seed and juice which further enhances its nutritional benefits to the body. Also, the methanol extract of the rind, seed and juice of *Passiflora edulis var. flavicarpa* contain important bioactive constituent and possess significant antimicrobial activity.

Conflicts of interest

I Rabiu Umar Aliyu Wasagu declare that they have no conflict of interest

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Table 1: Results of Proximate Compositions of the juice, rinds and seeds of *Passiflora edulis var. flavicarpa.*

Parameters (%Composition)	Juice	Rind	Seed
Crude protein	5.90 ± 0.07^{b}	5.40 ± 0.21^{c}	8.90 ± 0.88^{a}
Carbohydrate	75.30 ± 0.06^{a}	53.60 ± 0.15^{b}	20.70 ± 0.06^{c}
Crude lipid	0.80 ± 0.17^{c}	3.30 ± 0.17^{b}	41.30 ± 0.33^{a}
Crude Fibre	0.80 ± 0.17^{b}	29.60 ± 0.33^{a}	27.40 ± 0.44^{a}
Moisture content**	ND	8.20 ± 0.44^{a}	6.60 ± 0.44^{b}
Ash content	1.33 ± 0.17^{c}	7.60 ± 0.17^{b}	17.00 ± 0.29^{a}
Total solid*	13.80 ± 0.06^{a}	ND	ND

Values are expressed as mean ± Standard Error of Mean of three replicates. Mean Value Having Different Superscript Letters in rows are significantly different ($p<0.05$) (One way ANOVA followed by Duncan's, multiple comparison test).

LEGEND: * g/100cm^3, ** value expressed as % wet weight.

Table 2: Antinutritional factors present in seed, rind, and juice of *Passiflora edulis var. flavicarpa.*

Antinutritional factors	**Concentration(mg/100g)**		
Sample	**Seed**	**Rind**	**Juice**
Phytate	0.47 ± 0.02^{a}	0.50 ± 0.01^{a}	0.17 ± 0.01^{b}
Oxalate	0.47 ± 0.02^{b}	0.78 ± 0.03^{a}	0.13 ± 0.01^{c}
Cyanide	0.07 ± 0.00^{a}	0.06 ± 0.02^{a}	0.05 ± 0.01^{a}
Tannins	0.07 ± 0.00^{a}	0.04 ± 0.00^{b}	0.02 ± 0.00^{c}

Values are expressed as mean ± Standard Error of Mean of three replicates. Mean Value Having Different Superscript Letters in rows are significantly different ($p<0.05$) (One way ANOVA followed by Duncan's, multiple comparison test).

Table 3: Qualitative phytochemical analysis of seed, rinds, and juice extracts of *passiflora edulis var. flavicarpa.*

S/No	**Phytochemicals**	**Observation**		
		Seed	Peel	Juice
1	Flavonoid	+	+	+
2	Alkaloid	+	+	+
3	Tannins	+	+	-
4	Saponins	+	-	+
5	Steroid	+	-	+
6	Anthraquinones	-	-	-
7	Volatile oil	+	+	+
8	Glycosides	+	+	-
9	Cardiac glycoside	-	-	+
10	Saponin glycoside	+	+	-
11	Balsam	+	+	+
12	Terpenes	+	-	-
13	Cyanogenic glycoside	+	-	-

+ = m present - = Absent

Table 4: Antibacterial activity of seed, rind and juice methanol extracts of *Passiflora edulis var. flavicarpa.*

Species	Extracts	Concentration of extract(mg/ml)		
		30	60	90
		Zone Of Inhibition(mm)		
	Seed			
Staphylococcus aureus		00	00	03
Salmonella spp		00	01	02
Escherichia coli		00	00	02
	Rind			
Staphylococcus aureus		00	00	06
Salmonella spp		02	04	06
Escherichia coli		00	00	00
	Juice			
Staphylococcus aureus		00	00	00
Salmonella spp		00	00	00
Escherichia coli		00	00	00

Key: Diameter of the cork borer is 6mm. Values greater than 6mm indicate some activity. Values less than 6mm show that there was no activity.

Table 5: Antifungal activity of methanol extract of seed, rind, and juice of *Passiflora edulis var. flavicarpa.*

Concentration(mg/ml)	Inhibition Zone(mm)		
	Seed	**Rind**	**Juice**
10	35.67±2.96^{a}	37.67±1.45^{a}	25.00±6.81^{a}
20	24.67±2.40^{b}	30.67±0.67^{b}	16.67±3.33^{a}
40	17.67±1.45^{c}	15.67±2.33^{c}	15.67±2.33^{c}
70	10.00±1.56^{b}	10.00±0.58^{b}	12.67±1.45^{a}
100	4.33±1.20^{b}	4.67±0.88^{b}	4.33 ±1.43^{a}

Values are expressed as mean ± Standard Error of Mean of three replicates. Mean Value Having Different Superscript Letters in rows are significantly different ($p<0.05$) (One way ANOVA followed by Duncan's, multiple comparison test).