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ANTIMICROBIAL ACTIVITY OF THE ETHANOLIC AND AQUEOUS EXTRACT OF PASSION FRUIT (*PASSIFLORA EDULIS* SIMS), IN THE ABSENCE AND PRESENCE OF $Zn(OAc)_2.2H_2O$.

R. C. Jagessar[1]*, A. Hafeez[2], M. Chichester[2] and Y. Crepaul[2]

[1]Department of Chemistry, University of Guyana, Turkeyen campus, Guyana, South America.

[2]Department of Biology, University of Guyana, Turkeyen campus, Guyana, South America.

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***Corresponding Author**
Dr. R. C. Jagessar
Department of Chemistry,
University of Guyana,
Turkeyen campus,
Guyana, South America.

ABSTRACT

The aqueous and ethanolic extract of mature passion fruit in the absence and presence of transition metal salts were subjected to antimicrobial studies using the Disc Diffusion Assay under aseptic conditions. From the ethanolic extract, a white solid crystallised out and its antimicrobial activity was investigated. The highest AZOI of 268.7 mm^2 was induced by sample 9 against *S. aureus*. The lowest AZOI of 21.6 mm^2 was induced by sample 6 against *C. albicans*. These quoted AZOI are exclusive of the reference AZOI. For the non-metal solution, the highest AZOI of inhibition was induced by the aqueous solution (0.026g/ml) against *P. aeruginosa* i.e *P. aeruginosa* was more susceptible. There seems to be an increased in antimicrobial activity of the ethanolic fruit extract as the concentration increases. For the aqueous extract of the white isolate, an increase in concentration generally resulted in a decrease in antimicrobial potency. Interestingly, there seems to be an increase in antimicrobial activity of the aqueous solution of the white ethanolic isolate in the presence of Zn (OAc).$2H_2O$ salt against pathogens in some cases and a decrease in another instance. Selective antimicrobial activity was observed for the fruit extracts in the presence and absence of $Zn(OAc)_2.2H_2O$. For all experiments conducted, antimicrobial activity seems to be less than that of the standard antibiotics: Ampicillin and Nystatin.

KEYWORDS: Antimicrobial, Passion fruit (*Passiflora edulis* Sims), Area of Zone of Inhibition, AZOI, asceptic conditions, *E.coli, S. aureus, K. pneumoniae and C. albicans*

INTRODUCTION

Research in the design and synthesis of antimicrobials will continue to be problematic on our planet, considering that bacteria and fungi develop resistance to antimicrobials over a period of time.[1-5] This results from indiscriminate use of commercial antimicrobial drugs for the treatment of infectious diseases and the current global antibiotic resistance.[1-5] Many synthetic drugs have several adverse side effects which are usually irreversible when administered and the cost of synthesizing drugs in most cases is an expensive endeavor.[1-5] Plants have a long therapeutic history over thousands of years and are still considered to be promising source of medicine in the traditional health care system.[6] Plants also have a wide variety of secondary metabolites some of which are antimicrobial.[7-9] Crude plants extracts have also demonstrated antimicrobial activity.[10-12]

Guyana flora is richly biodiversified and its organic and aqueous extracts have been shown to possess potent and selective antimicrobial activity to date, compared with standard antibiotics: penicillin, nystatin and ampicillin.[13-17]

Clinical trials are an expensive endeavour and developing countries like Guyana are yet to develop the collaborative scientific framework for research. This paper reports the antimicrobial activity of the edible Passion fruit, *Passiflora edulis* Sims (*Passifloraceae*) in the absence and presence of $Zn(OAc)_2.2H_2O$.

There is always an urgent need to synthesise new antimicrobials, considering that bacteria develop antimicrobial resistance. This results from indiscriminate use of commercial antimicrobial drugs for the treatment of infectious diseases and the current global antibiotic resistance.[1-5] Many synthetic drugs have several adverse side effects which are usually irreversible when administered and the cost of synthesizing drugs in most cases is an expensive endeavor.[1-5] In addition, before a drug is declared a safe antibiotic, it has to be clinically screened. This is an expensive endeavour for a developing country and requires state of the art technology to be realized. The use of tropical edible fruits as antimicrobial agents would eliminate the need for clinical trials and would prevent the side effects encountered with synthetic drugs.

The objectives of this research project were to select fruits and vegetables that have strong suspected folklore antimicrobial activities. To conduct selective solvent extraction of *P. edulis,* using solvents of increasing polarity. To conduct antimicrobial assay on *P. edulis* in the absence and presence of $Zn(OAc)_2.2H_2O$ using the Disc Diffusion Assay

LITERATURE REVIEW

Literature review reveals that little or no work has been done with regards to the antimicrobial activity of passion fruit, *P. edulis* in the presence and absence of transition metal salts (Zn and Cu). However, there is one report on the the antimicrobial activity of the stems and leaves of another species, *P. ligularis* Ajuss.[18] No report has been mentioned on the effect of transition metal salts on *P. edulis*.

Passion fruit is cultivated mostly in the tropics. The fruit is round to oval and usually yellow at maturity, with a soft to firm, juicy interior filled with numerous seeds. The fruit is both eaten and juiced or blended with other fruit juice to enhance aroma.[19-22] It has several medicinal uses. These include: It boosts the immune system, it protects against cancer, heart diseases and premature ageing. It keeps skin hydrated and glowing. It improves eye health, aids in blood circulation in the body. Its beneficial in improving heart health, increases bone mineral density and bone strength. It also provides relief from constipation, facilitates healthy digestion of food and regulation of bowel movements. It reduces the risk of macular degeneration, cataracts and night blindness.[19-22]

Transition metal salts such as zinc and copper play fundamental roles in human biological systems. Heavy metals such as copper (Cu), iron (Fe), manganese (Mn), cobalt (Co) and zinc (Zn) are important micronutrients of plants, whereas potassium (K) is an important macronutrient. These elements play important roles in the plant physiology and affect the surrounding environment. 9% of Eukaryotic proteins bind various metals and 40% of all enzyme catalysed reactions involve metals such as Mg, Zn, Fe, Mn, calcium(Ca), cobalt (Co), Cu, nickel (Ni), molybdenum (Mo), tungsten (W), sodium (Na), potassium (K) and vanadium (V).[24-25] Cu is a prerequisite for many enzyme processes, proper photosynthesis, manufacture of lignin (cell walls) and in grain production. Zinc, is essential for optimum crop growth. Its deficiency causes various adverse effects on growth and yield of crops. It is also involved in formation of chlorophyll, carbohydrates, in several dehydrogenises, proteinese and peptidase enzymes. It promotes growth hormones (auxin) and starch formation. It also

responsible for the biosynthesis of cytochrome: a pigment and maintain plasma membrane integrity and synthesis of leaf cuticle.

Passion fruit is rich in polyphenols.[19-22] The fruit also contain prunasin and other cyanogenic glycosides in the peel and juice.[21] Passion fruit oil is composed mainly of linoleic acid (77%) with smaller amounts of oleic acid (15%) and palmitic acid (10%). It also contains vitamin C (36%), dietary fiber (42%), B vitamins riboflavin (11%) and niacin (10%), iron (12%) and phosphorus (10%) in significant percentages of the daily value.[22]

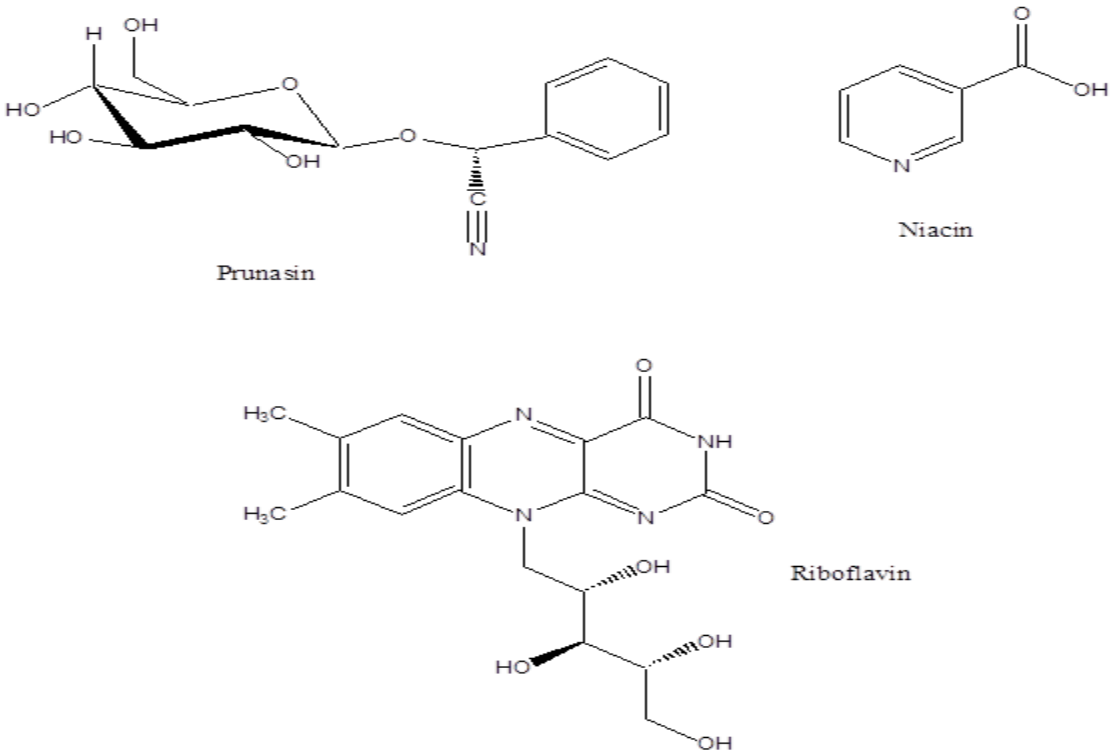


Fig. 1.0. Some of the natural products constituents of passion fruit, *Passiflora edulis Sims.*[19-22]

METHODOLOGY

The methodology involves the collection, washing, drying and solvent extraction of the cut fruits, antimicrobial studies on the extracts of the fruits, statistical computation and graphical analyses of antimicrobial results.

MATERIALS AND METHOD

(a) Collection, treatment of plant material and solvent extraction: Green passion fruits were purchased from a vendor at the Bourda market in the municipality of Georgetown, Guyana. They were washed, rinsed with distilled water and allowed to air dry. The entire

fruits were weighed (2.380kg), sliced vertically in two and placed in two extraction jars. This was followed by selective extraction using solvents of increasing polarity: n-C_6H_{14}, CH_3CH_2OH and water. From the hexane extracts, solvents were removed in *vacuo*, resulting in a light yellow viscous oil of 1.5g (0.06% yield). The ethanol extract, yellow brown in nature constituted a weight of 155.5g (6.53%) yield. The aqueous extract, light yellow in colour constituted a concentration of 1.6g/ml. From the ethanolic extract, a white solid (1.5g) was crystallised and recrystallised.

Solutions for antimicrobial studies

Varying amounts of ethanolic, aqueous extracts and the white solid that crystallised were taken out, weighed and made up to the requisite concentration of 0.015mg/L, 0.05mg/L and 0.1 mg/L using the respective solvents of ethanol and water.

Microorganisms used

The human pathogenic microorganisms used for the antimicrobial activity were: *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginiosa* and the fungus *Candida albicans.* All microorganisms were collected from Georgetown Public Hospital, GPHC. All microorganisms obtained were cultured in a pure *Luria-Bertani* broth.

Luria –Bertani broth (LB broth) is a rich medium used to culture bacteria such as *E.Coli and S.aureus*. To make it, tryptone (10g), yeast extract (5g) and sodium chloride (10g) were measured and placed in a 1L cylinder. Distilled water was added to make up the 1L solution and the mixture was poured and re-poured until the contents were dissolved. The pH of the solution was adjusted to 7.4 using NaOH. 3mL each of LB broth was placed in 56 test tubes. The tubes were plugged with cotton wool foil and wrapped with aluminium foil over each top. The tubes were placed into a beaker and autoclaved at 121^0C for 2h. These tubes were used in the dilutions experiments.

Preparation of the media

23 grams of the powdered media (Muller Hinton) were dissolved in 1000ml sterile distilled water in a conical flask. The weighed amount was mixed properly then allowed to dissolve by heating over a water bath. The conical flask was then plugged with cotton wool and wrapped in aluminum foil, then autoclaved at 121°C for 15 minutes. The sterilized medium was then poured into the sterilized petri-plates and allowed to cool and solidify.

Antimicrobial activity (Saklani et al, 2011,[23-25])

The antimicrobial activity of passion fruit extracts against pathogenic microorganisms: *E. coli, S. aureus, K. pneumoniae, C. albicans and P. aeruginosa* was investigated using the Disc Diffusion assay under aseptic conditions. Antimicrobial investigations were done on the ethanolic and aqueous extract of an isolate, a white compound isolated from the ethanolic extract and in the presence of aqueous solution of transition metal salts such as zinc and cuprous acetate of varying percentage (1 to 10%). The ethanolic extract was prepared in concentration of 0.015g/ml, 0.051g/ml, 0.1g/ml. The aqueous solution was prepared in concentration of 0.0026g per ml, 0.0052 g per ml. The Zn acetate solution was prepared as a 1 and 10% respectively. The copper acetate solution was prepared as 0.01%, 1.0% and 10.00% respectively. For each microorganism, a reference experiment was conducted. Ampicillin and Nystatin were used for bacteria and fungal species respectively. For each experiments, investigations were done in triplicates and the diameter (DZOI) was measured and area of zone of inhibition, AZOI computed. Data were statistically analyzed[25-26] to find the mean diameter of the zone of inhibition, DZOI, standard deviation, validity of precision and the accuracy of the zone of inhibition, for each extract at different concentrations.

Antimicrobial tests were carried out using the Agar Disc diffusion Method as follows:

Agar Disc Diffusion Method

The antimicrobial activity of the crude ethanolic extracts and the white isolate compound was determined using the Disc Diffusion method assay. Using this method, 40g of Mueller Hinton Agar (MHA) was placed into 1000ml of distilled water. This was mixed thoroughly. The mixture was then heated with frequent agitation while in a conical flask. The mixture was boiled for one minute to completely dissolve the agar powder. It was then autoclaved at 121ºC for 15 minutes. The molten agar was then poured into 90mm sterile Petri dishes, to a depth of 4mm. These plates were allowed to cool and refrigerated for use the following day. The plates were labeled and inoculated with the respective bacterial colonies. Three discs, impregnated with the antimicrobial plant extracts at appropriate concentrations were placed on the MHA plates. Four separate plates were prepared in a similar manner for the positive controls for the bacterial strains respectively. The plates containing the bacterial colonies were incubated for 24hrs and 48 hrs at 37ºC for bacterial and fungal species respectively.

Data Analyses

Data were statistically analyzed[25-26] to find the mean diameter of the zone of inhibition, DZOI, standard deviation, validity of precision and the accuracy of the zone of inhibition, for each extract at different concentrations. Two-Factor ANOVA with replication was used to analyse whether significant differences exist in the diameter of zone of inhibition between extracts and organisms.

RESULTS

Table 1.0. Weight of fruit, volume of solvent added, concentration and % yield of extract per solvent type extract.

Weight of ground plant material	Type of extract	Volume of solvent added (ml)	State of extract	weight/concentration of extract	% yield of extract
2.38kg	n-C_6H_{14}	6.9L	Light yellow viscous oil	1.5g	0.06
2.38kg	ethanol extract	9.2L	dark yellow, light brown	155.44g	6.53
2.38kg	aqueous extract	3.2L	yellow	1.6g/ml	------

Table 2.0. DZOI, mean DZOI with standard deviation and AZOI induced by respective samples in the absence of $Zn(OAc)_2.2H_2O$ against pathogenic microorganisms.

Sample	Organism	Diameter of ZOI (mm)	Mean Diameter of ZOI (mm)	AZOI (mm^2)
1	*E. coli*	11, 7, 9, 6	8.25 ± 2.22	53.4
2	*E. coli*	11, 13, 10, 0	8.5 ± 5.8 0	56.7
3	*E. coli*	16, 9,7,6	9.5 ± 4.51	70.9
Reference	*E.coli*	31, 29, 28	29.33 ± 1.53	675.3
1	*K. pneumoniae*	15, 12, 5, 0	8 ± 6.78	50.2
2	*K. pneumoniae*	12, 8, 7, 10	9.25 ± 2.22	67.2
3	*K. pneumoniae*	9, 11, 7, 0	6.75 ± 2.69	15.9
Reference	*K. pneumoniae*	23, 35, 36	31.33 ± 5.91	770.6
1	*S. aureus*	10, 7, 5.5, 5	6.88 ± 1.95	32.2
2	*S. aureus*	9,, 11, 7, 7	8.5 ± 5.98	56.7
3	*S. aureus*	10, 14, 10, 7	10.25 ± 2.87	82.5
Reference	*S. aureus*	36, 27, 28	30.33 ± 4.93	722.1
1	*P.aeruginosa*	12 ,10, 8, 6	9.0 ± 2.58	63.6
2	*P.aeruginosa*	10, 8, 13, 8	9.75 ± 2.36	74.6
3	*P.aeruginosa*	7, 9, 8, 16	10 ± 4.08	78.5
Reference	*P.aeruginosa*	19, 16, 15	16.67 ± 2.08	218.1
1	*C. albicans*	25, 11, 11, 9	14.0 ± 7.39	153.9
2	*C. albicans*	12, 11, 7,8	9.5 ± 2.38	70.9
3	*C. albicans*	11, 17, 7, 9	11 ± 4.32	94.9
Reference	*C. albicans*	25, 26, 27	26 ± 1.0	530.7

Aqueous solution of white isolate at 0.026g/ml (4)	*P. aeruginosa*	5, 14, 15, 17, 18.5	13.8 ± 4.54	149.5
Aqueous solution of white isolate at a concentration of 0.052g/ml (5)	*P. aeruginosa*	9, 11, 11, 10	10.25 ± 0.96	82.5
Reference	*P. aeruginosa*	14, 18, 19	17 ± 2.65	226.9

Table 3.0 DZOI, mean DZOI with standard deviation and AZOI induced by respective samples in the absence of $Zn(OAc)_2.2H_2O$ against pathogenic microorganisms.

Sample	Organism	Diameter of ZOI (mm)	Mean Diameter of ZOI (mm)	AZOI (mm^2)
Aqueous solution of white isolate 0.026g/ml (4)	*K.pneumoniae*	15, 8, 9, 8	10 ± 2.16	78.5
Aqueous solution of white isolate at 0.052g/ml (5)	*K.pneumoniae*	9, 10, 0,, 6	6.25 ± 4.66	30.7
Reference	*K.pneumoniae*	23, 24, 25	24 ± 1.0	452.2
Aqueous solution of white isolate at a concentration of 0.026g/ml (4)	*S. aureus*	10, 13, 9, 9	10.25 ± 1.89	82.5
Aqueous solution of white isolate (5) at a concentration of 0.052g/ml	*S. aureus*	12, 15, 11, 13	12.75 ± 1.71	127.7
Reference	*S. aureus*	30, 35, 34	34 ± 2.65	907.5
Aqueous solution of white isolate at a concentration of 0.026g/ml (4)	*E. coli*	11, 9, 7, 10	9.25 ± 1.76	67.2
Aqueous solution of white isolate at a concentration of 0.052g/ml (5)	*E. coli*	8, 11, 10, 7	9 ± 1.83	63.6
Reference		29, 25, 24	26 ± 2.65	530.7

Table 3.0, Cont'd.

Sample	Organism	Diameter of ZOI (mm)	Mean Diameter of ZOI (mm)	AZOI (mm^2)
Aqueous solution of white isolate (5) at a concentration of 0.026g/ml	*C. albicans*	13, 10, 10, 11	11 ± 1.41	94.9
Aqueous solution of white isolate (5) at a concentration of 0.026g/ml	*C. albicans*	13, 10, 9, 8	10 ± 2.16	78.5

Table 4.0. DZOI, mean DZOI with standard deviation and AZOI induced by respective samples in the presence of $Zn(OAc)_2.2H_2O$ against pathogenic microorganisms.

Sample	Organism	Diameter of ZOI	Mean Diameter of ZOI	AZOI (mm^2)
6	*E. coli*	5, 7, 10, 5	6.75 ± 1.85	35.8
7	*E. coli*	11, 7, 9, 10	9.25 ± 1.71	67.2
8	*E. coli*	12, 11, 12, 10	11.25 ± 1.66	99.4
9	*E. coli*	13, 13, 10, 14	12.5 ± 1.73	122.7
10	*E. coli*	7, 11, 9, 7	8.5 ± 1.92	56.7
6	*K.pneumoniae*	0, 0, 0, 0	0.00 ± 0.00	0.000
7	*K.pneumoniae*	10, 11, 5, 5	7.75 ± 3.65	38.5
8	*K. pneumoniae*	12, 9, 15, 11	11.75 ± 4.33	108.4
9	*K. pneumoniae*	14, 16, 10, 8	12.00 ± 3.65	113.0
10	*K. pneumoniae*	17, 13, 11, 14	13.75 ± 2.90	148.4
6	*S. aureus*	6, 6, 6, 6	6 ± 0.00	28.3
7	*S. aureus*	9, 7, 10, 7	8.25 ± 1.5	53.4
8	*S. aureus*	22, 18, 17, 19	19.0 ± 2.16	283.4
9	*S. aureus*	17, 20, 19, 18	18.5 ± 1.29	268.7
10	*S. aureus*	7, 6, 7, 6	6.5 ± 0.33	33.2

Table 5.0. DZOI, mean DZOI with standard deviation and AZOI induced by respective samples in the presence of $Zn(OAc)_2.2H_2O$ against pathogenic microorganisms.

Sample	Organism	Diameter of ZOI (mm)	Mean Diameter of ZOI (mm)	Area of Zone of Inhibition, AZOI (mm^2)
6	*P. aeruginosa*	6,6,6,6	28.26 ± 0.00	28.3
7	*P. aeruginosa*	7,5,0,5	14.18 ± 2.98	14.2
8	*P. aeruginosa*	8,10, 13, 15	103.82 ± 3.61	103.8
9	*P. aeruginosa*	8, 10, 11, 13	86.55 ± 2.52	86.6
10	*P. aeruginosa*	13, 15, 17, 19	200.96 ± 2.58	200.9
6	*C. albicans*	7, 5, 9, 0	5.25 ± 6.69	21.6
7	*C. albicans*	7,5,7,6	6.25 ± 2.74	30.7
8	*C. albicans*	0,6, 9, 11	6.5 ± 4.68	33.2
9	*C. albicans*	11, 11, 6, 7	8.75 ± 2.63	60.1
10	*C. albicans*	7, 5, 5, 5	5.5 ± 1.0	23.8

Key:

Sample 1: 0.015g/ml of ethanolic extract

Sample 2: (0.050g/ml) of ethanolic extract

Sample 3: (0.1g/ml) of ethanolic extract

Sample 4: 0.026g/ml of aqueous solution of isolated compound

Sample 5: (0.052g/ml) of aqueous solution of isolated compound

Sample 6: 1% solution of zinc acetate in aqueous extract of fruit

Sample 7: 10.0 % solution of zinc acetate in distilled water

Sample 8: (reference sample): 1.0g of zinc acetate in 10ml of aqueous solution

Sample 9: 0.1g of zinc acetate in 10 ml of aqueous solution of unknown isolate

Sample 10: 0.11g of zinc acetate in 10 ml of aqueous solution of unknown isolate

Table 6.0. A comparison of the antimicrobial potency of the fruit extract versus that of standard antibiotics, Ampicillin and Nystatin.

Pathogenic Microorganisms	Reference Antibiotics	AZOI of Reference Antibiotics (mm^2)	Highest AZOI of Fruit extracts/isolate (mm^2)	% Potency of Fruit extracts/isolate relative to standard antibiotics
E. coli	Ampicillin	675.3	70.9	10.4
S. aureus	Ampicillin	722.1	82.5	11.4
C. albicans	Nystatin	530.7	153.9	28.9
P. aeruginosa	Ampicillin	226.9	149.5	15.2
K.pneumoniae	Ampicillin	770.6	67.2	8.7

Table 7.0. Comparison of zones of inhibition between organisms and samples using ANOVA Two Factor without Replication ($p < 0.05$ = insignificant).

Data Analyses

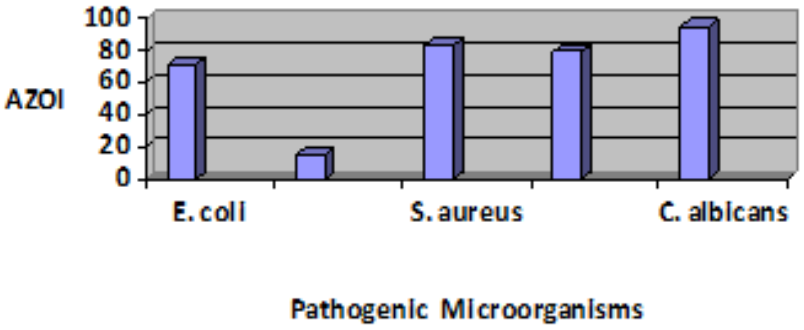
Comparison	F value	F critical	p value	Significance
Between Samples	2.7	1.39	1.72	Significant
Between Organisms	6.5	2.41	5.78	Significant

Table 8.0. shows comparison of zones of inhibition when different extracts are applied to microbial cultures using ANOVA single factor ($p < 0.05$ = insignificant).

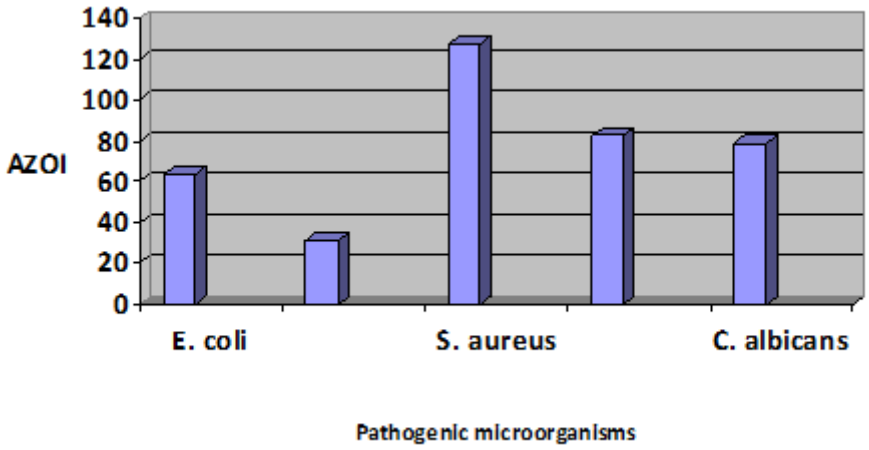
Comparison	F value	F critical	P value	Significance
Between samples	4.13	2.24	0.001	Not significant

Table 9.0. Atomic Absorption Spectroscopic Analyses of the ethanolic and aqueous extract of *P. edulis.*

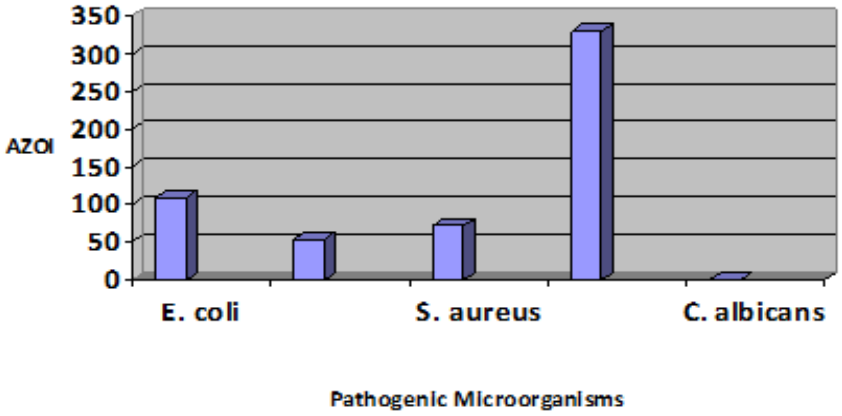
	Cu mg/kg	Zn (mg/kg)	Pb (mg/kg)	Fe (mg/kg)	Co (mg/kg)	Al (mg/kg)	Cl^- (mg/kg)	SO_4^{2-} (mg/kg)
Ethanolic Extract	74.5	15.6	ND	2.04	67.7	ND	42540	15875
Aqueous Extract	ND	ND	ND	0.56	7.60	139	1418	553



Graph 1.0. Antimicrobial activity of fruit extract against pathogenic microorganisms.



Graph 2.0: Antimicrobial activity of aqueous solution of ethanolic isolate against selective pathogenic microorganisms.



Graph 3.0: Antimicrobial activity of aqueous solution of ethanolic isolate in the presence of $Zn(OAc)_2.2H_2O$.

Fig. 2.0

Fig. 3.0

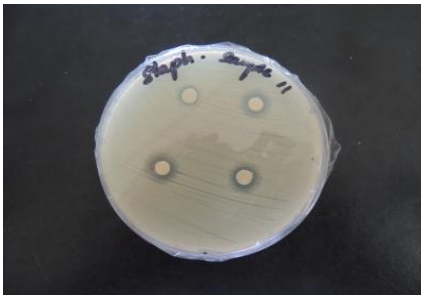


Fig. 4.0

Fig. 5.0

Zone of inhibition induced by sample 9, Fig.2.0 against *E.coli*, sample 2, Fig. 3.0 against *C. albicans*, sample 11 against *S. aureus*, Fig. 4.0 and by sample 12 against *S. aureus*, Fig. 5.0.

DISCUSSION

The diameter of zone of inhibition, DZOI and area of zone of inhibition, (AZOI) were used as indicators of the fruit extracts antimicrobial potency. The mean diameter of zone of inhibition, DZOI with its standard deviation (SD) is expressed in Table 2.0 to Table 5.0. The passion fruit extract in the absence of transition salts induced AZOI, ranging from 16.0 to 150.0 mm^2. In the presence of transition metal salts, AZOI, ranges from 14.0 to 268.7 mm^2. The highest AZOI of 268.7 mm^2 was induced by sample 9 against *S. aureus*. The lowest AZOI of 21.6 mm^2 was induced by sample 6 against *C. albicans*. The above quoted AZOI are exclusive of the reference AZOI.

There seems to be an increase in antimicrobial activity of the ethanolic fruit extract as the concentration increases. To support this, against *E. coli*, AZOI of 53.4 mm^2 and 70.85 mm^2 were obtained at a concentration of 0.015g/ml and 0.1g/ml of ethanolic extract for sample (1) and (3) respectively. For the aqueous extract of the white isolate, an increase in concentration generally resulted in a decrease in antimicrobial potency. For example, against *P. aeruginosa*, AZOI of 149.49 mm^2 and 82.5 mm^2 were obtained at a concentration of 0.026 and 0.052g/ml

respectively, Table 2.0. An exception to this being against *S. aureus*. With the latter, AZOI of 82.5 mm^2 and 127.6mm^2 were obtained at a concentration of 0.026 and 0.052 g/ml respectively, Table 3.0. Graph 1 shows the antimicrobial activity of fruit extract against pathogenic microorganisms. Graph 2.0 shows the antimicrobial activity of the aqueous solution of ethanolic isolate against selective pathogenic microorganisms. Graph 3.0 shows the antimicrobial activity of the aqueous solution of ethanolic isolate in the presence of $Zn(OAc)_2.2H_2O$.

For all experiments conducted, antimicrobial activity seems to be significantly less than that of the standard antibiotics: Ampicillin and Nystatin. Against, *S. aureus*, the antimicrobial activity of the ethanolic extract at the highest concentration of (0.1g/ml) was 11.42% of that of the Reference standard, Ampicillin. Against *C. albicans*, it was 28.9%.

Interestingly, there seems to be an increase in antimicrobial activity of the aqueous solution of the white ethanolic isolate in the presence of Zn (OAc).$2H_2O$ salt against *K.pneumoniae*. With the latter, AZOI of 78.5 mm^2 and 30.7 mm^2 were obtained for the aqueous extract in the absence of the salt at a concentration of 0.026mg/ml and 0.052g/ml, Table 3.0. In the presence of the salt, AZOI of 113.0 mm^2 and 148.4 mm^2 were observed at the same respective concentration against *K. pneumoniae* Table 4.0. A similar trend was noted for *E. coli* and *S. aureus* at the lower concentration of administration, Table 3.0. Against other pathogenic microorganism such as *P.aeruginosa* and *C. albicans*, there was a decrease in antimicrobial activity. It is anticipated that the white isolate will chelate/complex with the transition metal salt which may have both a positive and negative effect on the antimicrobial activity.

Antimicrobial selectivity was also observed for the fruit extracts in the presence and absence of transition metal salts. Antimicrobial selectivity is an important factor to be taken into consideration when designing a drug for its therapeutic functions. It is also important in preventing antimicrobial resistance, currently, a global problem. As an example, AZOI of 16.0 mm^2 was induced by the ethanolic extract at a concentration of 0.1g/ml of against *K. pneumonia*, Table 2.0. Compared against *S. aureus*, AZOI of 82.5mm^2 was observed at the same concentration of fruit extract i.e a selectivity factor of 0.2. For the transition metal salts, AZOI of 283.39 mm^2 was observed against *S. aureus* at a concentration of 10% aqueous Zn(OAc). $2H_2O$. In comparison, AZOI of 108.4 mm^2 and 33.2 mm^2 was observed against *K. pneumoniae* and *C. albicans* respectively at a concentration of 10% aqueous Zn(OAc). $2H_2O$,

Table 4.0 and Table 5.0. Fig.2.0 shows Zone of Inhibition, ZOI induced by sample 9.0 against *E.coli*. Fig. 3.0 shows ZOI induced by sample 2.0 against *C. albicans*, Fig. 4.0 shows ZOI induced by sample 11 against *S. aureus* and Fig 5.0 shows ZOI induced by sample 12 against *S. aureus*.

The ethanolic and aqueous extract of *P. edulis* were subjected to Atomic Absorption Analyses Spectroscopic Analyses, AAS. The results are shown in Table 9.0. AAS analyses of the ethanolic extract of *P. edulis* revealed selective presence of cations. Cu, Zn, Fe and Co are detected. There is no detection for Pb and Al. Of the anions, Cl^- showed a higher value of 42,540 mg/kg compared to 15, 875 mg/kg. The aqueous extract of *P.edulis* revealed no detection for Cu, Zn and Pb. However, there was detection for the presence of Al and Co, with Al registering a higher value of 139 mg/kg. Of the anions, Cl^- registered a higher value of 1418 mg/kg in comparison to 553 mg/kg detected for SO_4^{2-}.

Table 8.0 shows that differences between samples were significant throughout, since the calculated p values is greater than 0.05. Table 9.0. shows that differences between samples were not significant throughout, since the calculated p-values are less than 0.05.

CONCLUSIONS

The aqueous and ethanolic extract of green passion fruit in the absence and presence of transition metal salts were subjected to antimicrobial studies using the Disc Diffusion Assay under aseptic conditions. From the ethanolic extract, a white solid crystallised out and its antimicrobial activity was investigated. The highest AZOI of 268.7 mm^2 was induced by sample 9 against *S. aureus.* The lowest AZOI of 21.6 mm^2 was induced by sample 6 against *C. albicans*. These quoted AZOI are exclusive of the reference AZOI. For the non-metal solution, the highest AZOI of inhibition was induced by the aqueous solution (0.026g/ml) against *P. aeruginosa* i.e *P. aeruginosa* was more susceptible. There seems to be an increased in antimicrobial activity of the ethanolic fruit extract as the concentration increases. For the aqueous extract of the white isolate, an increase in concentration generally resulted in a decrease in antimicrobial potency. Interestingly, there seems to be an increase in antimicrobial activity of the aqueous solution of the white ethanolic isolate in the presence of Zn (OAc).$2H_2O$ salt against pathogens in some cases and a decrease in another instance. Selective antimicrobial activity was observed for the fruit extracts in the presence and absence of $Zn(OAc)_2.2H_2O$. For all experiments conducted, antimicrobial activity seems to be less than that of the standard antibiotics: Ampicillin and Nystatin. Nevertheless, the

ethanolic and aqueous extracts of mature passion fruit can be used as a natural antibiotics against a range of bacteria induced diseases.

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