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Antimicrobial Activity of the Ethanolic and Aqueous Extract of *Vicia faba* L. (Fabaceae) at Three Different Concentrations in the Absence and Presence of $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$

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Abstract

The aqueous and ethanolic extract of *Passiflora edulis* and *Vicia faba* L. (Fabaceae) exhibited antimicrobial activity against the pathogenic microorganisms: *E. coli*, *S. aureus*, *K. pneumoniae* and *C. albicans*. This was evaluated using the Disc Diffusion Assay under aseptic conditions. Antimicrobial activity wasn't induced by the solvent, ethanol nor water. The highest AZOI was 153.9 mm² and the lowest 12.56 mm². Negligible Zone of Inhibition, ZOI were observed in several instances. The aqueous extract of the fruit also induced negligible Zone of Inhibition, ZOI. In comparison to the reference, Ampicillin and Nystatin, these values are less. As the concentration of the metal salt, $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ and ethanolic extract increases, there seem to be a variation in antimicrobial activity. $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ appears to intensify the antimicrobial activity of the plant ethanolic and aqueous extract. $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ in the absence of any extracts exhibited antimicrobial activity. The AZOI range from 47.2 mm² to 117.8 mm². Antimicrobial selectivity was also observed in several instances.

Keywords: Antimicrobial activity; Pathogenic microorganisms; Disc Diffusion Assay; Area of Zone of Inhibition; Zone of Inhibition; $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$

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. Also, using the incorrect dosage of the antimicrobials. 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 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 [10-17].

Guyana flora is richly biodiversified and it's 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].

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 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 conduct selective solvent extraction of *Vicia faba* L. using solvents of increasing polarity and to investigate the antimicrobial proficiency on *Vicia faba* L. ethanolic and aqueous extract against pathogenic microorganisms in the absence and presence of $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$, using the Disc Diffusion Assay

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) [18,19]. 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 dehydrogenases, proteinase 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

Literature review reveals that there is only one report on the antimicrobial activity of *Vicia faba* L. (Fabaceae). This was conducted on the seeds. Antimicrobial activity of the crude seed extract was carried out against *E. coli* and *B. subtilis* [20]. Phytochemical screening and antioxidant properties have been reported for *Vicia faba* L. extracts [21]. The applicability of extracts from fava bean seeds (*Vicia faba* L.), as natural coagulants, was investigated [22].

Two new antimicrobial plant peptides related to γ -thionine were isolated from *Vicia faba* L, broad beans [23]. Fava beans are very low in saturated fat, cholesterol and sodium. They are also a good source of dietary fiber, protein, phosphorus, copper, manganese, and a very good source of Folate [22-27]. Fava beans are very high in protein, rich source of dietary fiber, high in phytonutrients such as isoflavone and plant-sterols. It also contains Levo-dopa or L-dopa, a precursor of neurochemicals in the brain such as dopamine, epinephrine and nor-epinephrine. For Parkinson Disease, it has also been reported to be a cure. It is also an excellent source of folates. Significant amounts of vitamin-B₆ (pyridoxine), thiamin (vitamin B₁), riboflavin and niacin have also been reported. It is also a fine source of minerals like iron, copper, manganese, calcium, magnesium. At 1062 mg or 23% of daily recommended levels, *Vicia faba* L are one of the highest plant sources of potassium [22-27].

Methodology

Collection and treatment of plant material

Vicia Faba L., Fava beans were purchased from a vendor at Bourda (Georgetown) market. They were washed, rinsed with distilled water and allowed to dry in air. The fruits were weighed (1425 g), cut in pieces horizontally and placed in two extraction jars. This was followed by selective extraction using solvents of increasing polarity: $\text{n-C}_6\text{H}_{14}$ (5100 ml), $\text{CH}_3\text{CH}_2\text{OH}$ (3400 ml) and water (1700 ml). The hexane extract was filtered to yield a light green extract which was dried over anhydrous Na_2SO_4 to yield a light green viscous extract as noted by the naked eye. This was followed by selective extraction using the more polar solvent, ethanol. The ethanol extract was also dried over anhydrous Na_2SO_4 . Solvents were removed in *vacuo* to yield a dark brown extract of weight (54 g). The aqueous extract, light yellow in colour constituted a concentration of 5.67×10^{-3} g/ml.

Antimicrobial studies

Antimicrobial activities were investigated against human pathogenic organisms, *E. coli*, *S. aureus*, *K. pneumonia* and *C. albicans* using the Disc Diffusion assay under aseptic conditions [28,29].

Solutions for antimicrobial studies

Varying amounts of ethanolic extracts of *Vicia faba* L were taken out, weighed and made up to the requisite concentration of 0.015 mg/L, 0.05 mg/L and 0.1 mg/L using ethanol. 10 ml of the aqueous extract was also

poured out into a sample vial and its antimicrobial activity investigated. $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ salt made up to the concentration of 0.015 mg/L, 0.05 mg/L and 0.1 mg/L was also investigated. In addition, $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ salt dissolved in the requisite aqueous extract of the fruit at concentration of 0.015 mg/L, 0.05 mg/L and 0.1 mg/L was also investigated.

Microorganisms used

The human pathogenic microorganisms used for the antimicrobial activity were: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and the fungus *Candida albicans*. All microorganisms were collected from Georgetown Public Hospital, GPHC. All bacterial cultures obtained were inoculated in a pure nutrient broth which was incubated at 37°C for 18 hrs. The fungal cultures were inoculated in peptone broth at 37°C for 48 hours.

1.3 g of nutrient broth was added to 100 ml of sterile water and mixed thoroughly. 10 ml of nutrient broth was added to a test tube. A streaking loop was formed and this was used to isolate the organism colonies from the pure culture. A loopful of organisms were added to the nutrient broth and mixed thoroughly.

For the peptone broth, 1.5 g of peptone broth was added to 30 ml of water and mixed thoroughly. 10 ml of peptone broth was added to three test tubes. A streaking loop was flamed and this was used to isolate the organism colonies from the pure culture. A loopful of organisms were added to the nutrient broth and mixed thoroughly to evenly distribute the colonies.

Preparation of the media

40 grams of the powdered media (Muller Hinton) were dissolved in 1000 ml sterile distilled water in a conical flask. The weighed amount was mixed properly then allowed to dissolve by heating over a water bath for several minutes. 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. The molten agar was then poured into 90 mm sterile Petri dishes, to a depth of 4mm. These plates were allowed to cool and refrigerated for use the following day.

Agar Disc Diffusion Method [25-27]

The antimicrobial activity of the crude ethanolic extracts and the white isolate compound was determined using the

Disc Diffusion method assay. The plates prepared above 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 24 hrs and 48 hrs at 37°C for bacterial and fungal species respectively.

Reference Experiments

To compare the diameter of zone of inhibition, DZOI induced by the bacteria: *E.coli*, *S. aureus* and *K. pneumoniae*, ampicillin capsules were used in concentration of 0.015 mg/L, 0.05 mg/L and 0.1 mg/L. The DZOI induced by *Candida albicans* were referenced to Nystatin tablets in concentration of 0.015 mg/L, 0.05 mg/L and 0.1 mg/L.

Control Experiments

For the control experiment, discs impregnated with the solvent ethanol and deionized water were applied to agar disc inoculated with the above pathogenic microorganisms

Data Analyses

Data were statistically analyzed [30,31] to find the mean diameter of the zone of inhibition, DZOI, standard deviation (SD), 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: Weight of fruit, volume of solvent added, concentration and % yield of extract per solvent type extract.

weight of ground plant material (g)	Type of extract	Volume of solvent added (ml)	State of extract	weight/ concentration of extract	% yield of extract
1425	n-C ₆ H ₁₄	5100	Light green	1.8 g	0.1
1425	Ethanol extract	3400	Dark brown	54 g	3.8%
1425	aqueous extract	3400	Light yellow	5.67×10 ⁻³ g/ml	

Table 2: DZOI, mean DZOI with standard deviation and AZOI induced by respective samples against pathogenic microorganism, *E.coli*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
Sample 1	(1)	-----		
Sample 2	(1)	10, 9, 9, 9	9.25	67.2
Sample 3	(1)	14, 12, 10, 12	12	113.0
	(2)	12, 13, 12, 10	8	50.2
Sample 4	1	8, 9, 7, 8	8	50.2
	2	7,6, 8, 6	6.75	35.8
	3	6, 7, 7, 7	6.75	35.8
Sample 5	1	9,9,7,11	9	63.6
	2	11,9,10,7	9.25	67.2
	3	10,9,9,9	9.25	67.2
Sample 6	1	9, 9,10,9	9.25	67.2
	2	8,9,9,8	8.5	56.7
	3	17,14,13,12	14	153.9
Sample 7	1	-----	-----	-----
	2	-----	-----	-----
	3	-----	-----	-----
Sample 8	1	-----	-----	-----
	2	-----	-----	-----
	3	-----	-----	-----

Table 2: cont'd. DZOI, mean DZOI with standard deviation and AZOI induced by respective samples against pathogenic microorganism, *E.coli*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
Sample 9	1	-----		
	2	-----		
	3	-----		
Sample 10	1	-----		
	2	-----		
	3	-----		
Sample 11	1			
	2			
	3			
Sample 12	1	7, 7, 6, 6,	6.5	33.2
	2	8, 7, 6, 6	6.75	35.8
	3	6, 6, 6, 6	4	12.6
Sample 13	1	7, 6, 6, 6	6.25	30.7
	2	7, 7, 6, 6	6.5	33.2
	3	8, 7, 6, 6	6.75	35.8
Sample 14	1	6, 6, 6, 6	6	28.3
	2	6, 6, 6, 7	6.25	30.7
	3	7, 7, 6, 6	6.5	33.2
Sample 15	1	6, 6, 7, 6	6.25	30.7
	2	8, 6, 9, 6	7.25	41.3
	3	6, 6, 6, 6	6.0	28.6

Table 3: DZOI, mean DZOI with standard deviation and AZOI induced by respective samples against pathogenic microorganism, *S. aureus*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
Sample 1				
Sample 2	1	14, 11, 10, 11	11.5	8461.2
	2	13, 8, 10, 12	10.75	90.7
Sample 3	1	10, 9, 11, 10	10.0	78.5
	2	11, 11, 9, 10	10.25	82.5
	3	11, 10, 10, 10	10.25	82.5
Sample 4	1	-----	-----	
	2	-----	-----	
	3	-----	-----	

Sample 5	1	9, 10, 12, 9	10.0	78.5
	2	11, 10, 9, 9	9.75	74.6
	3	9, 10, 11, 10	10.0	78.5
Sample 6	1	11, 11, 12, 10	11.0	94.9
	2	12, 10, 11, 12	11.25	99.4
	3	13, 10, 13, 11	11.75	108.4
Sample 7	1	-----	-----	-----
	2	-----	-----	-----
	3	-----	-----	-----
Sample 8	1	7,	7	38.5
	2	-----	-----	-----
	3	7		38.5

Table 3: Cont/d, DZOI, mean DZOI with standard deviation and AZOI induced by respective samples against pathogenic microorganism, *S. aureus*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
Sample 9	1	7, 6, 6, 6	6.25	30.7
	2	7, 7, 6, 6	6.5	33.2
	3	7, 8,	7.5	44.2
Sample 10	1	6, 7	6.5	33.2
	2	-----	-----	-----
	3	8, 6, 6	6.67	34.9
Sample 11	1	-----	-----	-----
	2	-----	-----	-----
	3	-----	-----	-----
Sample 12	1	7, 6, 7, 7	6.75	35.8
	2	6, 7, 7, 6	6.5	33.2
	3	7, 6, 7, 7	6.75	35.8
Sample 13	1	6, 8, 6, 6	6.5	33.2
	2	7, 9, 8, 6	7.5	44.2
	3	6, 6, 6, 7	6.25	30.7
Sample 14	1	7, 6, 6, 6	6.25	30.7
	2	6, 6, 6, 6	6.0	28.3
	3	7, 7, 6, 6	6.5	33.2

Table 4: DZOI, mean DZOI with standard deviation and AZOI induced by respective samples against pathogenic microorganism, *K. pneumoniae*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
Sample 1				

Sample 2	1	11, 11, 11, 12	11.25	99.4
	2			
	3			
Sample 3	1	12, 12, 13, 11	12	113.0
	2	11, 12, 12, 12	11.75	108.4
	3			
Sample 4	1	-----		
	2	-----		
	3	-----		
Sample 5	1	11, 9, 9, 11	10.0	78.5
	2	13, 11, 12, 12	12	113.0
	3	11, 9, 8, 10	9.5	70.8
Sample 6	1	13, 13, 11, 13	12.5	122.7
	2	11, 11, 11, 13	11.5	103.9
	3	14, 13, 14, 12	13.25	137.8
Sample 7	1	-----		
	2	-----		
	3	-----		
Sample 8	1	-----		
	2	7,7,7,7	7.0	38.5
	3	7,7,7,7	7.0	38.5

Table 4: cont'd. DZOI, mean DZOI with standard deviation and AZOI induced by respective samples against pathogenic microorganism, *K. pneumonia*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
Sample 9	1	7, 6,6,6	6.25	38.7
	2	7,6,6,6	6.25	38.7
	3	7, 6,6,7		
Sample 10	1	-----		
	2	-----		
	3	-----		
Sample 11	1	7,7,7,7	7.0	38.5
	2	7,7,7,7	7.0	38.5
	3	-----		
Sample 12	1	-----		
	2	-----		
	3	-----		
Sample 13	1	7, 7, 6, 6	6.5	33.2
	2	6, 6, 6, 6	6.0	28.3
	3	7, 6, 6, 7	6.5	33.2

Sample 14	1	7, 7, 6, 6	6.5	33.2
	2	6, 6, 6, 6	6.0	28.3
	3	7, 7, 8, 7	7.25	41.3
Sample 15	1	7, 7, 6, 6	6.5	33.2
	2	6, 7, 6, 6	6.25	30.7
	3	7, 6, 6, 7	6.5	33.2

Table 5: DZOI, mean DZOI with standard deviation and AZOI induced by respective samples against pathogenic microorganism, *Candida albicans*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
Sample 1	1	7,6,6,6	6.25	30.7
	2	8,9,7,7	7.75	47.2
Sample 2	1	10, 10, 9, 8	9.25	67.2
	2	12, 9, 8, 8	9.25	67.2
Sample 3	1	13,9,10,10	10.5	86.6
	2	12,11,14,12	12.25	117.8
	3	13,13,11,11	12.0	113.0
Sample 4	1	7,6,6,7	6.5	33.2
	2	8,6,7,8	7.25	41.3
	3	7,8,7,6	7.0	38.5
Sample 5	1	11,8,9,8	9	63.6
	2	11,13,10,10	11	94.9
	3	10,10,9,9	9.5	70.9
Sample 6	1	14,12,13,10	12.25	117.8
	2	13,11,18,11	13.25	137.8
	3	11,12,14,11	12.0	113.0
Sample 7	1	-----		
	2	-----		
	3	-----		
Sample 8	1	7,7,7,7	7.0	38.5
	2	6,6,6,6	6.0	28.3
	3	-----		

Table 5: cont'd DZOI, mean DZOI with standard deviation and AZOI induced by respective samples against pathogenic microorganism, *C. albicans*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
Sample 9	1	-----		
	2	7,7,7,7	7.0	38.5
	3	7,7,7,7	7.0	38.5

Sample 10	1	-----		
	2	8,8,8,8	8.0	50.2
	3	-----		
Sample 11	1	-----		
	2	-----		
	3	-----		
Sample12	1	7, 7,7,7	7.0	38.5
	2	7,7,6,7	6.8	35.8
	3	6,6,6,6	6.0	28.3

Table 6: cont'd DZOI, mean DZOI with standard deviation and AZOI induced by reference samples against pathogenic microorganism, *S. aureus*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
16	3	35, 27, 25, 25	28	615.4
	2	25, 24, 23, 25	24.3	463.5
	1	30, 30, 30, 30	30	706.5
17	1	28, 30, 29, 30	29.3	673.9
	2	29, 27, 30, 29	28.8	648.9
	3	35, 35, 35, 35	35	961.6
18	1	32, 30, 32, 30	31	754.4
	2	33, 30, 28, 28	29.8	694.8
	3	30, 30, 30, 30	30	706.5

Table 7: cont'd DZOI, mean DZOI with standard deviation and AZOI induced by reference samples against pathogenic microorganism, *E.coli*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
16	1	26, 26, 33, 24	27.25	582.9
	2	25, 24, 26, 23	24.5	471.2
	3	29, 29, 30, 27	28.75	648.9
17	1	30, 30, 30, 30	30.0	706.5
	2	29, 33, 30, 30	30.5	730.3
	3	40, 39, 40, 40	39.75	1240.4
18	1	35, 33, 30, 30	32.0	803.8
	2	30, 30, 30, 30	30.0	1256.0
	3	30, 45, 45, 50	37.5	1103.9

Table 8: cont'd DZOI, mean DZOI with standard deviation and AZOI induced by reference samples against pathogenic microorganism, *K. pneumoniae*.

Sample (single or combined)	Plate number	Diameter of zone of Inhibition (mm)	Mean Diameter of Zone of Inhibition (mm)	AZOI (Area of Zone of Inhibition) (mm ²)
16	1	13, 16, 14, 13	14.0	153.9
	2	15, 14, 14, 14	14.25	159.4
	3	16, 14, 12, 14	14.0	153.9
17	1	10, 15, 16, 18	14.75	170.8
	2	16, 17, 18	17.0	226.9
	3	16, 19, 18, 15	17	226.9
18	1	20, 19, 20, 19	19.5	298.5
	2	20, 20, 20, 20	20.0	314.0
	3	22, 22, 23, 21	22	379.9

Key:

Sample 1: 0.1g of Zn(OAc)₂.2H₂O in 10 ml of water

Sample 2.. 0.5g of Zn(OAc)₂.2H₂O in 10 ml of H₂O

Sample 3. 1.0g of Zn(OAc)₂.2H₂O in 10 ml of H₂O

Sample 4. 0.1g of Zn(OAc)₂.2H₂O in 10 ml of aqueous extract of fruit

Sample 5. 0.50g of Zn(OAc)₂.2H₂O in 10 ml of aqueous extract of fruit

Sample 6. 1.0g of Zn (OAc)₂.2H₂O in 10 ml of aqueous extract of fruit

Sample 7. aqueous extract of fruit at one concentration

Sample 8. 0.1g of the ethanolic extract in 10ml of ethanol

Sample 9. 0.5g of the ethanolic extract in 10 ml of ethanol

Sample 10. 1.g of the ethanolic extract in 10 ml of ethanol

Sample 11. 0.5g of the ethanolic extract in 10 ml of ethanol

Sample 12. 2.5g of the ethanolic extract in 10 ml of ethanol

Sample 13. 0.15g of the ethanolic extract in 10 ml of ethanol

Sample 14. 0.62g of the ethanolic extract in 10 ml of ethanol

Sample 15. 1.1g of the ethanolic extract in 10 ml of ethanol

Sample 16. 0.1 g of the ampicillin tablets in 10 ml of ethanol

Sample 17. 0.5g of the ampicillin tablet in 10 ml of ethanol

Sample 18 1.0g of the ampicillin tablet in 10 ml of ethanol

Sample 19 0.1g of the nystatin tablet in 10 ml of ethanol

Sample 20 0.5g of the nystatin tablet in 10 ml of ethanol

Sample 21 1.0 g of the nystatin tablet in 10 ml of ethanol

Table 9: Control Experiment: AZOI induced by solvent deionized water and ethanol

Pathogenic microorganisms	DZOI induced by H ₂ O (mm)	DZOI induced by ethanol (mm)
<i>E. coli</i>	< 5	< 5
<i>S. aureus</i>	< 5	< 5
<i>K. pneumoniae</i>	< 5	< 5
<i>C. albicans</i>	< 5	< 5

Table 10: Shows the ANOVA result for *E. coli* against all the samples.

	F calculated	P value	F critical	Result
Samples	1.02062759	0.504498212	3.025472724	Not significant

Table 11: Shows the ANOVA results for *S. aureus* against all the samples.

	F calculated	P value	F critical	Result
Samples	39.99212186	7.07357E-08	2.637123558	Significant

Table 12: Shows the ANOVA results for *K. pneumoniae* against all the samples.

	F calculated	P value	F critical	Result
Samples	11.20413268	0.000486146	3.025472724	Significant

Table 13: Shows the ANOVA results for *C. albicans* against all the samples.

	F calculated	P value	F critical	Result
Samples	9.500127818	0.000933314	3.025472724	Significant

Table 14: Shows the ANOVA results for *S. aureus* references (Ampicillin).

	F calculated	P value	F critical	Result
Samples (16-21)	1.450690363	0.306253895	5.14325285	Not significant

Table 15: Shows the ANOVA results for *E. coli* references (Ampicillin).

	F calculated	P value	F critical	Result
Samples (16-21)	3.640055418	0.092224998	5.14325285	Not significant

Figure 1: Zone of Inhibition induced by (a) sample 2 and (b) sample 6 against *S. aureus* and *K. pneumoniae* respectively.

A Plot of AZOI versus pathogenic microorganism for Sample 6.

A plot of AZOI versus Pathogenic microorganism for Sample 12.

Table 16: Shows the ANOVA results for *K. pneumoniae* references (Ampicillin).

	F calculated	P value	F critical	Result
Samples (16-21)	24.81713329	0.001254373	5.14325285	Significant

Table 17: Shows the ANOVA result for Samples 1-3 against all the selected microbes.

	F calculated	P value	F critical	Result
Samples 1-3	4.392912705	0.066821625	5.14325285	Not significant
Microbes	1.081759049	0.43649814	5.409451318	Not significant

Table 18: Shows the ANOVA result for the aqueous extract samples (4-7) against all the selected microbes.

	F calculated	P value	F critical	Result
Samples 4-7	14.84869207	0.003491868	4.757062663	Significant
Microbes	0.636886329	0.618214779	4.757062663	Not significant

Table 19: shows the ANOVA result for the Ethanolic extract samples (8-15) against all the selected microbes.

	F calculated	P value	F critical	Result
Samples 8-15	1.76258985	0.185317078	2.913358179	Not significant
Microbes	2.311402525	0.115028414	3.238871517	Not significant

Table 20: Atomic Spectroscopic Analyses of the Ethanolic and Aqueous extract of *Vicia fabia*.

Sample Description	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	101	13.6	ND	1.88	65.1	ND	28360	17675
Aqueous	ND	ND	ND	ND	10.2	134	2836	499

Discussion

Antimicrobial properties were shown by the aqueous and ethanolic extract of the fruit, *Vicia faba* L. (*Fabaceae*) in the absence and presence of Zn (OAc)₂.2H₂O. Sample (1) to (3), constitutes Zn (OAc)₂.2H₂O in varying concentration. Sample (4)-(6), aqueous extract of fruit in varying concentration of Zn(OAc)₂. 2H₂O. Sample (7), aqueous extract of fruit at one concentration. Samples (8)-(15) represents ethanolic extracts of fruit at varying concentration. Samples (16-21) represents reference experiments against the pathogens, bacteria, (16)-(18) and fungus, *C. albicans*, 19-21.

The results, Tables 1-8 indicates that the fruit can be used for its nutritional properties in addition to its antimicrobial activities. The highest Area of Zone of Inhibition, AZOI of 153.86 mm² was induced by sample 6 against *E.coli* (Table 2). In comparison, Sample 3.0, induced a lower AZOI of 113.0 mm². This shows that that Zn (OAc)₂.2H₂O did amplified the antimicrobial activity of the aqueous fruit extracts. Sample (7), aqueous extract of fruit at one concentration showed negligible AZOI against *E.coli*.

The lowest non-zero AZOI of 28.3 mm² was induced by sample 14 against *S.aureus* (Table 3). Negligible AZOI was induced by sample (7), (8), (9), (10) and (11) against *E.coli*. Samples (4), (7), (11) against *S. aureus* also exhibited negligible AZOI. Negligible AZOI were seen for samples (4), (7), (10) and (12) against *K. pneumoniae* and samples (7) and (11) against *C. albicans*. Figure 1: (a) shows Zone of Inhibition, ZOI induced by sample 2 against *S. aureus* and (b) sample (6) against *K. pneumoniae*, respectively.

Antimicrobial selectivity was also evident. Antimicrobial selectivity is very important factor in the design and synthesis of an antibiotic, as it prevents antimicrobial resistance. The latter is currently of global concern as its responsible for antibiotics becoming impotent and useless.

In terms of antimicrobial selectivity, sample 4 display AZOI of 50.2 mm² against *E. coli*, whereas negligible AZOI was noticeable against *S. aureus*. Likewise, sample 12 shows AZOI of 35.77 mm² against *S. aureus*, whereas negligible AZOI was obtained against *K. pneumoniae*. Sample 11 shows negligible AZOI against *E.coli* and *S. aureus* and *C. albicans* but AZOI of 38.5 mm² against *K. pneumoniae*.

Increasing the concentration of the metal salts, Zn(OAc)₂.2H₂O increased the AZOI, whereas for the ethanolic extract, there seem to be a variation of AZOI against the pathogens. For example, sample 13 showed AZOI of 33.2 mm², 44.2 mm² and 30.7 mm² against *S. aureus* at concentration of 0.15 g/ml, 0.62 g/ml and 1.1 g/ml respectively (Table 3).

Zn (OAc)₂.2H₂O in the absence of any extracts exhibited antimicrobial activity. The AZOI range from 47.2 mm² to 117.8 mm², samples (1)-(3).

Samples (1), (2), (3), (5) and (6) were used to compare the effect of Zn(OAc)₂.2H₂O on the antimicrobial activity of the aqueous extract of the fruit. It is evident that the Zn(OAc)₂.2H₂O did amplify the antimicrobial activity of the aqueous extract of the fruit in most cases. For example, against *E.coli*, sample 1 exhibits negligible AZOI. In comparison, sample (4) exhibits AZOI of 50.2 mm². Sample 3 exhibits AZOI of 113.0 mm² against *K. pneumoniae*, whereas sample (6) exhibits AZOI of 122.7 mm².

The control experiment indicates that negligible zone of inhibition, ZOI and AZOI were obtained by the solvent against the pathogenic microorganisms: *E. coli*, *S. aureus*, *K. pneumoniae* and *C. albicans* (Table 9). This indicates that the antimicrobial properties exhibited by the ethanolic and aqueous extracts of fruit 2 is due to its chemical constituents and is not a solvent effect.

The Reference Experiment (Table 8) indicates that significantly higher AZOI was induced by the reference samples, Ampicillin and Nystatin at varying concentration in comparison to the ethanolic and aqueous extracts of fruit, *Vicia faba* L..

The results were statistically analysed for the mean, standard deviation, SD. In addition, A One way single factor ANOVA analysis was conducted to compare the effect of the different samples (Samples 1-15) against *E. coli*, *S. aureus*, *K. pneumoniae*, *C. albicans* etc. Against *E. coli* (Table 10). A P value >0.05 was observed, indicating no statistical difference between the different samples used. Against, *S. aureus* (Table 11), a P value <0.05 was calculated indicating that there is a statistical difference between the different samples used. Against *K. Pneumoniae*, Table 12 shows a P value <0.05 was calculated, indicating that there is a statistical difference between the different samples used. Against, *C. albicans* (Table 13), a P value <0.05 was calculated, indicating that there is a statistical

difference between the different samples used.

One way single factor ANOVA analyses were also conducted to compare the effect of Ampicillin (Sample (16)-(18) as the reference samples and the results are presented in Tables 14-16. Against *S. aureus*, Table 14, a P value >0.05 was observed indicating that there was no statistical difference between the different concentrations of ampicillin used against the bacteria. Using Ampicillin as the reference (Sample 16-21) against *E. coli*, Table 15, a P value >0.05 was observed, indicating that there was no statistical difference between the different concentrations of ampicillin used against *E. coli*. Using Ampicillin as the reference (Sample 16-21) against *K. pneumoniae*, Table 16, a P value <0.05 was observed, indicating that there exists statistical difference between the different concentrations of ampicillin used against *K. pneumoniae*.

A one way ANOVA analyses was conducted on samples (1)-(3), where $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ was added to 10 ml of water. It was done to compare if there was any statistical difference between the different samples used regardless of the pathogen and also if there was any statistical difference between the different selected pathogens used. The results are presented in Table 17. For both cases, the P values was >0.05, indicating that amongst the three samples which contained the $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ in 10 ml of water, there was no statistical differences between the different concentrations of substance used nor any differences between the different selected pathogens.

For samples (4)-(7), in the first case, a P value <0.05 was observed indicating that there is a statistical difference between the different concentration of aqueous extract used in which $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ is dissolved.. However, there is no statistical difference recorded between the different selected pathogens, since the P values > 0.05 (Table 18).

For samples (8)-(15) in both cases, the P values was >0.05, so it can be concluded that there was no statistical differences between the different concentrations of ethanolic extract used nor any differences between the different selected pathogens (Table 19).

Atomic Absorption Spectroscopic Analyses were conducted on the ethanolic and aqueous extract of *Vicia fabia* and the results are presented in Table 20. The results indicates that for the ethanolic extract, the concentration of Cu was the highest, 101 mg/kg and Fe the lowest, 1.88

mg/kg. There was no detection for Pb and Al. For the anions, Cl^- exhibited the highest concentration of 28,360 mg/kg. For the aqueous extract, Al exhibited the highest concentration of 134 mg/kg whereas Co was the lowest. There was no detection for Cu, Zn, Pb and Fe. For the anions, Cl^- exhibited the highest value of 2836 mg/kg, whereas SO_4^{2-} was the lowest, 499 mg/kg. Also, there was selective presence for metal cations and anions in the ethanolic and aqueous extract. For example, consider Cu and Fe in the ethanolic extract, the selectivity ratio being (53.72: 1). For the aqueous extract, consider Al and Co, the selectivity ratio is 134/10.2 is 13.14: 1.

Conclusion

The aqueous and ethanolic extract of *Passiflora edulis* and *Vicia faba* L. (*Fabaceae*) exhibited antimicrobial activity against the pathogenic microorganisms: *E. coli*, *S. aureus*, *K. pneumoniae* and *C. albicans*. Antimicrobial activity wasn't induced by the solvent, ethanol nor water. The highest AZOI was 153.9 mm² and the lowest 12.56 mm². In comparison to the reference, Ampicillin and Nystatin, these values are less. As the concentration of the metal salt and ethanolic extract increases, there seem to be a variation in antimicrobial activity. Antimicrobial selectivity was also observed in several instances. Atomic Absorption Spectroscopy, AAS indicates selective presence of the cation and anion in the ethanolic and aqueous extract. For the ethanolic extract, Cu records the highest and SO_4^{2-} the lowest. For the aqueous extract, there was no detection for Cu, Zn, Pb and Fe. Of the anions, Cl^- records the highest value. No toxic metal ions are present, so the extract should be safe to use as a topical applicants.

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