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Antimicrobial Activity of *Hylocereus polyrhizus* (dragon fruit) ethanolic flesh and peel extracts: discovering potential natural antibiotics

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Abstract

Antimicrobial activity of ethanolic extract of the peel and pulp of dragon fruit, *Hylocereus polyrhizus* was investigated using the disc diffusion assay under aseptic conditions. The diameter of Zone of Inhibition (DZOI) was used as an indicator of the antimicrobial potency. Both ethanolic peel and pulp extract showed significant antimicrobial properties. The largest diameter of zone of inhibition (DZOI) was induced by the dragon fruit pulp against *Candida albicans* (DZOI: 15.7mm). The lowest DZOI of 7.89mm was induced against *Pseudomonas aeruginosa*. On the average, the DZOI induced by the ethanolic peel extract was greater than that of the ethanolic pulp. The peel is the first line of defence against pathogens compared to the fruit pulp. The solvent ethanol had negligible DZOI (< 5mm) against the pathogens. With the exception against *E.coli*, the DZOI induced by reference drugs/positive control in Ampicillin and Nystatin were lower than that of either the ethanolic peel and pulp extract. Anova two-way analyses without replication indicated that there is no significant difference between the DZOI induced by the peel and pulp of dragon fruit versus selected pathogens as $p = 0.405 > 0.05$, $F (1.29) < F_{crit} (6.38)$. Also, there was no significant difference in DZOI amongst the pathogens as induced by peel and pulp of dragon fruit, $p = 0.24 < 0.05$, $F (1.94) < F_{crit} (7.71)$. Thus, the dragon pulp and peel extract can be used as potential antibiotics targeting specifically *Klebsiella pneumoniae* and *Candida albicans* induced infections. However, further research is necessary to validate results.

Keywords: *Hylocereus polyrhizus* (dragon), peel, pulp, diameter of zone of inhibition, selected pathogens, Anova

Introduction

Research in the design and synthesis of antimicrobials will continue to proliferate on our planet, considering that Bacteria and fungi develop antimicrobial resistance 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]. Antimicrobial resistance is defined as the resistance against pathogenic microorganisms such as bacteria and fungi [6]. Increasing antimicrobial resistance (AMR) presents a major threat to public health because it reduces the effectiveness of antimicrobial treatment, leading to increased morbidity, mortality, and health care expenditure [7]. According to the World Health Organization (WHO, 2025), over 3500 people die every day due to antibiotic-resistant diseases [8-10].

Bacteria develops antimicrobial resistance via several mechanisms. These include the production of enzymes that will destroy certain prime selective groups in the drug molecule that are necessary for it to act. For example, bacteria produce the enzyme, β-lactamase that will destroy the β-lactam ring of penicillin. The latter is necessary for the antibacterial effect of the drug, penicillin. Other methods bacteria have evolved to protect against the drug, include an increase efflux of the drug across the bacterial membrane, the protection of bacterial ribosomes via processes such as methylation, induced by the enzymes methylase, decrease influx of the drug across bacteria [11].

Many synthetic drugs have been used over the years but have several adverse side effects which are usually irreversible when administered and the cost of synthesizing drugs in most cases is an expensive endeavour [1-5]. Synthetic drugs are also not environmentally friendly. Natural antimicrobials overcome all these problems. 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 [12]. Plants also have a wide variety of secondary metabolites, some of which are antimicrobial [13-15]. Crude plants extracts have also demonstrated antimicrobial activity [16-28].

Antimicrobial resistance is a global health threat. In 2019, it was responsible for 1.27 million deaths worldwide. Persons of all age groups and regions were affected. However, middle and low income countries were mostly affected [10]. Drug shortage which is on the increase globally, can exacerbate the spread of drug resistant pathogens like *E.coli* and methicillin- resistant *Staphylococcus aureus* [10]. There is no system in place to monitor, and report priority antimicrobial resistant pathogens in Guyana, although one has been drafted [19].

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 synthetic antibiotics: penicillin, nystatin and ampicillin [20-28]. The objectives of this research were to determine the antimicrobial properties of the ethanolic extracts of peel and pulp of

dragon fruit, *Hylocereus polyrhizus*, with the ultimate aim to develop/ discover another natural antibiotic. Four (4) strains of bacteria and 1 fungus namely; *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, Pseudomonas *aeruginiosa* and *Candida albicans* were investigated, using the Agar Disc Diffusion Method. To the best of knowledge, the antimicrobial activity of dragon fruit has never been reported in the Western world. This research aim at filling that gap. Recently, in regions four and ten in Guyana, the cultivation of dragon fruit (*Cucurbitaceae* family) has been iniated [29].

Dragon fruit, also called pitahaya or pitaya, is the fruit of several species in the cactus genus *Selenicereus*, is a native to the southern regions of Mexico and the Pacific coast of Guatemala, Costa Rica, and El Salvador. In the 21st century, its cultivation has expanded to regions including Vietnam, China, Colombia, and Southeast Asia [30-31]. Figureure. 1.0. is a morphological display of dragon fruits harvested and sole locally in Guyana.



Figure. 1.0. Morphology of dragon fruit, *Hylocereus polyrhizus*

The juice of a dragon fruit can be used to flavor and colour beverages, pastries, or candy.The flowers can be eaten as a vegetable or steeped as a tea [32-36].

In terms of Natural products constituents, Betacyanin, a natural product with potential antioxidant properties, has been isolated from *Hylocereus polyrhizus* peel and flesh, Figureure.2.0. Its widely used in the food, cosmetic and pharmaceutical applications. However, it is sensitive to heat and light, presenting challenges in extraction and processing [37].

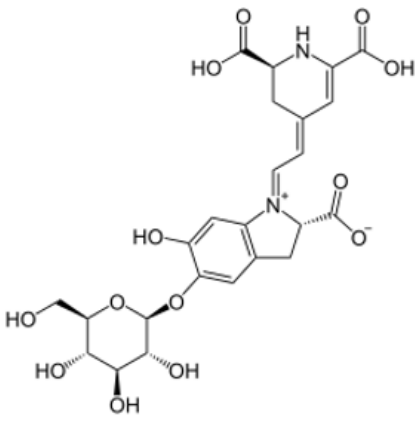


Figure. 2.0. Betacyanin

Literature Reviews

A total of thirty one (31) research articles were reviewed, using the Google Search Engine to identify research gaps. Most of the relevant research were conducted in India, China, Taiwan, Spain, Phillipines, Malaysia etc. Most of the research focused on the ethanolic extract of the peel of dragon's fruit. Two articles focused on the peel and pulp of dragon fruit, whereas the others focused on either the peel or pulp. The pathogenic bacteria investigated were *E.coli, S.aureus* and in some instances, methicillin resistant *staphylococcus aureus* (MRSA). Two articles mentioned study against *Bacillus subtillis* and *Salmonella typhi*. A wide range of statistical analytical tools were used to analyse results. Based on the literature review, there is little or no report on the antimicrobial activity of the dragon fruit in Guyana and the rest of the Caribbean and Western World[38-52].

Methodology

Experimental Design

The Experimental design includes a Factorial Complete Randomized Design. The factorial design was appropriate because the experiment included multiple independent factors: (A) plant part (flesh vs peel) and (B) microorganism type (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*). This results in a × 2 × 5 factorial structure plant component, microorganism). Each combination was used as a treatment. For example, peel-*Klebsiella pneumoniae*, flesh-E. coli, Peel-*Candida albicans*. As a result, there were five (5) randomly allocated combinations that were repeated three times to get 15 replicate petri dishes and this was done for a negative and positive controls, the peel and pulp of the flesh. This yielded a total of 60 plates. This design enables for the examination of both main effects i.e if peel extracts are more effective than flesh extracts and interaction effects. Randomization guarantees that uncontrolled variables are fairly distributed across treatments, whereas factorial analysis makes better use of resources by producing detailed data on how numerous factors interact [53].

Material and Apparatus

Petri dishes, a spirit lamp filled with ethanol (for sterilizing inoculating loop and tweezer and for keeping the surround air free of contamination), tweezer(for transferring punched out discs), cotton wool wrapped in paper(for plugging opening of conical flask to prevent escape of heat), Mueller Hinton Agar, MHA, autoclave, Nutrient broth/ Luria bertani broth (for sub culturing microorganisms), laminar airflow(working in a controlled environment), inoculating loop(for transferring microorganism unto agar plate.

Preparation of plant extracts

The dragon fruits were purchased locally, washed, dried aerially. Peel and flesh (mesocarp) were removed. (287.14 g) of peel and (692g) of pulp were weighed and sequentially extracted with hexane to remove the lipophilic components. The hexane was filtered off and dried over anhydrous sodium sulphate. After extraction with hexane, the residual dragon fruit peel and flesh were extracted with ethanol twice. Solvents were then filtered off, dried over anhydrous sodium sulphate and removed *in vacuo* to produce a viscous material. 0.5g of the ethanolic extracts of the peel and pulp of the fruit were poured into two separate vials and were made up to the requisite concentration.

Solutions for antimicrobial studies

A 5% solution of the ethanolic extract of peel and pulp of each fruit were made in separate vials. Punched out sterilise discs were added to the vials containing the extracts for about one hour and then aseptically transferred with a tweezer to the agar plates (streaked with the microorganism) at equidistant from each other. There were four discs per plate.

Preparation of the media- MHA

46 g of Mueller Hinton Agar (MHA) was placed into 1200 ml of distilled water. This was mixed thoroughly then heated with frequent agitation while in a conical flask. The mixture was boiled for one minute to completely dissolve the agar powder, then autoclaved at 121 °C for 15 minutes. The molten agar was poured into 90mm sterile Petri dishes, to a depth of 4mm. These plates were exposed to UV rays for 20 minutes to be sterilized then allowed to cool and refrigerated for use the following day. The plates were dried in a dryer and sterilized with UV rays for 20 minutes before use.

Antimicrobial activity

The antimicrobial activity of the crude ethanolic extract of the peel and fruit of dragon fruit was determined using the Disc Diffusion method assay. The plates were labelled and inoculated with the respective bacterial colonies. Four (4) discs, impregnated with the antimicrobial plant extracts at 5% concentrations were placed on the MHA plates. Separate plates were prepared in a similar manner for the positive and negative controls for the bacterial and fungal strains respectively. The plates containing the bacterial colonies were incubated for 24 hours and 48 hours at 37°C for bacterial and fungal species respectively. It was anticipated that if the extract were antimicrobial, they would diffuse outwards on the agar. Larger the area of zone of inhibition, the greater is the antimicrobial proficiency of the extract. After the required time, the diameter of zone of inhibition was measured [53-56]. The area of zone of inhibition, AZOI, was computed, from which the antimicrobial proficiency of the extract was evaluated. The above experiment was repeated for the positive control: Ampicillin for bacteria and Nystatin for fungus. 0.5g of the peel and fruit extracts were used in all cases. The experiment was repeated for the control experiment using ethanol.

A total of 60 plates were used to assay the ethanolic extracts of peel and pulp of fruit. These were allocated as follows: 3 x 5 (ethanolic peel extract) + 3 x 5 (ethanolic pulp extract) + 3 x 4 (ampicillin extract) + 3 x 1 (nystatin extract), (3 x 5) plates for the ethanol control.

Results

Table 1.0. Antimicrobial activity of ethanolic extract of dragon fruit peel vs selected pathogens

Pathogen	Mean ZOI (mm) with SD	AZOI (mm^2)
Escherichia coli	12.33 ±3.61	119.47
Candida albicans	12.89 ±5.67	130.47
Staphylococcus aureus	12.89 ±3.26	130.47
Pseudomonas aeruginosa	12.67 ±4.90	126.01
Klebsiella pneumoniae	13.44 ±3.13	141.96

Table 2.0. Antimicrobial activity of ethanolic extract of dragon fruit pulp vs selected pathogens

Pathogen	Mean DZOI (mm) with SD	AZOI (mm^2)
Escherichia coli	9.33 ±4.27	68.42
Candida albicans	15.67 ±3.54	192.77
Staphylococcus aureus	9.67 ±6.12	73.39
Pseudomonas aeruginosa	7.89 ±6.41	48.88
Klebsiella pneumoniae	12.56 ±3.32	123.81

Table 3.0. Antimicrobial Activity of ethanolic extract (Control) vs selected pathogens

Pathogen	Mean ZOI ± SD (mm)	AZOI (mm^2)
Escherichia coli	1.33 ± 4.00	1.40
Candida albicans	0.78 ± 2.33	0.48
Staphylococcus aureus	2.00 ± 4.00	3.14
Pseudomonas aeruginosa	1.56 ± 3.09	1.90
Klebsiella pneumoniae	0.78 ± 2.33	

Table 4.0. Antimicrobial Activity of Ampicillin vs selected pathogens.

Pathogen	Mean ZOI with SD (mm)	AZOI (mm^2)
Escherichia coli	35.44 ±2.60	986.70
Pseudomonas aeruginosa	9.33±2.29	68.42
Klebsiella pneumoniae	5.44 ±4.19	23.28
Staphylococcus aureus	0.00 ±0.00	0

Table 5.0. Antimicrobial Activity of ethanolic extract in Nystatin vs selected pathogens

Name of Pathogen	Mean diameter of ZOI, DZOI with Standard Deviation	Area of zone of Inhibition, AZOI(mm2)
Candida albicans	0.00 ± 0.00	0.00
Candida albicans	11.67 ± 0.71	106.96
Candida albicans	2.33 ± 11.67	4.26

Table 6.0. Relative antimicrobial potency of ethanolic peel and fruit extracts vs. Ampicillin

Pathogen	Type of plant ethanolic extract	Mean ZOI (mm) with SD	Ampicillin	Mean ZOI (mm) with SD	Relative potency of ethanolic plant extract to Ampicillin
Klebsiella pneumoniae	Peel	13.44 ± 3.13	*Klebsiella pneumoniae*	5.44 ± 4.19	2.47
Candida albicans	Pulp	15.67 ± 3.54	*Candida albicans*		

Table 7.0. Relative antimicrobial potency of ethanolic peel extract vs ethanolic pulp extract

Pathogens	ΔDZOI (mm)	Relative antimicrobial potency of ethanolic peel extract vs ethanolic pulp extract vs ethanolic pulp extract against pathogens
Escherichia coli	3.0	24.33
Staphylococcus aureus	3.22	24.9
Pseudomonas aeruginosa	4.78	37.7
Klebsiella pneumoniae	12.56	6.55

Table 8.0. Two Way Anova analyses without replication for ethanolic peel vs ethanolic pulp extract of dragon fruit vs. selected pathogens

ANOVA						
Source of Variat	SS	df	MS	F	P-value	Fcrit
Peel vs Pulp	22.04464	4	5.51116	1.29076	0.405316	6.388233
Pathogens	8.281	1	8.281	1.939481	0.236143	7.708647
Error	17.0788	4	4.2697			
Total	47.40444	9				

Figure.3.0. Graph showing Area of Zone of Inhibition Vs. Pathogen for dragon fruit Peel

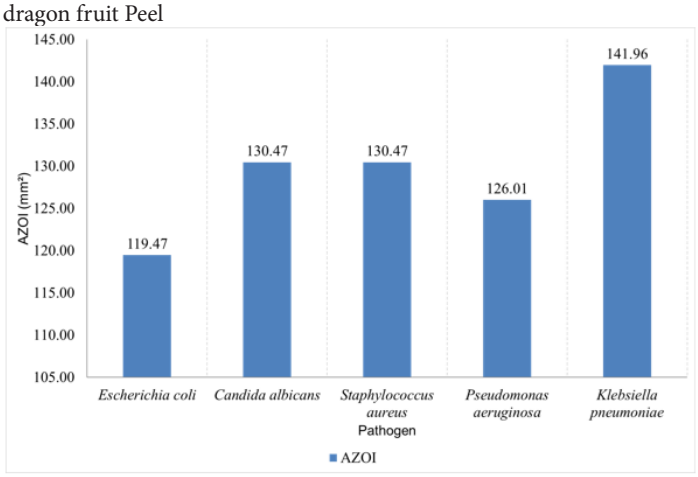


Figure. 4.0. Graph showing Area of Zone of Inhibition Vs. Pathogen for dragon fruit Pulp

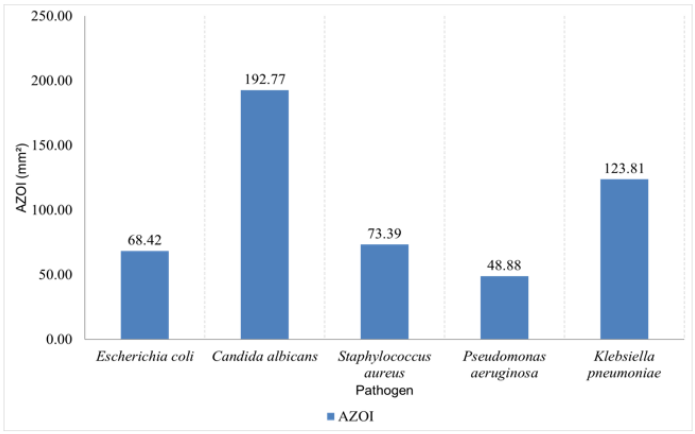


Figure. 5.0. Graph showing Area of Zone of Inhibition Vs. Pathogen for Ethanol

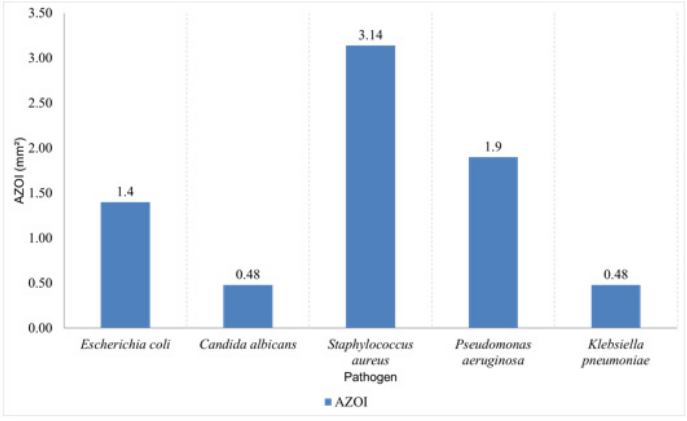


Figure. 6.0. Graph showing Area of Zone of Inhibition Vs. Pathogen for Ampicillin

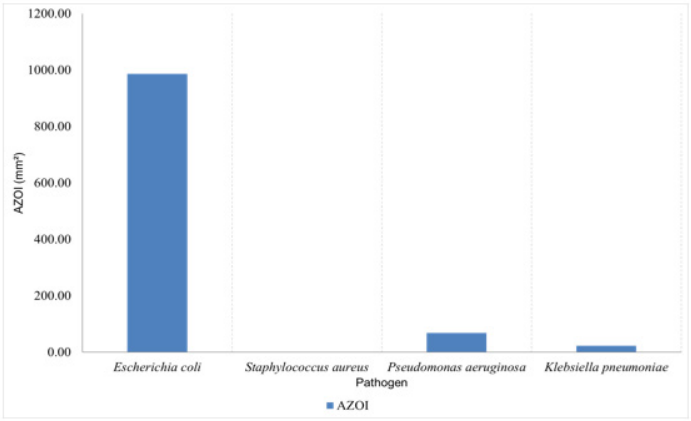


Figure. 7.0. Graph showing Area of Zone of Inhibition Vs. Pathogen for Nystatin

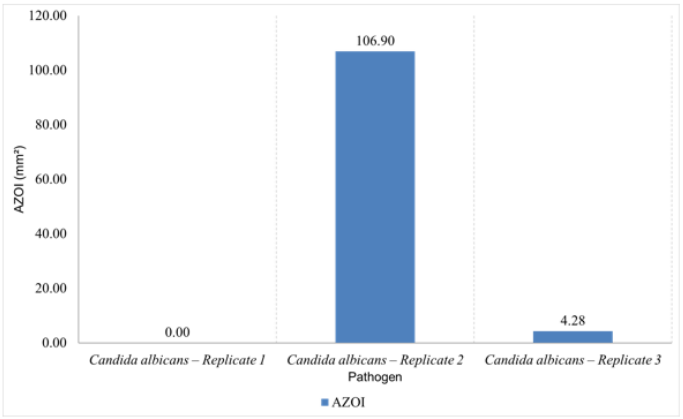


Figure. 8.0. Graph showing Comparison of Area of Zone of Inhibition of Peel and Pulp of dragon fruit and Ethanol Extracts vs. Pathogens

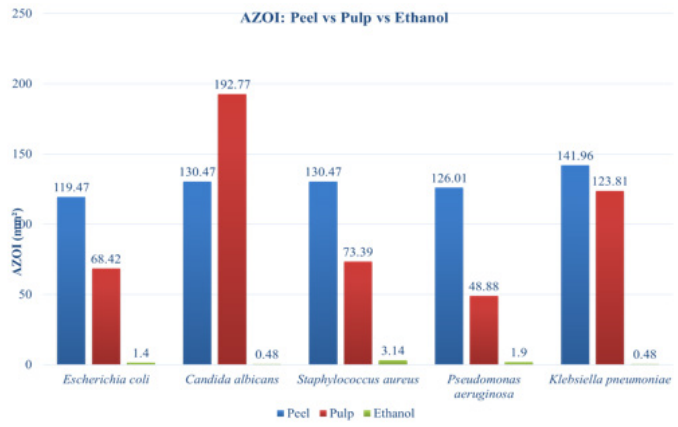
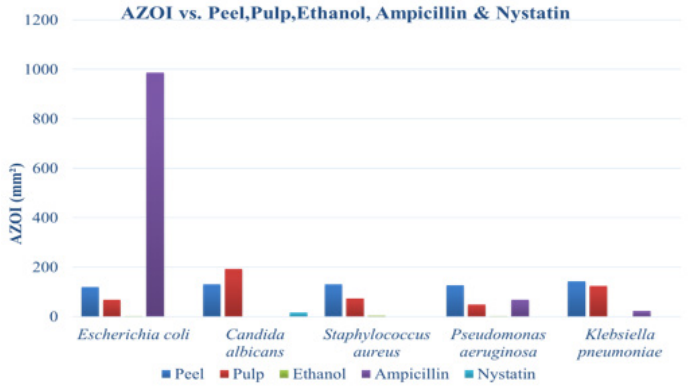


Figure. 9.0. Graph showing Comparison of Area of Zone of Inhibition of Peel and Pulp of dragon fruit extracts, Ethanol, Nystatin and Ampicillin vs. Pathogens



Discussion

Antimicrobial activity was investigated using the Disc Diffusion Assay (DDA). The diameter of zone of inhibition (DZOI) and corresponding AZOI were used as indicators of fruit extract antimicrobial activity. The diameter of zone of inhibition (DZOI) with standard deviation (SD) and Area of Zone of Inhibition are computed and presented in Tables 1.0-5.0. The ethanolic peel and pulp extracts were found to have significant antimicrobial properties. Table 1.0. shows the antimicrobial profile of the ethanolic extract of dragon peel vs selected pathogens. The largest diameter of zone of inhibition, DZOI (13.44 ± 3.13 mm) was induced against *K. pneumoniae* and the lowest of 12.33 ± 3.61 mm was induced against *E.coli*. This corresponds to an AZOI of 141.96 mm² and 119.47 mm² respectively. The DZOI range from 12.33 ± 3.61 mm to 13.44 ± 3.13mm. The peel extract was of equal magnitude against *C. albicans* and *S.aureus* (DZOI = 12.67 mm ± 4.90). Antimicrobial activity seems to follow the trend: *K. pneumoniae* > *C. albicans* = *S.aureus* > *P. aeruginosa* > E. coli. For the peel extract, the DZOI range from 12.33 ± 3.61 mm to 13.44 ± 3.13. Figure 10. shows the DZOI induced by the peel ethanolic extract vs. *Candida albicans*.

Table 2.0 demonstrates the antimicrobial activity of the ethanolic extract of dragon fruit pulp versus selected pathogens. The highest DZOI of 15.67 mm ± 3.54 was obtained against *C. albicans* and the lowest of 7.89 ± 6.41 mm against *Pseudomonas aeruginosa*. Antimicrobial activity follows the sequence: *C. albicans* > *K. pneumoniae* > *S.aureus* > *E.coli* > P.aeruginosa. For the ethanolic pulp extract, the DZOI range from 7.89 ± 6.41 mm to 15.67 ± 3.34 mm.

With the exception against *C. albicans*, it seems that the antimicrobial activity of the peel is greater than that of the pulp. The peel is the first line of defence against pathogens, protecting the fleshly pulp or meso carp of the fruit. Hence, its expected that it should show a greater antimicrobial activity.

Table 3.0 shows the antimicrobial activity of the peel ethanolic extract vs selected pathogens. Negligible DZOI (< 5mm) were obtained, indicating that the antimicrobial activity is not a solvent ethanolic effect, but is due to the phytochemical constituents of the fruit peel.

A positive control in Ampicillin was used to compare its antimicrobial activity with that of the ethanolic peel and pulp extract. All pathogens, with the exception of *E.coli* (DZOI = 35.44 ± 2.60 mm) showed DZOIs which were less than that of *K. pneumoniae* and *C. albicans*, induced by the ethanolic peel and fruit extracts, Table 4.0-Table 5.0. Table 6.0 shows the relative antimicrobial potency of the ethanolic peel and fruit extracts vs. Ampicillin. This is the first example of natural antibiotics that have demonstrated antimicrobial potency, greater than standard antibiotics. Figure 10.0 shows the DZOI induced by the ethanolic peel extract of the dragon fruit against *Candida albicans*.

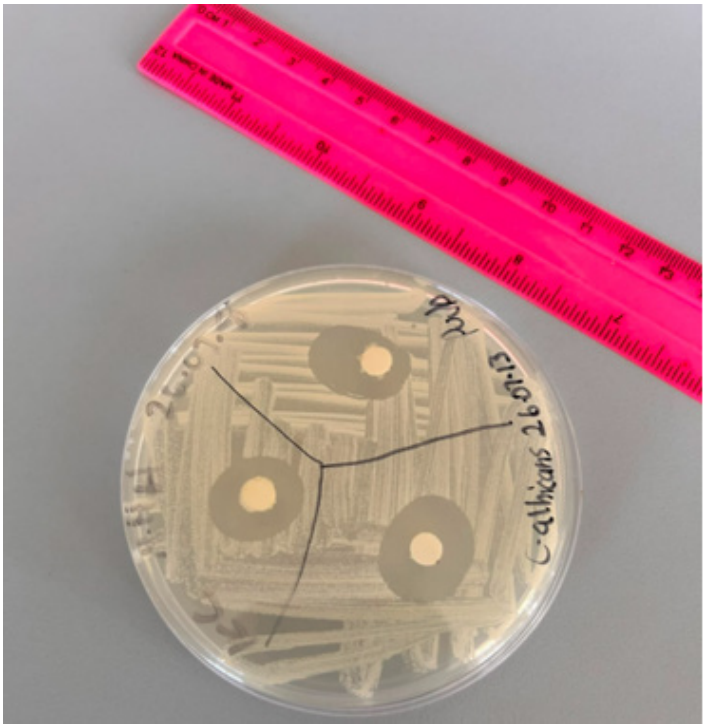


Figure. 10.0. The DZOI induced by the peel ethanolic extract vs. *Candida albicans*

Anova two-way analyses without replication was used to determine whether there is a significant difference between the DZOI induced by the peel and pulp of dragon fruit versus selected pathogens and also amongst pathogens. There was no significance difference between the DZOI induced by the peel and pulp of dragon fruit ethanolic extract against the pathogens as p = 0.405 > 0.05, F (1.29) < Fcrit (6.38). Also, there was no significant difference in DZOI amongst the pathogens as induced by peel and pulp of dragon fruit, p = 0.24 < 0.05, F (1.94) < F crit (7.71).

Conclusion

Dragon fruit ethanolic peel and pulp extracts were found to have significant antimicrobial activity, with the peel more potent than the pulp. Also, with the exception against *E.coli*, both peel and pulp were found to be more antimicrobial than standard antibiotics: Nystatin and Ampicillin. The control solvent, ethanol had negligible antimicrobial activity against the selected pathogens. Thus, the ethanolic extracts of the peel and pulp of dragon fruit can be used as future antibiotics against *Klebsiella pneumoniae* and *Candida albicans* induced infection. Anova two-way analyses without replication, indicated that there was no significant difference between the DZOI induced by the peel and pulp of dragon fruit versus selected pathogens.

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Declaration of work

Prof. R. Jagessar continual vision of exploring for rare natural antibiotics is noted. He is also the author of this paper.

Kezia Cossiah was responsible to pursue the theoretical and practical as-

pect of the research under Prof. R. Jagessar supervision.

Conflict of Interest

The author declare no conflict of interest.

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