

Phytochemical Screening, Nutrient Analysis, Anti-termite and Antimicrobial Activity of *Citrus paradisi* Peel Powder

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Authors' contributions

This work was carried out in collaboration between all authors. Authors UOE, RUBE, UME, GMI, EEE and ENM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors UOE and EEE managed the literature searches, analyses of the study performed the spectroscopy analysis and authors UOE, EEE and RUBE managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Citrus paradisi like most other plants has been shown to contain a number of secondary metabolites with enormous potentials. However, tonnes of the peels are discarded each year. The aim of this study was therefore to evaluate the peels powder for phytochemicals, nutrients, anti-nutrients, antimicrobial and anti-termite activity. Phytochemical analysis from ethanolic and aqueous extracts, nutrients, anti-nutrients, elemental, vitamins, anti-termite and antimicrobial activity were analysed using standard techniques. Results of the phytochemical screening and quantification showed the presence of reducing compounds (18.40%), polyphenols (5.76%), alkaloids (2.42%), glycosides (2.54%), saponins (1.50%), tannins (0.34%) and flavonoids (8.70%). The anti-nutrients (mg/100 g) present were total oxalate (42.75) hydrocyanic acid (3.93), soluble oxalate (24.20) and phytate

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(0.40). Elemental analysis (mg/100 g) showed the presence of Na(6.45), K(264.20), Ca(78.40), Mg(118.30), Fe(10.90), Zn(3.30), Cu(2.60) and P(172.51). Furthermore, Vitamin A, B, C and E were also found in the peels with vitamin C(3.24 mg%) and E (3.43 mg%) being the most abundant. Proximate composition showed the presence of moisture (11.86%), ash (3.97%), protein (10.71%), fat (6.64), fibre (7.55%) and carbohydrate (71.86%). The peels powder also showed anti-termite activity with complete mortality after 4 hours of exposure. However, an equal combination of the peels and the permethrin based insecticide was the fastest in killing the termites. The isolates obtained were *Esherichia coli*, *Proteus*, *Pseudomonas* and *Staphylococci* species. Antimicrobial sensitivity testing showed zones of inhibition of 10 mm to 20 mm on all the isolates. Given antimicrobial and anti-termite potentials, there is a need for more studies that will elucidate the exact nature of these bioactive components.

Keywords: Termites; antimicrobial; peels; *Citrus paradis*; nutrients.

1. INTRODUCTION

As the population of the world continues to increase from every continent of the world, there are concerns about food security, environmental pollution and increasing microbial resistance to routinely used antibiotics. Despite advances in the drug design and in genomics very few antibiotics have become commercially available from the 1970s and resistance is on the increase [1]. As a result of these challenges, alternatives have been sought that are more environmentally friendly. These alternatives majorly utilize living organisms such as microorganisms and plants derived products.

Termites are highly destructive polyphagous insect pests of crop plants, which damage green foliages, seedlings, woods, fibers, and other household cellulose based materials. Most of the termite species attack crop plants, significantly reducing yields and heavily infest post harvest stored products. Most of field termites live in huge mounds, invade green vegetation and dry biomass. Both worker and soldier termites harm non-seasoned commercial wood and its formed materials. Whether it is a rural area or an urban domestic site, termite menace is everywhere and wooden parts of building are not spared [2]. However, for controlling termite population and its menace in the field, various synthetic pesticides such as chlorodane, cypermethrin, hydroquinone and indoxcarb have been used [2-5]. Sadly, they are slowly or non-biodegradable [2]. Due to their longer residual persistence in the environment, these were proved highly toxic to non-target organisms in the ecosystem [3-5]. Approximately 30% of the world's agricultural food produce are lost to pests attacks, it is not surprising then that hundreds of such chemicals make their way into the market despite the fact that they are toxic and recalcitrant.

The biopesticide market is dominated by *Bacillus thurigiensis* at the moment. Plants based products are also on the increase around the world. The main advantage of biopesticides is their safety to non-target organism, biodegradability and their specificity, which permits the use of small dosages, hence avoiding pollution caused by conventional pesticides [6]. In addition to being less harmful than chemicals, biopesticides have been of great value in integrated pest management (IPM) strategies where its use has greatly reduced the use of chemicals, while improving crop yields.

Citrus paradis otherwise called Shaddock or commonly called grape fruits are fruits that are less cultivated in this part of the country despite the fact that their therapeutics and nutritional properties are well known [7]. Based on laboratory studies, the juice have been shown to have antiviral and anticancer effects amongst other properties [7,8]. The dried peels have been shown to have antioxidant and antimicrobial activities [9]. The aim of this project was therefore to determine the anti-termite and antimicrobial activities of *Citrus paradis* peels in addition to their phytochemicals, nutritional and proximate analysis.

2. MATERIALS AND METHODS

2.1 Collection of Fruit

The peels of the *Citrus paradis* used in this study were collected in June 2016 from Obong University, Obong Ntak, Etim Ekpo LGA, Akwa Ibom State and was identified at the University of Calabar Botanical Garden, Cross Rivers State.

2.2 Collection of Termites

Workers termites were collected from an ant hill on campus. These were then identified at the University of Uyo Zoology Department.

2.3 Collection and Identification of Clinical Isolates

The clinical isolates used in this study were obtained from the Microbiology laboratory of the University of Uyo Teaching Hospital and were characterized using morphological and biochemical tests as earlier described [10-12]. The isolates were *Esherichia coli*, *Pseudomonas* spp, *Staphylococcus* spp and *Proteus* spp.

2.4 Preparation of Peel Extracts and Powder

Freshly harvested Shaddock fruits (grape) were peeled using a clean stainless knife into a clean basin. The peels were then oven dried for 3 hour at 60°C. After drying, the peels were then ground into a powder using a mortar and pestle, and stored in universal bottle at room temperature (25°C) and away from moisture. The aqueous and ethanolic extracts were then prepared as previously described [13,14]. Briefly, 20 g of the peel powder were extracted in 100 ml of distilled water and 100 ml of 90% ethanol, respectively. These were then allowed to stand for 72 hours and then heated in a water bath at 70°C to evaporate the solvents. This was done till the extracts became slurry. These were then kept in sample bottles and stored at 4°C in a refrigerator with an aluminium foil wrapped around the sample bottles until required for use.

2.5 Phytochemical Screening

The extracts were screened for the presence of phytochemicals as previously described [14-16].

2.6 Proximate Composition Analysis

The powdered sample was analyzed for food composition according to the Association of Analysis Official Analytical Chemists [17]. These included moisture, crude fiber, ash, crude protein, crude fat, and carbohydrate.

2.7 Moisture

About 5 g of freshly obtained peel powder was dried in an oven to a constant temperature of 70°C. The moisture content was expressed as loss in weight obtained after cool weighing.

2.8 Ash Content

About 5 g of each of the dried peel powder was placed in a crucible and heated to 550°C in order

to burn off the organic components. The crucible with its contents was cooled in a desiccator and weighed. The ash was then expressed as a percentage of the original dry weight of the sample.

2.9 Crude Protein

This was done using the micro-Kjedahl method. The protein nitrogen in 5 g of the sample was converted into ammonium sulphate by digestion with concentrated sulphuric acid in the presence of copper sulphate catalyst with the liberation of ammonia. The liberated ammonia was collected in boric acid double indicator solution. Nitrogen was the quantified by titration using standard HCl. The nitrogen was multiplied by a factor of 6.25 to derive the protein content.

2.10 Crude Fat

Crude fat was extracted from the sample by using about 10 g of the samples with petroleum ether and Soxhlet extractor apparatus. The weight of the fat was obtained after evaporating off the solvent from the extract gave the crude fat in the samples.

2.11 Crude Fibre

About 5 g of the defatted sample was used to determine the fibre contents in both samples using separate exhaustive extraction by acid digestion, filtration and base digestion. The acid digestion was done using 1.25% of H₂SO₄ while the base digestion was done using 1.25% of NaOH. Both steps were boiled for 30 minutes. Following the base digestion, the residues were washed off using 95% methanol. The residue was eventually ignited at 550°C. Fibre content was then expressed as a percentage using lost on ashing and initial weight.

2.12 Carbohydrate

The carbohydrate content was obtained as the difference from 100 of crude protein, fat, ash, and fibre.

2.13 Determination of Vitamins

Vitamins A, E, B and C were determined according to methods previously described [18,19].

2.14 Determination of Mineral Elements

The mineral elements were determined by the dry ash extraction method of AOAC (1995) at the Central Laboratory of Faculty of Agriculture, Wildlife and Forestry, University of Calabar, Calabar, Nigeria.

2.15 Estimation of Anti-nutrients

The peel powder was also examined for anti-nutrients such hydrocyanic acid, phytic acid and oxalate. These were done as previously described [20].

2.16 Hydrocyanic Acid (HCN)

Exactly 10 g of the sample was soaked in 300 ml in distilled water for about 4 hours for the liberation of the cyanide. The liberated cyanide was steam distilled into 20 ml (2.5% w/v) NaOH. To the mixture, 8 ml of NH_4OH was added to the distillate before titrating with 0.02M AgNO_3 to a faint and permanent turbidity. 1 ml of 0.02 AgNO_3 gave 1.08 mg of HCN.

2.17 Phytic Acid

Two grams of the sample was extracted with 0.5M HCl. Ferric chloride was used to precipitate the phytic acid to ferric phytate. NaOH solution was then added to further precipitate it into sodium phytate. This was afterward digested with an acid mixture (equal portion of concentrated H_2SO_4 and HClO_4). The liberated phosphorus was then quantified colorimetrically at 620 nm after colour development with molybdate solution.

2.18 Oxalate

About 2.5 g of the sample was extracted with dilute HCl. The oxalic acid in the extract was precipitated with calcium chloride as calcium salts. The precipitated oxalate was washed with 25% H_2SO_4 and dissolved in hot water before titrating with KMnO_4 .

2.19 Antimicrobial Sensitivity Test

The antimicrobial sensitivity test was carried out using agar disk diffusion methods previously described [21,22]. Briefly, a manual borer was used to obtain disks of 5 mm in diameter from Whatman filter paper No 1. The discs were heated in an oven for 30 minutes at 100°C to sterilize them. After sterilization, triplicate discs were soaked in each of the extracts (aqueous

and ethanolic) for 15 minutes, allowed to air dry and were gently placed on Muller Hinton Agar plates inoculated with the test organisms using spread plate technique. The plates were then incubated at 37°C overnight. Following incubation, the diameters of the zones of inhibitions were measured in millimeter and the mean and standard deviation obtained.

2.20 Anti-termite Activity of the Powder

The anti-termite activity of the grapefruit peel was also tested. A known commercial insecticide with permethrin (0.6%) as active agent was used as positive control and sand as negative control. Briefly, exactly 0.2 g of the peel powder was weighed out and introduced into a sample bottle holding 10 worker termites in triplicates. This was also done with the commercial insecticide and the sand. To another sample bottle, 0.1 g each of the peel and insecticide was weighed and mix together and introduced into the bottle containing the termites. The set-ups were then monitored for mortality every half hour for 4 hours.

2.21 Statistical Analysis

Replicate readings obtained were analyzed for significance using Analysis of Variance (ANOVA) at 95% significance level. All the analyses were done using Microsoft Excel 2007 Version. The results were then presented as mean \pm sd (standard deviation). Probability values less than 0.05 were considered significant.

3. RESULTS

The result of the proximate composition of the studied *C. paradisi* sample presented in Table 1 indicates that the peel powder contains carbohydrate (71.86 ± 0.02), moisture (11.86 ± 0.02), crude fat (6.64 ± 0.02), crude fibre (7.55 ± 0.01) and ash (3.97 ± 0.01) in descending order. The minerals examined were sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Copper (Cu), and Phosphorus (P) and the results are presented in Table 2. The most abundant mineral was potassium (264.20 ± 0.10) followed by phosphorus (172.50 ± 0.01), and magnesium (118.34 ± 0.02). The rest were calcium (78.40 ± 0.10), iron (10.90 ± 0.10), sodium (6.45 ± 0.01), zinc (3.30 ± 0.10) and copper (2.60 ± 0.02). Analysis of variance of the replicate readings of the proximate composition and the mineral composition showed significance ($p < 0.05$).

The result of the vitamins analysis is presented in Table 3. From the result, the most abundant vitamins were C and E with values of 3.24 ± 0.02 and 3.43 ± 0.02 mg%, respectively. The least abundant vitamins were A 138.06 ± 0.20 $\mu\text{g}/\text{dl}$ and B 257.14 ± 0.02 mg/dl in ascending order. Analysis showed significance ($p < 0.05$). The results of the anti-nutrient is presented in Table 4 and it shows the presence of phytate, hydrocyanic acid, soluble oxalate and total oxalate and in mg/100 g were 0.40, 3.93, 24.20 and 42.75, respectively.

Furthermore, the results of the phytochemical screening with ethanolic and aqueous extracts are presented in Table 5 while that of the quantification is presented in Table 6 as the Mean \pm SD (standard deviation) of replicate readings obtained. The screening reveals that the *Citrus paradisi* is very rich in a variety of phytochemicals such as alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds and polyphenols. However, phlobatannins, anthraquinones and hydroxymethyl anthraquinones were absent. Polyphenol was present in much excess in the ethanolic extract and in excess in the aqueous extract. Saponins and tannins were the least abundant. On quantification, reducing compounds gave a value of 5.76 ± 0.01 mg %, while polyphenol was (18.40 ± 0.10) followed by flavonoids (8.70 ± 0.10). The rest were saponins $1.50 \pm 0.02\%$ and alkaloids (2.42 ± 0.02) %. Analysis of the replicate readings showed significance ($p < 0.05$).

The result of the antimicrobial sensitivity is presented in Table 7. The range of inhibition observed was 10.00 ± 0.01 to 20.00 ± 0.01 mm on the test isolates. However, the aqueous and ethanolic extracts of the peels powder did not show any *E. coli* and *Proteus spp.*

Table 1. Proximate composition *Citrus paradisi* peels

Parameters	Composition (%)
Crude Protein	10.71 ± 0.01^a
Fat	6.64 ± 0.02
Ash	3.97 ± 0.01
Crude Fiber	7.55 ± 0.01
Carbohydrate	71.86 ± 0.02
Moisture	11.86 ± 0.02

^aMean \pm SD and P value < 0.05 and is significant

As shown in the Fig. 1 and Table 8, the peel powder has potential as an anti-termite. The

negative control which was sand showed zero anti-termite activity even after four hours. However, peel powder when used alone gave 100% mortality at 4 hours while the chemical based insecticide when used alone and synergistically with the peel powder had almost similar mortality of 100% at 3 hours.

Table 2. Elemental composition of the *Citrus paradisi* peel

Minerals	Composition (mg/100 g)
Sodium (Na)	6.45 ± 0.01^c
Potassium (K)	264.20 ± 0.10
Calcium (Ca)	78.40 ± 0.10
Magnesium (Mg)	118.34 ± 0.02
Iron (Fe)	10.90 ± 0.10
Zinc (Zn)	3.30 ± 0.10
Copper (Cu)	2.60 ± 0.02
Phosphorus (P)	172.51 ± 0.01

^cMean \pm SD and P value < 0.05 and is significant.

Table 3. Vitamins composition of *Citrus paradisi* peel

Minerals	Quantity
A ($\mu\text{g}/\text{dl}$)	$^{a}138.06 \pm 0.20$
B(mg/dl)	257.14 ± 0.02
C (mg %)	3.24 ± 0.02
E (mg %)	3.43 ± 0.02

^aMean \pm SD and P value < 0.05 and is significant.
mg/dl is equivalent to mg/100 ml and $1(\mu\text{g}/\text{dl}) = 0.001\text{mg}/100\text{ml}$

Table 4. Anti-nutrient composition of the *C. paradisi* peels

Anti-nutrient	Composition (mg/100 g)
Hydrocyanic	3.93 ± 0.01^d
Total oxalate	42.75 ± 0.02
Soluble oxalate	24.20 ± 0.10
Phytate	0.40 ± 0.10

^dMean \pm SD and P value < 0.05 and is significant

4. DISCUSSION

Tonnes of grapes are produced and consumed yearly around the globe for their health and therapeutic benefits. After consumption, the peels are usually discarded even though some therapeutic and nutritional facts also exist about them as well. Studies around the world have shown that the peels, seeds, juice and leaves are very rich in bioactive compounds [7-9]. The proximate analysis in our study indeed indicates that the powdered peels of *Citrus paradisi* is very rich in a plethora basic nutrients such as

carbohydrate, proteins, fat, moisture, fibre and ash. The most abundant was carbohydrate 71.86±0.02% while the least was ash 3.97±0.01%. Although, it does not make the dish of most delicacies as do most vegetables, our results show that the peels of the Shaddock under study is far richer in protein than most vegetables. In a recent study by Ebana et al. (2016) where the proximate composition of leaves of *Lasianthera africana* and *Dennettia tripetala* were examined. The protein content of the leaves was reported as 5.70±0.06% for *D. tripetala* and 19.86±0.06% for *L. africana* placing our study plant peels in between the protein contents of these two commonly used vegetables in Southern Nigeria. Despite being so rich in nutrients, the peels have never been utilized by man as a direct or indirect source of food. However, domestic animals like goats eat them anywhere they see the peels being discarded. In another study the proximate components of the flavedo, albedo and seeds of *Citrus sinensis* were much lower than the protein found in our study [23].

Table 5. Phytochemical Screening of *Citrus paradisi* peels

Phytochemicals	Ethanol extract	Aqueous extract
Alkaloids	++	+
Glycosides	+	++
Saponins	+	+
Tannins	+	+
Flavonoids	++	+
Reducing Compounds	++	+
Polyphenol	+++	++
Phlobatannins	-	-
Anthraquinones	-	-
Hydroxymethyl Anthraquinones	-	-

Key: + = Present, ++ = Present in Excess, +++ = Present in much Excess and - = Absent

Elemental composition analysis showed the presence of sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Copper (Cu), and Phosphorus (P). The most abundant element being potassium (264.20±0.10 mg/100 mg) and the least was copper (2.60±0.02 mg/100 mg). The results contained in this study are higher than that reported previously by Ebana et al. [15] for *Cola rostrata* and *C. nitida* seeds and pods. The presence of copper is consistent with an earlier study that also found copper in the fruit juice [24].

Table 6. Quantification of phytochemicals of *Citrus paradisi* peel

Phytochemicals	Mean±SD (%)
Alkaloids	2.42±0.02 ^a
Glycosides	2.54±0.01
Saponins	1.50±0.10
Tannins	0.34±0.01
Flavonoids	8.70±0.10
Polyphenol	18.40±0.10
Reducing compounds (mg %)	5.76±0.02

^a Triplicate readings are presented as Mean±SD, P value < 0.05 and significant

Table 7. Antimicrobial sensitivity (mm) of the aqueous and ethanolic extracts (Mean±SD)

Isolates	Ethanolic extracts	Aqueous extracts
<i>Proteus species</i>	-	10.50±0.01
<i>Escherichia coli</i>	20.00±0.01	-
<i>Pseudomonas</i>	13.00±0.02	10.00±0.01
<i>Staphylococcus</i>	10.00±0.01	14.50±0.01

Key: - = no inhibition

The most abundant vitamins were C and E (% mg) with values of 3.24±0.02 and 3.43±0.02, respectively. The least abundant vitamins were A and B in ascending order. The abundance of vitamins in the peels is also consistent with the presence of the same vitamins in the juice of pink fruit juice. Wood (2005) reported vitamin contents of fruit juice to be vitamin C (44.03 mg), vitamin B1 (0.05 mg) and vitamin A (59.33 mg). Anti-nutrient composition of the peels reveals that it contains allowable limits of anti-nutrient. Hydrocyanic acid was the second least anti-nutrient. The total oxalate reported in our study was far less than that reported in the seeds and skins of ready to eat green and golden Kiwifruit in New Zealand [25]. In another study by Alexander et al. [26] they found the concentration of HCN in cassava to be in the range of 300 to 2,360 (mg/kg) which is far less than what we found in our study. Interestingly, the amount of HCN reported in our study is lower than the allowable oral toxicity standard of 50 to 90 mg of WHO [27]. The amounts of all the anti-nutrients examined in our study were equally less than that reported for commonly eaten vegetables such as *Vernonia amygdalina* (Bitter leaf), *Cochorous olitorius* (Ewedu), *Talinum triangulare* (Water leaf), *Moringa oleifera* (Drum stick) and *Telfairia occidentalis* (Pumpkin leaf) [20,26]. The amount of the vitamin C obtained in the quantity analyzed is much lower than the daily recommended levels by FAO/WHO [28].

The phytochemical screening reveals that the *C. paradisi* is very rich in a variety of phytochemicals such as alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds and polyphenols. However, phlobatannins, anthraquinones and hydroxymethyl anthraquinones were absent. Reducing compounds gave a value of 5.76 ± 0.01 mg %, while the highest was polyphenol (18.40 ± 0.10) followed by flavonoids (8.70 ± 0.10). The rest were saponins $1.50 \pm 0.02\%$ and alkaloids $2.42 \pm 0.02\%$. The findings in this study is consistent with the reports of Oikeh et al. [23] who reported the presence of alkaloids, flavonoids, saponins, tannins, steroids and cardiac glycosides in *Citrus sinensis* fruit wastes.

Termite activity of the peels reveals that the peels have potentials as anti-termites. The worker termite were killed at varying times with the test powder and positive control

insecticide powder used in this study. The negative control which was sand showed zero anti-termite activity even after four hours. However, the permethrin-based insecticide was the fastest in killing the termites. When used synergistically, the killing time and number of mortality recorded was similar to the insecticide used alone. The mortality of the peel powder was in between that of the positive control and combination of peel powder and insecticide powder.

The results of the antimicrobial sensitivity indicate that in addition to the anti-termite activity shown by the peel powder, the ethanolic and aqueous extract have antimicrobial activity. The range of inhibition observed was 10.00 ± 0.01 to 20.00 ± 0.01 mm on the test isolates. However, the aqueous and ethanolic extracts of the peels powder did not show any activity against *E. coli* and *Proteus* spp, respectively.

Table 8. Termite mortality activity of peel powder

Time (hours)	Negative control (sand)	Positive control (0.2 g insecticide powder)	0.2 g peels	Mixture (0.1 g of insect powder and peels)
0.5	0 (0%)	4 (40%)	2 (20%)	5(50%)
1	0 (0%)	6(60%)	3(30%)	7(70%)
2	0(0%)	10(100%)	5(50%)	10(100%)
3	0(0%)	10(100%)	7(70%)	10(100%)
4	0(0%)	10(100%)	10(100%)	10(100%)

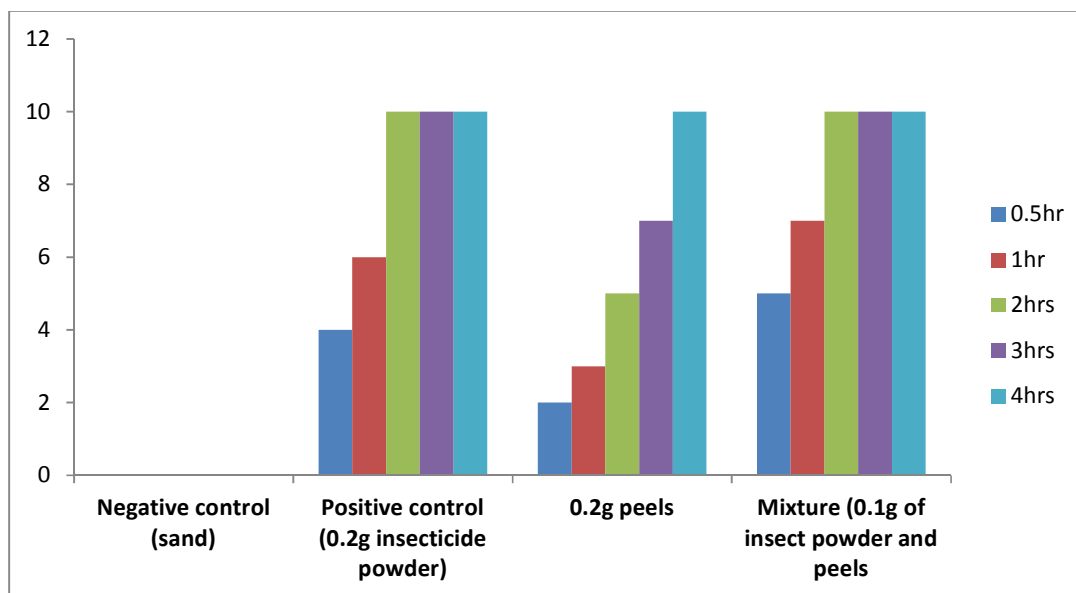


Fig. 1. Multiple bar chart showing the mortality and concentration of the test and control powders

Srividhya et al. [9] reported the efficacy of various peel extract against pathogens causing intestinal disorders. In their study, the aqueous extract of *Citrus paradisi* Mac fad (Grapefruit), *Citrus sinensis* (Orange), *Citrus limon* (Lemon), and *Citrus aurantifolia* (Lime). The zones of inhibition observed in their study ranged from 4 mm to 16 mm on *Shigella* spp, *Salmonella* spp and *Escherichia coli*. Their zones of inhibitions were almost similar to our results.

5. CONCLUSION

The present study has revealed that usually discarded peels of Shaddock or grape used in this study is rich in basic nutrient such as protein, carbohydrate, fiber, ash, moisture, vitamins and minerals. Anti-nutrient analysis shows the presence of allowable levels of anti-nutrients such as HCN, phytate, soluble and total oxalates. Phytochemical screening revealed that it is also abundant with secondary metabolites or phytochemical bases that could be exploited for bioactive compounds. The excellent activity shown against clinical isolates anti-termite activities shown by the peel powder show a lot of promise and calls for more research.

ETHICAL APPROVAL

Ethical approval was sought for and obtained from the university research and ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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