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Phytochemical Composition, Antioxidant and Antibacterial Properties of Pummelo (*Citrus maxima* (Burm.)) Merr. against *Escherichia coli* and *Salmonella typhimurium*

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Abstract

The antioxidant and antibacterial activities of the phytochemical constituents of the pericarp, mesocarp and segment membrane crude ethanolic extracts of Pummelo (*Citrus maxima* (*Burm.*)) fruit were were tested against *Escherichia coli* and *Salmonella typhimurium*. Preliminary phytochemical test revealed the presence of phenols, tannins, saponins expressed as catechine quivalent (CE)/100ml and flavonoid expressed as gallic acid equivalent (GAE)/100ml. The order of which was as follows pericarp > segment membrane > mesocarp. The strongest antioxidant activity was obtained by the pericarp extract (29.64 expressed as % lipid peroxidation). The differences in the measured amount of phytochemicals and antioxidant activity among the three sample extracts were found to be significant. In terms of antimicrobial activity, the pericarp, mesocarp and segment membrane extracts generated zone of inhibitions measuring 17.10, 18.00 and 17.03 mm for *S. typhimurium*, respectively at 100% concentration. *E. coli* was noted to be inactive in all three sample extracts at 100% concentration. The capacity of *E. coli* to counteract the inhibitory effect of the phytochemicals contained in the pummelo extracts may be attributed to its rough corrugated cell wall and thick periplasmic space as opposed to the smooth curved and barely seen periplasmic space of *S. typhimurium*. However, no significant correlation was detected among the phyto-

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chemical content, antioxidant and *in vitro* antimicrobial activities of the sample extracts against *S. typhimurium.*

Keywords

Pummelo; Antibacterial; Phytochemical; *E. coli*; *S. typhimurium*

1. Introduction

Escherichia coli and *Salmonella typhimurium* are two common gram negative bacterias frequently causing gastrointestinal infection in both animals and humans. Many of the cases of *E. coli* and Salmonella infections in humans are food-borne in nature. The food borne infections caused by *E. coli* and *S. typhimurium* are of great consequence on the state of public health as they add to the causes of morbidity and mortality. However, the previously well-recognized factors determining the cause, distribution and control of these enteric gram negative bacteria have changed through time. Emerging pathogens were reported to increase in prevalence, and they become associated with new food vehicles and cause systemic diseases with long term complications and lastly, antibiotic resistance. The resistance of *E. coli* and *S. typhimurium* to different classes of antimicrobial agents has been documented worldwide [1]-[4].

In the context of the growing interest in public health, the issue of food safety cannot be over emphasized. Interest towards the study of antimicrobial extracts derived from new economic plants and its possible expanded use in securing food security recently flourished as a result of many reports on the safeness and health benefits of natural versus processed food items, discovery of new pathogens described to possess the ability to resist antibiotics and issues on the misuse of traditional antibiotics to name a few. Though various citrus species have been the subjects of several researches concerning antimicrobial activity, little or no work at all has been done on determining the chemical composition and the antibacterial potential of pummelo (*Citrus maxima* (Burm.) Merr.), in particular the inedible fruit parts usually just cast aside. Pummelo is a common citrus species in the country, thought to be the local version of grapefruit. In view of previous findings, ascertaining the high antimicrobial and anti-oxidant activities of a number of phytochemicals inherent in plant food like citrus and the fact that all citrus have similar complex structure regardless of cultivars, pummelo can therefore be a potential replacement for synthetic preservatives therefore providing multiple benefits to consumers by way of furthering its possible usage in the fields of medicine, therapeutics and food technology [5] [6].

Examining the possible resistance of gram negative pathogens like the *E. coli* and *S. typhimurium* to natural antimicrobial potential of pummel fruit extracts may shed some light on some of the important mechanism acquired to resist many antimicrobial agents. This information is fundamental to effectively control its growth and minimize if not totally eliminate the high burden of infectious disease it might cause to the population.

The objectives of this study were to identify the phytochemical content of the extracts and to determine the antimicrobial activity of the pummelo inedible fruit parts extract against *E. coli* and *S. typhimurium*.

2. Material and Methods

2.1. Collection of Fruit Materials

The pummelo was obtained from a local supermarket. The mature (140 - 160 days from fruit set and light green to yellow color) and medium size classification (651 - 900 g) of the *Nenita* variety of pummelo was used [7].

2.2. Preparation of Fruit Extracts

The pummelo pericarp, mesocarp and segment membrane were washed individually with clean sterile water. The parts were cut into small pieces and were soaked overnight in ethanol. The following day, the slurry were blenderized, filtered and evaporated using a rotary evaporator under reduced pressure at 70˚C. The obtained extracts were secured in flasks and stored at 4˚C.

2.3. Antibacterial Assay

Two Gram negative pathogenic bacteria, *S. typhimurium* and *E. coli* were used as test organisms. Pure cultures were prepared and obtained from Biotech, University of the Philippines Los Banos.

The sensitivity of the isolated and sub-cultured test organisms to pomelo extracts was carried out using the cup cylinder method. Ten ml of sterile Mhueller Hinton agar (MHA) was placed in sterile plates and solidified. Five ml of top agar seeded with test organism (equivalent to #0.5 McFarland) was placed onto base agar and solidified. Sterile cup cylinders were placed on top of top agar. One-tenth ml of the supernatant broth of the crude extract was deposited into cup cylinder positioned on assay plates which were incubated for 24 hr at 37˚C. Zones of inhibition were measured using digital Vernier caliper

Different dilutions of the plant extracts were prepared in the order of 0.1 g/ml (10%), 0.3 g/ml (30%), and 0.5 g/ml (50%). The resulting inhibition zones were measured in millimeters and recorded against the corresponding concentration. Three replicates were carried out for each extract against each of the test organism. Kanamycin sulfate was the antibiotic used as postive test control [8].

2.4. Determination of Cell Wall Structure

Transmission electron microscopy (TEM) was used to study the cell walls of the two Gram negative test organisms. The specimens were embedded in resin for it to be thin sectioned. The test organisms were evaluated with JEM 1220 TEM operating at an acceleration voltage of 80 kV. The specimens were prepared at BIOTECH-UPLB while the TEM was conducted at Research Institute of Tropical Medicine (RITM) at Alabang, Philippines.

2.5. Chemical Analysis

The pH and total soluble solid content of the extracts was measured using a pH meter and refractomer, respectively. For titratable acidity, ten (10) ml of extracts were titrated with 0.1 N NaOH to an endpoint of 8.1 [9]. Analysis was done in triplicates.

2.6. Determination of Phytochemical Content and Anti-Oxidant Activity

2.6.1. Antioxidant Activity

An aliquot of 2.9 mL of 10^{-4} 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) solution in methanol was added to 25 µl of the extract and 75 µl of water. The mixture was shaken in a vortex mixer and allowed to stand in the dark for 30 minutes. The absorbance was measured at 517 nm. A reagent blank and a positive control using different concentrations of butylatedhydroxytoluene (BHT) were also prepared as in the sample [10].

Antioxidative activity (AOA) was measured using the formula shown below:

$$\text{AOA} = \frac{\text{Absorbance blank} - \text{Absorbance sample}}{\text{Absorbance blank}} \times 100$$

The antioxidant activity was expressed as percent lipid peroxidation. The higher the percentage, the lower was the antioxidative activity.

2.6.2. Phenols

Total phenolics were determined colorimetrically using Folin-Ciocalteau reagent [10]. About 2.8 ml distilled water was added to 0.2 ml extract, which was then mixed thoroughly. One ml of 0.2 M Na_2CO_3 and 0.2 ml of Folin-Ciocalteau phenol reagent were added. The solution was placed in boiling water for 15 minutes. The absorbance was read at 710 nm after cooling. The results presented were mean values of triplicate measurements and expressed as % total phenols.

2.6.3. Tannins

Total tannins analysis was conducted using the modified vanillin method. One ml extract was mixed with 5 ml vanillin reagent, incubated for 20 minutes at 30˚C and the absorbance was read at 500 nm. The results presented were the mean values of triplicate measurements and expressed as % tannins. A standard curve was prepared using catechin to compute for the amount of tannins in the sample [9].

2.6.4. Flavonoids

The total flavonoid concentration was measured using a colorimetric assay [11]. One mL of appropriately diluted sample was added to a 10 mL volumetric flask containing 4 mL of ddH_2O. At time zero, 0.3 ml of 5% $NaNO_2$ was added to each volumetric flask; at 5 min, 0.3 mL of 10% $AlCl_3$ was added; at 6 min, 2 ml of 1 M NaOH was added. Each reaction flask was then immediately diluted with 2.4 ml of dH_2O and mixed. Absorbances of the mixtures upon the development of pink color were determined at 510 nm relative to a prepared blank. The total flavonoid contents of the samples are expressed in milligrams gallic acid equivalent per 100 ml sample.

2.6.5. Saponins

The saponin concentration was measured using a colorimetric assay [12]. Five hundred (500) mg of ground samples was mixed with 5 ml of 80% ethanol. The mixture was subjected to 50˚C temperature with intermittent shaking for 15 minutes. After cooling the mixture, it was centrifuged at 3000 rpm for 10 minutes. The extraction was repeated twice and the supernatants were pooled. The total volume was adjusted to a total of 15 ml with 80% ethanol.

The polyvinylpolypyrollidone (PVPP) mini column was prepared and submerged in water overnight to keep PVPP hydrated. The supernate was passed in the column. The first columns of eluents were removed with traces of water. Two ml eluate was collected. For every test tube, 0.10 ml aliquot was treated with 0.5 ml glacial acetic acid. After shaking, 3.0 ml freshly prepared Lieberman-Blurchard reagent was added and heated in a 90˚C - 100˚C water bath for 30 minutes. The absorbance was read at 450 nm. The results were mean values of triplicate measurements. The standard to be used was saponins from gysophylla.

2.6.6. Alkaloids

For alkaloids, 200 mg extract mixed with 10 ml distilled water were filtered. Two ml filtrate mixed with 1% HCI were subjected to steaming. One ml filtrate was collected and added with 6 drops of Mayor's reagent/ Wagner's reagent. Presence of cream/brown and red-orange precipitate indicated the presence of different alkaloids [9].

2.6.7. Terpenoid

Terpenoid was determined by mixing 1 mg of the extract with 2 ml chloroform and concentrated sulfuric acid. Appearance of reddish brown color indicated the presence of terpenoids [9].

2.7. Statistical Analysis

One way ANOVA was used to examine the mean differences of the chemical constituents among the samples. Least significant difference (LSD) procedure was applied to compare means. Likewise ANOVA was performed to determine the significance of the measured log reduction and zone of inhibition values of the different extracts against the test organisms at varying exposure time and concentrations. Correlation analysis was applied to measure the degree of association among the phytochemicals, antioxidant and the *in vitro* antibacterial activities of the sample extracts.

3. Results and Discussion

3.1. Phytochemical Content and Anti-Oxidative Activity

The detected phytochemicals and antioxidant activity in all three extract samples were found to be significantly different (**Table 1**).

Though varied in shape and size, the complex structure of citrus fruit is quite similar regardless of cultivar. The fruit surface termed as pericarp consists of exocarp and outermost mesocarp. Included in the exocarp are the cuticle-covered epidermis and the parenchyma cells. Lipids and waxes whose main function is to prevent water loss from peel and sustain ample water content within tissue are synthesized in the epidermal cells. The pericarp extract showed the highest amounts of phenol, tannins, saponins (expressed as mg catechin equivalent per 100 ml) and flavonoids (expressed as mg gallic acid equivalent per 100 ml) and antioxidant activity (% LP).

The mesocarp extract displayed the least amount of phytochemical content and antioxidant activity compared

with the pericarp and segment membrane extracts.

The phytochemical content and antioxidant activity of the segment membrane were second to that of pericarp. Terpenoids were noted to be present in all three extracts whereas alkaloids proved positive in the pericarp and segment membrane extracts but not in the mesocarp extract. In terms of antioxidant activity represented by % lipid peroxidation, low antioxidant activities were noted in the mesocarp and segment membrane extracts. Akin to the phytochemical analysis, the pericarp extract displayed the highest antioxidant activity among the three sample extracts as illustrated by the low peroxidation percentage. The antioxidant activity of the extract samples followed the order pericarp > segment membrane > mesocarp. The synergistic action of antimicrobial and antioxidant agents present in the sample extracts will not only aid in preservation but also suppress development of off-flavor that is in the end beneficial in further processing of food.

The sample extracts did not inhibit the growth of *E. coli*. The effect of increasing concentration on the inhibitory potential of the tested extracts was found to be significant for *S. typhimurium* at $P < 0.05$. The *in vitro* antimicrobial activity showed a satisfactory effect as compared with the standard antibiotic. Increasing the concentration of all the extracts from 10% - 50% did not enhance the antimicrobial activity. In all three extracts namely, pericarp, mesocarp and segment membrane, the inhibition zone induced was more or less the same averaging at 8 mm in diameter. However at 100%, the extent of bacterial inhibition approximately doubled (17 - 18 mm in diameter) for all the extracts (**Table 2**).

The standard antibiotic, Kanamycin Sulfate, used in the study is a wide spectrum antibiotic that belongs to bactericidal aminoglycoside group. The marked significant difference between the extent of bacterial growth inhibition of the extracts and the standard antibiotic could be explain by the fact that active compounds in the sample extracts were much lower than the standard antiobiotic and if the active compounds were to be isolated, purified and applied in similar amount as the standard, it would most likely deliver the same antimicrobial activity as the standard.

3.2. Antibacterial Activity of the Extracts

These results agreed with previous studies that have reported the inhibitory effect of phytochemicals from various plant food against different microorganism. Despite the moderate antimicrobial activity, as illustrated by the

Table 1. Phytochemical composition and antioxidant activity of *Citrus maxima* (Burm.) Merr. fruit portion extracts.

Extract Sample	Total Phenol mg CE/100ml	Tannins mg CE/100ml	Flavonoids mg GAE/100ml	Saponins mg CE/100ml	Anti-oxidant activity % LP	Alka-Loids	Terpe-Noids
Pericarp	25.71^{a}	40.47^{a}	31.48^{a}	345.39^{a}	29.64^{c}	Positive	Positive
Mesocarp	13.94^{c}	7.94^{c}	11.65^{c}	79.03^{c}	75.95^{a}	Negative	Positive
Segment Membrane	18.46^{b}	21.02^{b}	20.17^{b}	147.93^{b}	71.54^{b}	Positive	Positive

Means values from triplicate samples in a column followed by different superscript are significantly different at $P < 0.05$.

Table 2. Zone of inhibition (diameter in mm) of *Citrus maxima* (Burm.) Merr. fruit portion extracts against *E. coli* and *S. typhimurium.*

<table>
<tr><th rowspan="3">Conc. (%)</th><th colspan="5">E. coli</th><th colspan="5">S. typhimurium</th></tr>
<tr><th colspan="3">Different Pummelo Extracts</th><th colspan="2">Control</th><th colspan="3">Different Pummelo Extracts</th><th colspan="2">Control</th></tr>
<tr><th>Pericarp</th><th>Mesocarp</th><th>Segment Membrane</th><th>$+^{*}$</th><th>$-^{**}$</th><th>Pericarp</th><th>Mesocarp</th><th>Segment Membrane</th><th>$+^{*}$</th><th>$-^{**}$</th></tr>
<tr><td>10</td><td>0</td><td>0</td><td>0</td><td rowspan="4">26.70</td><td rowspan="4">0</td><td>8.10ac</td><td>8.13b</td><td>8.10c</td><td rowspan="4">28.06</td><td rowspan="4">0</td></tr>
<tr><td>30</td><td>0</td><td>0</td><td>0</td><td>8.17a</td><td>8.16ab</td><td>7.95b</td></tr>
<tr><td>50</td><td>0</td><td>0</td><td>0</td><td>8.23a</td><td>8.23ab</td><td>8.17c</td></tr>
<tr><td>100</td><td>0</td><td>0</td><td>0</td><td>17.10ac</td><td>18.00b</td><td>17.03c</td></tr>
</table>

*Antibiotic-Kanamycin Sulfate (1000 ppm); **Methanol. Means with the same letter in a row are not significant.

zone of inhibition ranging 35% - 39% less than the standard antibiotic at 100% concentration, the inhibitory effect of the extracts together with its phytochemical content and antioxidant activity proved to be interesting. Referring to literatures, the impervious characteristic of the Gram negative strains against the sample extracts were attributed to their structural differences. The thick polysaccharide outer membrane possessed by the Gram negative bacteria protects the cell wall by restricting the entry of these plant compounds which trigger loss of chemiosmotic control [13] [14]. However, contrary to previous reports, results obtained in this study showed that the sample extracts do not have selective antimicrobial activity on the basis of cell-wall differences between the two Gram-negative test bacteria. At various concentrations, the extracts showed considerable amount of inhibition against *S. typhimurium. E. coli* was observed to be resistant to all the sample extracts at all concentrations. At 100%, the mesocarp extract produced the highest zone of inhibition against Salmonella (18 mm), followed by the pericarp extracts (17.10 mm). Least measures of zone inhibition for the *S. typhimurium* were gathered from the segment membrane extract (**Figure 1**).

3.3. Cell Wall Structures

The discrepancy of antimicrobial activity may be attributed to the structure of its cell wall. The TEM micrographs of *S. typhimurium and E. coli* revealed smooth straight curved and rough corrugated cell walls, respectively (**Figure 2**). The alternating folds or ridges component in a corrugated design imparts durability and sturdiness in structure. The rough corrugated cell wall structure of *E. coli* might have provided a more rigid and stronger barrier that resists the forceful penetration of substances into the cell. The observed corrugated cell wall of the *E. coli* specimen used in the study may be the reason why the bacteria remained resistant to the crude ethanolic pomelo fruit extracts. In contrast, the smooth straight curved cell wall of *S. typhimurium* provided a weak barrier offering little or no resistance against the entry of substances such as the crude ethanolic pummelo fruit extracts into the cell causing possible interference to growth and metabolism hence the detected inhibitory effect of the extracts to the bacteria. Aside from the difference in cell wall structure, a distinct disparity in the periplasmic space of the two tested Gram negative bacteria was noticed. Based on the electron microscopy images (**Figure 3**), the periplasmic space of *E. coli* looked thick whereas in *S. typhimurium*, the periplasmic space

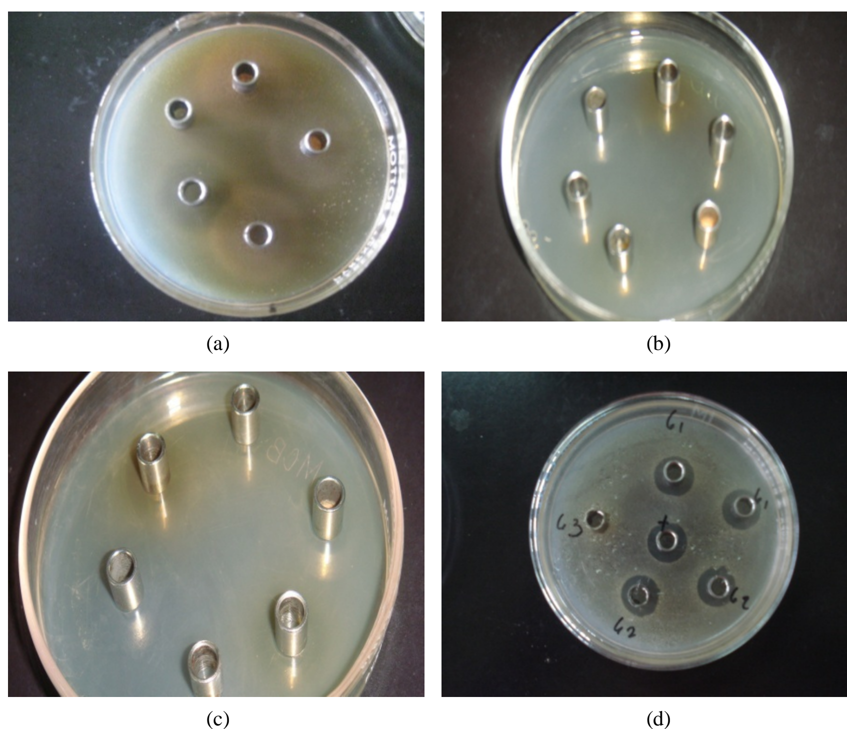

Figure 1. Zone of inhibition of extracts from *Citrus maxima* (Burm.) Merr. fruit parts at 100% concentration, (a) *S. typhirium*; (b) *E. coli* showing no inhibition; (c) control negative and (d) control positive.

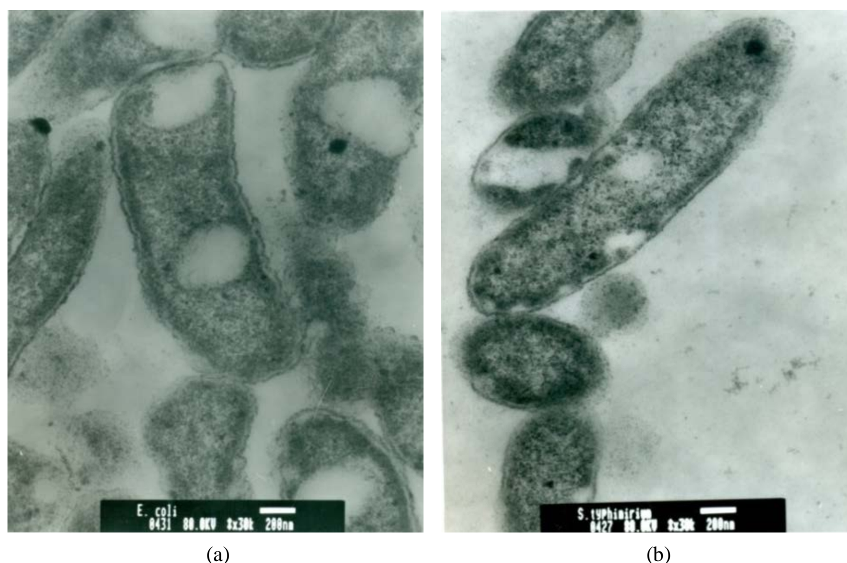

Figure 2. Comparison of the cell walls of *E. coli* B 1825 and *S. typhimurium* B 1741. (a) Corrugated cell wall of *E. coli* B 1825. Bar = 200 nm; (b) Straight curved cell wall of *S. typhimurium* B 1741. Bar = 200 nm.

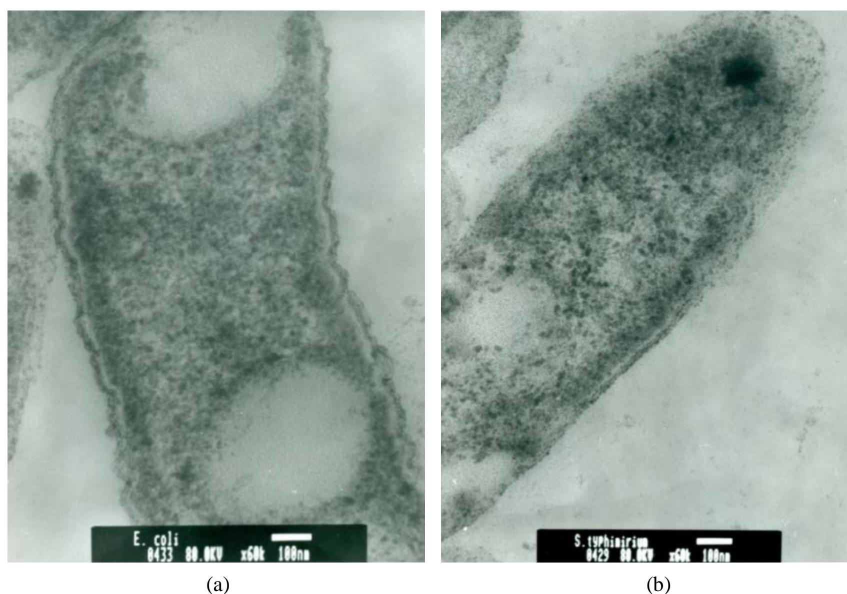

Figure 3. Comparison of the periplasmic space of *E. coli* B 1825 and *S. typhimurium* B 1741. (a) Thick periplasmic space of *E. coli* B 1825. Bar = 100 nm; (b) Thin periplasmic space of *S. typhimurium* B 1741. Bar = 100 nm.

was barely visible. The periplasmic space situated between the inner and outer membrane of the Gram negative bacteria houses a chain of loose network of murein as well as various types of proteins. Proteins in the periplasmic space possess great importance since it relate to cell protection, growth and metabolism [15]. And so a thick periplasmic space suggests the presence of more enzymes involved in hydrolysis, degradation, electron transport and alteration of substances toxic to the cell hence offering more protection to the cell. Some other factors like different amounts, concentrations and mechanism of action of chemicals in the extract similar to the functions of many antibiotics against wide spectrum of microorganism, strains and serotypes of test organism use, the selected methodology to determine the antimicrobial activity and may also have affected the outcome of the experiment.

3.4. Correlation Analysis

The correlation analysis among the phytochemical content, antioxidant and invitro antibacterial acitivity, was noted to be insignificant (**Table 3**). Based on the results obtained from the *in vitro* antibacterial activity using the cup cylinder method, the mesocarp extract displayed the highest inhibitory effect against all test organisms whereas the pericarp and segment membrane extracts produced more or less similar antibacterial effect against same test organisms. In relation to phytochemical investigation, the order of rank in decreasing effectivity was as follows pericarp > segment membrane > mesocarp.

As described in previous studies, the phytochemical contents and antioxidant activity of each of the sample extracts significantly correlates to their antimicrobial property which indicates that the phytochemical constituents and antioxidant activity are likely to contribute to the antimicrobial potential of the extracts. Various studies on antimicrobial activity of diverse plants reported that high amount of phytochemicals demonstrated the great inhibitory effect against different microorganisms. It was suspected that the variant antimicrobial activity of studied traditional plant may be attributed to the disparity of mixture of phytochemicals which in effect influence the rate through which it penetrates the cell wall and membrane structures of microorganisms [2] [13] [14] [16] [17]. Hence, the differences in the antibacterial activity of the pummelo fruit part extracts could be attributed to the varying amounts of phytochemicals contained in each studied fruit part.

Results of this study agreed with previous studies that have reported the inhibitory effect of phytochemicals from various plant food against different microorganisms [5] [18]-[20].

4. Conclusions

The phytochemical investigation of the crude ethanolic extract of the pericarp, mesocarp and segment membrane of pummel (*Citrus maxima* (Burm.)) Merr. fruit indicated the presence of phenols, tannins, flavonoid, saponins and terpenoids. Presence of alkaloids were detected in the pericarp and segment membrane extracts but was absent in the mesocarp extract. The pericarp extract contained the most amounts of phenols, tannins, flavanoids and saponins followed by segment membrane whereas the extract from mesocarp displayed the least amount among the three. The same ranking was noted in terms of antioxidant activity.

In general, the sample extracts exhibited moderate antibacterial activity. The pericarp, mesocarp and segment membrane extracts generated zone of inhibitions measuring 17.10, 18.00 and 17.03 mm for *S. typhimurium*, respectively at 100% concentration. *E. coli* was noted to be inactive in all three sample extracts at 100% concentration. The rough corrugated cell wall and thick periplasmic space of *E. coli* as opposed to the smooth curved and barely seen periplasmic space of *S. typhimurium*may be attributed to be capacity of the Gram negative bacteria to counteract the inhibitory effect of the phytochemicals contained in the pummelo extract.

An insignificant correlation was detected among the phytochemical content, antioxidant and *in vitro* antimicrobial activities against *S. typhimurium*. The mesocarp extract, described to have the least amount of phytochemicals and high antioxidant activity compared with the other two sample extracts, delivered the utmost inhibitory effect against *S. typhimurium*, The *in vitro* antimicrobial effect of pericarp and segment extracts were observed to be relatively similar although the phytochemical content and antioxidant activity of segment membrane were a bit higher than the mesocarp extract.

This investigation indicates a mild antibacterial effect of the different pummelo fruit part extracts. Though the

Table 3. Correlation coefficients of the interaction between phytochemical analysis and *in vitro* anti bacterial activity of the extracts from the pericarp, mesocarp and segment membrane of *Citrus maxima* (Burm.) Merr. fruit against *S. typhimurium*.

Phyto-Chemical/Anti-Oxidant	Pericarp	Mesocarp	Segment Membrane
Phenol	−0.41^{ns}	−0.05^{ns}	−0.08^{ns}
Tannins	−0.37^{ns}	−0.05^{ns}	0.36^{ns}
Flavanoids	−0.42^{ns}	−0.13^{ns}	−0.18^{ns}
Saponins	−0.07^{ns}	0.26^{ns}	0.32^{ns}
Antioxidant	−0.32^{ns}	0.28^{ns}	−0.34^{ns}

^{ns}Not significant at alpha 5%.

inhibitory effect was meager, due probably to insufficient antibacterial constituents present in each of the sample extracts, its comparative activity merits attention. Further exploration to improve its potential as an alternative organic source of preservative was suggested.

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References

[1] Friedman, M., Henika, P.R., Levin, C.E. and Mandrell, R.E. (2004) Antibacterial Activities of Plant Essential Oils and Their Components against *Escherichia coli* 0157:H7 and *Salmonella enterica* in Apple Juice. *Journal of Agricultural Food Chemistry*, **52**, 6042-6048.

[2] Geornaras, I., Yoon, Y., Belk, K.E., Smith, G.C. and Sofos, J.N. (2007) Antimicrobial Activity of e-Polylysine against *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* in Various Food Extracts. *Journal of Food Science*, **72**, M330-M334. http://dx.doi.org/10.1111/j.1750-3841.2007.00510.x

[3] Azu, N.C. and Onyeagba, R.A. (2008) Antimicrobial Properties of Extracts of *Allium cepa* (onion) and *Zingiber officinale* (Ginger) on *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*. *The Internet Journal of Tropical Medicine*. www.Ispub.com/IJTM/3/2/11056

[4] Duan, J. and Zhao, Y. (2009) Antimicrobial Efficiency of Essential Oil and Freeze-Thaw Treatments against *Escherichia coli* 0157:H7 and *Salmonella enteric Ser Enteritidis* in Strawberry Juice. *Journal of Food Science*, **74**, M131-M137. http://dx.doi.org/10.1111/j.1750-3841.2009.01094.x

[5] Cowan, M.M. (1999) Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*, **12**, 564-582.

[6] Ladiya, M. (2008) Citrus Fruit Biology. Technology and Evaluation. Elsevier Academic Press, Amsterdam.

[7] Sarian, Z.B. (2009) Good Reasons for Growing Pummelo. Agriculture Magazine. www.mb.com.ph/articles/193178/goodreasons-growing-pummelo

[8] BAM (2001) *Bacteriological Analytical Manual.* Merck: Microbiological Manual.

[9] AOAC (1990) Association of Analytical Official Chemist. 15th Edition, Association of Official Analytical Chemists, Inc., Washington DC.

[10] Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. (1998) Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products. *Journal of Agricultural and Food Chemistry*, **46**, 4113-4117. http://dx.doi.org/10.1021/jf9801973

[11] Zhishen, J., Mengcheng, T. and Jianmimg, W. (1999) The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects Onsuperoxideradicals. *Food Chemistry*, **64**, 555-559. http://dx.doi.org/10.1016/S0308-8146(98)00102-2

[12] Sudesh Jood, B.M. and Chauhan, A.C. (1986) Saponin Content of Chickpea and Blackgram: Varietal Differences and Effects of Processing and Cooking Methods. *Journal of Science Food and Agriculture*, **37**, 1121-1124. http://dx.doi.org/10.1002/jsfa.2740371111

[13] Du, W.X., Olsen, C.W., Avena-Bustillos, R.J., McHugh, T.H., Levin, C.E., Mandrell, R. and Friedman, M. (2009) Antibacterial Effects of All Spice, Garlic and Oregano Essential Oils in Tomato Films Determined by Overlay and Vapor-Phase Methods. *Journal of Food Science*, **74**, M390-M397. http://dx.doi.org/10.1111/j.1750-3841.2009.01289.x

[14] Naz, S., Siddiqi, R., Ahmad, S., Rasool, S.A. and Sayeed, S.A. (2007) Antibacterial Activity Directed Isolation of Compounds from *Punica granatum*. *Journal of Food Science*, **72**, M341-M345. http://dx.doi.org/10.1111/j.1750-3841.2007.00533.x

[15] Beveridge, T.J. (1999) Structures of Gram Negative Cell Walls and Their Derived Memebrane Vesicles. *Journal of Bacteriology*, **181**, 4725-4733.

[16] Ennajar, M., Bouajila, J., Lebrihi, A., Mathieu, F., Abderraba, M. and Raies, A. (2009) Chemical Composition and Antimicrobial and Antioxidant Activities of Essential Oils and Various Extracts of *Juniperus phoenicea* L. (Cupressacees). *Journal of Food Science*, **74**, M364-M371. http://dx.doi.org/10.1111/j.1750-3841.2009.01277.x

[17] Mkaddem, M., Bouajila, J., Ennajar, J., Lebrihi, A., Mathieu, F. and Romdhane, M. (2009) Chemical Composition and Antioxidant Activities of *Mentha longifolia* L. and *M. viridis* Essential Oils. *Journal of Food Science*, **74**, M358-M363. http://dx.doi.org/10.1111/j.1750-3841.2009.01272.x

[18] Cottingham, J., Heggers, J., Gusman, J. P., Reagor, J., McCoy, L., Carino, E., Cox, R. and Zhao, J.G. (2002) The Effectiveness of Processed Grapefruit-Seed Extract as an Antibacterial Agent: II. Mechanism of Action and *in Vitro*

Toxicity. *Journal of Alternative and Complementary Medicine*, **8**, 333-340. http://dx.doi.org/10.1089/10755530260128023

[19] Cvetnic, Z. and Vladimir-Knezevic, S. (2004) Antimicrobial Activity of Grapefruit Seed and Pulp Ethanolic Extract. *Acta Pharmaceutica*, **54**, 243-250.

[20] Dao, T., Wong, G.L., Lundin, R.Y. andMahoney, N. (2001) Identification of Benzethonium Chloride in Commercial Grapefruit Seed Extracts. *Journal of Agricultural Food Chemistry*, **49**, 3316-3320. http://dx.doi.org/10.1021/jf010222w