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Phytochemical screening, antimicrobial and antioxidant activity determination of *citrus maxima* peel

Abstract

Background: The scientific name of pomelo is *Citrus maxima*. Malaysia is popular for growth of" *Citrus maxima*" with the local name of pomelo. Pomelo is helpful in boosting body immune system, prevent weight gain and as anti-aging agent. Pomelo is also useful in preventing constipation as to promote digestion and peristalsis. Pomelo contains a huge amount of potassium (an effective vasodilator) and vitamin C (an antioxidant). Maceration and Soxhlet extraction of peel of *Citrus maxima* were carried out with n-hexane. Anti-microbial activity of *Citrus maxima* was followed by"Well Diffusion" method and comparing the clear inhibition zone of standard antibiotic and the extracts on "Mullen-Hinton agar". Anti-oxidant activity of *Citrus maxima* was carried out by using DPPH radical-scavenging activity of total phenol assay. The qualitative test for alkaloids confirmed in all the extracts. The presence of terpenoids observed in filtrate and residue after maceration while terpenoids were absent in the Soxhlet sample. Presences of Flavonoids were found only in residue. Total phenolic compounds present in the hexane extract and found that higher the sample concentration, the higher the GAE value obtained.

Keywords: *Citrus maxima,* soxhelt, maceration, hexane, phytochemical, antimicrobial, antioxidant

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Introduction

Citrus maxima (known as Pomelo in Malaysia) is one of the citrus species, belongs to family *Rutaceae*, grown widely in tropical and subtropical climates of Southeast Asia, Taiwan, China, India and Philippines.[1,2] The suitable condition for the growth of pomelo is the free–froth condition because they are subtropical nature and there should be small variation in the temperature differences. The pomelo tree is perennial shrub which can grow for a longer period of time and their height are around 5-15 meters tall.[3] The pomelo peel appears in greenish-yellow or pale yellow colour while the pulp consists of various colour.[4] *Citrus maxima* (known as Pomelo in Malaysia) exhibits some similarities with grapefruit like these are from the same kingdom, order and family and from similar genus. As a result, peoples always miss understand pomelo as grapefruit because of their similar outer appearance. However, there are some differences which can distinguish both of them such as their scientific name, place to grow, health benefits, colour of peel and taste of the pulp. The scientific name of grapefruit is *Citrus paradisi* and pomelo is *Citrus maxima*.[5] Malaysia is popular for growth of pomelo while grapefruit usually grows in China.[6] Pomelo is responsible in boosting our immune system, preventing weight gain and as an anti-aging agent while grapefruit aims in controlling the blood sugar level in diabetic patients and helps patient's relief from insomnia.[7–10] Each part of pomelo exhibits various benefits like hot leaf decoction can be applied on swellings. Fruits juice can be used as a febrifuge, a kind of medicine exert its function in reducing the fever. The seeds of pomelo plays a role in curing the coughs and dyspepsia.[11,12] Furthermore, fruits of pomelo also useful as cardio-tonic. In addition, fruit of pomelo is useful in preventing constipation problem and improve digestion and peristalsis.[13,14]

Method

Collection and preparation of *Citrus Maxima* peels

The pomelo was cut to separate the peels. After slicing, the peels washed by distilled water to remove the unwanted impurities.[11] The whole pomelo peels was weighed before and after. After slicing, the pomelo peels were washed by distilled water to remove the impurities present.[12] The pomelo peels were kept in an air-tight bag and placed inside the refrigerator to protect from microbial contamination or growth.[13,14]

Maceration

The extraction of pomelo peels carried out through maceration process.1.5L of n-hexane was prepared and poured carefully into the round bottom flask. The cleaned pomelo peels which cut in strip form inserted into the 2L round bottom flask to immerse themselves into the n-hexane. The mixture was shaken from time to time. The duration for maceration process was around seven days. After filtration two layers were observed in the filtrate. One layer was solvent extract while other layer was oil globules present in the pomelo peels. The mixture of two layers was separated.

Soxhlet extraction

The hot percolation was conducted in extracting the chemical constituents present in the pomelo peels. The fresh pomelo peels 300g cut into small species wit 300mL of hexane. Once no changes of solvent colour observed, the extraction process stopped.

Evaporation

The extracts were evaporated by using rotatory evaporator. The temperature of rotator evaporator set to 60°C. The evaporated extracts were removed and transferred into China dish. The China dish was heated by placing on water bath at 68°C for further evaporating. The extracts were kept in the refrigerator to protect the extract from contamination or growth of bacteria. Dilution of extract sample was carried out before phytochemical process started. 1ml of extract solution was prepared and inserted into the 50ml volumetric flask. The n-hexane was added to make up of 50ml of solution. The total phenolic content was determined on soxhelt extraction.[15]

Results

Table 1

Table 1 Results of phytochemicals present in extracts obtained by maceration and soxhlet extraction

No.	Analysis Test	Maceration		Soxhlet
		Filtrate	Residue	
1	Alkaloids	+	+	+
2	Reducing sugars	-	-	+
3	Saponins	-	-	-
4	Terpenoids	+	+	-
5	Anthraquinones	-	-	-
6	Glycosides	-	-	-
7	Tannins	-	-	-
8	Flavonoids	-	+	-
9	Carbohydrates	-	-	+

+ = presence, - = absence

Detection of total phenolic content (TPC)

Different concentrations of Soxhlet extract (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0mg/ml) were prepared.0.5 ml of different concentrations of extract solution and also control (methanol, without extract) were added into individual centrifuge tubes.[15] These were individually mixed with 2.5ml of 0.75%w/v Sodium bicarbonate solution and 2.5ml of 1%v/v Folin-Ciocalteau reagent. The mixtures were vortexed for few seconds and left to stand in the dark at room temperature for 15 minutes. The absorbance was measured at 765nm against a blank by using UV-visible spectrophotometer.[16–19] The total phenolic content was expressed as milligrams of Gallic acid equivalents per grams (mg GAE/g).[20,21] All the steps above were repeated for maceration extract and also Gallic Acid solution of different concentrations. The data for both extraction methods were collected and tabulated. The standard curve was prepared.[17,22–24]

Gallic acid (standard solution)

(Table 2)(Table 3)

Table 2 Standard solution concentration and absorbance values

Concentration (mg/ml)	Absorbance (WL765nm)
0.1	0.105
0.2	0.126
0.4	0.169
0.6	0.25
0.8	0.294
1	0.377

Table 3 Sample solution concentration and the absorbance values

Concentration (mg/ml)	Absorbance (WL 517nm)
0.1	0.13
0.2	0.146
0.4	0.179
0.6	0.334
0.8	0.45
1	0.535

Methodology

Inoculums of different strains (Bacillus subtilis, Staphylococcus pyogenes, Pseudomonas *aeruginosa* and Escherichia coli) were prepared by transferring one colony of each test organism to 5 ml of Mueller-Hinton broth in sterilized individual universal bottles. Mueller-Hinton broth was used to prepare inoculum. The concentration of inoculum was standardized by comparing with McFarland standards.[25] 0.5 McFarland standards was prepared by mixing 9.95ml 1% sulphuric acid in Mueller-Hinton broth and 0.05ml 1% Barium chloride in distilled water in order to estimate bacterial density which is 1.50x 108CFU/ml. The broth was incubated at 37°C for 24 hours. The turbidity of the bacterial suspension was standardized by comparing to 0.5 McFarland standards.[26] 24 petri dishes were prepared and filled with molten Mueller-Hinton agar and kept in cold room for one night. The petri dishes were then taken out and labeling was done by using marker pen in laminar airflow cabinet. With the Bunsen burner on, one strain of the prepared inoculum was spread evenly over plate with sterile loop.[17] A standard cork borer of 6 mm diameter was used to cut 5 uniform wells on the surface of the agar. A40µl of different concentration of Soxhlet extract, negative control and positive control (Ciprofloxacin 5µg) were introduced in the different well. The plates were incubated at 37°C for 24 hours. Zone of inhibition was measured to the nearest millimeter (mm) and mean was calculated (Table 4) (Table 5).[26–30]

Antioxidant activity of citrus maxima peel in n-hexane extract

Methodology

Different concentrations of Soxhlet extract (0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0mg/ml) were prepared. The 1.0 ml of different concentrations of extract solution and control (methanol, without extract) were added into individual centrifuge tubes.[23–25] They were individually mixed with 5.0 ml of methanolic solution of DPPH

reagent. The mixtures were vortexed for few seconds and left to stand in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm against a blank by using UV-visible spectrophotometer. The percentage of DPPH scavenging activity was calculated using the following equation:[26–29]

Radical scavenging (%) = $[(A_0-A_1/A_0)\times 100]$

Where,

A_0 is the absorbance of the control.

A_1 is the absorbance of the sample extracts

All the steps above were repeated for maceration extract and also BHT solution of different concentrations.[30,31] The 50% inhibitory concentration value (IC50) for both extracts were calculated and it is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.[32] The data for both extraction methods were collected and tabulated.[33]

DPPH scavenging assay

Table 6

Table 4 Antimicrobial susceptibility test

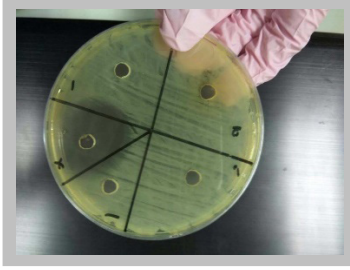
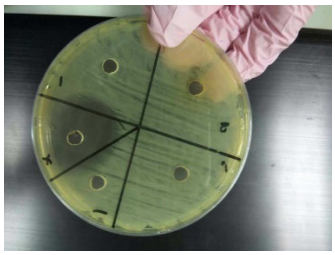
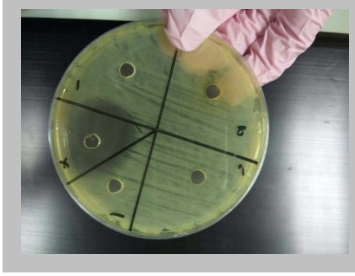
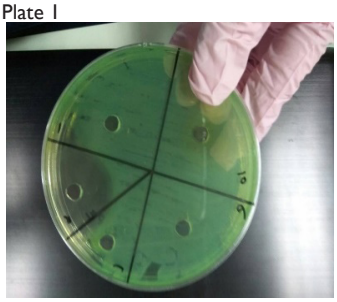
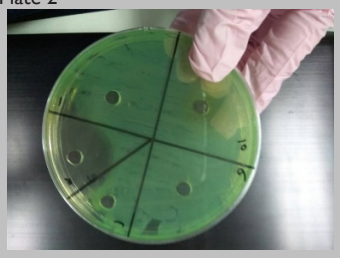
Microorganisms	Results
Escherichia coli Plate 1 	Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.4 cm.
Plate 2 	Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.3 cm.
Plate 3 	Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.5 cm.
Pseudomonas aeruginosa Plate 1 	Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.2cm.

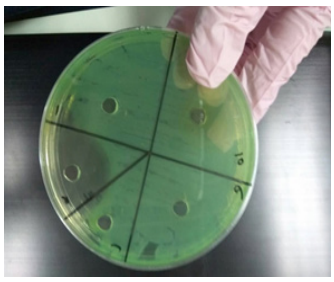
Figure continued..

Plate 2



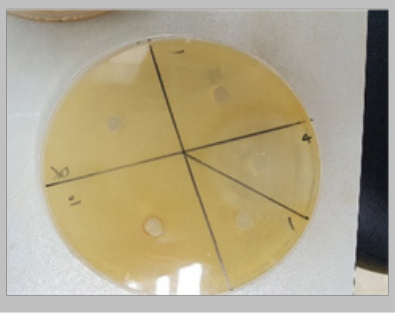
Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.4cm.

Plate 3



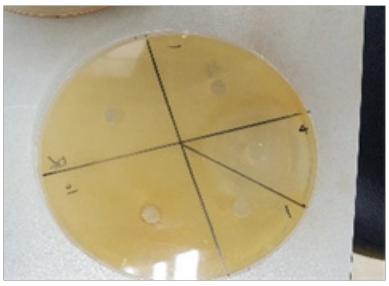
Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.5cm.

Bacillus Subtilis
Plate 1



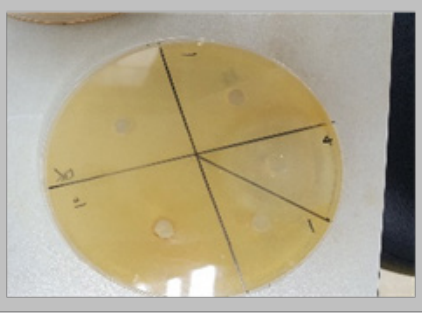
Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.3cm.

Plate 2



Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.4cm.

Plate 3



Negative result shown. No zone of inhibition observed. The zone of inhibition of positive control was 2.6cm.

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Table 5 Antimicrobial activity of maceration residue in n-hexane extract of different concentrations

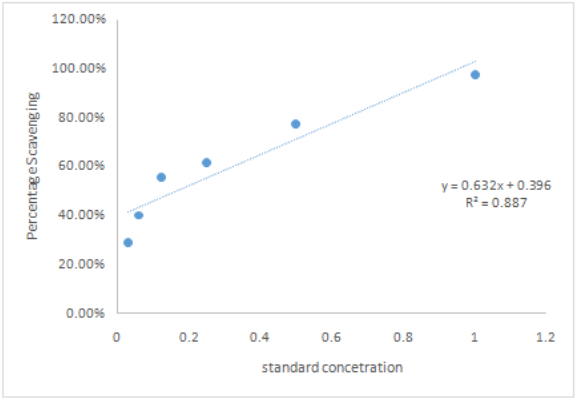
Microorganism	Extraction process	Conc. of n-hexane extracts (mg/ml)	Zone of inhibition observed on Mullen-Hinton Agar, mm in diameter	Ciprofloxacin (positive control), mm in diameter	Sterile Distilled water (Negative control),mm in diameter
Bacill us subtilis	Maceration	1	-	2.4±0.2	-
		5	-	2.6±0.2	-
		10	-	2.3±0.2	-
Staphylococcus pyogenes	Maceration	1	-	2.5±0.2	-
		5	-	2.4±0.2	-
		10	-	2.5±0.2	-
Escherichia coli	Maceration	1	-	2.4±0.2	-
		5	-	2.3±0.2	-
		10	-	2.5±0.2	-
Pseudomonas aeruginosa	Maceration	1	-	2.4±0.2	-
		5	-	2.6±0.2	-
		10	-	2.3±0.2	-

Table 6 Concentration of BHT, their absorbance and percentage scavenging

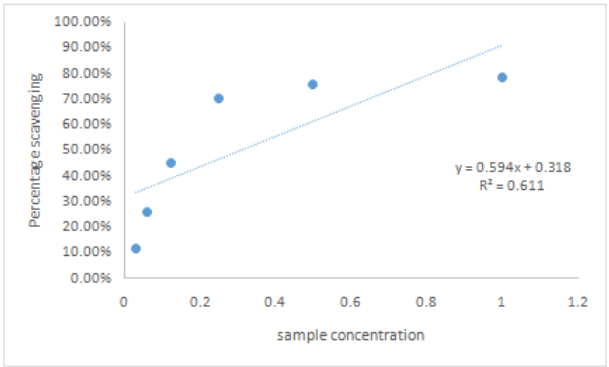
Concentration mg/ml	Absorbance (WL517)	Percentage scavenging (%)
0.03125	0.394	29.13%
0.0625	0.332	40.29%
0.125	0.245	55.93%
0.25	0.212	61.87%
0.5	0.124	77.70%
1	0.013	97.66%

Results and discussion

Phytochemical screening or preliminary test is the first procedure carried out to provide information and knowledges to researchers about the presence of phytochemical compounds in the tested plants. Carbohydrates, proteins, amino acids, vitamins and lipid are primary metabolites present in the plant. The secondary metabolites found in the plant are tannins, saponins, alkaloids, Flavonoids, phenolic and phenol group, glycosides and anthraquinones.[2,10,13,15,21] In this study, the presence of primary metabolite such as carbohydrates and reducing sugars and secondary metabolites like glycosides, alkaloids, terpenoids, saponins, tannins, anthraquinones and flavonoids investigated through standard procedures.[18–20] TPC (total phenolic content) is one of the methods used in evaluating the antioxidant activity of pomelo peels by investigating their total phenolic contents. The blue colour complex formed had the ability in absorbing the radiation and allowed quantification process carried out. TPC present in the hexane extract was defined in term of GAE and can be calculated by using the equation y=0.3008x+0.064, R2=0.9884. Results of the TPC in various concentration of hexane varied from 0.0006 to 1.18 mg GAE/g. The higher the sample concentration, the higher the GAE value obtained. By comparison, total phenolic content of macerated ethanolic extract showed high value which indicated better antioxidant activity (Graph 1)(Graph 2).



Graph 1 Graph of percentage scavenging against standard concentration.



Graph 2 Graph of percentage scavenging against sample concentration.

From the Graph 1 and Graph 2 plotted the relationship of the absorbance of sample and sample concentration studied. The linear line observed in the graph stated that they were directly proportional to each other. Higher the concentration of sample, higher the absorbance

which lead to higher gallic acid equivalent values. The absorption of phosphor molybdenum depended on the concentration of phenol compounds present in the plant extract. The higher the concentration of sample prepared, the higher the phenol content extracted by n-hexane, the higher the absorption rate of phosphor molybdenum in Folin-Ciocalteu reagent, the higher blue intensity observed which lead to higher absorbance value obtained.[31–37]

Antimicrobial susceptibility testing conducted in determining the antimicrobial resistance and sensitivity of microorganism toward the antibiotics applied. Through the antimicrobial susceptibility testing, the antimicrobial effect of phytochemical compound present in the plant extract can be studied by observing the zone of inhibition. The presence of zone of inhibition observed to determine the ability of plant extract in inhibiting the growth of particular microorganism strains.[29–37] Negative result obtained in all tested bacterial strains by using plant extract prepared in n-hexane solvent.[2] There was no zone of inhibition observed in each concentration of plant extract prepared. Plant extract prepared in three different concentrations which were 1mg/ml, 5mg/ml and 10mg/ml by mixing the plant extract with sterile distilled water. The agar media prepared for the antimicrobial susceptibility testing was Mullen-Hinton agar which is a normal agar media used in several studies due to its broad range of inhibition. As a result, the plant extract unable to inhibiting the growth of tested bacterial strains and show no response to them.[38,39]

Antioxidant activity was tested *Citrus maxima* and two types of the tests were carried out

A. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay used to evaluate the antioxidant activity of the pomelo peel extract prepared in n-hexane extract by measuring the scavenging capacity of DPPH present in the tested solvent extract. The scavenging capacity of DPPH can be determined by the spectrophotometer at 517nm because using of this wavelength showed the maximum absorption band of DPPH. Once the stable DPPH radical formed by oxidation, the discoloration of deep purple colour occurred and yellow colour product observed. This condition showed the DPPH radical scavenging happened and their scavenging capacity can be determined by using the spectrophotometer at 517nm. Compared to other antioxidant test, the DPPH scavenging assay was an antioxidant method conducted commonly due to their highly selectivity. The percentage scavenging of BHT and *Citrus maxima* extract can be calculated through following equation:

$$\% \text{ Scavenging of test sample} = \frac{\text{control absorbance} - \text{test absorbance}}{\text{control absorbance}} \times 100$$

B. From the result obtained, the percentage scavenging of BHT and *Citrus maxima* extract shown was calculated. The concentration of BHT and *Citrus maxima* prepared were 0.03125, 030625, 0.125, 0.25, 0.5 and 1mg/ml. The percentage scavenging of the BHT ranged from 29.13% to 97.66%. For the Citrus maxima peel, the percentage scavenging of *Citrus maxima* peel ranged from 11.86% to 78.58%. The higher the concentration of BHT and *Citrus maxima* peel extract, the lower the absorbance obtained due to the more and more DPPH radical scavenged by the phytochemical compound extracted from the Citrus maxima peel extract. Besides that, the IC_{50} values of DPPH radicals in BHT and *Citrus maxima* peel extract found to be 78.47 and 83.63mg/ml. Their IC_{50} values calculated. The high values obtained in *Citrus maxima* peel extract indicated the pomelo peel exhibited high antioxidant properties. BHT used as standard solution in this study. The use of BHT in this study as standard solution in determining the antioxidant activity of tested plant extract.[23,24]

Conclusion

Form this research conducted, it may be concluded that *Citrus maxima* can exhibit antioxidant activity. The presence of phytochemical such as alkaloids, terpenoids and flavonoids in the plant extract. *Citrus maxima* also proved to exhibit anti-microbial activity toward several microorganism strains although the negative result shown in this research conducted. There are several factors which can affect their antimicrobial activity such as resistance of tested bacterial strains, variation of temperature of preparation condition and low concentration of phytochemical present in the plant extract. As a result, further study should be carried out to study their antimicrobial activity.

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Conflict of interest

Author declares that there is no conflict of interest.

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