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Evaluation of In-Vitro Antioxidant and Antimicrobial Activities of the Various Parts of Benincasa hispida

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Abstract: *Benincasa hispida* (wax gourd), a fruit that consumed by many Asians, belongs to the family curcubitaceae. The present study was under taken to investigate the antioxidant and antimicrobial activities of the pulp, peel and seed extracts of wax gourd. The various parts of wax gourd were extracted with water. Antioxidant estimation of the extracts were analysed for the total phenolic content (TPC) measured using Folin-Ciocalteu reagent assay. The antioxidant activity (AO) of various parts of wax gourd were evaluated using ferric reducing antioxidant potential (FRAP), 2,2-diphenyl-1-picryl hydrazyl (DPPH) and β-carotene bleaching assays. The antimicrobial activity of the extracts was tested against six Gram-positive and seven Gram-negative bacteria, one yeast and two mold using the disc diffusion method. The seed extract of wax gourd showed the highest TPC (207.9 mg Gallic acid equivalent (GAE)/g extract weight) while AO of peel, pulp and seed of wax gourd were 21.73, 26.71 and 35.06 mM Trolox equivalent (TE)/g extract weight, respectively as determined by FRAP assay. As for EC_{50} values of DPPH assay were 165.42 (seed), 195.17 (pulp) and 392.21 µg/ml (peel). AO based on β-carotene bleaching assay were 34.39% (peel), 76.27% (pulp) and 90.22% (seed). The various parts of wax gourd extracts as determined by all AO assays were correlated with TPC and the value varies from $-0.999 \leq r^2 \leq 0.874$. The antimicrobial activity of the extracts showed an inhibition towards Gram-negative bacteria (*Salmonella typhimurium, Pseudomonas aeruginosa, Proteus vulgaris, Serratia liquefaciens, Cronobacter muytjensii, Shigella boydii* and *Serratia marcescens*) compared to Gram-positive bacteria, however, there are no inhibition towards yeast and mold for all extracts. Results may suggested that seed extract of wax gourd possesed strong antioxidant and antibacterial activities. Hence, they had the potential as natural preservatives in food, cosmetic and pharmaceutical industries for applications.
Keywords: Benincasa hispida, Total phenolic content, Antioxidant activity, Antimicrobial .

Introduction

Natural antioxidant mainly from fruits and vegetables have gained interest among consumers and scientists because various epidemiological[1,2] and laboratory studies shown that frequent consumption of natural antioxidant may reduce the cardiovascular disease[3] and cancer[4,5]. Three major groups that are related to defensive effects of natural antioxidant and antimicrobial in fruits and

vegetables are vitamins, polyphenols and carotenoids[5]. Plant secondary metabolites such as polyphenols, have properties including antioxidant, antimugenic, anticarcinogenic, antiflammatory and antimicrobial effects that might preventing diseases[5].

Benincasa hispida (Thunb) is one of the species of cucurbitaceae family that are grown primarily for its fruits and also has a great potential for functional food production[6]. In Malaysia, only two cultivar (round shape and elongated) of wax gourd are grown for commercial purposes. The peel and seed residue is the primary waste fraction. Several studies have already realized that the pulp and seed of the wax gourd has many beneficial effects for example the peel has antioxidant activity and capable for inhibition of angiotensin converting enzyme (ACE)[7] while the seed has angiogenic effect, anti tumor, used for the treatment of syphilis, cardiovascular diseases and antioxidant[8,9,10,11]. However, the antimicrobial activities of peel, pulp and seed of wax gourd have not been investigated.

Antioxidants are the compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reaction[12], while antimicrobial are compound or substance that kills or slows down the growth of bacteria and fungus (mold and yeast). The ability polyphenolic compounds to act as scavengers of free radicals besides their antioxidant and antimicrobial properties raising the possibility of their function in food and pharmaceutical application.

The aim of this research were to compare the antioxidant activity (AOA) using different types of assays namely DPPH, FRAP and β- carotene bleaching and to investigate the antimicrobial activity of peel, pulp and seed of wax gourd extracts by using disc diffusion method.

Experimental

Plant materials

Wax gourd fruits were harvested at matured stage and were purchased from a local market in Selayang, Selangor, Malaysia.

Extractions

Fruit extracts for ascorbic acid analysis were obtained by homogenizing 1 g of wax gourd tissue (peel, pulp and seed) in 5 ml 80% acetone until uniform consistency. The homogenates were centrifuged at 2,500 rpm at 4 °C for 20 minutes. The peel,pulp and seed of wax gourd extracts for total phenolic and antioxidant activity were prepared according to the method of Duh and Yen (1997)[13]. Each part of wax gourd was extracted with distilled water for 20 minutes and the ratio between samples to extraction medium was 1:5. The mixture was then filtered through a filter paper (Whatman No. 4) using a buchner funnel. After filtration, the filtrate was evaporated by using rotary evaporator (R-20, Buchi, Switzerland). The extracts were later dried completely using a lyophilizer (ALPHA 1-4 LO plus, CHRIST freeze dried).

Antioxidant estimations

Total phenolic content (TPC)

Total phenolic content was determined by Folin-Ciocalteau method, which was adapted from Singleton and Rossi (1965)[14]. 0.1 ml of an aliquot of the extract was added to 0.5 ml Folin-Ciocalteau reagent in a test tube and then mixed well using a vortex. The mixture was allowed to react for 3 minutes then 1.5 ml of 7.5 % w/v Na_2CO_3 and 7.9 ml distilled water was added and mixed well. The solution was incubated at room temperature (23°C) in the dark for 2 hour.After 2 hours incubation at room temperature,the absorbance was read at 765 nm using a spectrophotometer. The amount of the total phenolic content was expressed in mg of gallic acid equivalent using the standard curve.

Antioxidant activity determinations

Scavenging activity on 2,2-diphenyl-2-picryl hydrazyl radical (DPPH assay)

The DPPH free radical scavenging activity of each sample was conducted according to the method described by (Braca *et al*., 2001)[15]. A solution of 0.1 mM DPPH in ethanol was prepared. The butylated hydroxytoluene (BHT) / butylated hydroxyannisole (BHA) combination and ascorbic acid were used as standards. The concentration of extracts and standards were prepared from 200 until 1000 µg/ml. An aliquot of 0.6 ml of each concentration of extracts and standards were added to 4.5 ml of ethanolic DPPH solution. The mixture was shaken vigorously and left to stand for 20 minutes at room temperature in a dark room. Absorbance was read using a spectrophotometer at 517 nm. EC_{50} value was determined from the plotted graph of scavenging activity against the concentration of extracts which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50 %[16].

Ferric reducing activity power (FRAP assay)
The FRAP assay was done according to Benzie and Strain (1996)[17]. The stock solutions included 300mM acetate buffer (3.1 g $C_2H_3NaO_2$, $3H_2O$ and 16ml $C_2H_4O_2$), pH 3.6, 10mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40mM HCl, and 20mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ solution and then warmed at 37°C before using. The extracts (150 ml) were allowed to react with 2850 µl of the FRAP solution for 1 hour in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 100 and 500 mM Trolox. Results are expressed in mM TE/g extract weight.

β-carotene bleaching assay
The β-carotene bleaching assay was determined by the spectrophotometric method at 470 nm adapted by Velioglu *et al*. (1998)[12]. This assay was carried out to measure the % antioxidant activity of extracts of each part. In this method combination of BHA/BHT at 200 µg/ml was used as the standard. 0.2 mg/ml of β-carotene was dissolved in chloroform, 0.02 ml linoleic acid and 0.2 ml Tween 20 were transferred into a 50 ml round bottom flask. Chloroform was then removed at room temperature under vacuum at reduced pressure using a rotary evaporator (R-20, Buchi, Switzerland) for 10 minutes. After evaporation, 50 ml of distilled water was added to
the mixture and shaken vigorously to form an emulsion. 2 ml aliquots of the emulsion was pipetted into 0.2 ml of extract, standard and water (as control) and immediately placed in a water bath (Memmert, Kuala Lumpur, Malaysia) at 50°C.The absorbance was read at 20 minutes intervals for 2 hours at 470 nm and the rate of bleaching of β-carotene was recorded to calculate the % antioxidant activity of each part of wax gourd extracts.

Evaluation of Antimicrobial Activity
Bacterial strains
The antibacterial activity of fruit peels extracts was evaluated using seven Gram-positive bacteria and seven Gram-negative bacteria. All the bacterial strains were obtained from Laboratory of Microbiology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia. The microorganism strains were inoculated in universal bottle containing nutrient broth at 37°C for 24 h in Tryptic Soy Broth (TSB) (Merck, Germany). The optical density (O.D) of the bacterial suspension was adjusted turbidometrically to 1.1 O.D at wavelength of 600nm.

Mold and Yeast strains
Mold and Yeast strains were obtained from Laboratory of Microbiology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia. The microorganism strains were inoculated in universal bottle containing potato dextrose broth at 30°C for 72 h in Tryptic Soy Broth (TSB) (Merck, Germany). The optical density (O.D) of the bacterial suspension was adjusted turbidometrically to 1.1 O.D at wavelength of 540nm.

Disc Diffusion Method
The screening of the extracts on antibacterial activity was carried out by determining the diameter zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method. The sterile discs impregnated with 25 µl of extract solution with varied concentration (25 to 100mg/ml extract) were placed in inoculated agar that has been swabbed with adjusted bacteria. The zones of growth inhibition around the discs were measured after 18 to 24 h of incubation at 37 °C. Chloramphenicol (10 µg/disc), Vancomycin (5 µg/disc), Penicillin G (1 unit), Streptomycin (10 µg/disc), Gentamicin (35 µg/disc) and distilled water were used as standard for bacteria and Nystatin (10 µg/disc) was used as standard for mold and yeast. The controls were prepared using the same solvents without extracts. The sensitivity of the microorganism species to the crude extracts was determined by measuring the sizes of inhibitory zones (including the diameter of disc) on the agar surface around the disks, and values <6.5 mm were considered as not active against microorganisms.

Statistical Analysis
All data were expressed as mean ± standard deviation. Data was analyzed using SAS. Pearson's correlation test was used to access connection between means. P-values < 0.05 were considered statistically significant. The EC_{50} values were calculated by linear regression analysis.

Results and discussion

Total phenolic content
The total phenolic content of the extract of the wax gourd measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent, where the Folin-

Ciocalteu method is based on oxidation/reduction on redox properties of antioxidant compound presence in the extract that can react with Folin-Ciocalteu and sodium carbonate produce blue color of phosphomolybdic-phosphotungstic phenol complex[14]. Thus, most concentrated blue color contains highest total phenolic content.

Total Phenolic Content (TPC) were significantly different among each parts of wax gourd tested (Table 1). The TPC showed higher content in seed (207.9 mg GAE/g extract weight) followed by pulp (184.9 mg GAE/g extract weight) and peel (74.83 mg GAE/g extract weight). This finding is not in agreement with Huang *et al*. (2005)[7], who reported that there was no significant difference in total phenolic content of various parts of wax gourd extracts. This may be due to different cultivated area, different species and different extraction methods used in the study. The high in total phenolic content in seed extract might be due to the presence of saponin[18], amino acid[19] and triterpenoids[20]. A study done by Baydar *et al*. (2006)[21] found that most plant by-products (fruit wastes) contain polyphenols with potential application as food antioxidant and Soong and Barlow (2004)[22] demonstrated that fruit seeds had higher phenolic content than edible portions.

Table 1:Total phenolic contents of various wax gourd parts
(result expressed as mg GAE/g extract weight).

Wax gourd parts	TPC mg GAE/g extract weight
Skin	74.83 ± 1.42^{C}
Pulp	184.9 ± 5.02^{B}
Seed	207.9 ± 11.21^{A}

Values are expressed as mean ± standard deviation (n = 3).

Means with different letters were significantly different at the level $p < 0.05$.

Antioxidant activity

Scavenging activity on 2,2-diphenyl-2-picrylhdrazyl radical (DPPH assay)

In order to provide antioxidant potential of the ability of each of wax gourd extracts to act as free radical scavengers or hydrogen donors, DPPH radical scavenging activity assay was carried out[23]. DPPH is a stable, nitrogen centered free radical which produces violet color in ethanol solution[24]. When the free radical have been scavenged, DPPH will generated its color to yellow because as odd electron of the radical becomes paired off in the presence of a hydrogen donor, the absorption intensity will be decreased and resulting discoloration with respect to the number of electron captured[25,26]. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups[27].

Figure 1 showed that the scavenging activity of all extracts (peel, pulp and seed) on DPPH radicals increased rapidly from concentration of 200 µg/ml until it became plateau after concentration of 600 µg/ml. EC_{50} value (the amount of antioxidant (AO) necessary to decrease the initial DPPH radical concentration by 50 percent (%) was determined from the plotted scavenging activity against the concentration of various parts of wax gourd extracts graph.

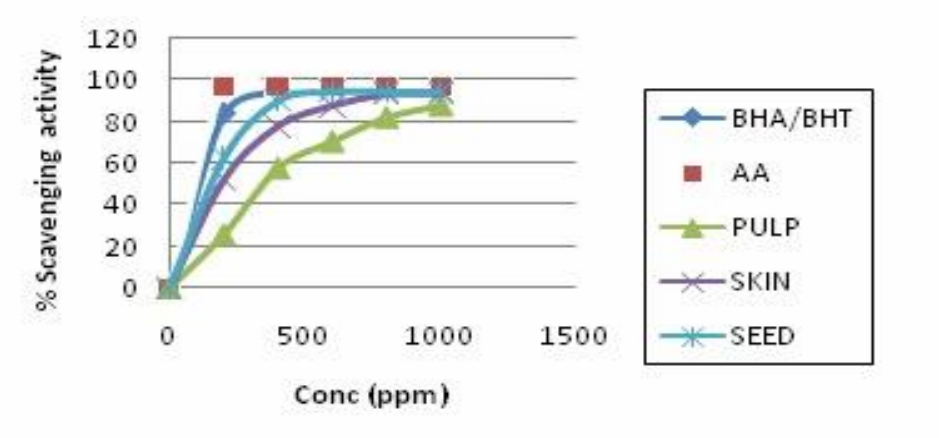

Figure 1. Scavenging effect of various parts of wax gourd extract on DPPH radicals. Values are expressed as mean ± standard deviation (n = 3). Ascorbic acid and BHAT/BHT were used as a standard.

Table 2: Antioxidant activity of various parts of wax gourd extracts as determined by the DPPH (EC_{50}), FRAP and β-carotene bleaching (% AA) assays.

Part of Wax Gourd extract	Antioxidant Activities		
	DPPH (EC_{50})[a]	FRAP[b]	B-Carotene bleaching assay[c]
BHA/BHT (Standard)	120.03 ± 0.18^{C}	Not applicable	94.91 ± 4.5^{A}
Ascorbic Acid (Standard)	104.03 ± 0.18^{C}	Not applicable	Not applicable
Peel	392.21 ± 20.16^{A}	21.73 ± 2.13^{B}	34.39 ± 4.92^{C}
Pulp	195.17 ± 20.04^{B}	26.71 ± 3.05^{B}	76.27 ± 13.41^{B}
Seed	165.42 ± 0.25^{B}	35.06 ± 5.68^{A}	90.22 ± 7.68^{A}

Values are expressed as mean ± standard deviation (n=3). Means with different letters were significantly different at the level $p<0.05$.

[a]Scavenging activity EC_{50} of various part of wax gourd extracts on DPPH radicals in µg/ml.

[b]FRAP assay result expressed as mM TE/g extract weight.

[c] Antioxidant activity (%) by β-carotene linoleate bleaching assay.

The lowest EC_{50} indicates the strongest ability of the extracts to act as DPPH scavengers (Azizah *et al*., 2007). From Table 2, each part of wax gourd extracts, pulp extract had the highest EC_{50} and there was no significant difference existed between the EC_{50} of seed and skin extracts. Sun and Ho (2005)[28] reported a significant correlation between total phenolics and scavenging ability of buckwheat extract on DPPH radicals. However, our study showed no correlation between scavenging ability and total phenolic content. A study done by Norshazila *et al*. (2010)[29] found negative correlation between scavenging activity and total phenolic content of selected seeds of Malaysian tropical fruits. This findings was in contrarily with the results obtained from β-carotene bleaching assay in this study and this might be due to the antioxidant activity measured by β-carotene bleaching assay was calculated based on the presence of any compound with anti oxidative activity while in DPPH, free radical scavenging activity was calculated based on presence of compound that was able to donate electron to scavenge DPPH radicals. Our results indicated that high scavenging ability on DPPH radicals might not be due to phenolic compounds in the seed extract of wax gourd.

Ferric reducing activity based on FRAP assay

The seed extract of wax gourd exhibited the highest antioxidant potential among the extracts (Table 2) based on the FRAP assay. However, there was no significant difference between pulp and skin extract on the antioxidant potential in FRAP assay.

In FRAP assay, reducing potential of an antioxidant reacting with ferric tripyridyltriazine (Fe3+-TPTZ) complex was measured and producing a colored ferrous tripyridyltriazine (Fe2+-TPTZ)[17,30]. The reducing properties are associated with the presence of compounds in the extracts breaking the free radical chain through donating a hydrogen atom[13]. The highest antioxidant potential of seed extract could be due to high in total phenolic content. In addition, there was positive correlation between FRAP assay and phenolic content (R^2=0.874) for each part of the wax gourd extracts. This result was in agreement with Benzie and Stezo (1999)[31] who found a strong correlation between total phenolic content and FRAP assay. A study done by Gardner *et al*. (2000)[31] for apple and pineapple juices had also shown that the synthetic free radical potassium nitrosodisulfonate (by using electron spin resonance) and Fe^{3+} (by using FRAP) showed strong correlation with phenolic content. Thus, in this study phenolic compound of seed extract of wax gourd exhibited high reducing power on Fe^{3+}-TPTZ. In addition, Rice *et al*. (1996)[32] reported that phenolic compounds have redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. The redox potential of phenolic compound plays an important role in determining the antioxidant capacity[32].

β-Carotene-linoleate bleaching assay

In this study, the percent antioxidant activity of the various part of wax gourd extract were measured by the bleaching of β-carotene. Table 2 shows the comparative β-carotene bleaching in percent antioxidant activity of the control, standard and sample extracts. The standard (combination of BHA/BHT) and seed extract of wax gourd did not show any significant and this indicates that the standard and the seed extracts were able to inhibit the discoloration of β-carotene, however the skin extract and the pulp extract of wax gourd showed a significant difference ($p<0.05$) as compared to the standard and seed extracts. The order was BHT/BHA>seed>pulp>peel.

Based on the percentage of antioxidant activity resulted from β-carotene bleaching assay, this study showed high correlation (0.989) between the percent antioxidant activity and total phenolic content for all of the extracts. Our findings is in agreement with Yen *et al*. (2004)[33] and Norshazila *et al*. (2010)[29], who reported that extracts with high amounts of total phenolic content also showed a high antioxidant activity.

Antimicrobial activity

In antimicrobial studies, the results (Table 4 and Table 5) indicated that peel, pulp and seed of wax gourd extracts had showed more inhibitory effects against gram negatives bacteria compared to gram positive bacteria and seed extract showed the most inhibition zone especially on *Cronobacter muytjensi, Shigella boydii and Serratia marcescens.* However, there are no inhibition for mold and yeast for all the wax gourd extracts. The standard antibacterial discs i.e., Chloramphenicol (10µg), Penicillin G (1 unit), Streptomycin (10µg), Vancomycin (5µg) and Gentamicin (30µg) showed a high inhibitory effects towards both Gram-positive and Gram -negative bacterias as showed in Table 3 and the standard for mold and yeast also showed high inhibitory effects towards *Aspergillus niger*, *Penicillium chrysogenum* and *Candida albicans*.

In the present study, seed extracts of wax gourd was found higher in both Gram-positive and Gram-negative bacteria as compared to the peel and pulp extracts. This may be due to the presence of miscellaneous compound such as steroid, alkaloids, triterpenoids and saponin[19] in the seed that has specificability to form pores in membranes cell of bacteria that are able to slow down the growth of bacteria[34]. In addition, based on total phenolic content (TPC) results, seed possessed higher phenolic content and in previous study by Uchikoba *et al.* (1998)[19] showed that gallic acid was found to possess clear inhibitory effects against several panthogenic intestinal bacteria.

Table 3: Zones of inhibition produces by Standard discs.

Bacteria	Standards (Inhibition zone ,mm)					
	Chloramphenicol	Penicillin G	Streptomycin	Vancomycin	Gentamicin	Distilled water
	10µg	1 unit	10µg	5µg	30µg	-
Gram-positive:						
Bacillus subtilis	20 ± 0.05	19 ± 0.04	23 ± 0.25	15 ± 0.12	26 ± 0.06	(-)
Staphylococcus aureus	16 ± 0.04	9 ± 0.05	13 ± 0.06	9 ± 0.23	20 ± 0.08	(-)
Micrococcus species	35 ± 0.04	27 ± 0.08	(-)	18 ± 0.04	23 ± 0.09	(-)
Staphylococcus epidermidis	20 ± 0.06	21 ± 0.1	12 ± 0.09	12 ± 0.07	33 ± 0.23	(-)
Staphylococcus xylosus	15 ± 0.07	21 ± 0.23	20 ± 0.34	12 ± 0.13	31 ± 0.43	(-)
Bacillus circulans	23 ± 0.08	(-)	10 ± 0.07	(-)	15 ± 0.54	(-)
Gram-negative:						
Salmonella typhimurium	18 ± 0.01	(-)	(-)	(-)	16 ± 0.05	(-)
Pseudomonas aeruginosa	7 ± 0.04	7 ± 0.04	7 ± 0.12	(-)	12 ± 0.04	(-)
Proteus vulgaris	10 ± 0.03	(-)	16 ± 0.86	12± 0.34	23 ± 0.03	(-)
Serratia liquefaciens	16 ± 0.05	(-)	24 ± 0.45	(-)	22 ± 0.06	(-)
Cronobacter muytjensii	26 ± 0.05	25 ± 0.23	23 ± 0.09	(-)	30 ± 0.45	(-)
Shigella boydii	15 ± 0.06	(-)	14 ± 0.32	17 ± 0.05	(-)	(-)
Serratia marcescens	19 ± 0.07	(-)	11 ± 0.05	(-)	13 ± 0.08	(-)

Values are expressed as mean ± standard deviation (n = 3).

Table 4: Zones of inhibition produces by peel, pulp and seed of wax gourd extracts.

Bacteria	Aqueous extracts samples (Inhibition zone ,mm)								
	Peel			Pulp			Seed		
	0.63 mg/ disc	1.25 mg/ disc	2.5 mg/ disc	0.63 mg/ disc	1.25 mg/ disc	2.5 mg/ disc	0.63 mg/ disc	1.25 mg/ disc	2.5 mg/ disc
Gram-positive:									
Bacillus subtilis	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Staphylococcus aureus	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Micrococcus species	(-)	(-)	(-)	(-)	(-)	(-)	7 ± 0.05	8 ± 0.04	15 ± 0.03
Staphylococcus epidermdis	(-)	6.4 ± 0.06	6.6 ± 0.07	(-)	(-)	(-)	(-)	(-)	(-)
Staphylococcus xylosus	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Bacillus circulans	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Gram-negative:									
Salmonella typhimurium	(-)	(-)	(-)	(-)	(-)	6.3 ± 0.08	(-)	(-)	6.5 ± 0.07
Pseudomonas aeruginosa	(-)	(-)	(-)	(-)	(-)	6.5 ± 0.09	(-)	(-)	6.5 ± 0.08
Proteus vulgaris	(-)	6.3 ± 0.05	6.5 ± 0.08	(-)	(-)	(-)	7 ± 0.04	8 ± 0.08	10 ± 0.10
Serratia liquefaciens	(-)	(-)	(-)	(-)	(-)	6.5 ± 0.09	6.5 ± 0.05	6.9 ±0.05	7.2 ± 0.05
Cronobacter muytjensii	(-)	(-)	(-)	(-)	(-)	(-)	12 ± 0.07	18 ± 0.04	20 ± 0.05
Shigella boydii	(-)	(-)	(-)	(-)	(-)	(-)	7 ± 0.08	8 ± 0.09	9 ± 0.06
Serratia marcescens	(-)	(-)	(-)	(-)	(-)	(-)	9 ± 0.09	9 ± 0.08	10 ± 0.07

Values are expressed as mean ± standard deviation (n = 3).

Conclusion

This investigation supports the view that various parts of wax gourd extracts are promising source of natural antioxidants. Among these parts, seed extract of wax gourd fruits showed very strong antioxidant properties in all *in vitro* antioxidant assays performed (DPPH, FRAP, and β-Carotene bleaching assays) and also possessed strong antibacterial inhibitory especially on Gram-negative bacteria. The high antioxidant and antibacterial activities of seed extract of wax gourd appeared to be attributed to its high phenolic content. Therefore, isolation and identification on individual active compounds in wax gourd seed and their *In vivo* antioxidant activities need to be investigated further.

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