Original article

Antioxidant capacity and antibacterial activity of different parts of mangosteen (*Garcinia mangostana* Linn.) extracts

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Antioxidant capacity and antibacterial activity of different parts of mangosteen (*Garcinia mangostana* Linn.) extracts.

Abstract – Introduction. Mangosteen (*Garcinia mangostana*) is a tropical fruit that is famous for its edible pulp. This edible pulp makes up only 30% of the total fruit, while the remaining pericarp and seed are regarded as waste. Facing the increasing public demand for naturally safe foods and products, our study aimed to elucidate the antioxidant capacity and antibacterial activity of different parts of mangosteen, including the pericarp, pulp and seed. **Materials and methods**. The antioxidant capacities of mangosteen's pericarp, pulp and seed extracts were determined using the ferric reducing antioxidant power (FRAP) assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, whereas antibacterial activity was determined using the disc diffusion method. **Results**. The pericarp extract exhibited higher antioxidant capacities than those of pulp and seed extracts, with a Trolox equivalent antioxidant capacity (TEAC) value of 122.00 $\mu M \cdot g^{-1}$ and ferrous sulphate equivalent antioxidant capacity (FEAC) value of 18.99 $mM \cdot g^{-1}$. All mangosteen extracts showed strong inhibition against *Staphylococcus aureus* ATCC11632, while only the pericarp extract was effective against *Bacillus cereus* ATCC10876. No inhibition against *Escherichia coli* ATCC10536 was observed. **Conclusion**. The outcomes of our study could facilitate future application of mangosteen waste as a biopreservative in the food industry.

Malaysia / *Garcinia mangostana* / fruits / fruit extracts / pericarp / seed extracts / fruit pulps / antimicrobial properties / antioxidants

Capacité antioxydante et activité antibactérienne d'extraits de différentes parties du mangoustan (*Garcinia mangostana* Linn.).

Résumé – Introduction. Le mangoustan (*Garcinia mangostana*) est un fruit tropical réputé pour sa pulpe comestible. Cette pulpe comestible ne représente que 30 % de l'ensemble du fruit, tandis que le péricarpe et les graines sont considérés comme des déchets. Pour répondre à la demande croissante du public pour les aliments et les produits naturellement sains, nous avons cherché à mesurer la capacité antioxydante et l'activité antibactérienne de différentes parties du mangoustan, dont le péricarpe, la pulpe et les graines. **Matériel et méthodes**. Les capacités antioxydantes des extraits du péricarpe, de pulpe et de graine du mangoustan ont été déterminées à l'aide de dosages FRAP et DPPH, alors que l'activité antibactérienne a été déterminée selon la méthode de diffusion sur disque. **Résultats**. L'extrait de péricarpe a montré des capacités antioxydantes plus élevées que celles des extraits de pulpe et de graine ; il a donné lieu à une capacité antioxydante en équivalent Trolox (TEAC) de 122.00 $\mu M \cdot g^{-1}$ et à une capacité antioxydante en équivalent sulphate ferreux (FEAC) de 18.99 $mM \cdot g^{-1}$. Tous les extraits de mangoustan ont montré une forte inhibition contre *Staphylococcus aureus* ATCC11632, alors que seul l'extrait de péricarpe a été efficace contre *Bacillus cereus* ATCC10876. Aucune inhibition n'a été observée contre *Escherichia coli* ATCC10536. **Conclusion**. Les résultats de notre étude pourraient encourager la future utilisation des déchets de mangoustan comme biopréservatifs dans l'industrie alimentaire.

Malaisie / *Garcinia mangostana* / fruit / extrait de fruit / péricarpe / extrait de graines / pulpe de fruits / propriété antimicrobienne / antioxydant

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1. Introduction

Mangosteen (*Garcinia mangostana* Linn.) belongs to the family of Clusiaceae (Guttiferae). Its fruit has a comparatively small edible portion. The pulp or flesh makes up 30% of the total fruit [1]. More often than not, the pericarp and seed are regarded as waste. To date, Thailand is one of the world's largest producers of mangosteen, producing approximately 240,000 t annually, with exports recorded at 15,000 t in 2006[1]. Hence, development of a sustainable way to utilise these large amounts of waste is crucial.

Exploiting plant extracts is both a traditional tool and a new trend in natural medicine, especially in development of plant-derived drugs and nutraceuticals [2]. Recent studies have demonstrated that dietary plants and fruits are rich sources of antioxidants and can contribute to protection from age-related diseases [3–6]. Evidence suggests that increased consumption of fruits contributes to improved health and well-being [3]. Recently, numerous studies revealed that the mangosteen pericarp is the main source of phytonutrients, such as anthocyanins, oligometric proanthocyanins and xanthones [7, 8, 11]. Research has thus gained insight into the beneficial properties of mangosteen for human health. A number of therapeutic benefits that are associated with anthocyanins and other antioxidants have been extracted from mangosteen, including cardioprotective, anti-inflammatory, anticarcinogenic [9] and antimicrobial properties [10]. Facing the increasing public demand for natural and microbiologically safe foods and products, our present study therefore aimed to elucidate the antioxidant capacity and antibacterial activity of different parts of mangosteen, including the pericarp, pulp and seed.

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2. Materials and methods

2.1. Sample preparation

A total of 24 ripe mangosteens (*Garcinia mangostana* Linn.) with weights ranging from 70–110 g, dark purple in colour and with no apparent physical damage, were randomly selected from a local supermarket in Selangor, Malaysia. The mangosteens were randomly divided into four replicates, with six mangosteens per replicate. The fruits were rinsed with sterile distilled water and manually separated into the pericarp, pulp and seed. The fruit parts were immediately sealed and stored at –80 °C in a freezer until use.

2.2. Extraction of phytochemicals in mangosteen

The extraction of phytochemicals in mangosteen pulp, pericarp and seed was adopted from Alothman *et al.* [12] and Babbara *et al.* [13] with minor modifications. The pericarp and seed were dried at 60 °C in an oven for 24 h prior to extraction. Five grams of dried pulp, dried pericarp and dried seed were respectively ground into fine powder using liquid nitrogen with a mortar and pestle. The powders were then extracted with 100 mL of 70% methanol at 40 °C for 3 h. The extracts were filtered with a clean muslin cloth and concentrated using a rotary evaporator (Buchi, Switzerland) at 45 °C for 1 h. The concentrates were then re-dissolved in 10 mL of 70% methanol and stored at –80 °C in a freezer until use.

2.3. Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was carried out according to the method of Alothman *et al.* [12] with minor modifications. In this assay, the FRAP reagent was prepared by mixing 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL of 4,6-tripyridyl-s-triazine (TPTZ, Fluka, Sigma Aldrich Co., Germany) solution in 40 mM HCL and 2.5 mL of 20 mM ferric chloride.

Subsequently, fifty µL of respective mangosteen extracts were mixed with 950 µL of FRAP reagent and incubated in a water bath at 37 °C for 4 min. Absorbance was then measured at 593 nm against a reagent blank. The ferric reducing antioxidant power of the sample was determined based on the ferrous sulphate (Merck, Germany) calibration curve (0.0 mM to 1.6 mM). Results were expressed as ferrous sulphate equivalent antioxidant capacity (FEAC), with mM ferrous sulphate Eq·g^{-1} sample dry weight (dw).

2.4. The 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging assay

The ability of antioxidants to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was carried out according to the modified method of Weecharangsan *et al.* [14]. The 0.004% DPPH solution (Sigma, USA) was prepared freshly by mixing 2 mg of DPPH with 50 mL of methanol. After that, fifty µL of respective sample extracts were mixed with 950 µL of DPPH reagent and incubated at room temperature for 30 min in the dark. Absorbance was then measured at 517 nm wavelength against a reagent blank. The radical scavenging activity of the sample was calculated based on the Trolox (Acroz Organic, USA) standard curve (0 µM to 700 µM). Results were expressed as trolox equivalent antioxidant capacity (TEAC), with µM Trolox·g^{-1} sample dw.

2.5. Bacterial culture preparation and maintenance

In our study, three bacterial cultures, namely *Staphylococcus aureus* ATCC11632, *Bacillus cereus* ATCC10876 and *Escherichia coli* ATCC10536, were tested. All cultures were maintained in Lubria broth (Merck, Germany). Cultures were propagated in Lubria broth or on Lubria agar and then incubated for 24 h at 37 °C prior to use. The cultures were maintained as frozen stock at –80 °C in Lubria broth supplemented with 20% of sterile glycerol.

2.6. Disc diffusion test

The 24-h-old bacterial culture was diluted with sterile Lubria broth to obtain an absorbance value of 0.5 at 600 nm wavelength using a UV-visible spectrophotometer (Biochrom Ltd., UK). Then, the bacterial culture was swabbed on a Lubria agar plate. Subsequently, filter paper discs (6 mm in diameter) containing sample extract (stock concentration of 0.5 mg·µL^{-1}) at different amounts (0–80 µL) were placed on the freshly swabbed plate. A disc with 70% methanol was used as the negative control, while the streptomycin disc was used as the positive control. The plate was then incubated at 37 °C for 24 h and the diameter of the inhibition zone formed was measured.

2.7. Statistical analysis

All assays were carried out in four replicates. The data were presented as the mean ± standard deviation of the mean. Analysis of variance was calculated using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS), version 9.1.3. Duncan's Multiple Range Test was used to compare the treatment means at $P < 0.05$.

3. Results and discussion

3.1. Antioxidant capacity of mangosteen extracts

In our study, the ferric reducing antioxidant power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to examine the antioxidant capacities of mangosteen pericarp, pulp and seed extracts. Antioxidant capacities of mangosteen pericarp extract were significantly higher ($P < 0.05$) than those of pulp and peel extracts (*table I*). The ferrous sulphate equivalent antioxidant capacity (FEAC) value of the pericarp extract was approximately 27-fold and 8.6-fold higher than that of pulp and seed extracts, respectively. The pericarp extract consisted of a high amount of electron donors which are able to reduce

Table I.
Antioxidant capacities of mangosteen extracts (pericarp, pulp and seed) determined using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays.

Mangosteen extracts	FEAC (mM FE·g^{-1} dw)	TEAC (μM TE·g^{-1} dw)
Pericarp	18.99 ± 4.43 a	122.00 ± 2.00 a
Pulp	0.70 ± 0.21 b	24.00 ± 4.00 c
Seed	2.20 ± 0.48 b	80.00 ± 22.00 b

FEAC: ferrous sulphate equivalent antioxidant capacity.

TEAC: Trolox equivalent antioxidant capacity.

a, b, c: values with different letters within a column are significantly different at $P < 0.05$.

the reduce ferric-tripiridyltriazine (Fe^{3+}–TPTZ) to a ferrous form (Fe^{2+}). The trolox equivalent antioxidant capacity (TEAC) value of the pericarp extract was 5-fold and 1.5-fold higher than that of pulp and seed extracts, respectively. These findings showed that the pericarp extract was able to scavenge a higher amount of DPPH free radicals than pulp and seed extracts.

Such findings are in accordance with previous studies carried out on other fruits, in which the peel and seed of the respective fruits showed a higher antioxidant activity than its edible pulp. For instance, the peel of pomegranate contains a higher antioxidant activity than its pulp [15]. Similarly, Guo *et al.* [16] reported that grape seed has higher antioxidant ability than its pulp and represents a rich source of proanthocyanidin, an efficient reactive oxygen species scavenging agent. More recently, Arazo *et al.* [17] reported that the peel extract of yellow mangosteen (*Garcinia tinctoria*) exhibited a higher scavenging activity of DPPH radical compared with the pulp extract. Nevertheless, Arellano-González *et al.* [18] found that coffee pulp contains hydroxycinnamic acids which could be a potential source of natural antioxidants.

Earlier studies of mangosteen are mainly focused on two parts, the edible pulp and pericarp. Several studies on the antioxidant capacity of the edible part of mangosteen have been reported [19, 20]. These authors often compared the antioxidant capacity of the edible pulp of mangosteen with other tropical fruits. Only a few studies have been reported on the antioxidant capacity of mangosteen pericarp [21, 22]. To the best of our knowledge, studies on the antioxidant capacity of mangosteen seed are scarce. Although several published works have been carried out on mangosteen pulp and pericarp, it is rather difficult to make a direct comparison with the current findings. This obstacle is mainly due to the variation in the extraction methods used, the antioxidant determination assays and the standard solutions used to quantify the antioxidant capacity. Development of a standard protocol to measure the antioxidant capacity of fruits is thus crucial. Nevertheless, the current findings have provided a clear overview of the antioxidant capacities in different parts of mangosteen, which might enhance the future utilisation of these fruit wastes.

3.2. Antibacterial activity of mangosteen extracts

The disc diffusion method was employed to determine the antibacterial activity of mangosteen extracts against two Gram-positive bacteria (*Staphylococcus aureus* ATCC11632 and *Bacillus cereus* ATCC10876) and one Gram-negative bacterium (*Escherichia coli* ATCC10536). All extracts showed positive antibacterial activity against *S. aureus* ATCC11632 (*table II*). The results indicated that the inhibitory activities of mangosteen

Table II.
Antibacterial activity of mangosteen extracts (pericarp, pulp and seed) against *Staphylococcus aureus* ATCC11632 determined using the disc diffusion method.

Volume of mangosteen extract (µL)	Diameter of inhibition zone (mm)		
	Pericarp	Pulp	Seed
0	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 e
20	2.17 ± 0.58 d	1.25 ± 0.50 c	3.50 ± 1.00 d
40	3.83 ± 0.19 c	1.94 ± 0.13 b	6.50 ± 1.92 c
60	4.50 ± 0.19 b	2.31 ± 0.47 b	9.00 ± 1.16 b
80	5.08 ± 0.42 a	4.50 ± 0.71 a	12.50 ± 1.92 a

a, b, c, d, e: values with different letters within a column are significantly different at $P < 0.05$.

extracts were dose-dependent. When the amount of the extract increased from 0 µL to 80 µL, a similar increment in the diameter of the inhibition zone was observed. Among the three extracts, the seed extract exhibited the strongest inhibitory activity against *S. aureus* ATCC11632, followed by pericarp and pulp extracts. The inhibitory activity of the seed extract (80 µL) against *S. aureus* ATCC11632 was at least two-fold stronger than that of peel and pulp extracts.

The current findings were in agreement with several reports in which the *Garcinia* species contain naturally occurring compounds which have a very strong antimicrobial activity against *S. aureus* [23]. The side effects and emergence of methicillin-resistant *S. aureus* (MRSA) have become a critical issue in recent years. MRSA is one of the most critical strains that are commonly found in places such as hospitals [24]. Several studies have reported that the mangosteen peel extract contains alpha-mangostin (one of the xanthone derivatives), which is effective against MRSA [25, 26]. Hence, the current findings are timely and significant, as the seed and pericarp extracts could be developed as an alternative treatment for MRSA.

Unlike *S. aureus* ATCC11632, only mangosteen's pericarp extract showed positive antibacterial activity against *B. cereus* ATCC10876 (data not shown). An inhibition zone of 2.06 mm in diameter was formed with the application of 80 µL of pericarp extract. The inhibitory strength of the pericarp extract against *B. cereus* ATCC10876 was at least 2.5-fold less than *S. aureus* ATCC11632. However, the control streptomycin was 4.4-fold and 1.8-fold stronger than *B. cereus* ATCC10876 and *S. aureus* ATCC11632, respectively.

There are only a few publications reporting the antimicrobial activity of mangosteen extracts against *B. cereus*. Sundaram *et al.* reported the effectiveness of mangosteen pericarp extract against *Bacillus subillis* [23], while Negi *et al.* reported that the pericarp extracts of *Garcinia cowa* and *Garcinia pedunculata* exhibited high inhibitory effects against *B. cereus* due to the presence of xanthones [26]. There is a high possibility that the antibacterial activity of mangosteen peel extract in this study might be due to the xanthones. Nonetheless, further studies should be conducted to clarify and identify the antibacterial compounds present in the mangosteen extracts.

All mangosteen extracts showed negative antibacterial activity against *E. coli* ATCC10536. In contrast, Sundaram *et al.* indicated that *E. coli* was moderately susceptible to mangosteen extracts [23]. However, the degree of susceptibility was significantly lower than that of the other Gram-positive bacteria tested. Negi *et al.* also showed that the antimicrobial activity from *G. cowa* and *G. pedunculata* extracts were higher in Gram-positive bacteria than in Gram-negative bacteria [26]. Similar

results were reported in different fruits, including grapefruit extracts [27].

4. Conclusion

Our present study suggested that mangosteen pericarp, pulp and seed extracts have different antioxidant capacities, in which pericarp and seed extracts exhibited a promising antioxidant potential. In general, mangosteen extracts showed a greater inhibitory activity against the Gram-positive bacteria *S. aureus* ATCC11632. The results of our study could facilitate future application of mangosteen waste.

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Capacidad antioxidante y actividad antibacteriana de extractos de diferentes partes del mangostán (*Garcinia mangostana* Linn.).

Resumen – Introducción. El mangostán (*Garcinia mangostana*) es una fruta tropical conocida por su pulpa comestible. La pulpa comestible solo representa un 30% del total de la fruta, mientras que el pericarpio y las semillas se consideran desechos. Para responder a la creciente demanda del público de alimentos y productos naturalmente sanos, tratamos de medir la capacidad antioxidante y la actividad antibacteriana de diferentes partes del mangostán, como el pericarpio, la pulpa y las semillas. **Material y métodos**. La capacidad antioxidante e los extractos de pericarpio, pulpa y semillas se determinaron mediante los métodos FRAP y DPPH, mientras que la actividad antibacteriana se determinó con el método de difusión en disco. **Resultados**. El extracto de pericarpio mostró capacidades antioxidantes más elevadas que los extractos de la pulpa y las semillas: el resultado fue de una capacidad antioxidante equivalente al Trolox (TEAC) de 122.00 $\mu M \cdot g^{-1}$ y de una capacidad antioxidante equivalente al sulfato ferroso de (FEAC) de 18.99 $mM \cdot g^{-1}$. Todos los extractos de mangostán presentaron una fuerte inhibición contra *Staphylococcus aureus* ATCC11632, mientras que únicamente el extracto de pericarpio fue eficaz contra *Bacillus cereus* ATCC10876. No se observó ninguna inhibición contra *Escherichia coli* ATCC10536. **Conclusión**. Los resultados de nuestro estudio podrían fomentar la futura utilización de desechos de mangostán como bioconservantes en la industria alimentaria.

Malasia / *Garcinia mangostana* / frutas / extractos de frutas / pericarpio / extractos de semillas / pulpa de frutas / propiedades antimicrobianas / antioxidantes