International Journal of Agricultural Technology 2018 Vol. 14(2):183-191
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The Bioactivities of Seed Coat and Embryo Extracts from Indian Gooseberry (*Phyllanthus emblica*)

Jaiboonma, A.[1], Janvikul, W.[2] and Poeaim, S.[1*]

[1]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, 10520; [2]Biomedical Engineering Research Unit, National Metal and Materials Technology Center, Pathumthani, Thailand.

Jaiboonma, A., Janvikul, W. and Poeaim, S. (2018). The bioactivities of seed coat and embryo extracts from Indian gooseberry (*Phyllanthus emblica*). International Journal of Agricultural Technology 14(2):183-191.

Abstract The research evaluated the bioactivities of seed coat and embryo extracts from Indian gooseberry (*Phyllanthus emblica* Linn). The crude methanolic extract was further successively extracted with hexane, ethyl acetate, *n*-butanol and water by liquid-liquid partitioning method. The total phenolic content (TPC) in each solvent-extracted fraction was determined by Folin-Ciocateu method. The ethyl acetate seed coat extract possessed the highest TPC with values of 439.09±5.49 milligrams gallic acid equivalent (mgGAE)/g extract. The antioxidant activity of each extract was examined using ferric-reducing antioxidant power (FRAP) and 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) assay. The ethyl acetate seed coat extract revealed 50% inhibitory concentration (IC_{50} value) at a concentration of 31.53±0.36 microgram/milliliter (µg/ml) and ferric-reducing antioxidant power with 751.92±5.22 milligrams ascorbic acid equivalent (mgAAE)/g extract. The antibacterial activity of each extract was performed using disc diffusion method. The ethyl acetate seed coat extract demonstrated the greatest bacterial inhibition against *Bacillus subtilis* ATCC 6633, *Bacillus cereus* DMST 5040, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* TISTR 1466, and *Micrococcus luteus* ATCC 9341. These results suggested that the seed coat extracted with ethyl acetate could be served as a natural source of the bioactive compounds and used in the preparations of products such as drugs, anti-aging supplements, and cosmetics.

Keywords: Bioactivity, Indian gooseberry, *Phyllanthus emblica*, Seed coat and Embryo

Introduction

Phyllanthus emblica Linn., commonly found in Indian gooseberry or makham pom in Thailand, belongs to the family Euphorbiaceae. The Indian gooseberry is widely grown in South-East Asia, especially in tropical forests (Dinesh *et al*., 2016). The fruits are generally used in herbal medicine as fever reducer, wound healing promoter, cough and sore throat reliever, and immune system inducer. It has been known that this fruit contains a high level of

[*] **Corressponding author:** Poeaim, S.; **Email:** poeaim@hotmail.com

vitamin C, an effective antioxidant. (Verma and Gupta, 2004; Namiesnik *et al*., 2013). In addition, the fruit, leaves, bark, and branch were sources of many bioactive compounds, including polyphenols, flavonoids, gallic acid, ellagic acid, and other bioactive compounds, which were found to inhibit the oxidation most efficiently (Kumar *et al*., 2014; Patel *et al*., 2016). Furthermore, the plant materials were scientifically reported for its anticancer, antitumor, antibacterial and anti-inflammatory activities (Ngamkitidechakul *et al*., 2010), and found to decrease dangerous diseases such as diabetes, coronary heart disease and rheumatoid (Baliga and Dsouza, 2011). However, there have been only a few reports on the bioactivities of Indian gooseberry seed coat and embryo extracts. Hence, the aims of this research were to investigate the total phenolic content, antioxidant and antibacterial activities of Indian gooseberry seed coat and embryo extracts. In addition, the total phenolic content in the extracts was qunrified. The results obtained from this preliminary study would be beneficial to the developments of novel medical products.

Materials and methods

Preparation of extracts from Indian gooseberry

Fresh Indian gooseberries were collected from Phrae province, Thailand. The whole fruits were dried, and fleshy parts were then grated. The seeds were dried at 40°C, which the seed coat were physically detached from the embryo. The dried seeds were ground and soaked three times with methanol by maceration at room temperature. After 7 days, the methanolic extract was filtered through a filter paper (Whatman No. 1), and the solution was subsequently concentrated using a rotary evaporator. The crude methanolic seed coat and embryo extract was dissolved with sterilized water and partitioned successively with hexane, ethyl acetate, *n*-butanol and water (Beedessee *et al*., 2012). Each of partitions was evaporated using a rotary evaporator and then stored in a desiccator until used.

Determination of total phenolic content

The total phenolic content (TPC) was determined by Folin–Ciocalteu method with some modifications (Quy *et al.,* 2014). Twenty five microliter (µl) of each extract sample at a concentration of 500 microgram/milliliter (µg/ml) was mixed with 125 µl of 10% Folin–Ciocalteu reagent solution in a well of a 96-well plate and incubated for 5 min. To each solution, 100 µl of 7.5% Na_2CO_3 solution was then added immediately, and the whole solutions were incubated for 60 min in the dark. The absorbances of the solutions were finally

measured at 765 nm. Gallic acid was used as standard compound, and total phenolic content was expressed as mgGAE (milligrams gallic acid equivalent)/g extract.

Antioxidant assays

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was prepared followed by Saeed *et al.* (2012) with some modifications. Twenty µl of each sample extract (250 µg/ml) was mixed with 180 µl of freshly prepared FRAP-TPTZ solution, and the whole solution was then incubated in the dark at room temperature for 6 min. The absorbances of the solutions were measured at 593 nm. The standard curve was constructed using ascorbic acid (0-100 µg/ml), and the reducing antioxidant power activity was expressed as mgAAE (milligrams ascorbic acid equivalent)/g extract.

$ABTS^{\bullet+}$ scavenging activity assay

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) scavenging activity assay was performed according to Saeed *et al*. (2012). $ABTS^{\bullet+}$ scavenging activity was measured by mixing 20 µl of each extract (0-150 µg/ml) with 180 µl of $ABTS^{\bullet+}$ solution, and the solution was incubated for 6 min. The absorbances of the solutions were subsequently measured at 734 nm. The $ABTS^{\bullet+}$scavenging activity was calculated using the following equation:

The $ABTS^{\bullet+}$scavenging activity (%) = [(absorbance of the control– absorbance of the sample) / (absorbance of the control)]×100

The data were presented as mean of triplicate. The concentration required for 50% inhibition of $ABTS^{\bullet+}$ (IC_{50} value) was determined graphically.

Assessment of antibacterial activity

Microorganisms, i.e., *Bacillus subtilis* ATCC 6633, *Bacillus cereus* DMST 5040, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* TISTR 1466, and *Micrococcus luteus* ATCC 9341, were obtained from the Deparment of Biology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. The antibacterial activities of the extracts were performed by the disc diffusion method (Meerungrueang *et al.*, 2014). The extract solutions (1,000 µg/disc) were loaded on sterilized filter paper discs of 6 mm in diameter and dried for 10 min. The discs were placed on the surface

of the previous inoculated Mueller-Hinton agar (MHA) and incubated for 24 h at 37°C. DMSO: deionized water (1:1 v/v) and gentamycin (10 µg/disc) were used as a negative control and a positive control, respectively. The clear zones of bacterial inhibition around the discs were to be observed.

Statistical analysis

The data were expressed as means±standard deviations (SD) of measurements from three independent replicates (n=3). The data were statistically analyzed by a statistical package program version 20.0 (SPSS 20.0) using Duncan's multiple range test (DMRT); $p<0.05$ was considered as statistically signifcant.

Results

Total phenolic content

The total phenolic contents of the seed coat and embryo extracts were estimated using gallic acid (0-150 µg/ml) as a standard compound. A linear calibration curve of gallic acid showed y=0.0085x, where x is the explanatory variable, y is the depentdent variable and the slope of the line is 0.0085 with R^2 value of 0.99 (the square of the correlation coefficient is R). The Indian gooseberry extracts were found to have various total phenolic contents, ranging from 64.52 to 439.09 mgGAE/g extract. As revealed in Table 1, the ethyl acetate extract prepared from the seed coat had the highest total phenolic content, with an average value of 439.09±5.49 mgGAE/g extract. The lowest content was observed in the hexane extract prepared from the embryo, with an average value of 83.00±4.50 mgGAE/g extract.

Antioxidant activities

Ferric-reducing antioxidant power (FRAP)

The ferric-reducing antioxidant power assay was used to determine the antioxidant activities of the extracts, which were calculated using the standard curve of ascorbic acid (y=0.0152x, R^2=0.99). It was found that the seed coat and embryo extracts from Indian gooseberry had the FRAP activities, ranging from 83 to 751 and 86 to 688 mgAAE/g extract, respectively. The ethyl acetate extract prepared from the seed coat possessed the highest FRAP activity that was 751.92±5.22 mgAAE/g extract, as revealed in Table 1.

$ABTS^{\bullet+}$ scavenging activity

The $ABTS^{\bullet+}$ scavenging activities of the extracts (0-150 µg/ml) are expressed as IC_{50} value at which the $ABTS^{\bullet+}$ scavenging activities of the seed coat and embryo extracts were found in the ranges of 31-134 and 37-67 µg/ml, respectively. The ethyl acetect extract prepared from the seed coat exhibited the highest $ABTS^{\bullet+}$ scavenging activity with IC_{50} value at 31.53±0.36 µg/ml (Table 1 and Figure 1).

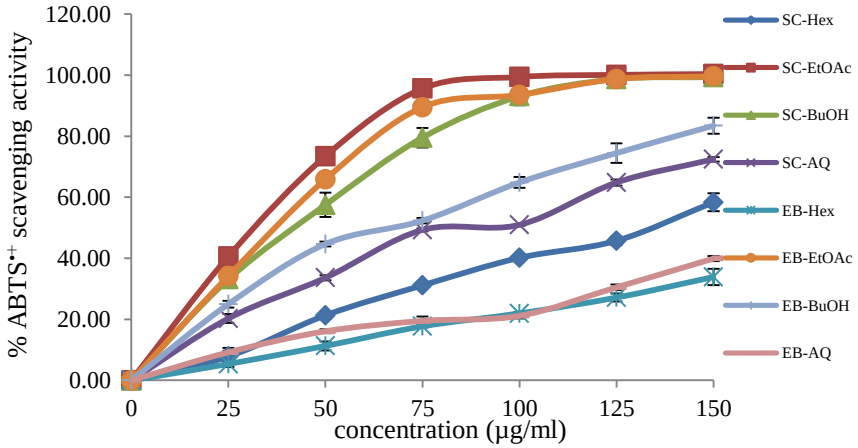

Figure 1. The $ABTS^{\bullet+}$ scavenging activity of the partitioning extracts from Indian gooseberry seed coat and embryo.

Antibacterial activity

The clear zones of bacterial inhibition of eigth extracts prepared in different solvents are presented in Table 2. All the extraction has overall

potential antibacterial activity. Apparently, the ethyl acetact extract prepared from the seed coat exhibited the greatest antibacterial activity against all bacteria tested,i.e., *Bacillus subtilis* ATCC 6633, *Bacillus cereus* DMST 5040, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* TISTR 1466, and *Micrococcus luteus* ATCC 9341, with the clear zone diameters of 15.27±0.56, 14.76±1.92, 16.80±2.21, 15.51±2.17, and 16.57±1.59 mm, respectively.

Table 1. Total phenolic contents, ferric-reducing antioxidant power and 50% inhibited concentration of $ABTS^{\bullet+}$scavening activities of seed coat and embryo extracts from Indian gooseberry

Indian gooseberry	TPC (mgGAE/g extract)*	FRAP (mgAAE/g extract)*	IC_{50} value (µg/ml)*#
			$ABTS^{\bullet+}$ scavenging activity
Seed coat-Hexane	126.41±2.60[e]	83.86±1.90[g]	134.71±4.61
Seed coat-EtOAc	439.09±5.49[a]	751.92±5.22[a]	31.53±0.36
Seed coat-BuOH	260.77±5.45[c]	525.87±5.93[c]	41.82±0.39
Seed coat-Aqueous	130.70±5.58[e]	114.59±3.19[e]	78.74±1.83
Embryo-Hexane	83.00±4.50[f]	86.45±3.59[g]	>150
Embryo-EtOAc	399.40±4.39[b]	688.16±6.69[b]	37.15±0.36
Embryo-BuOH	198.60±3.28[d]	305.82±5.13[d]	67.06±0.92
Embryo-Aqueous	64.52±3.44[g]	97.44±1.59[f]	>150

*The data are expressed as means±standard deviation (SD), three replicates (n=3).
#IC_{50} value was the inhibited concentration scavenged by 50%.
The letters a-g within the same column indicate the statistical significances at $p<0.05$.

Table 2. The clear zones of bacterial inhibition of the extracts prepared from the seed coat and embryo of Indian gooseberry.

Indian gooseberry	The clear zone diameters observed around test discs (mm)±SD*				
	B. subtilis	***B. cereus***	***M. luteus***	***S. epidermis***	***S. aureus***
Seed coat-Hexane	7.83±0.57[b]	10.01±1.66[c]	8.76±0.95[c]	9.53±0.53[c]	11.25±1.98[b]
Seed coat-EtOAc	15.27±0.56[a]	14.76±1.92[a]	16.57±1.59[a]	16.80±2.21[a]	15.51±2.17[a]
Seed coat-BuOH	12.47±1.02[a]	12.35±1.22[b]	13.59±1.05[b]	14.25±2.50[b]	16.30±2.40[a]
Seed coat-Aqueous	7.22±0.65[b]	6.62±0.35[d]	7.04±0.14[d]	NA	9.15±0.80[c]
Embryo-Hexane	6.77±0.41[b]	9.23±1.05[b]	7.34±0.32[d]	8.26±0.83[c]	7.17±1.15[d]
Embryo-EtOAc	14.22±1.12[a]	14.61±1.00[a]	16.19±1.46[a]	16.34±2.62[a]	16.25±1.56[a]
Embryo-BuOH	13.91±1.39[a]	11.73±0.74[b]	13.63±0.89[b]	13.42±0.78[b]	15.48±0.79[a]
Embryo-Aqueous	6.74±0.33[b]	6.76±0.22[d]	6.35±0.22[d]	NA	10.95±0.36[b]
Gentamycin (10 µg/disc)	23.53±0.54	29.08±1.99	23.16±1.24	30.01±0.83	29.47±0.93

*Each value was presented as mean±standard deviation (SD), three replicates (n= 3).
The letters a-d within the same column indicate the statistical significances at $p<0.05$.
NA represented no the clear zone inhibition

Discussion

The obtained results were in good accordance with the findings reported by Mishra and Mahanta (2014) that was the extracts from the seed coat of Indian gooseberry possessed high total phenolic contents. In addition, the ethyl acetate extract prepared from the seed coat had the highest total phenolic content, similar to the result disclosed in the literature (Mishra *et al*., 2015). The antioxidant activity of Indian gooseberry was previously researched by Nadheesha *et al*. (2007); it was reported that its seed had a higher antioxidant activity its fruit. The polyphenolic compounds present in the seed were likely contributed to the high antioxidant activity.

From these obtained results, the relationship between the total phenolic content and antioxidant activity of the seed coat and embryo extracts were determined, to confirm that the polyphenolic compounds were really attributed to the antioxidant activity found in Indian gooseberry, as reported previously (Cai *et al*., 2004; Wong *et al*., 2005). In addition, the major compound of the seed coat might contain polyphenols compounds, such as catechin acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, gallic acid, quercetin acid, and vanillic acid (Mishra *et al.*, 2015).

Antimicrobial activity of the extracts from seed coat and embryo of Indian gooseberry, was measured against selected bacteria, including bacterial food poisoning and an intestinal pathogen. It was previously reported that the extract from the Indian gooseberry seed possessed the the antimicrobial activity against *S. aureus* and *B. cereus* (Dabur *et al*., 2007; Priya *et al*., 2012).

In this study, the total phenolic content, antioxidant activity, and antibacterial property of the seed coat and embryo of Indian gooseberry were investigated. The bioactivities of the extracts prepared from the seed coat were found to be higher than those of the extracts prepared from the embryo. Furthermore, among all the solvents used, the ethyl acetate extract from the seed coat was found to be the most potent. Further studies on the identification of polyphenolic compounds present in the extracts are to be conducted to confirm the functionality present in Indian gooseberry.

Acknowledgements

This study was supported by King Mongkut's Institute Technology of Ladkrabang (KMITL), National Metal and Materials Technology Center (MTEC), and partially funded by Graduate Institute of Science and Technology (TGIST), Thailand.

References

Baliga, M. S. and Dsouza, J. J. (2011). Amla (*Emblica officinalis* Gaertn), a wonder berry in the treatment and prevention of cancer. European Journal of Cancer Preventio 20:225-239.

Beedessee, G., Ramanjooloo, A., Aubert, G. and Eloy, L. (2012). Cytotoxic activities of hexane, ethyl acetate and butanol extracts of marine sponges from mauritian waters on human cancer cell line. Environmental Toxicology and Pharmacology 34:397-408.

Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life sciences 74:2157-2184.

Dabur, R., Gupta, A., Mandal, T. K., Singh, D. D., Bajpai, V., Gurav, A. M. and Lavekar, G. S. (2007). Antimicrobial activity of some Indian medicinal plants. African Journal of Traditional Complementary and Alternative Medicines 4:313-318.

Dinesh, M., Roopan, S. M. and Selvaraj, C. I. (2016). Photocatalytic degradation of nitrophenol using biologically active *Phyllanthus emblica* seed extract. Journal of Photychemistry and Phytobiology B 161:273-278.

Kumar, A., Singh, A. and Singh, B. (2014). Assessment of therapeutic potential of *Phyllanthus emblica* (Amla): a natural godsend. International Journal of Cell Science and Biotechnology 3:4-14.

Meerungrueang, W. and Panichayupakaranant, P. (2014). Antimicrobial activities of some Thai traditional medical longevity formulations from plants and antibacterial compounds from *Ficus foveolata*. Pharmaceutical Biology 52:1104-1109.

Mishra, P., Dutta, N. and Mahanta, C. L. (2015). Partial extraction and identification of phenolics in Amla (*Emblica officinalis*) seed coat powder. Journal of Food Science and Technology 52:6990-7001.

Mishra, P. and Mahanta, C. L. (2014). Comparative analysis of functional and nutritive values of amla (*Emblica officinalis*) fruit, seed and seed coat powder. American Journal of Food Technology 9:151-161.

Nadheesha, M. K. F., bamunuarachchi. A., Edirisinghe, E. M. R. K. B. M. and Weerasinghe, W. M. S. K. (2007). Studies on antioxidant activity of Indian gooseberry fruit and seed. Journal of Science of the University of Kelaniya Sri Lanka 3:83-92.

Namiesnik, J., Vearasilp, K., Kupska, M., Ham, K. S., Kang, S. G., Park, Y. K, Barasch, D., Nemirovski, A. and Gorinstein, S. (2013). Antioxidant activities and bioactive components in some berries. European Journal of Food Research and Technology 237:819-829.

Ngamkitidechakul, C., Jaijoy, K., Hansakul, P., Soonthornchareonnon, N. and Sireeratawong, S. (2010). Antitumour effects of *Phyllanthus emblica* L.: induction of cancer cell apoptosis and inhibition of *in vivo* tumour promotion and *In vitro* invasion of human cancer cells. Phytotherapy Research. 24:1405-1413.

Quy, D. D., Artik, E. A., Phuong, L. T., Lien, H. H., Felycia, E. S., Suryadi, I. and Yi-hsu, J. (2014). Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatic*. Journal of Food and Drug Analysis 22:296-302.

Patel, S. S. and Goyal, R. K. (2012). *Emblica ofcinalis* Geart: A comprehensive review on phytochemistry, pharmacology and ethnomedicinal uses. Research Journal of Medicinal Plant 6:6-16.

Priya, G., Parminder, N. and Jaspreet, S. (2012). Antimicrobial and antioxidant activity on *Emblica officinalis* seed extract. International Journal of Research in Ayurveda and Pharmacy 3:591-596.

Saeed, N., Khun, M. and Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. Complementary and Alternative Medicine 12:221.

Wong, C., Li, H., Cheng, K. and Chen, F. (2006). A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chemistry 97:705-711.

Verma, R. C. and Gupta, A. (2004). Effect of pre-treatments on quality of solar-dried amla. Journal of Food Engineering 65:397-402.

(Received: 10 August 2017, accepted: 25 November 2017)