ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF *Garcinia prainiana* STEM BARK

Shaari Daud[1], Khairil Syazwan Salim[1], Aiza Harun[1*], Zati Ismah Ishak[1], Zurhana Mat Hussin[1], Ahmad Faris Seman@Kamarulzaman[1]

[1]*Faculty of Applied Sciences*
Universiti Teknologi Mara UiTM Pahang, 26400 Bandar Jengka

[*]*Corresponding author: aizaharun@uitm.edu.my*

Abstract

Finding new compounds with medicinal value from natural products is gaining popularity as they involve fewer side effects on the human body than synthetic drugs. The natural sources that provide promising antioxidants are usually phenolic compounds and triterpenoids. To date, investigations of phenolic and triterpenoid compounds from the bark of *Garcinia prainiana* unfortunately have not been well documented. Therefore, the bark of *G. prainiana* from Felda Jengka 1 Pahang was utilised in this research work. The aim is to evaluate the antioxidant potency of *G. prainiana* extracts through DPPH dot blot assays and radical scavenging activity. The total phenolic content (TPC) from the bark of *G. prainiana* was also determined using the Folin-Ciocalteu method. Crude extracts of the stem bark of *G. prainiana* were prepared through consecutive cold-soaking using four different types of organic solvents. The results reveal that the ethyl acetate and methanol extracts exhibited promising antioxidant activity, with IC50 values of 97.9631 µg/ml and 125.0517 µg/ml, respectively. Meanwhile, ethyl acetate extracts showed the highest TPC value of 112.2086 mg GAE/g sample. These research findings are beneficial for medicinal and pharmaceutical purposes because it provides basic scientific information that would assist in the development of drug discovery.

Keyword: Antioxidant activity, total phenolic content, *Garcinia prainiana*

Introduction

The genus *Garcinia* (Guttiferae) is widely distributed in the subtropical and tropical regions of West and East Africa, Central and South America, and South East Asia (Salleh et al., 2017). About 400 species are distributed across Asia, about 49 of which have been reported in Malaysia. The most widely known species of *Garcinia* in the Indo-Malay region is *Garcinia Mangostana*, known locally as mangosteen or "manggis". The genus *Garcinia* has its own ethnopharmacological activities, from which people have traditionally benefited. Recently, several studies have been conducted on the genus *Garcinia* to identify whether it possesses anti-cancer, hyperglycaemic, anti-diabetic (Ali et al., 2017) or antiviral properties (Junaidi, 2017).

Previous studies on the biological activity of *G. prainiana* have shown some significant findings. The types of biological activities of this species have been assessed using total phenolic content, DPPH, antibacterial and cytotoxic tests. Shamsul et al. (2013) reported that the methanol extract of leaves and stem bark produced the highest total phenolic content, expressed as gallic acid and (±)-catechin equivalents, as well as the highest value of ascorbic acid and butylated hydroxytoluene (BHT) equivalents for forming the phosphomolybdenum complex in the total antioxidant assay. Researchers have also

reported that methanol extracts from the stem bark resulted in the highest free radical scavenging activity, with an IC_{50} value of 74.5 µg/mL, while the isolated compound morelloflavone revealed a strong free radical scavenging activity, with an IC_{50} value of 15.7 µg/mL via an antioxidant assay on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Asang et al. (2018) reported the antioxidant and antibacterial activity of the leaves, fruit and twigs of *G. prianiana*. They concluded that all the parts showed moderate antioxidant and antibacterial activity against selected pathogenicbacteria. However, the authors did not report any uses of the *G. prianiana* bark.

Currently, research that explores other types of *G. prainiana* extracts remains limited compared with studies of other *Garcinia* species. Therefore, the current authors initiated this research into the antioxidant activity and total phenolic content (TPC) of four types of extracts of *G. prainiana* stem bark.

Materials and Methods

Plant material and extracts preparation

The stem bark of *G. prainiana* was collected from Felda Jengka 1 Pahang. Large chunks of *G. Prainiana* stem bark was finely ground using a grinder. The fine *G. Prainiana* powder was consecutively soaked with hexane, chloroform, ethyl acetate and methanol. The process of soaking and filtering to produce the extracts was performed three times for each solvent. A rotary evaporator was utilised to evaporate the solvent until the crude extracts formed. The crude extracts were collected and kept safely before use.

Thin layer chromatography

Thin-layer chromatography (TLC) was conducted to determine the best developing solvent to elute the compounds in each extract. A TLC plate was drawn and cut into 3 cm × 5 cm sections. A baseline was drawn on the plate to indicate the starting line where the spot/line of the sample was dropped. Then, about 2 mL of the solvent system was added to a TLC chamber and the TLC plate was placed into the chamber for the compounds to be eluted. Later, UV light was used to assist in observing the separation and elution of the compounds. A long UV wavelength was used to observe the spot as this showed a clear visualisation of the eluted compounds when applied. Random combinations of solvents with certain ratios were trialled to identify the best developing solvent to separate the compounds. The best developing solvent was needed as the correct solvent system would be used later in the column chromatography and screening tests.

Screening Tests for Alkaloids, Phenolics and Terpenoids

The presence of alkaloids in the crudes of each extract of *G. Prainiana* King was identified through spraying Dragendorff's reagent. Dragendorff's reagent is a solution of potassium bismuth iodide composed of basic bismuth nitrate ($Bi(NO_3)_3$), tartaric acid and potassium iodide (KI) (Raal et al., 2020). A small amount of sample from each crude was dropped onto the TLC plate, which was put into the TLC chamber with the correct solvent system to elute the compounds. Later, Dragendroff's reagent was sprayed onto the TLC plates and the formation of an orange colour was observed on each plate, indicating the presence of alkaloids (Raal et al., 2020).

The test to detect the presence of phenolics was conducted through spraying ferric chloride ($FeCl_3$) solution. The ferric chloride solution was prepared by dissolving solid ferric chloride in methanol and water (Sharma et al.,1998). A positive indication that phenolics are present in the crude samples occurs when a dark blue colour forms on the sprayed TLC plate.

Meanwhile terpenoid detection was conducted by dropping the prepared vanillin/H_2SO_4 reagent on TLC plate after which it was heated using a heating mantle at 110 ^oC The

formation of pink, blue, grey or purple color after heating indicates the presence of terpenoids in the crude extract (Al-Jumaily & Al-Amiry, 2012).

A qualitative test for antioxidants was performed by spraying a prepared DPPH reagent onto the TLC plate of crude extracts. A change to yellow from the purple of the DPPH reagent indicates the presence of antioxidants in the crude extracts (Cieśla et al., 2012).

Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy

Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy (ATR – FTIR) was used to determine the functional groups of the compounds in the crudes. In the infrared (IR) spectrum of each crude, only the region above 1500 cm^{-1} (or diagnostic region) was analysed to determine the functional groups.

Antioxidant activity

a. Semiquantitative dot blot assay

A dot blot assay is a simple yet rapid test to evaluate antioxidant activity in a sample. A dot blot assay utilised a TLC plate cut into a specific size and containing a block of squares measuring 1.5 cm × 1.5 cm. Before performing the assay, a series of sample solutions of different concentrations were prepared, ranging from 100 mg/mL – 0.024 mg/mL for each crude of *G. prainiana.* A capillary tube was used to aid in transferring a very small amount of each sample onto the squares drawn on the TLC plate. Later, the TLC plate was sprayed with 0.05% DPPH solution after it had been dried. Colour changes from purple to yellow were observed and recorded. The same method was applied to every crude of *G. prainiana* and the standard ascorbic acid. For each crude, three trials were performed.

b. DPPH radical scavenging activity

DPPH radical scavenging activity is a method of quantitatively determining antioxidant activity with a UV/Vis spectrophotometer. The antioxidant activity of the crude samples was measured as a decrease of DPPH. A series of *G. prainiana* extract solutions were prepared, ranging from 400 µg/mL – 12.5 µg/mL. For every 1 mL of each concentration, 3 mL of 0.004% DPPH solution (refer to Appendix 1) was added and mixed. Each sample solution was then shaken vigorously and placed in a dark place for about 30 minutes. Later, each sample was placed in a cuvette and then inside the UV/Vis spectrophotometer at 517 nm. The absorbance reading was recorded. For each crude/extract sample, three or triplicate trials were conducted. Then, analysis of DPPH radical scavenging activity was conducted on all four crude samples, as well as the ascorbic acid standard. The radical scavenging activity percentages were calculated according to the following equation:

$$\text{Scavenging activity (\%)} = (A_{control} - A_{sample})/A_{control) \times 100$$

whereby $A_{control}$ = absorbance of DPPH control

A_{sample} = absorbance of extract/standard

Total Phenolic Content (TPC)

Total phenolic content tests were conducted to evaluate the level and presence of phenolics in the samples tested. A series of gallic acid solutions with different concentrations ranging from 1000 ppm – 31.25 ppm was prepared before testing to develop a calibration curve. The absorbance of each gallic acid solution was measured by a UV/Vis spectrophotometer and recorded for further use.

Meanwhile, for each extract sample, 1000 ppm concentrations were prepared. Then, 0.5 mL of 1000 ppm of each sample was mixed with 2.5 mL of Folin-Ciocalteu reagent. Then, 2 mL of prepared sodium carbonate was added to the sample solutions and incubated for 30 min.

Each sample solution was then put into a cuvette and placed inside a UV/Vis spectrophotometer with a 765 nm wavelength to measure the absorbance, which was recorded. The blank for the test was prepared using the same method; however, the sample extract was not included.

Results and Discussion

Detection of Alkaloids, Phenolics, Terpenoids and Antioxidant.

To detect alkaloids, Dragendorff's reagent was used to detect the compounds. A positive indication of alkaloids in a crude extract leaves an orange spot on the TLC plate after spraying with Dragendorff's reagent. For the hexane and chloroform crudes, the orange precipitate could be seen clearly on the TLC plates after spraying, indicating the strong presence of alkaloids in both crudes. Meanwhile, for the ethyl acetate crude, the orange spot could still be visualised but not as strongly as with both the hexane and chloroform crude. However, no formation of an orange spot was observed on the TLC plate of methanol crude. Thus, it was concluded that the methanol crude extract of *G. prainiana* contained no alkaloid compounds.

For the detection of phenolic compounds, ferric chloride ($FeCl_3$) solution was utilised to aid the screening test. The formation of dark blue spots on a TLC plate after it was sprayed with $FeCl_3$ solution gave a positive indication of the presence of phenolics in the crude. The hexane and chloroform crude extracts possessed almost no dark blue spots on the TLC plates. However, the ethyl acetate and methanol crudes produced a contrasting result. Dark blue spots were clearly observed to be abundant on the TLC plates, which showed a positive indication of phenolic compounds.

Vanillin/H_2SO_4 reagent was used to detect terpenoids. Instant heating, using a heating mantle of developed TLC plates that had been previously sprayed with Vanillin/H_2SO_4 reagent, is essential for observing any colour formation. Pink, blue, grey or purple are the colours expected to appear on TLC plates if extracts contain terpenoid. The terpenoid compounds were not present in the hexane and chloroform extracts as none of the expected colours formed on the TLC plates. However, for the ethyl acetate and methanol crude extracts, the expected colours were clearly seen on their TLC plates, which indicated the presence of terpenoid compounds.

The DPPH reagent was purposely used to screen the presence of antioxidants. The colour changes of the DPPH reagent from deep purple to yellow indicated a positive result of antioxidant existence. When the TLC plates of the hexane and chloroform extracts were sprayed with DPPH, there was just a trace of yellow on the TLC plates, with less intense colour on the baseline of the plates where the crude sample was spotted. Nevertheless, ethyl acetate and methanol extract showed remarkable results, whereby the formation of yellow colour could be clearly seen on almost all parts of the TLC plates. This result revealed that ethyl acetate and methanol crude extracts behave as potent antioxidants. **Table 1** illustrates the presence of phytochemicals in *G. prainiana* stem bark.

Table 1 Phytochemicals in *G. prainiana* stem bark

Extract	Phytochemicals		
	Alkaloid	Phenolic	Terpenoid
Hexane	+++	-	-
Chloroform	+++	-	-
Ethyl acetate**	+	+++	+++
Methanol**	-	+++	+++

+++ : presence in high amount; + : presence in low amount; - : not present; ** : good antioxidant

Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy.

Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy (ATR – FTIR) was used to determine the functional groups of the compounds in the crudes. In the infrared (IR) spectrum of each crude, only the region above 1500 cm^{-1}, also known as the diagnostic region, was analysed to determine which functional groups were present. **Table 2** summarises the types of functional groups found in each extract of *G. prainiana*.

In the hexane crude IR spectrum, 1628.58 cm^{-1} shows a weak intensity, with the broad shape indicating the presence of aromatics or C=C groups in the sample. This appeared to be in the range of 1600 – 1680 cm^{-1} region, which is equivalent to the aromatics and C=C groups that should appear in the IR spectrum. A peak also appeared at 1740.94 cm^{-1}, which also shows weak intensity and a broad shape, which might be C=O groups. Even though the signal was not very strong, the presence of C=O groups could not be rejected as the crudes may have contained other compounds that interfered with the IR spectrum reading. In addition, three peaks appeared at the 2870.32 cm^{-1}, 2926.27 cm^{-1} and 2952.14 cm^{-1} regions, which might indicate the presence of alkane C-H groups in the sample tested. The peaks showed strong intensity and narrow-shaped peaks, which meant the presence of the C-H groups. The aldehydes or ketones group might be the major compounds that are abundant in the hexane crude base of the IR spectrum. However, this analysis was based on the *G. Prainiana* hexane crude, so many different compounds with different functional groups might have been present in the crude.

According to the IR spectrum of the chloroform crude extract, a narrow and weak peak appeared at 1711.39 cm^{-1}. The intensity was not strong enough to confirm that it belonged to carbonyls C=O groups; however, it still could not be neglected because the signal might have been interfered with by other compounds. Two peaks with medium intensity and narrow shapes appeared closer to each other at the region of 2871.08 cm^{-1} and 2928.38 cm^{-1}; these might be belong to the alkane C-H groups in the sample crude.

In terms of the ethyl acetate crude extract, the absorption of a weak and narrow signal was detected at the 1519.37 cm^{-1} region in the IR spectrum, which indicated the presence of aromatics C=C groups. The presence of C=C alkene was revealed from the peak signal at the 1635.83 cm^{-1} region. Furthermore, another two peaks appeared closer to each other at 1712.66 cm^{-1} and 1736.09 cm^{-1}, possibly belonging to carbonyls C=O groups. The signals of these absorption peaks almost overlapped due to the combination of various compounds in the ethyl acetate crude extract. Another two peaks also formed nearer to each other, with medium intensity and narrow shapes, at 2984.13 cm^{-1} and 2939.25 cm^{-1}. Both absorption peaks were due to sp2 C-H stretching and sp3 C-H stretching. Also, a medium to weak intensity peak with a broad shape appeared between the 3100 cm^{-1} – 3800 cm^{-1} region, which indicated the presence of a hydroxyl OH group.

According to the IR spectrum of the methanol crude, the peak with medium intensity and a narrow shape at 1636.46 cm^{-1} might indicate the presence of a conjugated carbonyl C=O group. Another two peaks, with medium to weak intensity and narrow shapes, belonged to the alkane C-H groups region and were at 2834.86 cm^{-1} and 2944.17 cm^{-1}. The broad absorption peak appearing at 3313.10 cm^{-1} belonged to the hydroxyl O-H group.

The IR analysis conducted for each *G. prainiana* crude was not conclusive as the samples were in crude form. This means that many compounds were abundant in the samples themselves. However, the IR data was still important and relevant as it provided basic information about certain groups of atoms that might be responsible for the antioxidant activity and total phenolic content.

Table 2 FTIR data for extracts of *G. prainiana*

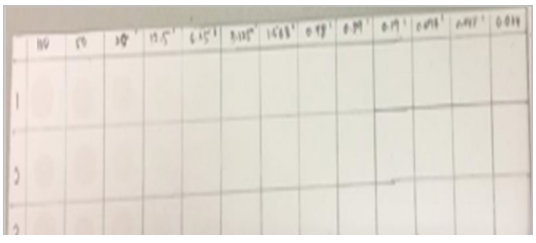
Type of extracts	Wavenumber, cm^{-1}	Type of functional group
Hexane	1628.58	C=C stretching
	1740.94	C=O stretching
	2870.32-2952.14	sp2/sp3 C-H stretching
Chlorofom	1711.39	C=O stretching
	2871.08-2928.38	sp2/sp3 C-H stretching
Ethyl acetate	1519.37	C=C aromatic
	1635.83	C=C stretching
	1712.66	C=O stretching
	2984.15-2939.25	sp2/sp3 C-H stretching
	3100-3800	O-H stretching
Methanol	1636.46	C=O stretching
	2834.86-2944.17	sp2/sp3 C-H stretching
	3313.10	O-H stretching

Semi-Quantitative Analysis by Dot Blot Assay

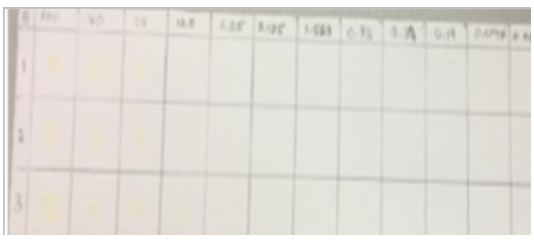
Quantitatively, a dot blot assay was conducted to ascertain the general antioxidant activity of the crudes of *G. prainiana* and was based on the concentration as the responding variable. The concentration at which the yellow colour first appeared on the marked TLC plate was regarded as the lowest concentration for DPPH inhibition, as illustrated in **Figure 1**. As depicted in **Table 3**, the ethyl acetate had the strongest antioxidant activity as it performed its DPPH inhibition at the lowest concentration, 0.195 mg/mL; followed by methanol, with a moderate concentration at 0.390 mg/ml applied for DPPH inhibition. The chloroform and hexane extracts apparently needed higher concentrations to inhibit DPPH radical activity. The dot blot assay result of ethyl acetate and methanol extract is consistent with the screening test of antioxidant of both extracts as illustrated in **Table 1**. The ethyl acetate extract and methanol extract work well as antioxidant source due to the presence of phenolic and terpenoid as depicted in **Table 1**.

Table 3 Antioxidant activity of from dot blot assay of *G. prainiana*

Type of extract	**Lowest concentration for DPPH inhibition,mg/mL**
Hexane	6.250
Chloroform	1.563
Ethyl acetate	0.195
Methanol	0.390

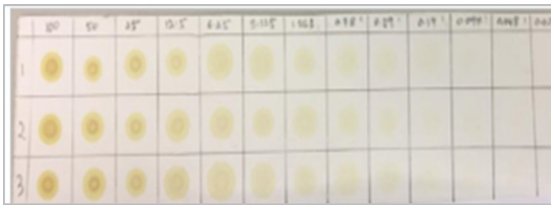


Acid ascorbic dot blot assay

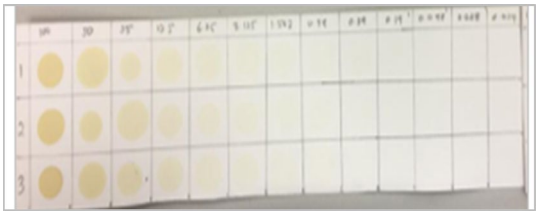


Hexane dot blot assay

Chloroform dot blot assay



Ethyl acetate dot blot assay



Methanol dot blot assay

Figure 1 Dot blot assay of *G. prainiana* stem bark

DPPH Radical Scavenging Activity

In the quantitative analysis of the antioxidant activity of the *G. Prainiana* crudes, a UV/Vis spectrophotometer was utilised to measure the absorbance for each crude extract. The IC_{50} values were calculated from the graph of the percentage of scavenging against concentration. The radical scavenging activity efficacy and IC50 values for all the crude extracts are depicted in **Figure 2** and **Table 4**.

According to **Table 4**, the ethyl acetate extract scavenged DPPH radical more efficiently, with IC50 values at a lower concentration of 97.9631 ug/ml. This means that the ethyl acetate extract only needed a lower concentration to scavenge 50% of the DPPH radical, compared to the methanol extract. The lower the IC50 value, the more readily the extract scavenged the DPPH radical and the better the antioxidant activity. In contrast, a high IC50 value indicated that the extract less readily scavenged 50% of the DPPH radical, resulting in low scavenging efficacy (Ramesh et al., 2015)

Figure 2 explains that the radical scavenging activity increases in a concentration-dependent manner. The higher the concentration of each extract, the higher the radical scavenging activity. Compared to lower concentrations, higher concentrations meant the extracts showed greater hydrogen-donating ability (Chung et al., 2006) and caused the DPPH solution to lose the characteristic deep purple colour and become yellow diphenylpicryl hydrazine faster (Ramesh et al., 2015).

Table 4 DPPH Inhibition concentration IC_{50} of crude extracts of *G. prainiana*

Extract/standard	IC_{50}, µg/mL
Hexane	3511.2644
Chloroform	806.9708
Ethyl acetate	97.9631
Methanol	125.0517
Standard ascorbic acid	7.0675

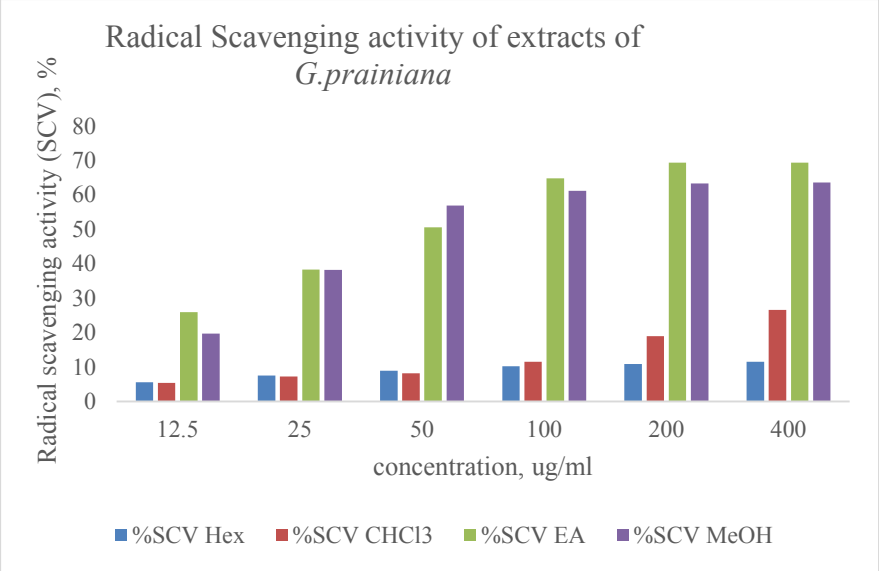


Figure 2 Radical scavenging efficacy of *G.prainiana* stem bark

Total Phenolics Content (TPC) of *G. prainiana* stem bark

In TPC testing using FCR, gallic acid was used as a standard for measuring the phenolic content of each crude extract. The TPC value was measured from the standard curve of gallic acid. **Table 5** records the TPC value for all the crude extracts of *G. prainiana*. As expected, the ethyl acetate extract exhibited the highest TPC value, at 112.2086 mg GAE/g sample, compared to the other extracts. The TPC values were not detected in the hexane or chloroform extracts, and this is consistent with the phenolic screening shown in **Table 1**. The higher TPC value of ethyl acetate explains its potent antioxidant activity effect, identified from the dot blot assay and radical scavenging activity. Conceptually, when any phenolic content extract reacts with the Folin-Ciocalteu reagent (FCR), the phenolic proton dissociates, leading to phenolate ion formation, which reduces the FCR to a blue colour. The findings shown in **Tables 4** and **5** suggest a direct correlation between the TPC and antioxidant activity, whereby the extracts with high phenolic content displayed higher scavenging activity and vice versa. Since the basic structural phenolic formula contains an OH group, a C=O group C=C and an aromatic ring, the TPC result is consistent with the FTIR result for the ethyl acetate extract, as depicted in **Table 2**.

Table 5 Total phenolic content (TPC) of *G. prainiana* extracts

Type of extract	TPC, mg GAE/g sample
Hexane	Not detected
Chloroform	Not detected
Ethyl acetate	112.2086
Methanol	68.7983

Conclusion

The ethyl acetate extract of *G. prainiana* displayed the highest antioxidant activity in vitro, with the lowest IC50 value of 97.9631 *u*g/ml, and it exhibited the highest TPC value of 112.2086 mg GAE/g sample. The higher activity of the ethyl acetate extract could be ascribed to its high phenolic content. Therefore, *G. prainiana* appears to be a promising source of antioxidant agents.

Acknowledgement

The authors would like to thank Dana Dalaman Negeri (DDN)- Dana Khas Lestari Phase 2

for the grant support. In addition, the authors would like to thank Faculty of Applied Sciences UiTM Pahang for the facilities provided throughout the research work.

Conflict of interest

The authors declare that there is no conflict of interest concerning the publication of this paper.

References

Al-Jumaily, E. F., & Al-Amiry, M. H. (2012). Extraction and Purification of Terpenes from Nutmeg (myristica fragrans). *Al-Nahrain Journal of Science, 15*(3), 151-160.

Ali, M., Paul, S., Tanvir, E., Hossen, M., Rumpa, N.-E. N., Saha, M., . . . Alam, N. (2017). Antihyperglycemic, antidiabetic, and antioxidant effects of Garcinia pedunculata in rats. *Evidence-Based Complementary and Alternative Medicine, 2017*.

Asang, G.S., Mohd Khairuddin,N.N., Alias, N., Ahmad, A., & Wan Taib, W.R. (2018). Antioxidant and Antimicrobial Activity of Different Plant Parts of Garcinia prainiana- an Endangered Plant. *International Journal of Engineering & Technology*, 7 (4.43),24-2.

Cieśla, Ł., Kryszeń, J., Stochmal, A., Oleszek, W., & Waksmundzka-Hajnos, M. (2012). Approach to develop a standardized TLC-DPPH test for assessing free radical scavenging properties of selected phenolic compounds. *Journal of Pharmaceutical and Biomedical Analysis, 70*, 126-135.

Chung, Y.,Chien, C., Teng,K. & Chou, S (2006).*Food Chemistry*, 97,418-425

Ee, G. C. L., Daud, S., Izzaddin, S. A., & Rahmani, M. (2008). Garcinia mangostana: a source of potential anti-cancer lead compounds against CEM-SS cell line. *Journal of Asian Natural Products Research, 10*(5), 475-479. doi:10.1080/10286020801948490

Junaidi, A. (2017). *Aktivitas Antivirus Ekstrak dan Fraksi Kulit Buah Manggis (Garcinia mangostana L.) Terhadap Enzim Neuraminidase Virus H5N1 Secara In Vitro.*

Raal, A., Meos, A., Hinrikus, T., Heinämäki, J., Romāne, E., Gudienė, V., . . . Chiru, T. (2020). Dragendorff's reagent: Historical perspectives and current status of a versatile reagent introduced over 150 years ago at the University of Dorpat, Tartu, Estonia. *Die Pharmazie-An International Journal of Pharmaceutical Sciences, 75*(7), 299-306.

Ramesh, D., Ramesh, D., Prakish Kekuda, T.R., Onkarappa, R., Vinayaka, K.S. & Raghavendra, H.L., (2015). Antifungal and radical scavenging activity of leaf and bark of Leea indica (Burm.f.) Merr. Journal of chemical and pharmaceutical research, 7(1), 105-110.

Salleh, W. M. N. H. W., Ahmad, F., Sirat, H. M., & Taher, M. (2017). Cytotoxicity of triterpenes from the leaves of Garcinia prainiana King (Guttiferae). *Marmara Pharmaceutical Journal, 21*(1), 127-131.

Shamsul O., Wan Mohd Nuzul Hakimi Wan S., Farediah A., Hasnah Mohd S., & Muhammad T. (2017). Cytotoxicity of triterpenes from the leaves of *Garcinia prainiana* King (Guttiferae). *Marmara Pharmaceutical Journal* 21, 127-131, DOI: 10.12991/marupj.259890

Sharma, O. P., Bhat, T. K., & Singh, B. (1998). Thin-layer chromatography of gallic acid, methyl gallate, pyrogallol, phloroglucinol, catechol, resorcinol, hydroquinone, catechin, epicatechin, cinnamic acid, p-coumaric acid, ferulic acid and tannic acid. *Journal of Chromatography A, 822*(1), 167-171.