

THE CHROMATOGRAPHIC SEPARATION OF *MANGIFERA* EXTRACT

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Abstract

The phytochemicals and biological properties of *Mangifera* species are documented. The main constituents of *M. pajang* include gallic, p-coumaric and ellagic acids. The fruits of *M. pajang* possess antioxidant, antibacterial and anticancer properties. The phenolic is mentioned as the compound that is responsible for the antioxidant property of *M. pajang*. In this study, the methanolic extraction of *M. pajang* and *M. indica* was performed. Based on thin layer chromatography (TLC), the presence of the phenolics in the samples could be confirmed. Comparative TLC was accomplished and the retention factor (Rf) of pyrogallol was recorded as 0.85. From the result, the TLC profiling of *M. pajang* and *M. indica* extracts were almost similar. In addition, the compounds of *M. pajang* extracts were analyzed from the ¹H-Nuclear Magnetic Resonance (NMR) spectroscopy. A mixture of ascorbic acid and a sugar moiety could be detected. On the other hand, the NMR signal for the metabolite of mangiferin was not observed. Therefore, the compound might not present in the sample.

Keywords: chromatography, extraction, spectroscopy, *Mangifera*

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Introduction

The *Mangifera* species (Anacardiaceae family) can be found locally. In Sabah, *Mangifera pajang* (*M. pajang*) or also known as bambangan, is considered as an iconic fruit. The plant is reported as a potential source for functional food and medicine among the indigenous people (Núñez Sellés *et al.*, 2002). From the literature reviews, the compositions of *M. pajang* such as carotenoids (Khoo *et al.*, 2010) and phenolics, such as gallic acid, p-coumaric acid and mangiferin (Rodríguez *et al.*, (2006) (Figure 1), could be identified from the pulp. In addition, the extracts showed antibacterial and anticancer (Che Rahim *et al.*, 2019) properties. *M. pajang* extracts also presented antioxidant properties. The pulp of *M. pajang* could be extracted with methanol in order to obtain polar, phenolic compound. The extracted compounds may then be subjected to liquid chromatography for separation and identification of the components. The chromatographic profiling of *M. pajang* could also be compared to *M. indica*, which is the common mango. It is expected that the chromatographic profiling of *M. pajang* and *M. indica* would be similar. Nevertheless, the concentrations of the biomolecules from the above *Mangifera* extracts might be different (Ali *et al.*, 2017).

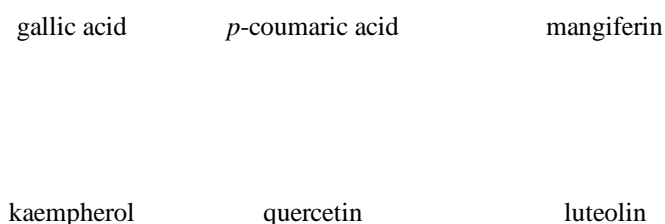


Figure 1. The chemical structures of the natural molecules from the *Mangifera* extract.

Methods

In this study, the literature search on *Mangifera* was conducted electronically (e.g. Science Finder, Medline, Scopus and Google Scholar). The articles were analyzed and reviewed (Boland *et al.*, 2014). The separation of the *Mangifera* extract was also presented.

Plant materials and chemicals

The plant sample of *M. pajang* was collected from Sabah, and identified by one of the authors (K. J. Jalani). A voucher sample of *M. pajang* (no. 012017SBH) was deposited in the laboratory of Faculty Pharmacy, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, Selangor. The plant material was dried and ground into fine powder prior to use.

The chemical that were used include methanol (MeOH), butanol (BuOH), acetic acid and distilled water. Freshly prepared sulphuric *p*-anisaldehyde (from Merck) was utilised as the spraying reagent, in order to stain and visualize any inactive compounds under the ultraviolet (UV) lamp. The glassware consisted of capillary tubes, small vials, filter funnel, forceps, beakers, spatula and stirrer. Thin layer chromatography (TLC) plates (5 x 10 cm, silica gel 60 F₂₅₄, Merck), preparative TLC glass plates (20 x 20 cm), analytical and preparative TLC tank (double trough) and UV chamber were used.

Extraction of *Mangifera* samples

The samples were extracted by using the simplest method of extraction which was maceration (infusion). They were labelled as R (reference solution, pyrogallol or benzene-1,2,3-triol from Merck), 1 (*M. indica* extract), 2 (Ahmad Tea[®] mango flavoured black tea), 3 (Boh[®] mango tea), 4 (pulp extract of *M. pajang*) and 5 (peel extract of *M. pajang*). They were all were soaked in MeOH, in a ratio of 1:10 (w/v). The beakers were placed in a benchtop sonicator (Starsonic 90 Easy) for 20 minutes. Later, the beakers were removed from the sonicator. Then, TLC was performed.

The High Performance Liquid Chromatography (HPLC) of *Mangifera* extract

The laboratory work involving the *M. pajang* extract was also conducted, by extracting and isolating the flavonoids via a reversed phased HPLC. The automated system comprised of solvent pump, a C18 column (Agilent, 5 μ m, 4.6 x 250 mm in 30°C) plus an ultraviolet detector ($\lambda = 280$ nm). The mobile phase was set at 1 mL/min, consisting of 2% acetic acid (CH₃COOH) and 0.5% mixture of acetic acid-acetonitrile (CH₃CN), (50:50 v/v). Every chromatogram was recorded for 60 minutes.

The Thin Layer Chromatography (TLC) of *Mangifera* extract

In the analytical scale, BuOH, acetic acid and distilled water in ratio of (4:1:1) were used as mobile phase. Once it moved over 6 cm from the sampling line, the plate was removed from tank and dried in air, before visualizing it under an UV light ($\lambda = 254$ and 365 nm). After the separation was completed, individual compounds appeared as spots that were vertically separated. The various spots were marked carefully. Each spot on the plate has a retention factor (R_f) which is equivalent to the distance travelled by sample over the total distance travelled by the solvent. The R_f of each spot on silica plate was observed. The TLC plate was stained by the spraying reagent, should there was an absence of spot after visualizing the plate under the UV light.

For preparative TLC, 20 cm x 20 cm glass plates, coated with silica gel 60 F₂₅₄ (Merck), was utilised as stationary phase. Similar with the analytical procedure, BuOH, acetic acid and distilled water in ratio of (4:1:1) were used as a mobile phase. By using a short pipette, the sample was spotted carefully on a silica glass plate as a thin line horizontal band instead of spot. The plate then was placed in the top-sealed chamber. After solvent run upward, the plate was removed. Then, 1 cm from the edge of glass plate was sprayed by anisaldehyde and three different bands were produced. These three bands were scrapped off by using a spatula. The three samples were labelled as 1 (bottom), 2

(middle) and 3 (top) with Rf value of 0.23 (46.5 mg), 0.33 (22.4 mg) and 0.90 (36.8 mg) respectively. Then, they were dissolved in MeOH and the silica was filtered out from the solution. In order to obtain the pure compounds, the solvent was evaporated. The isolated compounds (46.5, 22.4 and 0.90 mg, respectively) were dissolved in the deuterated methanol (CD₃OD) before they were subjected to NMR analysis on a Bruker 500 UltrashieldTM spectrometer.

Result and Discussion

From the literatures, pharmacological characteristics of the *M. pajang* extract was studied (Ahmad *et al.*, 2015; Mirfat *et al.*, 2016). The total phenolic and flavonoid contents in the fruit extracts were correlated with the anti-oxidant activity. The fruit kernel of *M. pajang* showed the highest phenolic content, when compared to the pulp and peel (Tangah *et al.*, 2017). For the free radical scavenging activity, the seed of *M. pajang* displayed the highest effect, followed by the peel and pulp (Abu Bakar *et al.*, 2009).

Liquid Chromatography

Both *M. indica* and *M. pajang* extracts displayed some interesting and coloured spots, when compared to the standard compound, pyrogallol (Figure 2). A preliminary screening via HPLC was also performed to investigate the peel and pulp extracts. Based on the chromatographic profile (Figure 3), methanolic peel crude extract may contain more phytochemical component. Both extracts gave some significant peaks, that were eluted at the retention time (R_T) = 19, 31 and 51 minutes. These constituents might include kaempferol, quercetin and luteolin (Figure 1).

These *Mangifera* extracts could also be sealed in a pouch and introduced as beverage. From the author's best knowledge, bambangan tea is not yet available from local or international market, when compared to other traditional products, e.g. roselle or pomegranate, and other fruit infusions, such as lemon and strawberry. This bambangan tea could be comprised of a pouch having a quantity of the dried *M. pajang* fruit pieces (ca. 2 g). It can be considered as a convenient pack for consumers to enjoy the benefit of this fruit, along with the more commonly prepared bambangan fruit pickles. The porous sachet could also function as a filter, as presented in an exemplary product (Jalani *et al.*, 2017). In addition to this form, bambangan fruit and its co-products could be used as ingredients of dietary fibre powder or could be incorporated into food products (e.g. biscuits and macaroni), in order to enhance their nutraceutical properties (Jahurul *et al.*, 2018, 2019).

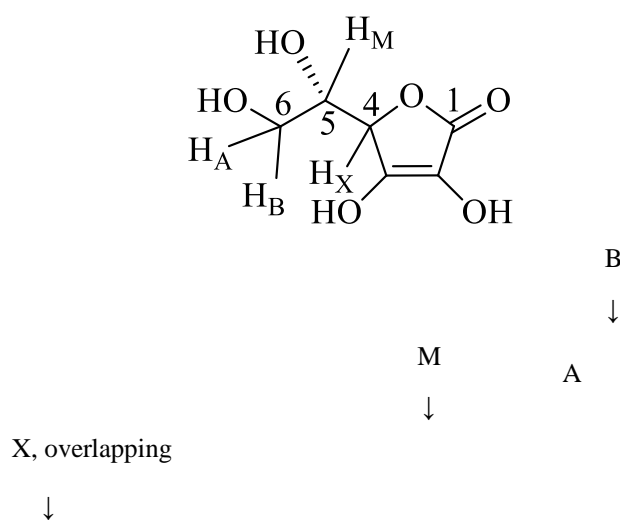
Label	Samples (left to right)
R	Pyrogallol
1	<i>M. indica</i> extract
2	Ahmad Tea [®] Mango Flavoured Black Tea
3	Boh [®] Mango Tea
4	<i>M. pajang</i> pulp extract
5	<i>M. pajang</i> peel extract

Figure 2. TLC of the *Mangifera* extracts.

(a) (b)

Figure 3. The chromatogram of the methanol extract of the *Mangifera* pulp (a) and peel (b) extract.**Nuclear Magnetic Resonance (NMR) Spectroscopy**

Figure 4 shows the $^1\text{H-NMR}$ spectrum (CD_3OD , 500 MHz) of compound 1. It presents the chemical shift of the protons in the compound. Based on the result, there are significant peaks at δ_{H} 0.9224, δ_{H} 1.3737, δ_{H} 1.9154, δ_{H} 3.4380, δ_{H} 4.1152, δ_{H} 4.6379 and δ_{H} 5.4084 ppm. Generally, this compound 1 is a non-phenolic and a non-carotenoid component of *M. pajang*. This is due to the absence of NMR signals that could represent the protons, either from a phenolic, or a carotenoid. According to Pavia *et al.*, (2009), these aromatic protons could produce signals in a range of the chemical shifts from δ_{H} 6.5 to δ_{H} 8.0 ppm. Furthermore, there is no signal within the region of δ_{H} 6.5 to δ_{H} 8.0 ppm, where the signal for aromatic compounds should be seen in most of constituents in the *M. pajang* such as gallic acid, *p*-coumaric acid and mangiferin (Figure 1).

Figure 4. The $^1\text{H-NMR}$ spectrum (CD_3OD , 500 MHz) of compound 1.

Nevertheless, the $^1\text{H-NMR}$ spectrum showed a multiplet peak between δ_{H} 3.6 to δ_{H} 3.9 ppm, indicating the presence of a sugar moiety (Sridhar *et al.*, 2005). As reported by Rodríguez *et al.*, (2006), mangiferin is one of the main compounds that can be found in the *M. pajang*. It possesses a sugar moiety in its structure. On the other hand, the NMR signal for a norathyriol (Figure 5), or the metabolite of mangiferin was not present, as well. Norathyriol or 1,3,6,7-tetrahydroxyxanthone is an

potential of methyl gallate isolated from Bambang (*Mangifera pajang*) in MCF-7 cell line. *Frontiers in Pharmacology*, Conference Abstract: International Conference on Drug Discovery and Translational Medicine 2018 (ICDDTM '18). doi: 10.3389/conf.fphar.2018.63.00149

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