Phytochemical Analysis of *Garcinia rubra*

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ABSTRACT

Garcinia rubra Merr. ('kamandiis') belongs to the Clusiaceae (Guttiferae) family and is one of the indigenous Garcinia species in the Philippines that has not been extensively studied yet. In this study, the phytochemical constituents in the crude leaf extracts of kamandiis was determined. Results showed that kamandiis contains carbohydrates, glycosides, tannins, phenolics, flavonoids, terpenoids and steroids. Carbohydrates and glycosides were found in ethanolic extract while tannins, phenolics and flavonoids were found in both ethanolic and ethyl acetate extracts. On the other hand, terpenoids and steroids were observed in dichloromethane (DCM) and hexane extracts. Results therefore suggest that kamandiis could be a source of these phytochemicals and may be further studied for its promising application in the pharmaceutical industry and alternative medicine.

KEYWORDS:

Garcinia rubra Merr.; kamandiis; Guttiferae; secondary metabolites; phytochemicals

INTRODUCTION

Garcinia belongs to the Clusiaceae (Guttiferae) family and have about four hundred species recorded (Soepadmo 1979 as cited by Wittayawannaku et al. 2010). Most members of this family are known for their edible fruits, timber, and some are used as alternative medicine. According to Biodiversity International Annual report of 2009, there are thirteen indigenous *Garcinia* species in the Philippines. Some species are well known and were proven to be very useful in many ways. Some of the *Garcinia* species with well documented medicinal use include *Garcinia cowa*, which is known to contain several bioactive compounds (Ritthiwigrom et al. 2013); *Garcinia cambogia*, which is known to contain hydroxycitric acid, a compound claimed to be an anti-obesity agent (Heymsfield et al. 1998); and *Garcinia mangostana*, amongst others.

Garcinia mangostana is one of the best known tropical fruits and is referred to as the 'queen of tropical fruits' and had been a subject of various researches due to its enormous remedial qualities. One component of *Garcinia mangostana* that received much attention lately is α-mangostin. Alpha-mangostin was observed to be effective in treating oxidative stress associated diseases such as cancer, diabetes and cardiovascular diseases (Ibrahim et al. 2014). It also contains prenylated xanthones that show *in vitro* cytotoxicity against certain lung cancer cell lines (Zhang et al. 2010). New geranylated biphenyl derivatives are also present that were shown to have antibacterial, anti-inflammatory and antifungal activities (Dharmaratne et al. 2003). It also contains flavonoids, terpenes, procyanidines and polyisoprenylated benzophenone derivatives like garcinol, xanthochymol and guttiferone isoforms that are known to have antioxidant, apoptotic, anti-cancer, anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, anti-ulcer, anti-protozoal, and histone acetylase transferase (HAT) inhibiting properties (Hemshekhar et al., 2011).

Numerous publications on the isolation and identification of bioactive compounds from other *Garcinia* species came out following the realization of the versatile uses of the secondary metabolites found in *Garcinia mangostana* (Joseph et al. 2005; Nilar et al. 2005; Rukachaisirikul et al. 2005; Sukpondma et al. 2005; Andrade et al. 2007; Guo et al. 2011; Ritthiwigrom et al. 2013; Jamila et al. 2015). In the Philippines, efforts had been initiated to study and determine compounds present in local *Garcinia* species that may have pharmaceutical applications. This study sought to contribute to this endeavor by conducting preliminary evaluation of

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the phytochemicals present in one of the indigenous *Garcinia* species found in the Philippines, the *Garcinia rubra.*

Garcinia rubra is native to the Philippines and is known as 'kamandiis' in Manila and Panay area, 'kamantiis' in Bikol, 'kandiis' in Sulu, and 'pagit' in Manobo. It is a simpodial tree that usually grows in primary forests at low altitude and flowers throughout the year. The tree usually grows up to about 10 m with about 90 cm diameter trunk. The bark of the young tree has rough and vertical lenticels. As the tree matures, the wood exerts pressure on the outer bark resulting in formation of new periderm that will eventually make the old layers break apart into scales (Figure 1a). The leaves are petiolated, with an undivided blade having pinnate veins and smooth margin, and grow in opposite sides of the stem (Figure 1b). The whole leaf comes in different sizes. It can grow to only about 6 cm long up to about 30 cm long. The flowers are unisexual and bright green. The fruits, which can be eaten raw, are flattened globose berry with about 3 cm in diameter, and green when unripe (Figure 1c). The fruits are usually used as souring agents of foods by locals. The seeds germinate slowly, which takes about six or more months.

(a) (b) (c)

Figure 1. *Garcinia rubra* Merr. or locally known as 'kamandiis': (a) the bark of an old tree showing scales; (b) mature leaves with an undivided blade having pinnate veins and smooth margin that grow in opposite sides of the stem; (c) green-colored mature fruit that turns yellow-orange when ripe.

MATERIALS AND METHOD

1. Plant collection and authentication

Kamandiis leaves were collected from Mt. Makiling, College, Los Baños, Laguna. The plant was authenticated at the botanical herbarium section of UPLB Museum of Natural History (MNH) and a voucher specimen (73205) has been deposited in Botanical Herbarium, University of the Philippines Los Banos, College, Laguna, Philippines.

2. Plant processing and extraction

The fresh leaves were washed under running water, shade dried at room temperature for two weeks, grounded and powdered using a Thomas Scientific Wiley® Mill, and sieved through a 500 µm mesh. Successive extraction of the secondary metabolites in the leaf sample is outlined in Figure 2. Twenty grams (20 g) of the powdered leaf was extracted by soaking it in 300 mL hexane for 24 h then filtered using a Whatman No. 1 filter paper. The extraction was repeated five more times. All hexane extracts were combined and concentrated at reduced pressure at 30 °C to recover Extract A. The residue left after extraction with hexane (Residue 1) was left in the fumehood for 30 min and mixed occasionally to remove traces of residual hexane. Hexane-free Residue 1 was then extracted with DCM using the same procedure outlined earlier to recover Extract B and Residue 2. DCM-free Residue 2, on the other hand, was extracted with EtOAc to yield Extract C and Residue 3. Lastly, EtOAc-free Residue 3 was extracted with EtOH to recover Extract 4 and Residue 4. The four extracts (Extracts A, B, C, and D) were evaluated for the presence of secondary metabolites by phytochemical qualitative reactions.

3. Phytochemical screening

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in different crude leaf extracts (Extracts A,

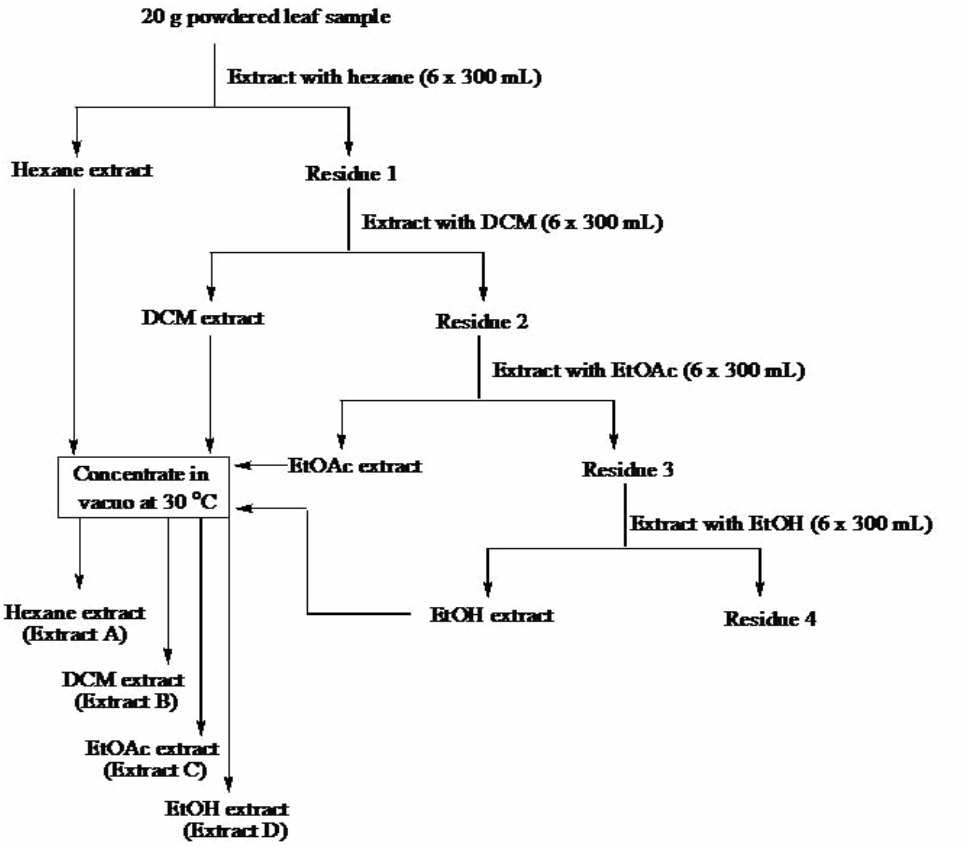

Figure 2. Schematic diagram for the successive extraction of secondary metabolites from the leaf sample of kamandiis using hexane, DCM, EtOAc, and EtOH to recover Extract A, Extract B, Extract C, and Extract D, respectively.

B, C and D) of kamandiis. The screening was done for alkaloids, carbohydrates, tannins, phenolics, flavonoids, anthraquinones, saponins, glycosides, terpenoids, and steroids according to Harborne (1973). Color changes of the solution or precipitate formation was used as analytical responses to these tests.

3.1. Test for alkaloids

3.1.1. Dragendorff's test

Dragendorff's reagent (a solution of potassium bismuth iodide prepared from basic $Bi(NO_3)_3$, tartaric acid, and KI) was added in separate test tubes containing Extracts A, B, C and D. Enough Na_2CO_3 was added into a separate test tube containing about 2 mL each of Extracts A, B, C, and D until the pH of the solution increased to pH 8-9. The pH of the solution was monitored using a pH paper. The alkaline mixture was then added with equal volume of $CHCl_3$. The mixture was shaken gently and allowed to stand. The layers were separated and the pH of the upper layer (aqueous layer) was lowered to pH 5 using 1% HCl (v/v) solution. The resulting mixture was then added with Dragendorff's reagent. The lower layer ($CHCl_3$ layer) was extracted again with about 2 mL of 1% HCl and the upper layer was again separated from the $CHCl_3$ layer, and added with Dragendorff's reagent. The formation of orange precipitate in the solution added with Dragendorff's reagent suggests the presence of alkaloid in the sample.

3.1.2. Hager's test

Each extract was added with 1 mL of Hager's reagent (1% picric acid). The formation of yellow precipitate confirms the presence of alkaloid.

3.1.3.Mayer's test

Each extract was added with 1 mL Mayer's reagent (mixture of 1.36% $HgCl_2$ and 5% KI). The formation of a dull white precipitate confirms the presence of alkaloid.

3.1.4. Wagner's test

Each extract was added with 1 mL Wagner's reagent (mixture of 2% iodine and 6% KI) along the sides of the test tube. The formation of a brown precipitate confirms the presence of alkaloid.

3.2. Test for carbohydrates

3.2.1. Molisch test

Each extract was added with 2 drops of 15% ethanolic α-naphthol. The mixture was shaken well and about 3 drops of concentrated H_2SO_4 was added slowly along the sides of the test tube. Appearance of a violet ring at the junction of the two liquids indicates the presence of carbohydrates.

3.2.2. Benedict's test

Each extract was added with 5 mL of Benedict's reagent (mixture containing 1.7% $CuSO_4$, 17% sodium citrate and 10% Na_2CO_3). The mixture was heated to boiling in a water bath for 2 min. A characteristic red colored precipitate indicates the presence of reducing sugar.

3.2.3. Fehling's test

Each extract was added with about 2 mL of a freshly prepared mixture of Fehling's reagent containing equal volumes of Fehling's solution A and Fehling's solution B. The mixture was then heated to 60 ^oC in a water bath. Appearance of a green suspension and a red precipitate indicates the presence of carbohydrates.

3.3. Test for glycosides

3.3.1. Bromine water test

Each extract was added with bromine water and mixed. Formation of a yellow precipitate indicates the presence of glycosides in the sample.

3.3.2. Borntrager's test

About 1 mL of each extract was boiled and added with 1 mL dilute H_2SO_4 in a test tube for 5 min, filtered while hot, and the filtrate cooled. The filtrate was shaken with an equal volume of DCM. The lower DCM layer was separated and shaken with half its volume with 10% NH_3. Production of a rose pink to red color in the ammoniacal layer suggests the presence of anthraquinone glycosides in the sample.

3.3.3. Keller-Killiani's test

About 1 mL of each extract was successively added with 5 mL distilled water and 2 mL glaci a l acetic acid containing a drop of $FeCl_3$ solution. About 1 mL of concentrated H_2SO_4 was poured down the side of the test tube. A color change to brown and the slight green color at the interface of the resulting layers indicates the presence of glycosides in the sample.

3.3.4. Froth test

Each extract was placed in a screw-capped test tube and added with 5 mL distilled water. The test tube was stoppered and shaken vigorously for about 5 min. Formation of a honey comb froth indicates the presence of saponins in the sample.

3.4. Test for tannins and phenolic compounds

3.4.1. Ferric chloride test

Each extract was added with 3 drops of 5% $FeCl_3$. The formation of intense green, purple, blue or black coloration suggests the presence of tannins in the sample.

3.4.2. Test for phenolic compounds

Each extract was added with 3 drops of freshly prepared $FeCl_3$ - $K_3Fe(CN)_6$ solution that is made up of equal volumes of 1% $FeCl_3$ and 1% $K_3Fe(CN)_6$ solution. The formation of intense green, purple, blue or black coloration suggests the presence of polyphenols in the sample.

3.5. Test for flavonoids

3.5.1. Shinoda test or magnesium hydrochloride reduction test

Each extract was added with few magnesium turnings and 2 drops of concentrated HCl. The appearance of red or green to blue color suggests the presence of flavonoids in the sample.

3.5.2. Zinc hydrochloride reduction test

Each extract was added with zinc dust and 2 drops of concentrated HCl. The appearance of red coloration after a few minutes suggests the presence of flavonoids in the sample.

3.5.3. Alkaline reagent test

Each extract was added with about 3-5 drops of 0.5 M NaOH. The change in color to yellow and

further to colorless after the addition of dilute HCl indicates the presence of flavonoids in the sample.

3.6. Test for terpenoids and steroids

3.6.1. Hirshonn reaction

Each extract was added with trichloroacetic acid and heated (40-50 °C) gently on a water bath. Formation of a red to purple coloration indicates the presence of terpenoids in the sample.

3.6.2. Test for steroid: Liebermann Burchard test

Each extract was added with 1 mL glacial acetic acid, 1 mL acetic anhydride and 2 drops concentrated H_2SO_4. Changes in color of the solution from red to blue to bluish green indicates the presence of steroids in the sample.

RESULTS

Summary of results for the phytochemical screening of kamandiis leaf extracts is shown in Table 1. Hexane and DCM extracts (Extracts A and B) only showed positive test for steroids and terpenoids while ethyl acetate extract (Extract C) showed positive test for tannins, phenolics and flavonoids. On the other hand, ethanolic extract (Extract D) showed positive test for tannins, phenolics, flavonoids, carbohydrates, and glycosides.

Table 1. Results of phytochemical screening of crude leaf extracts of kamandiis

Phytochemicals		Extracts			
		Hexane	DCM	EtOAc	EtOH
Alkaloids	Dragendorff's test	-	-	-	-
	Hager's test	-	-	-	-
	Mayer's test	-	-	-	-
	Wagner's test	-	-	-	-
Carbohydrates	Molisch's test	-	-	-	++
	Benedict's test	-	-	-	+
	Fehling's test	-	-	-	+
Glycosides	Bromine water test	-	-	-	++
	Borntrager's test	-	-	-	+
	Keller-Killiani's test	-	-	-	+
	Froth test	-	-	-	+
Tannins and phenolics	Ferric chloride test	-	-	++	+++
	Test for phenolics	-	-	++	+++
Flavonoids	Shinoda test	-	-	++	+++
	Zinc HCl reduction test	-	-	+	++
	Alkaline reagent test	-	-	+	++
Terpenoids	Hirshonn's test	+++	++	-	-
Steroids	Liebermann Burchard's test	++	+	-	-

+++: present in high amount ; ++: present in moderate amount; +: present in low amount; - : not detected

DISCUSSION

All extracts gave negative result to alkaloids by Dragendroff's, Hager's, Mayer's, and Wagner's tests. Similar results on the undetectable levels of alkaloids in most *Garcinia* species were observed except for few species that reported positive for alkaloids to contain some. Some of these *Garcinia* species that were observed to contain alkaloids are *Garcinia gummicutta* (Maridass et al. 2010), *Garcinia kola* (Ebana et al. 1991; Braide et al. 2003; Kagbo 2010; Ghamba et al. 2012), *Garcinia lucida* (Fotie et al. 2007) and *Garcinia mangostana* (Torres et al. 2015).

On the other hand, the presence of carbohydrate in the EtOH extract of kamandiis was confirmed by the result of the Molisch test. The presence of reducing sugars in the same extract was also verified by Benedict's and Fehling's test. No detectable amount of carbohydrate was observed in other extracts that was extracted using solvents less polar than EtOH.

Similarly, only ethanolic extract showed positive test for glycosides by bromine water test. The same extract also showed positive test for the presence of specific glycosides such as anthraquinone, cardiac and saponin glycosides by Borntrager's, Keller-Kiliani's and froth test, respectively. Results in this part is consistent with previous reports on various glycoside-containing *Garcinia* species such as *Garcinia kola* (Ebana et al. 1991; Okwu and Morah 2007), *Garcinia spicata* (Konoshima and Ikeshiro 1970), *Garcinia gracilis* (Supasuteekul et al. 2016), *Garcinia buchananii* (Stark et al. 2014), *Garcinia dulcis* (Deachathai et al. 2005) and *Garcinia mangostana* (Du and Francis 1977; Mahabusarakam and Wiriyachitra 1987). Specific glycosides such as anthraquinone glycosides were also found in *Garcinia kola* (Asaolu 2003; Adejumo et al. 2011) and *Garcinia mangostana* (Rajakannu et al. 2015) while cardiac glycosides had also been reported in *Garcinia kola* (Adegboye et al. 2008; Ebana et al. 1991; Kagbo and Ejebe 2009; Yakubu et al. 2011), *Garcinia cambogia* (Krishnamoorthy et al. 2014), and *Garcinia lancifolia* (Chowdhury and Handique 2012). Cardiac glycosides are used in the strengthening of a weakened heart and treatment of cardiac arrhythmia (Ebana et al. 1991). On the other hand, saponins were also found in other *Garcinia* species such as *Garcinia kola* (Ebana et al. 1991; Adegboye et al. 2008; Yakubu et al. 2011), *Garcinia mangostana* (Rajakannu et al. 2015; Torres et al. 2015) and *Garcinia lancifolia* (Chowhury and Handique 2012). Saponins are compounds that can interact both to polar and nonpolar substances. It can be used as an emulsifying agent and is known to have antioxidant, anti-cancer, anti-inflammatory, antihyperglycemic and antifungal properties (Aiyelaagbe and Osamudiamen 2009).

Test for tannins and phenolic compounds using solutions of $FeCl_3$ also confirmed the presence of such compounds in the ethyl acetate and ethanolic extracts of the plant. These results are consistent with a number of literature supporting the presence of these compounds in most *Garcinia* species (Torres et al. 2015; Rajakannu et al. 2015; Yakubu et al. 2011; Obolskiy et al. 2009; Mahabusarakam and Wiriyachitra 1987; Ebana et al. 1991; Gontijo et al. 2012; Deachathai et al. 2005; Baggett et al. 2005; Naldoni et al. 2009; Adegboye et al. 2008; Chowhury and Handique 2012). Harbone (1984) reported that phenolic compounds commonly exist as glycosides and are usually extracted as water soluble fractions of phenolic compounds. This may support the observation that no detectable amount of the said compounds were observed in both the hexane and DCM extracts. The glycosides that exist in *Garcinia rubra* are also likely to be in form of phenolic glycosides. Polyphenenolic glycosides such as tannins, on the other hand, are reported to have an antibacterial, anticancer, antiviral and even some were reported to have an anti-HIV property (Heslem 1989).

Appreciable amounts of flavonoids were also found in both EtOAc and EtOH extracts of kamandiis. Similar results were also observed in various *Garcinia* species (Carpenter et al. 1969; Mahabusarakam and Wiriyachitra 1987; Ebana et al. 1991; Baggett et al. 2005; Deachathai et al. 2005; Adegboye et al. 2008; Castardo et al. 2008; Yakubu et al. 2011; Gontijo et al. 2012; Rajakannu et al. 2015; Torres et al. 2015). Compounds belonging to this class have shown to exhibit biological activities and were known to have anticancer (Chahar et al. 2011), anti-allergic (Kawai et al. 2007), anti-inflammatory (Serafini et al. 2010), antimicrobial (Cushnie and Lamb 2005) and antiviral properties (Orhan et al. 2010).

The presence of terpenoids and steroids in hexane and DCM leaf extracts were also consistent with the published reports on some *Garcinia* species. Steroids are important in medicine because of their structural resemblance with sex hormones and could serve as a starting material for the synthesis of biological

hormones (Okwu 2001) while terpenoids can be used as template in the development of terpenoid-derived drugs for disease therapy and prevention (Wang et al. 2005).

In conclusion, kamandiis contains phytochemicals such as carbohydrates, tannins, phenolics, flavonoids, glycosides, terpenoids, and steroids. The general classes of phytochemicals present in *Garcinia rubra* leaves were also observed to be present in other *garcinia* species. The results of the present investigation therefore, could provide evidence that the crude extracts of *Garcinia rubra* could be considered for quantitative phytochemical characterization, purification, and identification of bioactive compounds that can be further studied for its potential application in pharmaceutical industry and alternative medicine.

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