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# A Cornucopia of Bioactive Compounds from Leaves Extract of *Garcinia mangostana*: A Contrastive Assessment of Phytochemical Constituents and Their Antioxidant Activities Influenced by Organic Solvents

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The phytochemical richness of *Garcinia mangostana* has garnered increasing interest due to its potent therapeutic potential. This study presents a comprehensive evaluation of the effects of different organic solvents, ranging from non-polar to highly polar, on the extraction efficiency, phytochemical composition, and antioxidant activities of *G. mangostana* leaf extracts. Solvents, including hexane, toluene, chloroform, acetone, ethanol, methanol, and water, were employed to investigate the variation in extractive value, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities via DPPH and ABTS radical scavenging assays. Results revealed that polar solvents, particularly ethanol and methanol, yielded the highest levels of phenolics (4588.5 ± 58.1 and 4261.9 ± 55.7 mg GAE/g DW, respectively) and flavonoids (2352.22 ± 48.1 and 2870.37 ± 51.5 mg QE/g DW, respectively), which correlated strongly with their superior antioxidant activities. Despite its low phenolic content, hexane extract exhibited moderate ABTS activity, suggesting the presence of lipophilic antioxidants. Water showed the highest extractive value (79 ± 1.23%) but was less effective in recovering phenolic-rich fractions. This study highlights the critical role of solvent polarity in modulating the phytochemical and biofunctional profile of *G. mangostana* leaf extracts, providing a valuable framework for optimizing solvent selection in natural product research and functional formulation development.

**Keywords**: Garcinia, phytochemical, biological activities, organic solvents

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Tropical provinces, particularly Southeast Asia countries, are the birthplace of a bountiful, unique diversity of plant species and varieties, many of which have been identified based on their therapeutic, medicinal, and nutritional properties [1], including trials, and provide an ideal setting for the plethora of tropical flora. These plants are treasured and immensely utilized, not just as a source of sustenance, but also for their remarkable phytochemical compositions that greatly benefit the world of food sciences, pharmaceuticals, nutraceuticals, and cosmetics [2]. To date, more than 250,000 plants have been documented worldwide with almost 25% of them being clinically used [3, 4] and plants belonging to the family *Clusiaceae* or *Guttiferacea* are unexceptional.

*Garcinia*, the largest genus family Guttiferaceae is natively distributed across tropical Asia, North East Australia, West Polynesia, and tropical America [5]. As they can be found scattered nearly everywhere from near seashores to mountain forests, *Garcinia* enriched with derivatives of polyphenols, flavonoids, bioflavonoids, polyisoprenylated benzophenones, triterpenes, and xanthones [6]. Such compounds are the star behind the scene for ample biological activities including antioxidant, antimicrobial, anti-inflammatory, anticancer, and anti-HIV activities [5, 7, 8]. *Garcinia mangostana* (GM) is one of the treasured tropical fruits in the spotlight as the "queen of tropical fruit" due to its remarkably unique and delectable tropical flavor [5, 9]. This type of fruit can be found in wide Asian regions such as Malaysia, Myanmar, Thailand, Philippines, Sri Lanka, and India [10]. Many exemplary literatures are well published on the beneficiary of various parts of the plants with the pericarp being extensively utilized as therapeutic medicine for many years in aiding sicknesses like trauma, skin infection, abdominal pain, dysentery, and wounds.

Most of these biologically active compounds can be accessed through the extraction process.

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Maximizing the number of compounds coupled with the highest biological activity demands an appropriate introductory step, and solvent selectivity is one of the factors mentioned. The choice of solvent is critically important as it affects the quality, efficiency, specificity, stability, and safety of the extracted compounds [11]. Organic solvents are historically the first choice and common solvents for efficiently removing various types of organic compounds from various matrices [12]. However, it is imperative to take utmost care to remove all extracting solvents from the filtrate since their left-over residues may be potentially harmful to the consumer's health even in traces [13]. Organic solvents can be categorized based on their solvent polarity and their selectivity in polarity is often significant due to the variability in solubility characteristics of high-value secondary metabolites that require solvents that can make them readily dissolve matching their chemical nature [14]. As such, recommending suitable extraction solvents for individual plant materials is generally difficult.

Based on the above-mentioned information of which is relevant to the current study, the reported literature has demonstrated a distinguished composition in secondary metabolites in *Garcinia*. It was, however, most of the research displayed prioritized the fruit and other non-edible pericarpin the screening and identification of bioactive compounds. The study on leaves of *Garcinia* remains scarce, despite the fact that the leaves posed significant secondary metabolites feasibly beneficial for human health. Keeping in view, the current study was designed with an aim to screen out the presence of different valuable phytochemicals, evaluate and analyze their antioxidant activities from the leaves of these *Garcinia* species. The outcome of this study may serve as a novel reference and clear direction for potential practical applications across the pharmaceuticals, food science, and sustainable industries.

## EXPERIMENTAL

### Chemicals and Materials

Healthy leaves of selected *Garcinia mangostana* were collected at Ladang Bersepadu, Ladang 10, Universiti Putra Malaysia (UPM). The plant material was identified and confirmed by a Plant Botanist. Voucher specimen was deposited in the Herbarium of Biodiversity, IBS, UPM. The leaves were dried in a mechanical, air-flow convection oven at 35°C, ground into powder using an electrical grinder, and stored at -20°C until analyzed. Chemicals used in this study are analytical reagents including ethanol, methanol, acetone, chloroform, acetonitrile, toluene, hexane, folin-ciocalteau reagent, sodium carbonate, gallic acid, sodium nitrite, aluminum chloride, sodium hydroxide, quercetin, DPPH radical reagent, ABTS reagent, potassium persulfate, and trolox.

### Characterization Methods

*Extraction Procedure*

Organic solvents with various polarities were utilized for small-scale extraction, including water, ethanol, methanol (polar), acetone, chloroform, acetonitrile (semi-polar), toluene, and hexane (non-polar). The direct maceration extraction was done in small quantities with a ratio of 1:10 (w/v), in which 0.5 g of fine powdered leaves was soaked in 5 mL of solvents in a 20 mL scintillation vial. The macerates were filtered with Whatman's no 1 filter paper after 24 hours of immersion at room temperature. The solvents were evaporated from the crude extracts, reducing the solvent concentration with the rotary evaporator. The semi-liquid crude extracts from the rotary evaporator flask were filtered with a 0.22 µm syringe filter to remove unwanted material. The final material was maintained in sterile airtight glass bottles and stored in a -20°C.

*Extractive Value*

The yield of extracts was calculated and expressed as a percentage and determined using the calculation formula mentioned by [15].

*Qualitative Screening*

All plant extracts from various solvents and analytical procedures were assessed for the existence of phytochemicals including phenolic, flavonoid, alkaloid, saponin, tannin, glycoside and etc. based on color detection [16].

*Total Phenolic Content Determination*

The content of phenolics, from the extract of *Garcinia* species, was evaluated using the Folin–Ciocalteau reagent-based assay, according to [17] with slight modification. A 2 mL of Folin–Ciocalteau reagent was added to the 200 µL of samples, and the solutions were allowed to stand for 10 min at 25°C. Later, 1 mL of sodium carbonate was added to the solutions, kept in total darkness for 20 min at room temperature. The absorbance reading of the blue color mixture was measured at 765 nm using a microplate reader ultraviolet-visible (UV) spectrophotometer (Shimadzu, Japan). Gallic acid was used as a standard for the calibration curve, and the samples were expressed as mg GAE/g plant dry weight.

*Total Flavonoid Content Determination*

Flavonoids were evaluated using the method by [17] based on the aluminium chloride assay with slight modification. 200 µL of extract was transferred into 10 mL volumetric flasks containing 1 mL of distilled water. Then, 0.3 mL of sodium nitrite ($NaNO_2$) solution (1:5, w/v), 0.3 mL of aluminium chloride

($AlCl_3$) (1:10, w/v) and 2 mL of sodium hydroxide (NaOH) solution (1M) were mixed into the sample. The absorbance reading of yellow color mixtures was measured at 510 nm by a microplate reader ultraviolet-visible (UV) spectrophotometer. The flavonoid value was expressed as mg quercetin/g plant dry basis.

*DPPH Scavenging Assay*

The antioxidant activity of the aqueous methanol extract of GM was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, based on the electron transfer reaction between the DPPH reagent and the plant extract according to the method [17]. A 0.2 mM solution of DPPH in pure methanol was prepared, and 1 mL of this solution was added to 1 mL of all extracts. The mixtures were shaken gently and allowed to stand for 30 min at room temperature. The absorbance for both positive control (DPPH solution) and samples was measured at 517 nm against methanol as a blank using an ultraviolet-visible (UV) spectrophotometer (Shimadzu-Japan). The inhibition percentage of the absorbance was calculated as follows:

$$\text{Inhibition \%} = \frac{(\text{absorbance of control} - \text{absorbance of control})}{\text{absorbance of control}} \; x\; 100$$

*ABTS Scavenging Assay*

The free radical scavenging activity of GM was evaluated by ABTS radical cation decolorization assay [18, 19]. $ABTS^+$ cation radical was yielded by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), then stored in the dark at room temperature for 12-16 hours before use. $ABTS^+$ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of aliquot of 100 µL extracts was added to 300 µL of ABTS reagent and was allowed to stand for 30 min at 23ºC before the absorbance was measured. An appropriate solvent blank was run in each assay. Trolox was used as standard and the percent inhibition was calculated as follows:

$$\text{Inhibition \%} = \frac{(\text{absorbance of control} - \text{absorbance of control})}{\text{absorbance of control}} \text{ x } 100$$

**Table 1.** Qualitative screening analysis of *Garcinia mangostana* across various organic solvent polarities.

| **Phytochemical compounds** | ***Garcinia mangostana*** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **$H_2O$** | **EtOH** | **MEOH** | **Ace** | **Chl** | **Ace** | **Tol** | **Hex** |
| Tannins | ++ | +++ | +++ | ++ | ++ | + | - | - |
| Saponins | ++ | +++ | +++ | ++ | ++ | + | - | - |
| Flavonoids | ++ | +++ | +++ | +++ | ++ | + | - | - |
| Phenols | ++ | +++ | +++ | +++ | ++ | + | - | - |
| Glucosides | ++ | +++ | +++ | +++ | ++ | + | - | - |
| Quinones | ++ | +++ | +++ | ++ | ++ | + | - | - |
| Terpenoids | ++ | ++ | ++ | ++ | ++ | + | + | - |
| Steroids | ++ | ++ | ++ | ++ | ++ | + | + | + |
| Anthraquinones | + | + | + | + | + | + | + | - |
| Phlobatannins | + | + | + | + | + | + | + | - |
| Anthracyanine | + | + | + | + | + | + | + | - |

+++ Highly concentrated, ++ Moderately concentrated, + Slightly concentrated, - Not detected

## RESULTS AND DISCUSSION

The qualitative phytochemical compositions of *Garcinia* extract obtained using eight solvents are demonstrated in Table 1. In this experiment, the findings of the preliminary screening study revealed that the Garcinia leaf, extracted with ethanol and methanol, contains high concentrations of plant secondary metabolites, including tannins, saponins, flavonoids, phenols, glucosides, and quinones. Despite the high crude extract obtained by water as the extractant, the phytochemicals were found to be moderately concentrated, notably lower than ethanol and methanol, suggesting limited water extraction efficiency in effectively extracting key compounds. Similar trends have been observed for fenugreek seeds, where water yielded more crude extract but ethanolic extract is contained higher phenolic concentrations [20]. Olive leaves (*Olea europaea* L.) demonstrated comparable findings where methanol-water and absolute methanolic extract had the highest total phenolic and flavonoid contents, whereas water extract had the lowest phenolic concentrations, reflecting lower efficiency of pure water for key phenolic compounds even though it effectively extracts other polar constituents.

Table 2 presents the extractive yields (in percent) with accumulation values of phenolics and flavonoids of GM as affected by different solvent polarities. From the table shown, the extraction of the ground ethno-medicinal leaf samples was performed using eight different solvents based on their increasing solvent polarity: water < methanol < ethanol < acetone < chloroform < acetonitrile < toluene < hexane. The extracts yielded ranged from 0.79 to 0.03 across various solvent polarities. Water extraction resulted in the highest extract yield followed by hexane which produced the lowest in producing crude extract. This result further indicated that optimal phytochemical extraction was achievable using high-polarity solvents, compared to low-polarity solvent systems. Plants generally possess low concentrations of bioactive molecules; therefore, optimal extraction of biomolecules from plants depends on the selection of a suitable extraction solvent. The composition of total phenolics (TPC) of *Garcinia* leaf extracted with various solvent polarities is well displayed in Table 1. While the initial execution of colorimetric detection may serve as an indication of the phytochemicals' availability in the extracts, the employment of subsequent quantification analysis feasibly yielded more reliable and actual information concerning the samples extracted with different solvent polarities. Corresponding to the qualitative results, methanolic and ethanolic extracts registered the highest ($P<0.05$) TPC values of 4261.9 ± 55.7 and 4588.5 ± 58.1, followed by the acetonic extract with a TPC value of 1754.7 ± 46.7. Similar progressions were observed in the TFC value of GM as extracted by various solvent polarities, whereby the highest value was recorded when GM was exposed to ethanol (2352.22 ± 48.1) and methanol (2870.37 ± 51.5), followed by hexane, yielding the lowest levels of flavonoids, including phenolics.

**Table 2.** Extractive value, total phenolics, and total flavonoids of *Garcinia mangostana* across various organic solvent polarities.

| Solvents | Extractive Value (%) | Total Phenolic Content (GA mg/DW g) | Total Flavonoid Content (QE mg/DW) |
|---|---|---|---|
| Water | 79 ± 1.23 | 280.4 ± 46.3 | 368.07 ± 44.5 |
| Methanol | 48 ± 0.59 | 4261.9 ± 55.7 | 2870.37 ± 51.5 |
| Ethanol | 32 ± 0.52 | 4588.5 ± 58.1 | 2352.22 ± 48.1 |
| Acetone | 16 ± 1.23 | 1754.7 ± 46.7 | 1143.54 ± 40.3 |
| Chloroform | 15 ± 1.11 | 227.7 ± 44.4 | 278.65 ± 44.2 |
| Toluene | 8 ± 0.34 | 56.6 ± 48.2 | 85.09 ± 40.1 |
| Hexane | 3 ± 0.12 | 39.1 ± 41.2 | 368.07 ± 35.3 |

Data are means ± standard deviation (SD) with three replications.

**Table 3.** DPPH and ABTS scavenging activity of Garcinia mangostana across various organic solvent polarities.

| Solvents | DPPH Scavenging Activity (%) | ABTS Scavenging Activity (%) |
|---|---|---|
| Water | 58.08 ± 0.24 | 36.60 ± 1.19 |
| Methanol | 59.25 ± 1.23 | 52.70 ± 0.11 |
| Ethanol | 66.15 ± 2.31 | 40.80 ± 0.71 |
| Acetone | 59.59 ± 0.59 | 44.90 ± 0.29 |
| Chloroform | 57.09 ± 0.47 | 31.11 ± 1.42 |
| Toluene | 54.25 ± 2.16 | 36.60 ± 1.52 |
| Hexane | 58.08 ± 2.13 | 52.70 ± 1.32 |

Data are means ± standard deviation (SD) with three replications.

The solvent polarities were also consistently influenced by the scavenging activities of GM. As a stable free radical, the employment of DPPH is known to be the most inexpensive, simple, and fast method to be utilized in determining the free radical scavenging activity of targeted samples. Furthermore, its 30-minute rapid reactivity allows the results to be repeated effectively and accurately, making the DPPH assay reliable in quickly screening a significant volume of samples and bioactive substances. Upon assessment at room temperature, the highest DPPH activity was observed in the ethanol extract, followed by acetone and methanol. These findings were well-aligned with high TPC and TFC values in Table 1 found in these extracts. Conversely, with the ABTS assay, which is more accommodating to both polar and non-polar constituents, the greatest scavenging activity was obtained in methanol and hexane extracts. It was suggested that the presence of lipophilic antioxidant compounds such as tocopherols, carotenoids, or phytosterols may contribute significantly to the ABTS radical neutralization, which explained the unforeseen increment of ABTS activity in the hexane extract [22]. This discrepancy concerning ABTS findings signifies the idea of inclusivity of other factors apart from phytochemicals, which predominantly contribute to the antioxidant actions in the extracts, including the nature of the compounds, solubility, and the complex matrix structure of the extracted compounds. The complementary action of DPPH and ABTS assays in this study revealed an enriching viability of antioxidants and phenolics in GM.

## CONCLUSION

It is concluded that different solvent polarities yield various responds in phytochemical compounds and antioxidant activities in *Garcinia* plants.

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