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Integrated Phytochemical Screening and Quantitative Antioxidant Evaluation (FRAP) of Garcinia Mangostana Pericarp Extracts: Unveiling Bioactive Constituents and Their Reducing Potential

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ABSTRACT

Introduction: *Garcinia mangostana* or mangosteen was traditionally used to treat various ailments especially gastrointestinal issues. This suggests the plant material contain high number of phytochemicals and potential natural antioxidant source. Garcinia mangostana, commonly known as mangosteen, is a tropical evergreen tree belonging to the family Clusiaceae, Angiosperms (Flowering Seed Plants) (dicotyledon), Autotrophic in nature with a hight of 25 meter. The fruit is best eaten fresh, because processing tends to ruin the flavour. It is sometimes eaten with sherbet or ice cream. Some Chinese like to eat the fruits, because they believe mangosteen has a cooling effect. Xanthones, including α-mangostin and γ-mangostin, have been extensively studied for their biological activity which includes anti-inflammatory, antimicrobial, and anticancer effects.

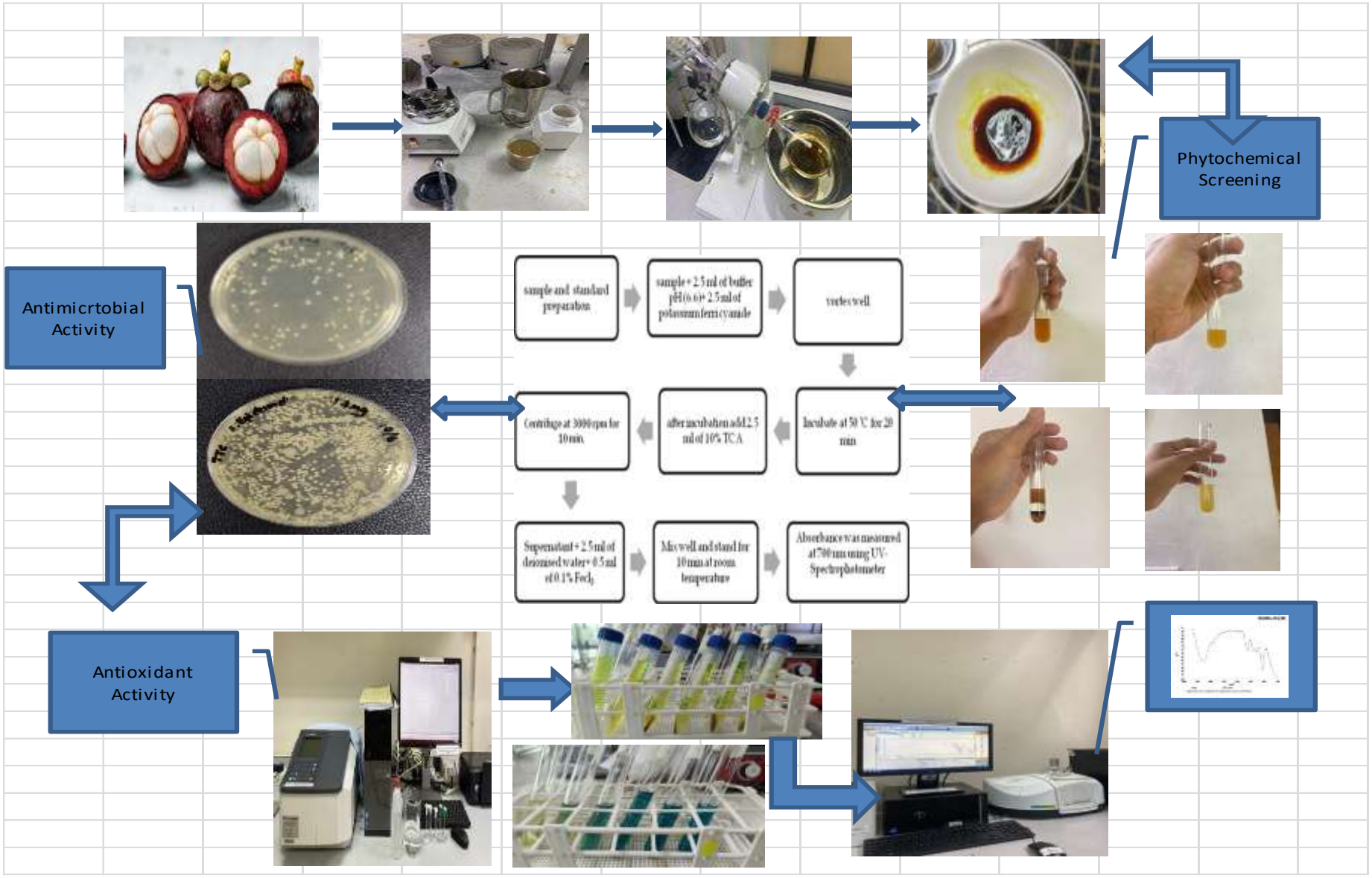
Objectives: The research aimed to investigate the phytochemicals existing in the pericarp of *G. mangostana* and to evaluate its antioxidant activity.

Methodology: Phytochemical screening was done including qualitative test for alkaloid, flavonoids, saponin and tannin. Characterization of the functional groups existing in plant extract was found using FT-IR spectral analysis. Ferric Reducing Antioxidant Power (FRAP) assay was used to evaluate its antioxidant capacity. This low-cost, basic, dependable approach produces consistent outcomes even without specific gear. Significantly, the FRAP test measures the overall reduction capability of antioxidants, so providing a more complete view of antioxidant capacity than methods as DPPH and ABTS that concentrate on particular radical scavenging. Its biologically relevance also arises from its capacity to replicate deteriorating conditions in biological systems, thereby enabling a more accurate portrayal of the antioxidant activity of in-vivo. These properties characterize FRAP as the recommended technique for thorough and consistent assessment of antioxidants.

Result and Conclusion: Majority of the constituents are alkaloid, flavonoid, saponin and tannin were confirmed. FTIR analysis revealed presence of hydroxyl, aromatic, aliphatic, and carbonyl-related functional groups. The antioxidant capacity was measured as 1.09 g AAE per gram of the extract. *G. mangostana* pericarp was confirmed to be a rich source of phytochemicals and antioxidants.

KEYWORDS: *Garcinia mangostana*; antioxidant activity; FRAP assay; antioxidant; plant extract; phytochemicals; mangosteen pericarp.

GRAPHICAL ABSTRACT



INTRODUCTION

Garcinia mangostana, commonly known as mangosteen, is a tropical evergreen tree belonging to the family Clusiaceae, Angiosperms (Flowering Seed Plants) (Dicotyledonous), Autotrophic in nature with a hight of 25 meter. This species is native to Southeast Asia, particularly found in countries such as Indonesia, Malaysia, Thailand, and the Philippines, where it thrives in tropical rainforest environments with a native habitat of terrestrial (Wang et al., 2016; Karim & Tangpong, 2018). It is difficult to cultivate this species for fruit, because seedling mortality rate is high and trees only begin to produce fruits after 8-15 years. The fruit of *Garcinia mangostana* is often referred to as the "Queen of Fruits" due to its exquisite flavor and numerous health benefits attributed to its rich phytochemical composition (Ibrahim et al., 2015; Karim & Tangpong, 2018). The fruit is characterized by a dark purple, round fruit is crowned by thick, persistent calyces (the outermost layer of the flower). The thick, fibrous rind protects white, fleshy segments that are arranged like a peeled mandarin orange. The flesh is juicy and sweet with a slightly acidic taste. The number of fleshy segments is proportional to the number of brown to grey, triangular lobes at the bottom of the fruit that is both aromatic and slightly acidic, making it one of the popular fruits consumed by consumers (Li, 2015; Lin et al., 2023).

The pericarp of *Garcinia mangostana* is particularly notable for its high concentration of xanthones, a class of polyphenolic compounds recognized for their potent antioxidant properties (Ibrahim et al., 2015; Gutiérrez-Orozco & Failla, 2013). Xanthones, including α-mangostin and γ-mangostin, have been extensively studied for their biological activity which includes anti-inflammatory, antimicrobial, and anticancer effects (Mohamed et al., 2014; Al-Massarani et al., 2013; Kharisma, 2024). These compounds are attributed to the fruit's usage in traditional medicine, where extracts from the pericarp are utilized to treat a variety of ailments, including abdominal pain, diarrhea, and skin conditions (Al-Massarani et al., 2013; Kharisma, 2024).

Figure 1: The fruit Garcinia mangostana or known as mangosteen.

Research has demonstrated that the antioxidant activity of *Garcinia mangostana* is comparable to that of many other fruits, including cinnamon and citrus essential oils (Ibrahim et al., 2015). The antioxidant properties are primarily attributed to the presence of xanthones, which play a crucial role in neutralizing free radicals and reducing oxidative stress in the body tissues (Gutiérrez-Orozco & Failla, 2013). This antioxidant capacity is related to its effect for promoting overall health and may help mitigate the risk of chronic diseases such as cancer and cardiovascular disorders (Wang et al., 2016; Karim & Tangpong, 2018).

In addition to its antioxidant benefits, *Garcinia mangostana* has shown promise in various therapeutic applications. Studies have indicated that extracts from the fruit can improve wound healing processes by promoting fibroblast proliferation and collagen formation, which are an important process for tissue repair (Qian

et al., 2021). Furthermore, the anti-inflammatory properties of xanthones have been linked to the modulation of immune responses, suggesting potential benefits in managing autoimmune conditions and inflammatory diseases (Fu et al., 2014). The consumption of *Garcinia mangostana* has also been associated with improvements in bone health. Comparative studies have highlighted the fruit's efficacy in enhancing bone density and alleviating symptoms related to bone loss (Chitra et al., 2017). This is particularly relevant for populations at risk of osteoporosis, such as postmenopausal women and older population. Moreover, the pharmacological potential of *Garcinia mangostana* includes its antimicrobial properties. Research has shown that extracts from the fruit exhibit significant activity against various pathogens, including bacteria and fungi, thereby supporting its use in traditional medicine for treating infections (Al-Massarani et al., 2013; Kharisma, 2024). The diverse range of bioactive compounds found in the fruit reveals the potential as a potent supplement source material for general health and for active bio compounds extracts.

Due to the increasing recognition of oxidative stress as a contributing factor to various chronic diseases, research involving oxidative stress and diseases have been carried out to understand it’s underlying principles and cure. Antioxidants are compounds that can neutralize free radical’s unstable molecules that can cause cellular damage and contribute to the pathogenesis of conditions such as cancer, cardiovascular diseases, and neurodegenerative disorders (Yang et al., 2011). The dietary intake of antioxidants, particularly from fruits and vegetables, has been associated with a lower risk of these diseases suggesting that a diet rich in antioxidants may play a protective role in maintaining health (Yang et al., 2011; Zhong et al., 2020).

Recent studies have emphasized the importance of assessing total antioxidant capacity (TAC) rather than focusing solely on individual antioxidant nutrients. Yang et al. highlighted that the synergistic effects of various antioxidants in the diet could provide a more comprehensive understanding of their health benefits (Yang et al., 2011). This approach aligns with findings from Wang, who reported that overall antioxidant intake is associated with reduced mortality rates among adults, particularly those with depression (Wang, 2023). Such evidence highlights the need to consider the interactions and combined effects of multiple antioxidants rather than attributing to a single nutrient in dietary assessments.

Apart from acting as anti-free radical, anti-oxidant also influences various biological processes that contribute to health. Mediterranean diet, characterized by high antioxidant intake, has been linked to a reduced risk of pancreatic cancer by Zhong et al. (2020) and Lucas et al. (2016). This highlights the potential for dietary antioxidants to mitigate the adverse effects of environmental factors on health. The potential for antioxidants to influence aging processes has also been a focal point of research. Goñi and Hernández-Galiot discussed the contribution of dietary antioxidants, particularly polyphenols, to anti-aging effects and overall health in elderly populations (Goñi & Hernández-Galiot, 2019). Their findings suggest that incorporating antioxidant-rich foods into the diet may help mitigate age-related decline in health and function, reinforcing the critical role of antioxidants in promoting longevity and quality of life.

Garcinia mangostana, commonly known as mangosteen, is a tree native to Southeast Asia. The fruit is characterized by a thick, dark purple rind and a juicy, white pulp that is both sweet and slightly tangy (Balunas et al., 2008; Zamarudin, 2023). Historically, mangosteen has been utilized in traditional medicine for its therapeutic properties, particularly in treating gastrointestinal issues such as diarrhea, dysentery, and ulcers, as well as for wound healing (Balunas et al., 2008; Aizat et al., 2019). The health-promoting properties of *Garcinia mangostana* are largely attributed to its rich composition of bioactive compounds, particularly xanthones, which are a class of polyphenolic compounds known for their potent antioxidant activities (Chitra et al., 2017; Gutiérrez-Orozco &

Failla, 2013). Research has demonstrated that these xanthones, including α-mangostin and γ-mangostin, exhibit various biological activities such as anti-inflammatory, antimicrobial, and anticancer effects (Zamarudin, 2023; Li et al., 2013). The pericarp of the fruit, which is often discarded, contains the highest concentration of these beneficial compounds, making it a valuable source for health applications (Ibrahim et al., 2015; Martínez et al., 2012). The increasing popularity of mangosteen in health supplements and functional foods reflects a growing interest in its potential health benefits. As research continues to uncover the various pharmacological effects of *Garcinia mangostana*, it is becoming evident that this tropical fruit not only satisfy the tongues but also offers abundance of health-promoting properties that warrant further exploration and utilization in dietary practices (Ibrahim et al., 2015; Qian et al., 2021).

Garcinia mangostana has been utilized in traditional medicine for centuries, particularly in Southeast Asia, where it is much used for its therapeutic properties (Qian et al., 2021; Karim & Tangpong, 2018). Historically, various parts of the *Garcinia mangostana* plant have been employed in traditional remedies. The pericarp, or rind, of the fruit is particularly notable for its medicinal applications. It has been used to treat a variety of ailments, including diarrhea, dysentery, skin infections, and wounds (Azam et al., 2021; Mohamed et al., 2014). The astringent properties of the mangosteen rind have made it a popular choice for addressing gastrointestinal issues, as it is believed to help alleviate symptoms associated with digestive disorders (Phumlek et al., 2022). Additionally, the anti-inflammatory effects of the compounds found in the pericarp have been utilized in traditional practices to manage inflammatory conditions and promote wound healing (Syam et al., 2014). The traditional applications of *Garcinia mangostana* are not limited to physical health; they also encompass mental well-being. In some cultures, the fruit is believed to have mood-enhancing properties, which may be attributed to its antioxidant effects that help mitigate stress and anxiety (Karim & Tangpong, 2018). This shows that the fruit has a high potential for a source of bioactive especially antioxidants which can be attributed to its therapeutic effects for use in traditional medicine.

In many Asian countries, the bark and fruit skin are used to treat diarrhea and dysentery. The rind contains a high concentration of tannin and is also used to treat dysentery. The Malays use a decoction of the roots to treat irregular menstruation. In Indonesia, the bark and leaves are considered astringent and also used to control fever. *Garcinia mangostana* is established to contain numerous bioactive compounds that contribute to its health benefits and medicinal properties. The primary bioactive constituents of this tropical fruit include xanthones, flavonoids, phenolic acids, and various other phytochemicals. Each of these compounds plays a significant role in the fruit's therapeutic potential. The sap of the mangosteen has been used as a black coloring agent to dye silk. It stains fabric and may also linger on your hands for some time as well.

One of the most notable groups of compounds found in *Garcinia mangostana* is xanthones, particularly α-mangostin and γ-mangostin. These compounds are recognized for their potent antioxidant properties, which help neutralize free radicals and reduce oxidative stress in the body (Yoshimura et al., 2015; Ibrahim et al., 2015). Research has shown that xanthones exhibit a variety of biological activities, including anti-inflammatory, antimicrobial, and anticancer effects (Le et al., 2015; Ratwita et al., 2019). Flavonoids are another important class of bioactive compounds present in *Garcinia mangostana*. These compounds are known for their antioxidant and anti-inflammatory properties, contributing to the overall health benefits of the fruit (Yoshimura et al., 2015; Ibrahim et al., 2015). Studies have indicated that flavonoids can enhance the immune response and protect against chronic diseases by reducing inflammation and oxidative damage (Ching et al., 2019; Chew & Lim, 2018). The

presence of flavonoids in mangosteen also supports its traditional use in treating various ailments, including infections and inflammatory conditions (Kharisma, 2024; Sultan et al., 2022).

Phenolic acids, such as chlorogenic acid and epicatechin, have also been identified in the extracts of *Garcinia mangostana* (Yoshimura et al., 2015; Ibrahim et al., 2015). These compounds are known for their antioxidant activity and potential health benefits, including cardiovascular protection and improved metabolic health. The antioxidant capacity of these phenolic compounds contributes to the fruit's ability to combat oxidative stress and may play a role in preventing chronic diseases (Yoshimura et al., 2015; Ibrahim et al., 2015). In addition to these primary bioactive compounds, *Garcinia mangostana* contains a variety of other phytochemicals, including saponins, tannins, and vitamins (Qian et al., 2021; Nufiarwan & Wulandari, 2019). Saponins have been shown to possess antimicrobial properties, while tannins contribute to the astringent taste of the fruit and may have additional health benefits, such as supporting digestive health (Qian et al., 2021; Nufiarwan & Wulandari, 2019). Vitamins A, C, and E found in mangosteen further enhance its nutritional profile, providing essential nutrients that support immune function and skin health (Qian et al., 2021; Nufiarwan & Wulandari, 2019). Alpha-mangostin, a xanthone extracted from the pericarp of *Garcinia mangostana* (mangosteen) is one of the key active bio-compound that is focused on research - emerging as a promising anti-cancer agent due to its multiple mechanisms of action. Current research highlights its ability to induce apoptosis, inhibit cell proliferation, and suppress metastasis in various cancer types, including breast, pancreatic, and cervical cancers. One of the primary mechanisms through which alpha-mangostin exerts its anti-cancer effects is the induction of apoptosis. Lee et al. (2017) demonstrated that alpha-mangostin activates reactive oxygen species (ROS) and the ASK1/p38 signaling pathway, leading to apoptosis in cervical cancer cells. This pro-apoptotic effect is further supported by findings that alpha-mangostin increases the expression of active caspases, which are an important mediator of the apoptotic process (Chang & Yang, 2012). Additionally, Huang et al. reported that alpha-mangostin induces endoplasmic reticulum stress and autophagy, which are associated with its ability to inhibit fatty acid synthase (FAS) and promote apoptosis in breast cancer cells (Huang et al., 2019).

In terms of its effects on cancer cell migration and invasion, alpha-mangostin has been shown to downregulate matrix metalloproteinases (MMP-2 and MMP-9), which are key enzymes involved in the metastatic process. Xu et al. (2014) found that alpha-mangostin suppresses the epithelial-mesenchymal transition (EMT) in pancreatic cancer cells by inhibiting the PI3K/Akt signaling pathway, thereby reducing cell migration and invasion. Similarly, Yuan et al. reported that alpha-mangostin inhibits lipopolysaccharide-induced invasion in pancreatic cancer cells by decreasing MMP-2 and MMP-9 expression (Yuan et al., 2013). Moreover, alpha-mangostin has demonstrated the ability to modulate various signaling pathways that are crucial for cancer progression. For instance, it has been shown to inhibit the ERK1/2 MAPK signaling pathway, which is often activated in cancer cells, thereby contributing to its anti-proliferative effects (Xu et al., 2014).

The antioxidant activity of *Garcinia mangostana* varies significantly due to several factors, including the part of the fruit used, extraction methods, and environmental conditions during growth. Research indicates that different components of the mangosteen exhibit distinct antioxidant capacities, primarily attributed to their unique phytochemical profiles. The pericarp of *Garcinia mangostana* is particularly rich in xanthones, flavonoids, and phenolic compounds, which are known for their potent antioxidant properties. Ibrahim et al. found that the antioxidant activity and total phenolic content differ across various parts of the fruit, with the pericarp showing the highest levels Ibrahim et al. (2015). Therefore, the pericarp is often the source material for extraction of bioactive compounds. The growth conditions of *Garcinia mangostana*, including soil quality, climate, and

agricultural practices, can also affect its antioxidant levels. Variations in these environmental factors can lead to differences in the phytochemical composition of the fruit, impacting its overall antioxidant capacity. For example, the presence of stressors such as drought or nutrient deficiency may enhance the production of secondary metabolites, including antioxidants, as a protective response (Qian et al., 2021).

The processing and storage of mangosteen products can lead to changes in antioxidant activity. Factors such as temperature, light exposure, and duration of storage can degrade sensitive compounds, reducing their efficacy. Research by Ibrahim et al. (2015) indicated that the antioxidant properties of mangosteen can diminish over time if not stored properly. Proper storage condition and handling is therefore important. When compared to other fruits, *Garcinia mangostana* exhibits competitive antioxidant activity. Studies have shown that its xanthone content provides superior antioxidant effects compared to many commonly consumed fruits, making it a valuable addition to diets aimed at combating oxidative stress (Ruankham et al., 2022).

Antioxidant assays are essential for evaluating the ability of substances to scavenge free radicals and reduce oxidative stress. These assays measure the antioxidant capacity of different compounds, including plant extracts, and can provide quantified information on the potency of a particular compound as an antioxidant. The FRAP assay quantifies the antioxidant capacity based on the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in an acidic medium. The resulting ferrous complex forms a blue color that can be measured spectrophotometrically at 593 nm. The intensity of the color is directly proportional to the concentration of antioxidants present in the sample (Payne et al., 2013; S & Kalava, 2021). This assay is particularly useful for assessing the total reducing power of antioxidants in food and biological samples.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating the free radical scavenging ability of antioxidants. In this assay, the stable DPPH radical accepts hydrogen atoms from antioxidants, leading to a discoloration that can be measured at 517 nm. The degree of discoloration is indicative of the antioxidant capacity of the tested sample (Gupta et al., 2011). This method is relatively simple and can be rapidly executed, making it favourable for screening of various materials.

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay measures the ability of antioxidants to scavenge the ABTS radical cation, resulting in a decrease in absorbance at 734 nm. This assay is compatible with both aqueous and organic solvent systems, making it versatile for different types of samples (Damgaard et al., 2014; Fan, 2024). The ABTS assay is often reported to yield higher antioxidant capacity values compared to the DPPH assay, particularly for hydrophilic and highly pigmented antioxidants.

METHODOLOGY

FRAP assay method is chosen to quantify the antioxidant activity of *Garcinia mangostana* L. extract in ethanol (70%), extracted using maceration method based on modified procedures. Dried ripe *Garcinia mangostana* pericarp is collected. The collected samples are rinsed to remove any contaminants and then dried. Drying is performed using an oven at a controlled temperature (around 40-60 °C) until the moisture content is sufficiently reduced (typically below 10%).

Figure 2: Processing dried pericarp of mangosteen

Once dried, the pericarp is ground into a fine powder using a mortar and pestle. The powdered pericarp is subjected to extraction with maceration, making sure the pericarp powder is fully submerged. A 200 grams of the powdered pericarp added with 800 mL of 80% ethanol in a one liter beaker. The mixture is then subjected to maceration for 5 days, with mixture being swirled and mixed intermittently to ensure complete extraction and to facilitate the extraction of bioactive compounds. Following extraction, the mixture is filtered through a muslin cloth first, then through a filter paper using vacuum filtration to separate the liquid extract from the solid residue. The liquid extract is then concentrated using a rotary evaporator to remove the solvent under reduced pressure yielding a concentrated extract. The final concentrated extract in thick liquid poured into an evaporating dish as shown in Figure 3 and Figure 4 respectively. It is further evaporated into solids facilitated by heating through placing it on a water bath at 30°C celcius. The final form of the extract would be used to make a stock solution of the extractas to qualify and quantify its photochemicals and antioxidant properties.

Figure 3: Concentration of extract with rotary evaporator.

Figure 4: Final evaporation after water bath

FRAP ASSAY OF *GARCINIA MANGOSTANA* L. PERICARP

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely used method to evaluate the antioxidant capacity of various substances, including plant extracts. There is different variation of FRAP assay, though the most common one is to use 2,4,6-Tri(2-pyridyl)-s-triazine or known as TPTZ that could bind reduced Fe^{2+} ions directly.

In this dissertation the use of potassium ferricyanide and ferric chloride is used to measure the ferric reduction power. The antioxidant contained in plant extract reduces potassium ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}). Then, ferrocyanide reacts with ferric chloride (Fe^{3+}) to form a Prussian blue-coloured complex, which can be measured at 700 nm. Higher antioxidant activity would cause a more intense blue colour to be measured via UV-V is spectrophotometer.

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely used method to evaluate the antioxidant capacity of various substances, including plant extracts. This assay measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in an acidic medium, resulting in a colour change that can be quantified spectrophotometrically at 593 nm.

The exact solutions are prepared fresh and are used at the very same day for FRAP assay to get the sample absorbance are shown in Table 1.

Table : Detail for freshly prepared solutions

Solution Name	Category	Concentration	Amount to Dissolve	Solvent	Final Volume	Notes
Disodium phosphate	Buffer	-	2.84 grams	Distilled water	100 ml	Phosphate buffer pH 6.6
Monosodium phosphate	Buffer	-	2.78 grams	Distilled water	100 ml	Phosphate buffer pH 6.6
Phosphate Buffer pH 6.6	Buffer	Mixed	37.5 ml disodium + 62.5 ml monosodium	Distilled water	200 ml	**Final buffer solution**
Ferric chloride	Reagent	0.1%	100 mg	Distilled water	100 ml	FRAP assay reagent
Potassium ferricyanide	Reagent	1%	1 gram	Distilled water	100 ml	FRAP assay reagent
Trichloroacetic acid	Reagent	10%	10 grams	Distilled water	100 ml	Corrosive - wear gloves
Extract solution (Stock)	Sample	10 mg/ml	100 mg powder	70% ethanol	10 ml	Prepare fresh before use
Extract solution (Dilution 1)	Sample	0.2 mg/ml	Serial dilution	Distilled water	10 ml	For FRAP assay
Extract solution (Dilution 2)	Sample	0.5 mg/ml	Serial dilution	Distilled water	10 ml	For FRAP assay

Extract solution (Dilution 3)	Sample	1.0 mg/ml	Serial dilution	Distilled water	10 ml	For FRAP assay
Extract solution (Dilution 4)	Sample	1.5 mg/ml	Serial dilution	Distilled water	10 ml	For FRAP assay
Extract solution (Dilution 5)	Sample	2.0 mg/ml	Serial dilution	Distilled water	10 ml	For FRAP assay
Ascorbic acid (Stock)	Standard	10 mg/ml	100 mg	Distilled water	10 ml	Prepare fresh before use
Ascorbic acid (Dilution 1)	Standard	0.2 mg/ml	Serial dilution	Distilled water	10 ml	Standard curve preparation
Ascorbic acid (Dilution 2)	Standard	0.5 mg/ml	Serial dilution	Distilled water	10 ml	Standard curve preparation
Ascorbic acid (Dilution 3)	Standard	1.0 mg/ml	Serial dilution	Distilled water	10 ml	Standard curve preparation
Ascorbic acid (Dilution 4)	Standard	1.5 mg/ml	Serial dilution	Distilled water	10 ml	Standard curve preparation
Ascorbic acid (Dilution 5)	Standard	2.0 mg/ml	Serial dilution	Distilled water	10 ml	Standard curve preparation

For each blank/concentration, 3 ml of blank/extract solution/ascorbic acid solution is mixed with 3 ml 0.2M phosphate buffer (pH 6.6) with 3 ml of 1% potassium ferricyanide in a boiling tube. The solution is vortexed for 5 minutes and incubated at 50°C for 20 minutes in a water bath. Afterwards, 3 ml of 10% TCA are added to each of the solution and centrifuged at 3000 RPM for 10 minutes. After that, pipetted out 1ml from the top layer and mixed with 10 ml distilled water with the addition of 0.5 ml 0.1% FeCl3. Waite the solution to stand for 5 minutes then measured the absorbance at 700 nm. The procedures for both extract solution and ascorbic acid are done in triplicate for each concentration is shown in Figure 5 and Figure 6.

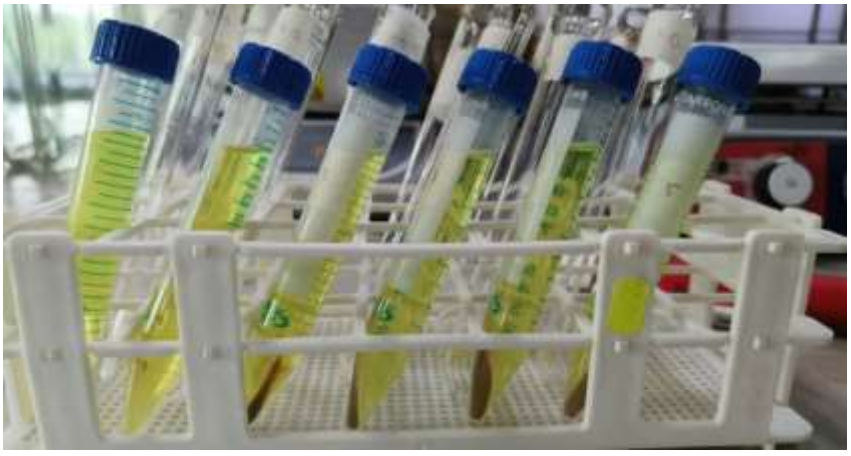


Figure 5: Extract in FRAP solution after centrifugation at 3000 RPM.

Figure 6: Extract after addition of Ferric Chloride.

The results are reported as Ascobic Acid Equivalent (AAE) per gram extract to express the antioxidant capacity of the *Garcinia mangostana* pericarp extract. Prepare a series of ascorbic acid dilutions (0.2 mg/ml, 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, and 2.0 mg/ml) by diluting the stock solution with distilled water. Measure the absorbance of each standard at 700 nm after completing the procedure described above and plot the absorbance against the concentration of ascorbic acid to plot standard curve. The antioxidant capacity of the *Garcinia mangostana* extract is calculated based on the absorbance readings and the standard curve. Plot the absorbance values of the ascorbic acid standards against their concentrations to create a standard curve. The equation of the line (y = mx + c) can be derived from the linear regression analysis, where: The absorbance recorded in an assay of sample extract is then inserted into the equation to get the antioxidant capacity of the sample expressed AAE. The results are reported as mg of Ascorbic Acid Equivalents (AAE) per gram of extract (mg AAE/g) to express the antioxidant capacity of the *Garcinia mangostana* pericarp extract.

Phytochemical Screening

Qualitative were done to identify the presence of alkaloid, flavonoid, saponin and tannin.

Screening for Alkaloid, Flavonoid, Saponin and Tanin as shown in the Table 2

Table 2: Alkaloid, flavonoid, saponin and tannin screening

Test	Sample Amount	Reagents/Procedure	Positive Result	Notes
Alkaloid test (Bourchardat reagent)	0.1 g extract	Add 2 drops ammonia + 5 ml chloroform, filter. Add 1 ml 2M sulfuric acid. Add Bourchardat Reagent	Orange-brown precipitate forms	Confirms presence of Alkaloids
Flavonoid test	Ethanolic extract	Add 1-5 drops concentrated hydrochloric acid (HCl)	Development of red colour	Red colour indicates presence of Flavonoids
Saponin test	0.1 g extract	Add 5 ml hot distilled water, filter. Vortex strongly and stand for 10 minutes	Stable foam formation	Stable foam confirms presence of Saponin
Tanin	0.1 g extract	Add to water. Add 10% ferric chloride solution	Dark blue or dark green precipitate	Colour precipitate confirms presence of Tannin

Test for Antimicrobial activity

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) is often described as the lowest concentration of compound required to prevent the visible growth of bacteria or also known as bacteriostatic. It represents the antimicrobial effectiveness of a compound. Broth dilution method is used to study the MIC. Broth dilution is based on the technique which the test tubes holding the identical volumes of broth with antimicrobial solution in incrementally increasing concentration are inoculated with known number of bacteria. In the context of this study, the antimicrobial solution was replaced with the Garcinia mangostana L. pericarp extract Anastasia et al. The density of bacteria suspension inoculated was made to be same as 0.5 McFarland standard, which is equal to 1.5×10^8 colony forming units (CFU/ml). The incubated broth was transferred and spreaded on the agar plate and the growth of the microorganism is recorded by observing turbidity on the surface of the agar plate. The lowest concentration where the turbidity disappear will be considered as the MIC Anastasia et al.

Preparation of bacteria selection and culture

Staphylococcus epidermidis and *Escherichia coli* were chosen as the test microorganisms. These bacterias were chosen because they are the most common bacteria that were found in our everyday life and can potentially cause diseases to humna being. 100 ml of nutrient broth was prepared by dissolving 1.3 g of nutrient broth powder in 100 ml of distilled water. Nutrient broth powder was stirred thoroughtly by using a glass rod to ensure that all the broth powder dissolved in the beaker. After that, 20 ml of the agar broth was transferrred to a 50 ml conical flask. The flask opening was stoppered by using cotton wool and covered by using aluminium foil. The steps above were repeated for 3 times to produce 4 conical flask containing 20 ml nutrient broth. The nutrient broth in conical flasks were sent to auoclave at 121 degree celcius for 120 minutes.After autoclave process, the bacteria which are *Staphylococcus epidermidis* and *Escherichia coli* was inoculated from the mother culture into the nutritent broth in the conical flask. After that,the bacteria culture was incubated in the incubator for 24 hours and they are ready to be used.

Preparation of Muller Hinton Agar plate

24.7g of Mueller Hinton agar powder was weighted and dissolved in 650ml of distilled water. The powder was mixed and dissolved completely with the help of a glass rod. The Mueller Hinton agar solution was filled into two 500ml screw cap bottle. After that, the bottles containing Mueller Hinton agar solution were sent to autoclave at 121 degree celcius for 120 mins. After the agar is out from autoclave, they are allowed to be cooled before pouring into the petri plates. The sterile agar solution was poured into 24 sterile petri plates to cover ½ to 2/3 of the plate. This process was carried out in laminar airflow cabinet to ensure the sterility and prevent the contamination of sterile preparation. After the agar completely solidfy, the plates were covered and stored in the refrigerator for further use.The steps mentioned above were repeated for 3 time to produce a total of 96 agar plate for further use.

Preparatin of Mueller Hinton Broth

5.46g of the Mueller Hinton broth powder was weighed and addded into a 500 ml conical flask. 260 ml of distilled water was added to completely dissolved the broth powder in the conical flask. The solution was mixed by using a glass rod to ensure that all the broth powder dissolved completely in the distlled water to produce a homogenous solution. The MH broth was transferred into test tubes to produce 3 test tubes containing 5 ml of broth solution and 21 test tubes contaning 9 ml of broth solution. The test tubes containing the broth solution were stoppered by using cotton wool and also covered with aluminium foil. After that. the test tubes containing broth solution were

sent to autoclave at 121 degree celcius for 120 minutes. The steps mentioned above were repeated for another 3 times to produce 4 set of setup. Each set of test tubes containing the broth solution was used for culturing of different bacteria.

Preparation of 0.5 McFarland standard

0.1 g of Barium chloride was dissolved in 10ml distilled water to produce 1% barium chloride solution. After that, 0.05ml of 1% barium chloride solution was mixed with 9.95 ml of 1% sulphuric acid to produce 10 ml of 0.5 McFarland turbidity standard. The prepared McFarland standard was kept in a universal bottle. The McFarland standard was kept in refrigerator for further use.

Preparation of Chloramphenicol antibiotic stock solution

Figure 7: Chloramphenicol antibiotic stock solution

10 mg of the chloramphenicol powder was weighed accurately by using electronic balance. The chloramphenicol powder was completely dissolved in 10 ml of distlled water to produce a 1mg/ml chloramphenicol stock solution. The chloramphenicol stock solution was kept in centrifuge bottle for further use. Figure 7 above shows chloramphenicol antibiotic stock solution.

Preparation of *Garcinia mangostana L. pericarp extract* stock solution

4 mg/ml of *Garcinia mangostana L. pericarp* stock solution was prepared by dissolving 400 mg of the plant extract obtained from the maceration process in 100 ml of ethanol solution (70%). The plant extract was able to dissolve completely in 70 % ethanol solution has minimum effect on the minimum inhibitory concentration (MIC) of the plant extract. The stock solution prepared was stored in universal bottle and stored in refrigerator for further use.

Broth dilution method procedure

For the broth dilution method for determining the minimum inhibitory concentration (MIC) of the *Garcinia mangostana L. pericarp extract*, 3 replicate will be performed for each bacteria strain. Firstly, the test tubes were labelled with the respective plant extract concentration and sequence of replication. 2 fold serial dilution will be performed and there will be 7 concentration of *pericarp extract* which are 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/ml respectively. The test tubes were also labelled with R1, R2, and R3 respectively to indicate the number of replication. Next, 4 ml of the 4mg/ml pericarp extract stock solution was transferred into the first test tube labelled with R1 4 mg/ml . From the first test tubes, 5ml of the solution will be transfered to the 2nd test tubes. The concentration of plant extract in the 2nd test tube was 2mg /ml. After that, 5ml of the solution was transferred from the 2nd test tube to the 3rd test tube, which was 1mg/ml. The step was repeated untill a test tube with 0.0625

mg/ml extract was produced. The same steps were repeated for the 2nd and 3rd replicates. No plant extract should be added into the blank, positive control and negative control. All of the steps above should be performed in a laminar air flow cabinet to ensure the sterility and prevent contamination of the preparation. Next, the culture of *E.coli* was added drop by drop to a sterile distilled water contained in a glass container with side-by-side comparison with 0.5 McFarland standard by using a micropippette. The bacterial culture was added untill the turbidity of the bacterial suspension was same as the 0.5 McFarland standard. With the help of a micropippete , 1ml of the bacterial suspension was transferred to each test tube, positive control and negative control. After that, 1ml of the chloramphenicol antibiotic suspension prepared was added into the positive control. The test tube with bacterial suspension was incubated in the incubator at 37 degree celcius for 24 hours. The steps above were repeated by replacing the *E.coli* with *Staphylococcus epidermidis.*

Spread plate technique

Firstly, the agar plate was labelled with the name, bacteria strain, date, and concentration of plant extract. Figure86 below shows the setup for spread plate technique. Next, 100 microlitre of the content from the incubated test tubes were transferred to the respective agar plate by using a 100 microlitre micropippete. Figure 9 below shows the trasnfer of broth solution to agar plate. The content of the test tube need to be stirred thoroughly before transferring the solution to the agar plate. After transferring to the plate, the solution was spreaded on the surface of agar plate with the help of the L-shaped glass rod. Figure 10 below shows the spreading of broth solution on the agar plate. The steps above were repeated for all the concentration in R1, R2, R3, blank, positive control ad negative control. This procedure was carried out in a laminar air flow cabinet to ensure the sterility and prevent the contamination of the products.

Figure 8: Setup for spread plate

Figure 9: Transfer of broth solution to agar plate

Figure 10: Spread the broth solution on the plate by using L-shape rod

FT-IR Spectral Analysis

FT-IR spectral analysis was done using the instrument FT-IR Spectrometer (Perkin Elmer Spectrum 2) using direct solid sample. Then the functional groups were identified based on the spectral analysis and wavebands

RESULTS AND DISCUSION

The antioxidant activity of *Garcinia mangostana* peel extract was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, with ascorbic acid used as the standard. Absorbance values were measured at 700 nm, and all samples were analysed in triplicates. The calibration curve constructed using ascorbic acid showed a linear relationship between concentration and absorbance, described by the equation:

$y = 0.985x + 0.035$ ($R2=0.999$)

where y is the absorbance and x are the ascorbic acid concentration (mg/mL). The average absorbance values for both extract and standard, along with standard deviations, are presented in Table 3. A graphical representation is shown in Figure 11 and Figure 12.

Table 3: Absorbance of Standard Ascorbic Acid and extract solution of different concentration

Concentration (mg/mL)	Standard Avg	Standard SD	Extract Avg	Extract SD
0.2	0.162	0.003	0.235	0.003
0.5	0.600	0.003	0.499	0.004
1.0	1.042	0.006	0.987	0.005
1.5	1.501	0.003	1.862	0.004
2.0	1.991	0.004	2.060	0.005

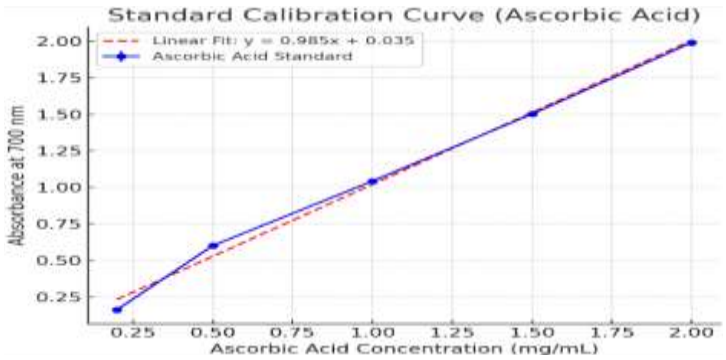


Figure 11: Standard calibration curve of ascorbic acid at 700 nm.

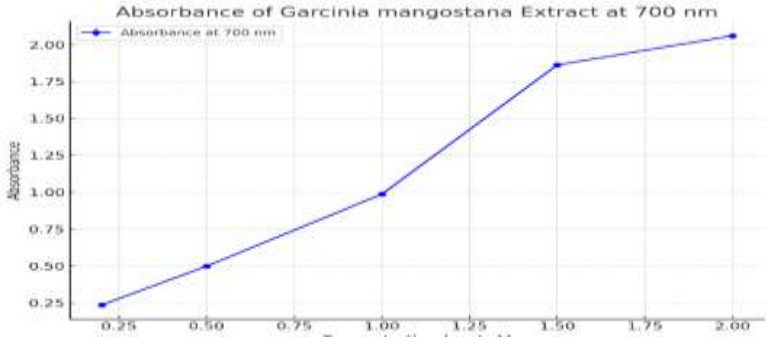


Figure 12: Absorbance curve of G. mangostana ethanolic extract solution at 700 nm.

Based on the calibration curve, the ascorbic acid equivalent (AAE) of the extract was calculated as shown in Table 4. From the standard curve equation or ascorbic acid, the equation regression equation from the curve can be rearranged. Absorbance

where x is the Ascorbic Acid Equivalent (mg/mL).

Table 4: AAE in mg/ml.

Extract Conc. (mg/mL)	AAE (mg/mL)
0.2	0.236
0.5	0.501
1.0	0.992
1.5	1.871
2.0	2.070

The AAE (mg/ml) were then converted into mg AAE per g extract as in Table 5

Table 5: g AAE per mg extract.

Extract Conc.	AAE (mg/mL)	g AAE per mg extract
0.2	0.236	1.18
0.5	0.501	1.002
1.0	0.992	0.992
1.5	1.871	1.247
2.0	2.070	1.035

The FRAP assay results indicate that Garcinia mangostana peel extract possesses high antioxidant potential, with a reducing capacity equivalent to 1091 mg of ascorbic acid per gram of dry extract. This strong ferric reducing ability reflects the presence of potent phytochemicals, especially polyphenols, xanthones (most prominent α-mangostin), and flavonoids, which are abundant in the pericarp of G. mangostana.

Compared to other plant-derived antioxidant sources, the observed AAE value is significantly high, supporting the traditional and commercial use of mangosteen peel in herbal medicine and supplementation. The nearly linear increase in absorbance with increasing extract concentration suggests dose-dependent antioxidant activity, which is consistent with literature findings on phenolic-rich plant extracts.

The FRAP assay, which measures the ability of antioxidants to reduce Fe^{3+} to Fe^{2+}, provides a good estimate of the extract's electron-donating capacity. While it does not reflect radical scavenging directly (as in DPPH or ABTS), it correlates well with total antioxidant potential in food and nutraceutical studies. The low standard deviation across triplicates indicates good reproducibility of the results and reliability of the method used.

In summary, the high AAE value suggests that Garcinia mangostana peel extract could be a promising natural antioxidant source for food, cosmetic, or pharmaceutical applications.

Antimicrobial acitivity against *Escherichia coli*

4 mg/ml 	2mg/ml 	1mg/ml 
0.5mg/ml 	0.25mg/ml 	0.125mg/ml 
0.0625 mg/ml 	Blank sample 	MHB +E.coli only 

MHB + E.coli +Chloramphenicol 		

Table 6: Growth of E. coli at 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 mg/ml Garcinia mangostana L. pericarp extract, blank, positive control and negative control

Antimicrobial activity against *S.epidermidis*

4mg/ml 	2mg/ml 	1mg/ml 
0.5mg/ml 	0.25mg/ml 	0.125mg/ml 

0.0625mg/ml 	Blank 	MH broth + *S. epidermidis* only 
MH broth + *S.epidermidis* +chloramphenicol 		

Table 7: Growth of S. epidermidis at 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 mg/ml Garcinia mangostana L. pericarp extract, blank, positive control and negative control

According to the result of the study, *Garcinia mangostana L. pericarp extract* was found to be having antimicrobial activitiy against E.coli. This is consistent with the finding of Sienna K. F et al whereby the *Garcinia mangostana L.* was suggested to be effective against the *E. coli*. Although there was a slight difference between the methodology used by Anastasia et al and this study, there is still validity in the correlation between this study and study performed by Anastasia et al. The minimum inhibitory concentration (MIC) of *Garcinia mangostana L. pericarp extract* against E.coli was found to be 4 mg/ml. It is because at 4mg/ml, the growth of E.coli was obviously inhibited to only around 100 colonies while in previous concentration which is 2mg/ml, there is turbidity shown all over the petri plate, which indicates visible growth of E.coli all over the plate. Based on the MIC, The minimum bactericidal concentration (MBC) was predicted to be 8mg/ml. In control wise, there is lack of inhibitory effect of chloramphenicol against *E.coli* most probably due to the development of antibiotic resistance in *E.coli* Ashraf A. M et al. According to the result of the study, *Garcinia mangostana L. pericarp extract* was found to be effective against *S. Epidermidis*. However, if compared to the inhibitory effect found in *E. coli*, the inhibitory effect of *Garcinia mangostana L.* to *S.epidermidis* was less significant as there are more bacterial colonies found on the surface of plate. Thus, it is coherent to *S.epidermidis* is less vulnerable to Garcinia mangostana L. extract even though it shares the same MIC with *E. coli.*

Based on this study, it was found that *Garcinia mangostana L. pericarp extract* was more potent on Gram-negative bacteria than on Gram-positive strains, thus acting as a broad spectrum. This can be validated by less number of bacterial colonies for *E.coli* and *S. Epidermidis* These findings are consistent with other reported research works such as the study by Sienna K. F et al and Ashraf A. M et al and this could be attributed to the presence of a lipophilic outer membrane consisting of lipopolysaccharide molecules with an affinity for lipophilic molecules.

FT-IR Spectral Analysis

The functional groups in Garcinia mangostana peel extract were identified using Fourier Transform Infrared Spectroscopy (FTIR). The FTIR spectrum is shown in Figure 13, and the characteristic absorption bands are listed in Table 8.

Table 8. Absorption bands and functional group assignment.

Wavenumber (cm^{-1})	Functional Group	Vibrational Assignment
3343	O–H (hydroxyl)	Broad stretching vibration of phenols/alcohols
2920	C–H (alkyl)	Stretching of aliphatic $–CH_2$ or $–CH_3$
1603	C=C (aromatic ring)	Aromatic skeletal vibration
1577	C=C / C=O (conjugated)	Aromatic or conjugated carbonyl stretching
1458	C–H (bending)	Bending vibration of $–CH_2/CH_3$
1276	C–O (phenol or ester)	Stretching of C–O bond in phenols or esters
1043	C–O (alcohol/ether)	C–O–C or primary alcohol stretching

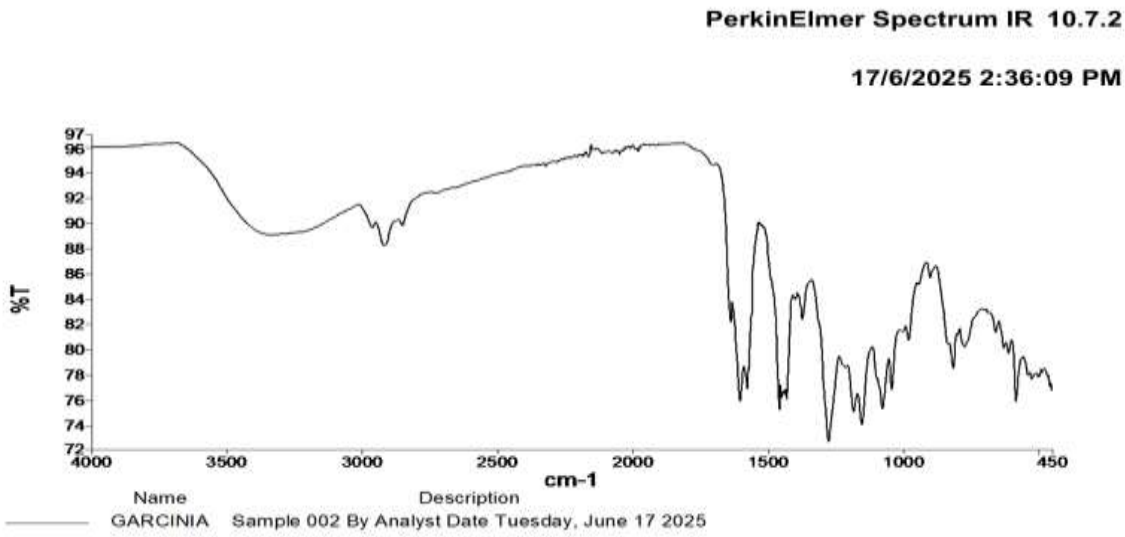


Figure 13 FT-IR Spectra of G. mangostana pericarp crude extract.

The FTIR spectrum provides insight into the types of functional groups present in the *Garcinia mangostana* peel extract. The broad peak at 3343 cm^{-1} corresponds to O–H stretching vibrations, indicating the presence of hydroxyl groups typically found in phenolic compounds, which are known for their antioxidant properties.

The peaks at 2920 cm^{-1} and 1458 cm^{-1} suggest the presence of aliphatic C–H groups, which may originate from methyl or methylene chains in secondary metabolites such as xanthones. Peaks at 1603 cm^{-1} and 1577 cm^{-1} are

characteristic of aromatic C=C and possibly conjugated C=O groups, confirming the presence of aromatic rings - consistent with the polyphenolic structure of compounds like α-mangostin

Furthermore, the absorption bands at 1276 cm^{-1} and 1043 cm^{-1} correspond to C–O stretching vibrations in phenols, esters, or ethers, which support the existence of flavonoids, tannins, and related compounds.

These functional group assignments support the FRAP antioxidant results, where the high reducing power (1091 mg AAE/g extract) is likely due to the phenolic and flavonoid content in the peel extract. The FTIR findings thus support the conclusion that *Garcinia mangostana* peel contains bioactive compounds with strong antioxidant potential.

CONCLUSION

This study had explored the phytochemical and antioxidant potential of *G. mangostana* pericarp to evaluate it's use as a source of natural antioxidant. Based on phytochemical screening, the compounds such as alkaloid, flavonoid, tannin and saponin are all known contributor to antioxidant activity. The result suggests the extract contained more compounds with different characteristics and potentials of use.

Based on FRAP assay, the graph showed a strong concentration-dependent increase in absorbance with derived antioxidant activity of 1091 mg ascorbic acid equivalent per gram of extract. This is a significant value and confirmed the extract of pericarp as effective antioxidant.

Lastly, the FTIR spectral analysis revealed the characteristics of functional groups of compounds present in the extract of the pericarp such as hydroxyl (O-H), aromatic (C=C) and carbonyl (C=O) groups which are associated with polyphenols, flavonoids and xanthone.

In summary, the results indicated that pericarp of *G. mangostana* is an excellent source of antioxidant and phytochemicals which support its application in various field including nutraceuticals, or in traditional medicine use.

The antimicrobial activity showed that *G. mangostana* pericarp has antimicrobial activity against *E. coli*, and *S. Epidermidis*. The MIC of *G. mangostana* pericarp for *E. coli*, and *S. epidermidis* was 4mg/ml.

FUTURE SCOPE AND LIMITATION

There are various ways for my research to be expanded, and to cover much more vast directions. In this thesis only few phytochemicals were screened qualitatively. Therefore, to use both quantitative and qualitative analysis would get more accurate data and to identify all of the phytochemicals existing in the pericarp extract. Measuring total phenolic, flavonoid and xanthone contents could correlate to their antioxidant activity in a quantitative measure.

The thesis was also centred on crude extract. There were too many compounds inside of the extracts that had different properties. With purification and fractionalisation of crude extract, various compounds can be separated and investigated individually. For example, based on literature one of the most prominent compounds found in *G. mangostana* is alpha mangostin. This compound was found to have many properties, and future research could be centred on the novel compound compared to only on the crude extract of pericarp which was well established in literature.

For the antioxidant assay, the thesis only used FRAP assay that utilised use of Potassium Ferricyanide instead of the more standard TPTZ due to chemical availability. The research could be improved by using multi-assay to

create a wider antioxidant profile would also give a better idea on its antioxidant property and be able to compare further with other natural antioxidants source.

The use of different kind of solvents also causes results to vary in literature. In the thesis 70% ethanol was used as the main solvent. However, there were many literatures that used other solvents including different alcohols and distilled water. An optimized method and solvent of extraction could be investigated in regard to extracting the compounds from the pericarp of *G. mangostana.* With different solvents and methods of extraction, the number of compounds extracted would vary thus affecting the end result. With the optimization, the antioxidant property of the the pericarp of the fruit could be more accurately investigated.

The research is centred on antioxidant due to its benefit to human health. However, only in vitro research was done in the thesis. Investigation in vivo to assess its relevance in biological system would be of greater importance in the future.

Related to relation to human health, the toxicological and pharmacological study of the extract and isolated compounds based on animal models would also be of relevance as the pericarp extract and powder were still used even in supplementation and traditional medicine.

Lastly, with the abundance of compounds in pericarp of the fruit, promoting utilization of waste materials could create long term benefit that would add more value to the usually discarded agrowaste. This would benefit the farmers, increase efficiency, lower cost, and create a connected community that promote health and environmental consciousness.

There are several limitations to this antimicrobial study. Firstly, the concentration of Garcinia mangostana L. pericarp extract is too dilute. This may affect the significance of the result. Secondly, the minimum bactericidal concentration (MBC) in the research was an approximate estimation. The accuracy is lower if compared to the standard method to determine MBC. Thirdly, some minor contamination was observed when performing antimicrobial activity. This may be due to some random error during preparation of Muller Hinton broth and agar plate.

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