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RESEARCH ARTICLE

ALPHA-MANGOSTIN QUALITY AND QUANTITY ANALYSIS IN NAKHON SI THAMMARAT MANGOSTEEN PERICARP USING THIN-LAYER CHROMATOGRAPHY

Siriluk Sintupachee[a*], Puttisan Rattanachoo[a], Suppawan Promproa[b]

[a] Program in Creative Innovation Science and Technology, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat 80280, Thailand.
[b] Program in Mathematics and Statistics, Faculty of Science and Technology, Nakorn Si Thammarat Rajabhat University, Nakhon Si Thammarat 80280, Thailand
[*]Corresponding Author Email: siriluk_sint@nstru.ac.th

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ABSTRACT

TLC (thin-layer chromatography) is a standard technique for simultaneously screening and monitoring chemical character in a large number of samples. The goal of this study was to explore if alpha-mangostin (a common secondary metabolite) could be detected in mangosteen pericarp phytochemical fingerprints and how much of it could be represented using a standard calibration curve. The antioxidant activity has been touted as a primary active ingredient in many commercial goods, including cosmetics and food supplements. To test for the antioxidant reaction to the DPPH onto the TLC plate, mangosteen pericarps were obtained from 12 orchards that were grown without pesticides in Cha-Uat (CU), Lan Saka (LS), and Phrom Khiri (PK) districts of Nakhon Si Thammarat. The samples were dried and powdered before being extracted with methanol using the reflux technique. After that, the TLC was utilized to determine the phytochemical fingerprint. The quality of phytochemicals from the LS orchards was found to differ from CU and PK samples, with different bands of compounds at R_F 0.2, 0.25, and 0.57. The average amount of alpha-mangostin in the 12 samples was not statistically different, according to a one-way analysis of variance with a *p-value* of 0.05. The average range of interest (ROI) intensity area of the antioxidant was investigated using a one-way analysis of variance with a *p-value* of 0.05 and repeated comparisons across the sample groups by Tukey's multiple comparison test. The average antioxidant reaction between the CU and PS group and the CU and PK group was significantly different.

KEYWORDS

alpha-mangostin, antioxidant, Khiri Wong, phytochemistry, DPPH

1. INTRODUCTION

Mangosteen brings money to growers every year during harvest season (July-November in Nakhon Si Thammarat) when it is exported to both domestic and international markets. According to data from the Department of Agricultural Extension, mangosteens accounted for two out of every four fruits (approximately 25,000 tons) exported from the province in the year 2020 (Ounlert et al., 2017; Pibul and Jawjit, 2021). In terms of taste and fruit characteristics, the mangosteen grown in the three districts of Cha-Uat (CU), Lan Saka (LS), and Prom Kiri (PK) is unique, especially in Kiri Wong (the sub-district in Lan Saka), which has Thailand's best climate (Ounlert et al., 2017; Suttirak and Manurakchinakorn, 2014). It has a distinct feature that portrays four various colors of mangosteen fruit depending on the stage of growth (Figure 1).

This is because the terrain of the province is in the highlands, with good weather all year, resulting in a diverse range of flora and the greatest conditions for mangosteen growth development (Ounlert and Sdoodee, 2015). Mangosteen is a fruit with a sweet white pulp covered in a thick purple outer shell that weighs three times as much as the inside flesh when harvested (Aizat et al., 2019). The processing of agricultural waste products, such as mangosteen pericarp, to produce active ingredients can be advantageous. As a result, it has a direct impact on the income of mangosteen producers. It is consequently vital to act as a source of raw materials for production and transformation into diverse goods. When alpha-mangostin is utilized as an ingredient in a variety of goods, it is commonly claimed that its bioactivity varies (Wezeman et al., 2015).

Figure 1: Color of the mangosteen development stage

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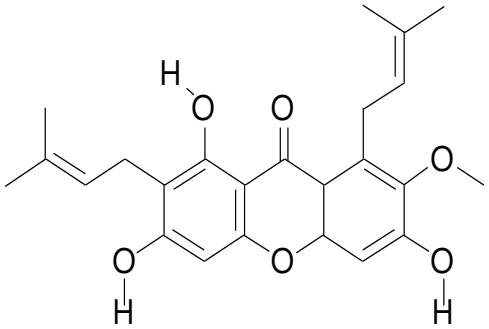

Figure 2: Chemical structure of alpha-mangostin

Farmers and government organizations are becoming more interested in processing mangosteen peels into a variety of goods and claiming the active element alpha-mangostin (Figure 2) in pharmacy, nutrition, and dietary supplement products (Ng et al., 2018; Nittayananta et al., 2018; Siriwattanasatorn et al., 2020; Sukatta et al., 2013; Xie et al., 2015). Alpha-mangostin is a plant secondary metabolite, by transferring the synthesis of key growth metabolic pathways into the secondary metabolic pathway, were produced during varied conditions to defend themselves from pathogens, wounds, or improper settings for growth and classed as a xanthone with the following qualities according to ancient folklore: It is mostly used to treat intestinal disorders, diarrhea, and chronic diarrhea (Pedraza-Chaverri et al., 2008). Antibacterial, antioxidant, and antifungal properties of medicinal antibiotics (Abdallah et al., 2016; Al-Massarani et al., 2013; Cheok et al., 2012; Febrina and Milanda, 2018a, 2018b; Machmudah, 2015; Tjahjani et al., 2014; Xie et al., 2015).

In the alpha-mangostin bioactivity assay as described using chemical techniques for plant isolating material to examine the influence of various components, it was established that isoprenoid phytochemicals are a substance in the essential oil group, and the phenolic group is a colorant (Gondokesumo et al., 2019; Herrera-Aco et al., 2019; Suttirak and Manurakchinakorn, 2014). Scientific methods, on the other hand, were utilized to determine the existence of alpha-mangostin in the raw material and explore its implications. It will increase community trust among consumers and producers.

TLC (thin-layer chromatography) is a well-known chromatographic technique for screening and separation of non-volatile mixtures. TLC can be used to monitor the progression of a reaction into a phytochemical profile similar to a fingerprint on a sheet, to identify chemical components in a mixture and determine the purity of a product. TLC has proven to be a useful technique in a range of applications as pesticide and insecticide residues in food, water, and soil are studied. The color composition of fibers is determined. Amino acid analysis, cosmetic contamination detection, active component concentration analysis, purity testing, and pharmaceutical and prescription identification are just a few of the services available (Chewchinda and Vongsak, 2019; Kumar et al., n.d.; Migas et al., 2020; Misra et al., 2009; Pratiwi et al., 2017). TLC is a speedier approach than column chromatography for screening plant compounds, identifying plant material, and determining marker content.

On TLC plates, the migration of a compound to a specific spot in the chromatogram is utilized to separate compounds, and the pictures are employed as a central feature to demonstrate similarities and differences. The capacity to analyze many samples grown in parallel on a plate and then re-evaluate in different ways with or without chemical derivatization is a significant advantage. Even biological activity testing can be performed on the same plate. The purpose of this study was to determine the phytochemical fingerprint and amount of the major active ingredient, alpha-mangostin, in mangosteen pericarp collected from the Cha-Uat (CU), Lan Saka (LS), and Phrom Khiri (PK) districts, as well as screening test for the antioxidant reactivity to DPPH on a TLC plate.

2. Materials and Methods

2.1 Mangosteen sample site

Mangosteens were obtained from 12 non-chemically cultivated mangosteen orchards from Cha-Uat (CU) (3 orchards), Lan Saka (LS) (5 orchards), and Phrom Khiri (PK) (4 orchards) between July and November 2020, when the mangosteen fruiting season was in full swing (Figure 3). The 12 mangosteen plantations from three districts were chosen because there have been reports of mangosteen output for both domestic and export. They produce almost a third of the province's products (Ounlert et al., 2017; Pibul and Jawjit, 2021). Farmers and entrepreneurs have exploited mangosteen peels from all three locations as raw materials for a variety of products. The sampling sites' latitude and longitude are represented in Figure 3.

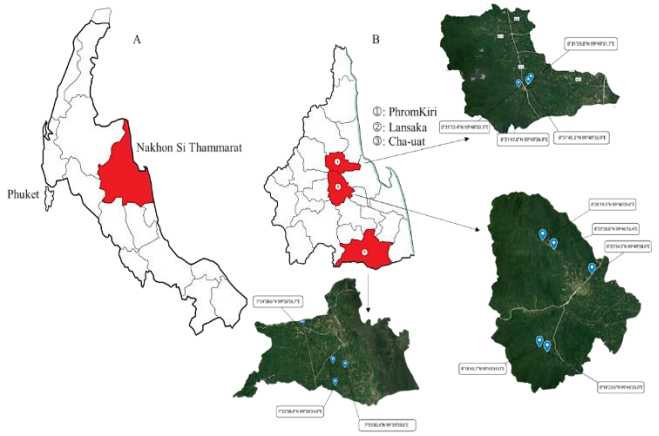

Figure 3: Nakhon Si Thammarat province and the sampling sites, A: presented the location of Nakhon Si Thammarat (in red) in the southern part of Thailand, B: showed where the sampling locations were in the districts of Cha-Uat, Lan Saka, and Prom Khiri.

2.2 Preparation and extraction of samples

All samples were transferred to the Nakhon Si Thammarat Rajaphat University's (NSTRU) Specialized Research Unit on Insects and Herbs for processing. Mangosteen fruit has been peeled and cuts into small pieces, then completely dried in an incubator at 50°C for two nights before being milled into powder. Three hundred milligrams of mangosteen powder were extracted in an extraction tube containing 10 ml methanol using the reflux method at 70°C for two hours, then vacuum dried the solution at 70°C and dissolved the extracts with 2 ml methanol (SyncorePlus, BUCHI), then centrifuged to remove the pellet. Only the clear supernatant was transferred to a new 2 ml microtube and TLC examination was performed. The quality and quantity analyses, as well as the antioxidant activity test (the experiment 2.3, 2.4, and 2.5), were conducted at Chulalongkorn University's Faculty of Pharmaceutical Sciences.

2.3 Thin-layer chromatography

2.3.1 Chemicals

European Pharmacopoeia reference standard for alpha-mangostin (Figure 1) Sigma-Aldrich provided (1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-en-1-yl)-9H-xanthen-9-one, CAS number 6147-11-1). (St. Louist, MO, USA). The mobile phase's chemical components, toluene, acetonitrile, ethyl acetate, and glacial acetic acid, were acquired as the analytical grade from Sigma. The antioxidant activity test utilized a reagent solution of 0.5 percent 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol obtained from Sigma-Aldrich.

2.3.2 Chromatographic state and apparatus

Using the LINOMAT5 (CAMAG), ten microliters of the extracts were spotted onto a 10 cm x 20 cm aluminum silica gel 60F_{254} TLC plate (Merck) versus five dilutions of the alpha-mangostin standard (Sigma) at 125, 250, 375, 500, and 625 µg per spot. The plate was then developed in a saturated TLC chamber with the mobile phase ratios of 35:5:15:0.15 for toluene, acetonitrile, ethyl acetate, and glacial acetic acid, respectively. The plate was allowed to develop up to an 80 mm distance before being withdrawn and blown to dry to terminate the chromatographic capillary force reaction. The experiment was done in triplicate.

2.4 Quality analysis

To examine the quality of the mangosteen pericarp by looking at the fingerprint of the phytochemical profile on TLC and separating those chemicals using a mobile solvent.

2.4.1 Detection and scanning

The CAMAG TLC scanning 3 and VisionCats 1.2 software (CAMAG) were used to densitometry the alpha-mangostin. The densitometric scanning was carried out in absorbance mode at a scanning speed of 40 mm/s, with a light source of deuterium and tungsten at 317 nm. Under 254 and 366

nm UV-transluminescent and documentation, the compound fingerprint of the mangosteen pericarp was observed.

2.5 Quantity analysis

A genuine alpha-mangostin standard was utilized as an indication on the TLC sheet, and a standard curve at various concentrations was used to calculate the amount of the medication we were interested in.

2.5.1 Alpha-mangostin standard preparation

Standard alpha-mangostin stock solution was produced in methanol at 1 $mg.ml^{-1}$ and diluted separately for working standard solution at 62.5, 125, 187.5, 250, and 312.5 $mg.ml^{-1}$, all of which were held at 4°C until use. The calibration curve was applied three times, then developed and scanned as described previously. The regression equation for the alpha-mangostin was calculated after the calibration curve was produced by plotting average peak areas vs the corresponding amounts.

2.6 DPPH activity reaction test

To examine the reaction of the alpha-mangostin found on the TLC plate to the DPPH was done for the antioxidant reaction, the separated fingerprint TLC plate was sprayed with 0.5 percent DPPH in methanol, which causes the material to turn yellow after 5 minutes at room temperature in the dark.

2.7 Statistical analysis

The standard calibration curve was used to estimate the amount of alpha-mangostin. The range of interest intensity (ROI) area of antioxidant activity on TLC plates was calculated using ImageJ software and integrated density sums of all pixels (Rueden et al., 2017). The statistical analysis was carried out and graphs were generated using the statistical software Prism 9® (Graphpad, CA, USA), which included a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A probability level of less than 0.05 was used to define statistical significance.

3. RESULTS

3.1 Quality analysis

3.1.1 Fingerprint optimization of chromatographic conditions

The mangosteen pericarp methanol extracts could be separated as toluene: acetonitrile: ethyl acetate: glacial acetic acid in the ratio 35:5:15:0.15, the fingerprint of the extract revealed the alpha-mangostin at Rf 0.67, compared to the authentic standard, which could be identified under the 254 and 366 nm UV wavelengths (Figures 4A and 4B). The phytochemical fingerprint has the same pattern characteristics as the others, however, there are variances in intensity under 254 nm UV light for all the methanol extracts (Figure 4A). The noteworthy characteristics of the mangosteen from Khiri Wong (LS1) and adjacent (LS2, LS3, and LS4 from Lansaka) reflected the district intensity at Rf 0.2, 0.25, and 0.57 (Figure 4B) differ from the sample from PK and CU at 366 nm and could not be seen at 245 nm. Spraying the NP-PEG reagent over all of the chemical constituent fingerprints on the TLC plate, which appeared yellow to light orange, was used to evaluate the flavonoid group (data not shown).

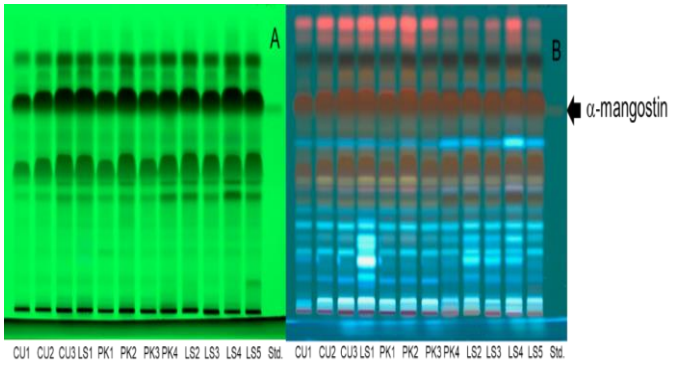

Figure 4: Phytochemical fingerprint of the mangosteen pericarp methanol extracts. A: the fingerprint under 254 nm UV-transluminescent, B: the fingerprint under 366 nm UV-transluminescent. CU1, CU2, and CU3 are the samples collected from Cha-Uat district, LS1, LS2, LS3, LS4, and LS5 are the samples collected from LanSaka district, PK1, PK2, PK3, and PK4 are the samples collected from Prom Kiri district. Std. is alpha-mangostin authentic standard and arrow indicated for the position of the alpha-mangostin.

3.1.2 Linear and range

The calibration curve was displayed in Figure 5A as a linear model, with a correlation coefficient (R2) of 0.9931. The alpha-mangostin standard had a concentration range that was pure, free of impurities and related compounds (Figure 5B). To show the characterization, the alpha-mangostin chromatogram was observed at a max wavelength of 317 nm (Figure 5C). The extracts' sample chromatogram was shown at Rf 0.67 ± 0.05. To fit in a region of the calibration curve, the extract was diluted by ten times, removing the low intensity of another element in the extract. The extracts' characteristic chromatogram appeared to be identical to the actual standard (Figure 5C).

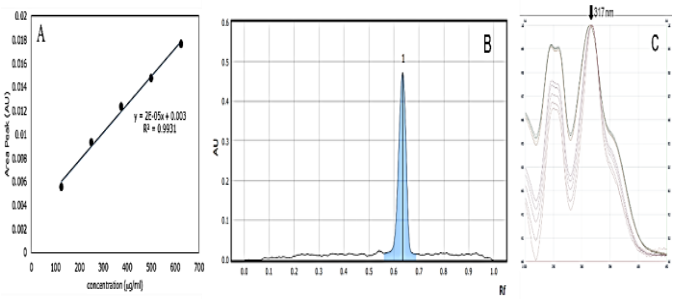

Figure 5: Alpa-mangostin standard calibration curve (A) and densitogram of alpha-mangostin scanned at λ = 317 nm (B) and spectrodensitogram of alpha-mangosteen scanned at λ range form 200-700 nm (C).

3.2 Quantity analysis

For the alpha-mangostin, the area peck of the scanning chromatogram versus the corresponding amounts was determined, as well as the regression equation. The average amount of alpha-mangostin content in pericarp extract was statistically examined using a one-way analysis of variance with a p-value of 0.05, and there was no significant difference between the sample and the location (Figure 6A). The average quantity of alpha-mangostin in pericarp extract was highest in LS4 (0.2482±0.2539 mg/100g) and lowest in PK1 (0.1908±0.1773 mg/100g) (Figure 7B). In the CU, LS, and PK districts, the average alpha-mangostin content was 0.2174±0.1857 mg/100g, 0.2310±0.1994 mg/100g, and 0.1997±0.1681 mg/100g, respectively (Figure 6B).

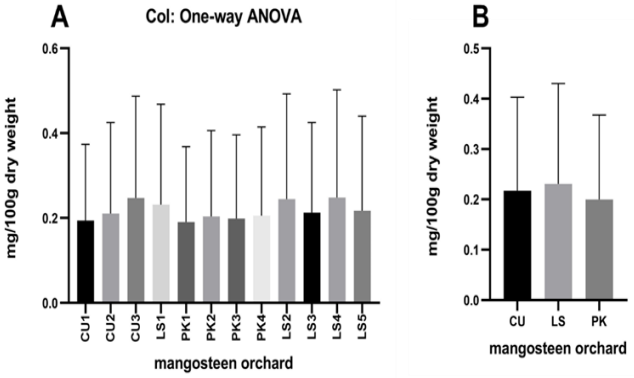

Figure 6: The amount of alpha-mangostin in mangosteen pericarp extract. (A) bar graph represents the mean amount of alpha-mangostin of 12 orchards. (B) bar graph represents the mean amount of alpha-mangostin of the orchards from the three districts.

3.3 Antioxidant activity

ImageJ was used to compute the area of the DPPH reaction on the TLC plate for the ROI intensity region of antioxidant activity relative to alpha-mangostin (Figure 7). The ROI intensity of alpha-mangostin in the pericarp of mangosteens from all 12 plantations was highest in LS4 orchard and lowest in CU1 orchard. Utilizing one-way ANOVA statistical analysis, the average intensity of the ROI of the DPPH response was investigated, and there was found to be a significant difference, as well as additional multiple-way ANOVA using Tukey's multiple comparison test and found to be significantly different (Figure 8A). The average intensity of CU, LS, and PK, which revealed significant differences between CU and LS, CU and PK, but not between LS and PK (Figure 8B). The average ROI intensities of CU, LS, and PK extracts are 26,512±6.04, 35,275.2±3.67, and 32,328±0.21, respectively (Figure 8B).

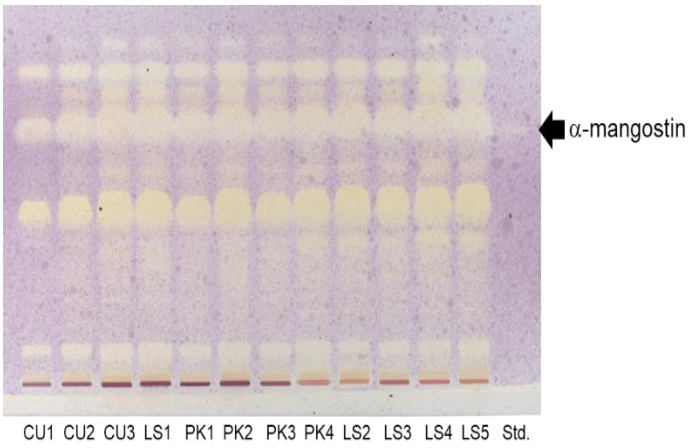

Figure 7: Phytochemical fingerprint of the mangosteen pericarp methanol extracts and the reaction to DPPH on TLC plate. CU1, CU2, and CU3 are mangosteen pericarp samples collected from Cha-Uat, LS1, LS2, LS3, LS4, and LS5 are the mangosteen pericarp from Lanska, PK1, PK2, PK3, and PK4 are the mangosteen pericarp from Prom Kiri. The Std. is alpha-mangostin authentic standard and arrow indicated for the position of the alpha-mangostin.

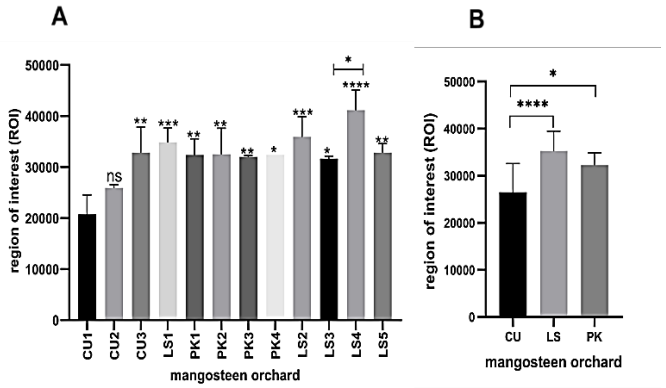

Figure 8: The alpha-mangostin labeling region of interest (ROI) intensity area of the mangosteen pericarp extract. (A) bar graph represents the mean content of the ROI of 12 mangosteen orchards. (B) bar graph represents the mean content of ROI of the orchards from three districts. The ^{ns} = $p > 0.05$, *= $p < 0.05$, ** = $p < 0.025$, *** $p < 0.001$, **** $p < 0.0001$.

4. Discussion

Mangosteen pericarps are used as raw materials for a variety of goods and claims that it contains vital chemicals, alpha-mangostin has become extremely popular. It's the product's principal ingredient. The invention of alpha-mangostin has simplified the detection of materials on a TLC sheet, which is a technique for comparing the quality and quantity of a substance of interest to a reference substance on a sheet containing many samples at the same time. TLC was utilized to explore active chemicals in the mangosteen pericarp, which were evaluated using standard curves that could be analyzed in several sample volumes at the same time. As a result, we have both quality in terms of the presence or absence of critical components, as well as a comparable standard that can immediately inform us if the samples we evaluated contain the compounds we need.

We performed alpha-mangostin on a TLC plate to test for preparation and to demonstrate its essential compounds. The solvent-mobile system can separate the alpha-mangostin from other phytochemicals at the R_F 0.67±0.05 compared to the previous study which indicated the alpha-mangostin at 0.37-0.46 using the toluene, ethyl acetate, and formic acid in various ratios (Kusmayadi et al., 2019; Kusmayadi and Adriani, 2018). According to the experiment, other molecules will be detectable beneath alpha-mangostin. This could be a valuable solvent system for screening additional compounds discovered in the pericarp of the mangosteen. In this investigation, there was no statistically significant difference in alpha-mangostin content between the 12 orchards and locations. The alpha-mangostin content in this experiment ranged from 0.1908 to 0.2482 mg/100g dry weight, according to the methanol extract and similar extraction technique, and other before was 4.67-31.50 ng/mg (w/w) (Abdallah et al., 2016; Andayani and Ismed, 2017; Chewchinda and Vongsak, 2019).

Microwave-prepared methanol extracts, on the other hand, were found to have a level that was two times higher than this study. However, the content of prior articles was determined using the standard's calibration curve from HPLC procedures (Kongkiatpaiboon et al., 2016; Rivero & Garibay, 2019; Yodhnu et al., 2009). For antioxidant activity, the antioxidant activity was calculated using the reaction area. The reactive ROI to DPPH expressed on the TLC plate varies greatly between the 12 orchards and locations. The intensity could indicate the strength of the antioxidant activity or the high concentration of the chemical that reacted with DPPH. According to the standard method for investigating the DPPH radical scavenging assay, the crude extract was used to calculate the percentage of scavenging, while the reaction on the TLC plate was used to express the intensity of the reaction, which was correlated to the position of the interested compound and could be seen in separate fingerprints (Ben Mansour et al., 2016; Ghosh et al., 2013).

The method for detecting such qualities on the TLC sheet, on the other hand, is a quick and straightforward way to observe a clear comparison. TLC procedures are simple, quick, and cost-effective, and the findings may be read promptly. However, there may be limitations on where alpha-mangostin can be found. There could be other compounds present, causing the DPPH reaction to being erroneous. In this experiment, screening for reactivity to DPPH versus standard produced preliminary data on samples reacting to DPPH compared to numerous samples at once and maybe a method to help solve this problem. The influence of screening alpha-mangostin quality and quantity on farmers and entrepreneurs selling their commodities may be that scientific procedures are more reliable.

5. Conclusions

In order to determine the quality and amount of alpha-mangostin in mangosteen pericarp, the TLC method was used. It was only able to compare fingerprints and the intensity of compounds identified in mangosteen pericarp in one of the 12 samples. In addition to being able to analyze the essential components of interest, multiple samples can be gathered at simultaneously. The substance content in the TLC research was 2 - 100 µL, which is a very little amount of the active component we were looking for, in the nanograms per 100 milligrams dry extract range. Antioxidant screening tests on the TLC sheet of the component of interest are instantly recognizable and may be compared directly. At the absolute least, it matches the location of the alpha-mangostin we're looking for, and it could lead to the discovery of more DPPH-reactive sites, allowing for more investigation. It is the foundational information used to make conclusions about subsequent research, whether it is in-depth research or a product review. The presence of active chemicals in mangosteen pericarp was confirmed using the TLC method. Continue to support producers in the sector, community, or community enterprise, as well as boost customer confidence in the sector, community, or community enterprise.

Authors Contribution

SS assisted with this study's research, data analysis, locating a publication to publish in, and finally writing and formatting the manuscript. PR was in charge of the research, mapping, and GIS gathering for the location. SP aided with the study and statistical analysis of the data. All of the authors were concerned about the research, reporting, article writing, editing, and, finally, permission for publishing.

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Khiri Wong, Lan Saka. This research had no such engagement from any funding source(s).

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