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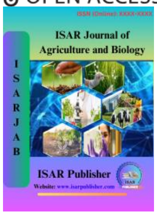

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Biochemical Profiling of Tropical Fruit Arils: Extraction and Quantification of Pigments and Nutrients

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Abstract: The investigation was on the bio profiling of selected tropical fruits for extraction and quantification of pigments and nutrients present in them. The study revealed significant variations in the biochemical constituents and pigments of different tropical fruit arils. The total phenolic content ranged from 1300 to 2200 mg GAE per 100 g, with longan and nutmeg arils exhibiting the highest levels. Anthocyanin content varied between 0.162 and 14.82 mg, with nutmeg aril containing the highest amount. Total carotenoid levels ranged from 0.294 to 5.758 mg, with longan showing the highest concentration. Protein content varied from 0.010 to 0.022 mg, being most abundant in pomegranate, while starch content ranged from 0.0062 to 0.0093 mg, with nutmeg containing the highest amount. The findings indicated that arils are rich in anthocyanins, proteins, phenols, and starch, offering various health benefits, including antioxidant, anti-inflammatory, and immunity-boosting properties. Coloured arils were found to contain higher anthocyanin levels, while white fleshy arils were richer in carotenoids. The consumption of arillated fruits was observed to serve as a natural remedy for depression, stress reduction, and fatigue relief, reinforcing their importance in a healthy diet.

Keywords: Aril, pigments, tropical fruits, biochemical profiling, phenol, starch, protein, cacao, longan, nutmeg. Passion fruit, pomegranate.

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Introduction

Tropical fruits are rich sources of bioactive compounds, including natural pigments and essential nutrients that contribute to their vibrant colours and health benefits. The arils, or edible portions of these fruits, contain a diverse array of carotenoids, flavonoids, anthocyanins, and other phytochemicals, which play significant roles in antioxidant activity and disease prevention. The extraction and quantification of these biochemical constituents are crucial for understanding their nutritional value, industrial applications, and potential health benefits. In nutmeg, the pericarp of non-translucent fruits contained higher levels of nitrogen, calcium, potassium, and boron compared to fruits with translucent arils (Pechkeo, 2007). Since calcium played a crucial role in maintaining cell wall integrity (Helper, 2005), its low content weakened the cell walls of the aril, making them unable to resist water influx. Foliar application of calcium chloride and boric acid increased the ratio of normal to translucent aril in mangosteen fruits, while top cutting, soil mulching, and calcium application also reduced the incidence of translucent arils (Chiarawipa, 2002).

Safa and Khazaei (2003) reported that the aril was the edible portion of pomegranate fruits, constituting 50–70% of the total fruit weight. It contained approximately 10% sugar (mainly fructose and glucose), 1.5% organic acids (including ascorbic, citric, and malic acids), and bioactive compounds such as anthocyanins and other phenolic compounds. Sudjaroen et al. (2012) observed that the skin and aril color of pomegranate genotypes depended on the cultivar, growing region, and ecological conditions during fruit maturation and ripening. Consequently, variations in skin color and thickness, water content, taste, and hardness were significant distinguishing features among different pomegranate genotypes.

The phenol contents and the antioxidant activities of 12 Chinese longan cultivars were studied (He et al., 2009). The polyphenols of longan pericarp and seed demonstrated significantly higher antioxidant capacities than did the pulp. The ferric reducing antioxidant powers of longan fruits were closely correlated with the polyphenol contents. The aril part of this passionfruit is a good source of ascorbic acid (vitamin C) and carotenoids, which are strong antioxidants. It has a rich flavor and is pleasantly aromatic (Lopez-Vargas et al., 2013; Seixas et al., 2014). The objectives of the study were to analyse the morphology of different fruit arils from tropical plants, extract pigments from the arils of fruits, and quantitatively evaluate selected biochemical constituents of the extracts. This study aims to explore efficient extraction methods and provide insights into the composition of pigments and nutrients in tropical fruit arils, contributing to the growing field of food science and natural product research.

Materials and Methods

The fruit arils for the present study were collected from Ernakulam and Nilambur which were the edible parts of the fruit. The fruits were collected and brought to the laboratory (Table 1). First of all, observed and studied the fruit morphological features like size, shape and colour. Photographs of the collected fruits were taken for further documentation. Aril from the fruit was separated.

Table 1: Fruits used for the study;

	Common Name	Scientific Name	Uses
1	Pomegranate	*Punica granatum*	Edible, used as medicine
2	Passion Fruit	*Passiflora edulis*	Edible, highly nutritive
3	Cacao	*Theobroma cacao*	Edible
4	Nutmeg	*Myristica fragrans*	Edible
5	Longan	*Dimocarpus longan*	Edible

The arils were subjected to pigment extraction, for estimation of chlorophyll a, b, total chlorophyll and anthocyanin. Estimation of phenol, protein and starch was also conducted to characterize the aril composition of fruits selected for study.

Pigment Extraction

A 0.5g portion of fruit aril was weighed, then ground into a fine pulp using a mortar and pestle while gradually adding 20ml of 80% acetone. The prepared sample was collected in a centrifuge tube and centrifuged for 3 minutes at 3000 rpm. The supernatant was then transferred into a labeled test tube. Following this, 1 ml of the centrifuged extract was transferred to another test tube and diluted to a total volume of 4 ml using 80% acetone. The absorbance of the solution was then measured at wavelengths of 645 nm, 662 nm, and 520 nm.

Estimation of Chlorophyll

Chlorophyll estimation was carried out by following protocol of Arnon (1949)

mg chlorophyll a /g of tissue=12.7 (A 662)-2.69(A 645) ×V/1000×W

mg chlorophyll b /g of tissue=22.9 (A645)-4.68(A 662) ×V/1000×W

mg Total chlorophyll / g of tissue=20.2 (A 645) +8.02(A 662) ×V/1000×W

Carotenoid = [1000(A 470) +3.27(Chl a-Chl b)/freshweightofsample×229] ×volume

Estimation of Anthocyanin

Quantification of Total Anthocyanin Content was done using the formula suggested by Lao and Giusti (2015). Total Anthocyanin content (mg/100g) =(100×A×DF×V) / 98.2 × X. Where,100 and 98.2 =A constant that takes the extinction coefficient and unit of conversions into consideration. A =Absorbance of sample at 535nm, DF =Dilution factor, V = The known volume anthocyanin extract was made up after extraction(ml) and X = The weight of tissue used for extraction(g).

Estimation Of Total Phenolic Content

The total phenol contents of different fractions of aril extracts prepared from different edible fruits and were determined using the modified version of Folin-Ciocalteau method. A 1g sample of tissue was weighed, and 10 ml of 50% ethanol was added. The mixture was homogenized using a motor and pestle. It was then centrifuged at 5000 rpm for 5 minutes, and the supernatant was collected. A 1 ml portion of the supernatant was mixed with 2 ml of distilled water. Subsequently, 1 ml of Folin reagent was added, and the mixture was incubated for 3 minutes. After incubation, 2 ml of 20% Na_2CO_3 solution was introduced to the sample, followed by mixing using a vortex mixer. The mixture was then placed in a boiling water bath for 1 minute. After cooling, the absorbance was measured at 650 nm using a spectrophotometer. A standard graph was prepared using different concentrations of phenol.

Determination Of Total Protein Content

The extracts obtained were subjected to protein quantification using Lowry's method. In this procedure, the protein sample was allowed to react with Folin's reagent and copper sulfate, forming a blue-colored complex. The color intensity was measured using a spectrophotometer at 660 nm. The concentration of the unknown sample was then determined from the standard or calibration graph, which depicted the absorbance values of a known protein sample. A standard graph was prepared using Bovine Serum Albumin as the protein sample.

Reagent A consisted of 2% sodium carbonate in 0.1N sodium hydroxide. Reagent B was prepared using 0.5% copper sulfate ($CuSO_4 \cdot 5H_2O$) dissolved in 1% potassium sodium tartrate. Fresh solutions were prepared by mixing stock solutions as needed. Alkaline copper solution, referred to as Reagent C, was obtained by combining 50 ml of Reagent A with 1 ml of Reagent B just before use. Diluted Folin's reagent, known as Reagent D, was prepared by diluting Folin-Ciocalteu reagent with an equal volume of 0.1N NaOH.

A 1 ml portion of the sample was pipetted out, and 5 ml of reagent C was added to all the test tubes, including the labeled blank and unknown. The contents of the tubes were mixed thoroughly by vortexing or shaking and were then left to stand for 10 minutes. Following this, 0.5 ml of reagent D was added rapidly, with immediate and thorough mixing. The tubes were incubated at room temperature in the dark for 30 minutes. Finally, the absorbance was recorded at 660 nm against the blank.

Estimation of Total Starch Content

Lugol's iodine solution was prepared by dissolving 10 g of potassium iodide in 100 ml of distilled water. Then, 5 g of iodine crystals were gradually added while shaking the mixture. The solution was filtered and stored in a tightly stoppered brown bottle. To carry out the procedure, 1 g of the sample was ground with 10 ml of distilled water using a motor and pestle. Afterward, 2 ml of starch solution and 2 ml of distilled water were added, followed by the addition of 0.5 ml of Lugol's iodine. The solution was thoroughly mixed, and its absorbance was recorded at 620 nm.

Results and Discussion

The biochemical profiling of tropical fruit arils revealed notable variations in the concentrations of chlorophyll, carotenoids, and anthocyanins, highlighting the unique pigment compositions of each fruit. Nutmeg exhibited the highest chlorophyll content, with chlorophyll a at 0.09624 mg/ml and chlorophyll b at 0.1985 mg/ml, far exceeding other samples. This suggests a higher photosynthetic pigment concentration, potentially influencing the colour and metabolic functions of the nutmeg aril. Carotenoid content varied significantly across the fruits, with pomegranate (9.42 mg/ml) showing the highest concentration, followed by longan (5.758 mg/ml) and cocoa (3.316 mg/ml). These pigments contribute to the vibrant hues and antioxidant properties of the fruits, reinforcing their potential nutritional benefits. The analysis also demonstrated a wide range of anthocyanin levels, with nutmeg (14.82 mg/ml) showing the highest concentration. In contrast, passion fruit displayed the lowest anthocyanin content (0.1629 mg/ml). Anthocyanins play a crucial role in the deep red and purple coloration of certain fruits and possess strong antioxidant properties, which may support anti-inflammatory effects and cardiovascular health.

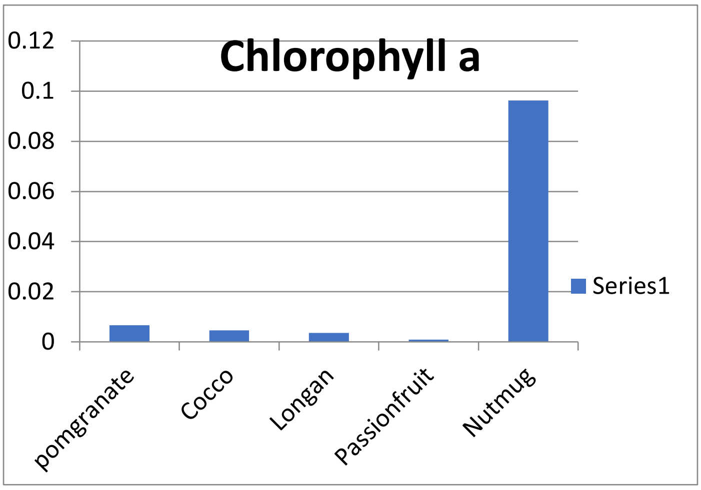

Fig 1: Chlorophyll a content in fruit arils

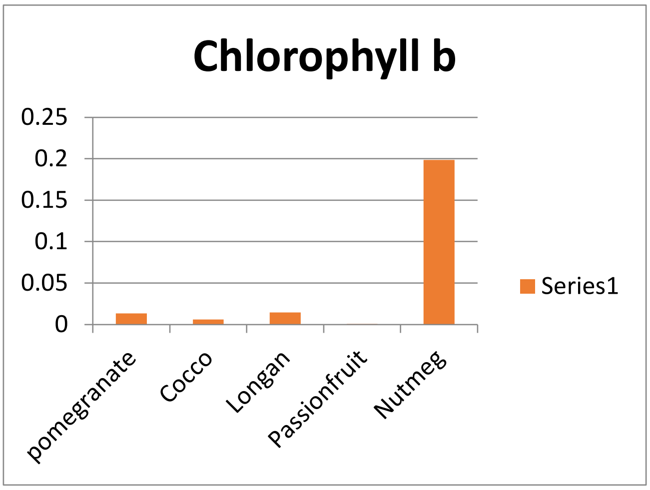

Fig 2: Chlorophyll b content in fruit arils

This biochemical variability suggests that tropical fruit arils differ widely in their pigmentation, which may impact their nutritional profiles, health benefits, and potential applications in food and pharmaceutical industries. Given their rich bioactive compound content, colored arils with high anthocyanin levels may offer enhanced antioxidant support, while white fleshy arils, richer in carotenoids, could be beneficial for vision and immune health. Overall, these findings emphasize the importance of including a variety of arillated fruits in the diet, as their distinct biochemical compositions contribute to various health-promoting properties such as boosting immunity, reducing inflammation, and alleviating stress and fatigue. Further studies could explore specific interactions between these compounds and human health, enhancing the understanding of their functional benefits.

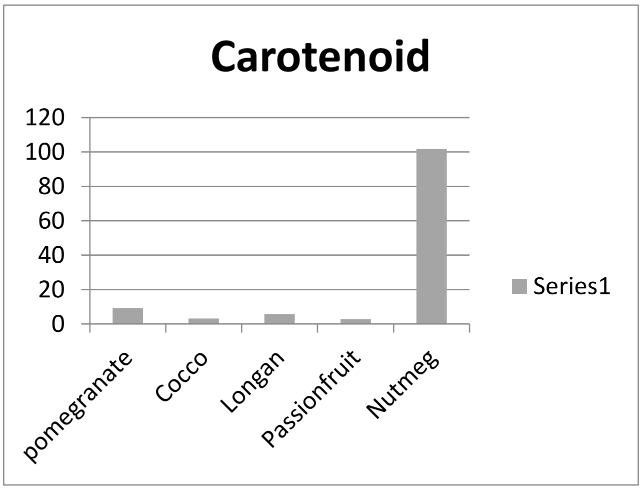

Fig 3: Carotenoid content of arils

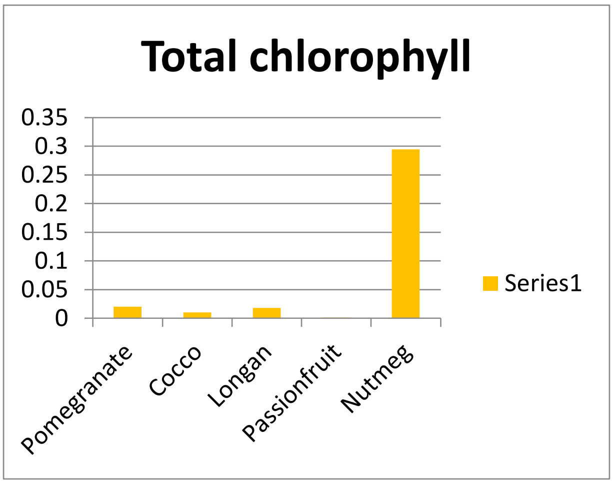

Fig 4:Total chlorophyll of arils

The phenolic content varied significantly across different tropical fruit arils, with longan (2160 mg/100g) exhibiting the highest concentration, followed closely by nutmeg (2040 mg/100g). These results suggest that longan and nutmeg arils may possess strong antioxidant properties, contributing to their potential health benefits. Pomegranate also demonstrated **a** high phenolic concentration (1700 mg/100g), reinforcing its well-documented role in cardiovascular health and anti-inflammatory effects. Cocoa and passionfruit showed relatively lower phenolic levels, with cocoa containing 1540 mg/100g and passionfruit the lowest at 1340 mg/100g**.** While these values are comparatively lower, they still contribute to the overall antioxidant capacity of these fruits. Phenolic compounds are known for their ability to neutralize free radicals, reduce oxidative stress, and promote overall well-being. Given their high phenolic content, these fruits could play a significant role in preventing chronic diseases and supporting immune function**.** The findings further emphasize the nutritional significance of incorporating a diverse range of arillated fruits into a balanced diet.

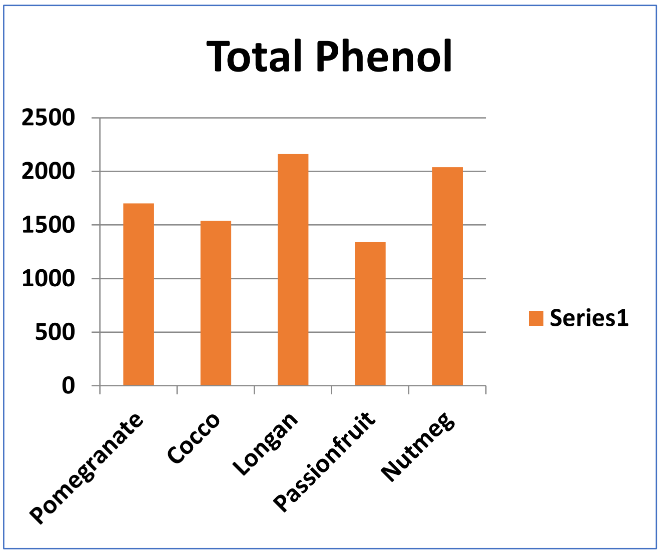

Fig 5: Total phenol of arils

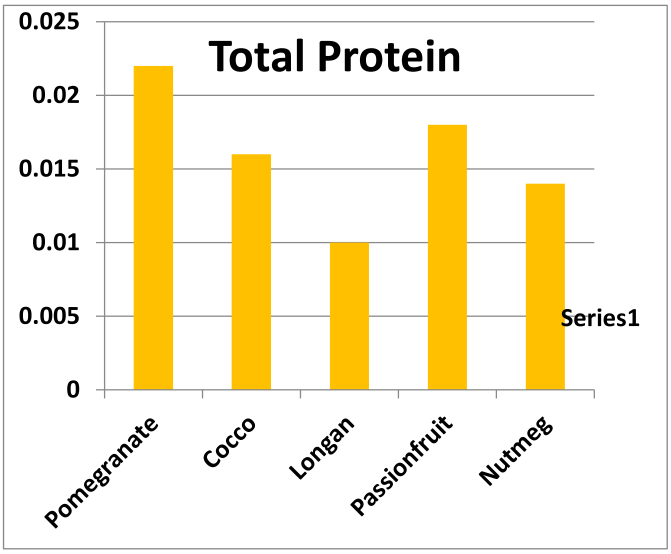

Fig 6 : Total protein of arils

The protein content in tropical fruit arils varied across different samples, with pomegranate exhibiting the highest concentration at 0.022 mg per 100 grams. This suggests that pomegranate arils could serve as a good dietary source of protein, contributing to muscle maintenance and metabolic functions. Passionfruit and cocoa also demonstrated moderate protein levels, at 0.018 mg per 100 grams and 0.016 mg per 100 grams, respectively. Their protein content, while lower than pomegranate, still plays a role in overall nutritional value, supporting cell repair and enzyme functions. Meanwhile, longan had the lowest protein concentration at 0.010 mg per 100 grams, followed by nutmeg at 0.014 mg per 100 grams. While these fruits are not primarily recognized for their protein content, they may offer other bioactive compounds and micronutrients beneficial to health. The variation in protein levels highlights the diverse biochemical composition of tropical fruit arils, suggesting that including a range of these fruits in the diet can enhance nutritional intake and support various health benefits.

The starch content varied across different fruit arils, with nutmeg having the highest concentration at 0.0093 mg per gram**,** followed by pomegranate (0.0089 mg per gram)**.** These values suggest that nutmeg arils contain more stored carbohydrates, which might influence their texture and nutritional profile. Cocoa and passionfruit showed moderate starch levels, at 0.0082 mg per gram and 0.0085 mg per gram, respectively. Their starch content contributes to their energy-providing properties and may affect their culinary applications. Longan exhibited the lowest starch concentration at 0.0062 mg per gram**,** indicating a lower carbohydrate content compared to the other samples. Despite this, longan is known for its high carotenoid concentration, making it valuable in terms of other nutrients. The variation in starch levels across these fruit arils suggests differences in their textural properties, digestion rates, and potential health benefits. Fruits with higher starch content may serve as energy sources**,** while those with lower starch levels might be preferred for their lighter, more fibrous composition**.**

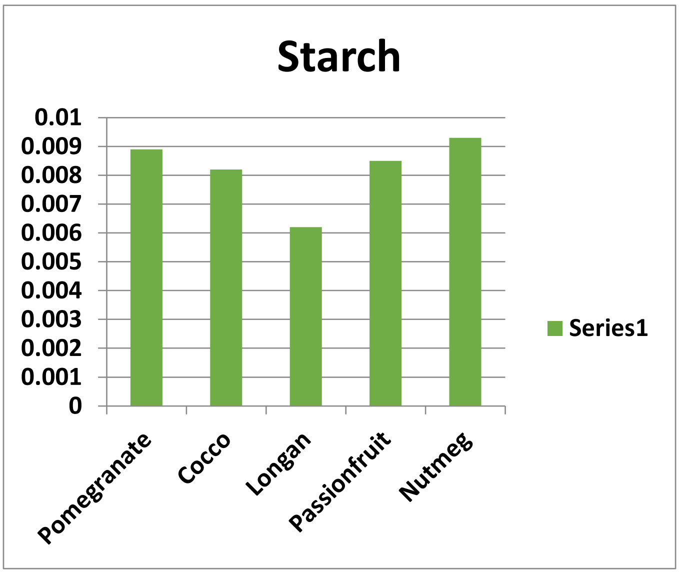

Fig 7: Total starch of arils

Summary and Conclusion

Fruits were recognized as sources of essential nutrients that nourished and enriched the human body with various mineral elements and vitamins, including potassium, dietary fiber, and vitamin C. The aril, an accessory covering of certain seeds, typically developed from the seed stalk and sometimes constituted over 52 percent of the total fruit weight, containing compounds such as sugars, anthocyanins, phenolics, vitamins, proteins, and minerals. Arils appeared in different colors depending on the fruit. The study of edible fruits with arils helped determine their quality, as they contained antioxidants and phenols. This project examined the pigment and biochemical composition of arils from different edible fruits, including pomegranate, cocco, longan, passionfruit, and nutmeg. The analysis involved the extraction of various pigments, including chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and anthocyanins, along with the quantitative evaluation of biochemical constituents such as phenols, proteins, and starch in fruit aril extracts. The investigation revealed significant variation in the biochemical constituents and pigments of different tropical fruit arils. The total phenolic content ranged from 1300 to 2200 mg GAE per 100 g, with longan and nutmeg arils exhibiting the highest levels. Anthocyanin content varied from 0.162 to 14.82 mg, with nutmeg aril containing higher anthocyanin levels than other samples. The total carotenoid content ranged from 0.294 to 5.758 mg, with longan showing the highest concentration. Protein content varied between 0.010 and 0.022 mg, with pomegranate being the richest source, while starch content ranged from 0.0062 to 0.0093 mg, with nutmeg containing the highest amount. The findings indicated that arils contained high levels of anthocyanins, proteins, phenols, and starch, which contributed to various health benefits in humans. They served as sources of fiber, antioxidants, and anti-inflammatory compounds, and helped boost immunity. Colored arils were found to contain high anthocyanin levels, whereas white fleshy arils were richer in carotenoids. The consumption of arillated fruits was observed to act as a natural remedy for depression, stress reduction, and fatigue relief. Studies suggested that the inclusion of arillated fruits in the diet contributed to a healthier lifestyle.

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