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Description of cover page

Garcinia quaesita Pierre, locally known as “Rath Goraka”, is an endemic member of the Clusiaceae family, occurs naturally in the lowland tropical rainforests of Sri Lanka. This evergreen hardwood tree has pendulous branches and typically attains a height of approximately 20 meters. *G. quaesita* Pierre exhibits a biannual flowering cycle, producing flowers and fruits. Its pinkish-white flowers emerge from the axils of upper leaves. The fruit of *G. quaesita* Pierre is small and globose with 7-13 deep grooves containing 6-8 seeds. The fleshy pericarp is initially green and sour, the young fruits mature into juicier and red. Analysis reveals the fruit’s richness in organic acids, primarily non-volatile, which confer notable antiseptic properties. It also contains glucose (15%), pectin, tartaric acid (10.6%), and phosphoric acid, mainly as calcium triphosphate. Moreover, the rind contains 16-30% (dry weight) of Hydroxyl Citric Acid (HCA), which competes with citric acid for the use as a substrate by citric acid lyase, inhibiting the synthesis of fatty acids.

(Photo courtesy: Hansaka Bandara, Sri Lanka)

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Research Article

Evaluation of phytochemical composition and novel bioactivities from fresh fruit of *Garcinia quaesita* Pierre

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ABSTRACT

The fruit rind of *Garcinia quaesita* Pierre is known as a condiment with therapeutic potential, including antioxidant, antihyperglycemic, and anti-proliferative effects. However, there has been a lack of in-depth investigation into its phytochemical composition and its activities related to anti-inflammatory, and anti-tyrosinase activities. In this study a 1:1 ethanol : water extract of fresh fruit of *Garcinia quaesita* was used. Preliminary phytochemical screening revealed the presence of numerous bioactive compounds, including alkaloids, polyphenols, flavonoids, glycosides, coumarins, anthraquinones, steroids, terpenoids, saponin, and xanthoproteins. The Total Phenolic Content (TPC) of the fruit extract of *G. quaesita* was 3053.25 ± 30.07 µg GAE g^{-1}, and Total Flavonoid Content (TFC) was 443.19 ± 4.76 µg CE g^{-1}. The extract exhibited moderate antioxidant activity (IC_{50} 238.14 ± 0.68 µg mL^{-1}) compared to ascorbic acid. In anti-inflammatory assays, compared to *O*-acetyl salicylic acid, the extract showed notably higher inhibitory effects against egg albumin denaturation and heat-induced hemolysis with lower IC_{50} values of 762.49 ± 1.70 µg mL^{-1} and 617.66 ± 1.75 ìg mL^{-1} respectively. Correlation analysis highlighted strong positive correlations between TPC and antioxidant (r=0.801) and anti-inflammatory (r=0.725 and r=0.605) activities, highlighting the role of TPC. At 4 mg mL^{-1}, the fruit extract of *Garcinia quaesita* Pierre exhibited lower inhibitory activity of tyrosinase with an IC_{50} value of 3.31 ± 0.04 mg mL^{-1}, than kojic acid. Additionally, the SPF value at 2 mg mL^{-1} was 15, indicating moderate UV protection. In conclusion, this study suggests the potential use of fresh *G. quaesita* fruit extract for functional foods and cosmetics, owing to its rich phytochemical composition and bioactivities, especially in antioxidative and anti-inflammatory domains.

Keywords: *Garcinia quaesita,* rath goraka, anti-inflammatory, anti-tyrosinase, UV protection

Abbreviations: *Garcinia* sp., Sri Lanka, biological activities, phytochemicals

INTRODUCTION

Garcinia quaesita Pierre, which is locally known as Rath Goraka, an endemic species belonging to the family Clusiaceae, occurs naturally in the lowland tropical rainforests in Sri Lanka (Pushpakumara *et al.,* 2007). It is an evergreen, hardwood tree with pendulous branches that typically reaches a height of around 20 meters. The fruit of *G. quaesita* is small and globose in shape with 7-13 deep grooves and the edible fleshy pericarp (rind) is filled with 6-8 seeds (Orwa *et al.,* 2009). In its initial stage, the young fruits are green in colour and sour in taste, which transforms into a juicier and red fruit as it ripens. The fruits are edible, but not consumed raw due to its high acidity levels. Dried

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fruit rinds of *G. quaesita* possess astringent and purgative properties (Liyanagamage *et al.,* 2020b). Decoction of fruit rinds are used internally to alleviate bowel complaints, chronic dyspepsia and rheumatism, while externally utilized as a wash for ulcers and a gargle for addressing weak and spongy gums (Pushpakumara *et al.,* 2007; Orwa *et al.,* 2009; Liyanagamage *et al.,* 2020b). In addition, fruit rind serves as a stomachic when taken internally, proving beneficial for anorexia (Orwa *et al.,* 2009; Liyanagamage *et al.,* 2020b). Studies have shown that hexane extract of dried *G. quaesita* fruit rind possesses antioxidant, antihyperglycemic and anti-proliferative properties (Pathiranage *et al.,* 2021; Liyanagamage *et al.,* 2020a,b). Bioassay-guided fractionation of hexane extract of *G. quaesita* has resulted isolation of Garcinol which has exhibited a remarkable antioxidant capacity exceeding ten-fold that of L-ascorbic acid and a moderate antihyperglycemic activity (Liyanagamage *et al.,* 2020a,b). Compositional analysis of the fruit has indicated that approximately 90% of its content comprises non-volatile compounds, primarily organic acids. Notably, hydroxy citric acid accounts for 16-30% of the fruit's composition, followed by tartaric acid at 10.6%, and phosphoric acid in the form of calcium triphosphate. Additionally, 15% of the fruit's composition is glucose, along with the presence of pectin (Pushpakumara *et al.,* 2007).

Nevertheless, many therapeutic effects and phytochemical profile of the fruit rind of *G. quaesita* remain relatively unexplored. To address this knowledge gap, the present study was carried out to analyze novel bioactivities associated with the hydroethanolic extract of fresh fruit rind of *G. quaesita*. This study contributes to a better understanding of the potential health-promoting properties of *Garcinia quaesita* fruit, thereby facilitating its effective utilization.

MATERIALS AND METHODS

Raw materials and sampling

Matured fresh fruits of *Garcinia quaesita* Pierre were collected in August 2021 from Mawathagama, located in the Northwestern Province of Sri Lanka (latitude: 7.4322° N, longitude: 80.4438° E). The fruit samples were authenticated by the Food Research and Development Institute at Kananwila, Horana.

Procedures

Preparation of the extract

The fruits were washed with tap water and then with deionized water to remove any dirt. The edible part of the fruit was separated from the seed, cut into small pieces, then ground to a pulp using a mechanical grinder. The constituents in the ground pulp of the edible part of the fresh fruit (200 g) was extracted into a mixture of water:ethanol (1:1, 5 L) by sonication at 40 °C for 2 hours. The resultant extract was then filtered using a cheesecloth. The crude material was re-extracted twice using ethanol [50% (v/v), 1 L] by sonication at 40 °C for 1 hour. The resultant extract was then filtered using a cheesecloth. The combined filtrates were concentrated using a rotary evaporator at temperature of 40 °C –50 °C, and then lyophilized to give a yield of 30 g of dry extract. The dry extract was purged with nitrogen and stored at 4 °C until further use. The freeze-dried extract was reconstituted with distilled water to give desired concentrations used in this study.

Preliminary phytochemical screening

The phytochemical compounds in the hydroethanolic extract of the *G. quaesita* fruit were evaluated using the following standard methods. They were carried out on 2% aqueous and organic extracts.

Test for alkaloids

Two drops of Wagner's reagent (prepared by dissolving 0.04 g of Iodine, 0.06 g of potassium iodide in 3 mL of distilled water) was added to 1 mL of the extract along the sides of the test tube. Appearance of reddish-brown colour is an indicative sign of the presence of alkaloids (Iqbal *et al.,* 2015).

Test for polyphenols

A few drops of 5% solution of lead acetate were added to 1 mL of extract. The appearance of yellow precipitate indicates the presence of polyphenols (Balamurugan *et al.,* 2019).

Test for flavonoids

A few drops of concentrated hydrochloric acid and

magnesium turnings were introduced to 0.5 mL of the extract. Immediate development of a red or pink colour is an indicative sign of the presence of flavonoids (Yadev *et al.,* 2011).

Test for anthocyanins

Two mL of aqueous extract was taken to which few drops of 2 N HCl was added, followed by the addition of ammonia, the conversion of pink-red into blue-violet indicates the presence of anthocyanins (Balamurugan *et al.,* 2019).

Test for cardiac glycosides (Keller-kilani test)

Two mL of extract was mixed with about 0.4 mL of glacial acetic acid containing traces of ferric chloride and 0.5 mL of conc. H_2SO_4 was added. A brown ring at the interphase indicates the presence of cardiac glycosides (Yadev *et al.,* 2011).

Test for anthraquinone glycosides

Few drops of conc. H_2SO_4 were added to 5 mL of extract, followed by the addition of 1 mL of diluted ammonia. The appearance of rose pink confirms the presence of anthraquinones (Iqbal *et al.,* 2015).

Test for coumarins

Three mL of 10% aqueous solution of NaOH was added to 2 mL of the extract. The production of yellow colour indicates the presence of coumarins (Balamurugan *et al.,* 2019.).

Test for tannins

A test tube was loaded with 5 mL of the extract, followed by the addition of 2 mL of 5% $FeCl_3$ solution. The appearance of a greenish-black precipitate is an indicative sign of the presence of tannins in the extract (Iqbal *et al.,* 2015).

Libermann-burchard's test for steroids and terpenoids

A solution containing the extract (50 mg) was mixed with 2 mL of acetic anhydride. Then, a gradual addition of 1 to 2 drops of concentrated sulfuric acid was performed, carefully down the inner wall of the test tube. The formation of a brown ring at the intersection of the two layers and turning the upper layer to green shows the presence of steroids. The formation of deep red colour indicates the presence of triterpenoids (Iqbal *et al.,* 2015).

Test for saponins

A small amount of the extract (5 mg) was diluted with distilled water to 10 mL. The suspension was shaken vigorously. The formation of frothing, which persists on warming in a water bath for 5 min is an indicative sign of the presence of saponins (Iqbal *et al.,* 2015).

Test for xanthoproteins

A few drops of nitric acid and ammonia were added to 1 mL of the extract. Reddish brown precipitate indicates the presence of xanthoproteins (Balamurugan *et al.,* 2019).

Determination of total phenolic content

Total soluble phenols in the samples were determined using the "Folin Ciocalteu" reagent. 1.25 mg mL^{-1} solution of gallic acid was prepared by dissolving 40 mg of the gallic acid powder in 10 mL solution consisting of 10% DMSO and 50 mM phosphate buffer solution. This initial solution was used to make a series of the following concentrations 0.25, 0.50, 0.75 and 1.00 mg mL^{-1} to obtain an external calibration curve. The extract/gallic acid standard (0.1 mL) was mixed with 0.15 mL of Folin Ciocalteu reagent and kept in dark for 5 minutes at room temperature, followed by the addition of 0.5 mL of 6% sodium carbonate and 0.4 mL of distilled water. This mixture was kept in dark for 1 hour at room temperature and the absorbance was measured at 765 nm using a microplate reader (Biotek, USA). The total phenolic content was calculated as µg mL^{-1} gallic acid equivalent (µg mL^{-1} GAE) by using gallic acid calibration curve (Hayat *et al.,* 2020).

Determination of total flavonoid content

Flavonoids present in the samples were determined by using $AlCl_3$ colourimetric method. The external calibration was created by using 500 µg mL^{-1} of catechin standard solution from which a serial dilution was performed to obtain a series of the concentrations 31.25, 62.50, 125 and 250 µg mL^{-1}. A volume of 0.2 mL of sample/standard was mixed with 0.05 mL of distilled water and 10 µL of 5% $NaNO_2$ and then the mixture was kept in the dark for 5 minutes. Next 15 µL of 10% $AlCl_3$ was added to the mixture, and it was kept in

dark for 6 minutes. Thereafter, 0.05 mL of 1 moldm^{-3} NaOH and 0.05 mL of distilled water were added to the mixture. The absorbance was measured at 510 nm using a microplate reader (Biotek, USA). The total flavonoids content was calculated as µg mL^{-1} catechin equivalent (µg mL^{-1} CE) by using catechin calibration curve (Eghdami and Sadeghi, 2010).

Evaluation of antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *Garcinia quaesita* fruit extract was determined according to the method described by Chatatikun and Chiabchalard with slight modifications (Chatatikun, 2013). Concentration series of the samples/ standard (ascorbic acid) consisting of 1000, 500, 250, 125, 62.5, 31.25, and 15.62 µg mL^{-1} were prepared using water. Sample/standard (200 µL) was added to DPPH (1 mg/mL, 50 µL) and incubated for 30 min in the dark at room temperature. Absorbance was measured at 517 nm using a microplate reader (Biotek, USA). The % inhibition was calculated using the equation equation (Chatatikun and Chiabchalard, 2013).

$$\%\ of\ inhibition\ =\ 100\left[1-\frac{A_{sample}}{A_{control}}\right] \quad \ldots (1)$$

where $A_{control}$ and A_{sample} are the absorbance values of the control and the sample respectively. Using the plot of % inhibition vs log concentration, IC_{50} value was calculated.

Determination of anti-inflammatory activity by egg albumin denaturation assay

2.5 mL solution was made which consisted of 1.4 mL of freshly prepared phosphate buffered saline of pH - 6.3, 1 mL of specific concentration of standard/extract, 0.2 mL of egg albumin extracted from hens egg. Specific concentrations were prepared separately for *Garcinia quaesita* as 200, 400, 600, 800 and 1000 µg mL^{-1} and 1 mL of distilled water was used as negative control while specific concentrations of 200, 400, 600, 800 and 1000 µg mL^{-1}. *O*-acetyl salicylic acid was used as the positive control. Then the mixtures were heated in water bath at 37ºC for 15 minutes and the temperature gradually increased up to 70ºC at which the samples were retained for 5 minutes. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm (Hasan *et al.,* 2019).

Percentage inhibition of protein denaturation was calculated by using the equation 1. Using the plot of % inhibition vs log concentration, IC_{50} value was calculated.

Determination of anti-inflammatory activity by HRBC membrane stabilization assay

Preparation of human red blood cells (HRBC) suspension

The assay was carried out according to the method described by Sakat *et al.* (2010) with slight modifications. Human blood was collected from healthy volunteers who had not taken any anti-inflammatory drug for 2 weeks prior to the experiment. The blood was centrifuged at 3000 rpm rate in heparinized centrifuge tubes and the supernatant was removed. This was repeated with an equal volume of normal saline until the supernatant was colorless. The red blood cells were reconstituted to a 10% v/v suspension with normal saline.

Heat induced haemolysis

HRBC (10% v/v, 200 µL) were added to 2.0 mL of different concentrations of the sample and standard anti-inflammatory drug *O*-acetyl salicylic acid (200, 400, 600, 800, 1000 µg mL^{-1}) in normal saline (consisting of 0.9% NaCl). The extracts were initially dissolved in DMSO (10%). The mixture without the plant extract was used as the negative control. The assay mixtures were gently mixed, incubated in a water bath at 56ºC for 30 minutes and cooled under running tap water. They were centrifuged at 3000 rpm rate and the absorbance of each supernatant was measured at 560 nm using a microplate reader (Biotek, USA). Each series was triplicated (Prasanth *et al.,* 2013). Percentage inhibition of haemolysis was calculated by using the equation 1. Using the plot of % inhibition vs log concentration, IC_{50} value was calculated.

Anti-tyrosinase assay

Preparation of reagents

Preparation of phosphate buffer solution (50 mM, pH 6.5)

50 mM phosphate buffer of pH 6.5 was prepared by dissolving 1.7011 g of KH_2PO_4 solid in 250 mL of

deionized water. The solution was placed in an ice bath and pH was adjusted to pH 6.5 using the pH meter.

Preparation of L-DOPA solution (12 mM)

12 mM solution of L-DOPA was prepared into a conical flask covered with aluminium foil by dissolving 59.16 mg of solid in 25 mL of 10% DMSO in 50 mM phosphate buffer (pH 6.5).

Extraction of tyrosinase enzyme

50 g mass of freshly plucked oyster mushroom was torn and transferred into a blender followed by the addition of 100 mL of 50 mM phosphate buffer. The mixture was filtered using a cheesecloth into a beaker immersed in an ice bath. The filtrate was centrifuged for 10 minutes at 3500 rpm under cold conditions. The supernatant was then transferred into a beaker, covered, and was stored in an ice bath (Velichkova *et al.,* 2015).

Enzyme activity assay

The activity of the enzyme solution was determined by following the formation of dopachrome spectrophotometrically at 30ºC. After the addition of 600 µL enzyme extract to a cuvette containing 1.4 mL of 50 mM phosphate buffer and 2.20 mL of L-DOPA, the solution was immediately mixed and the increase in the absorbance at 475 nm (indicating the formation of dopachrome) was recorded with the microplate reader. The initial rate was calculated from the linear part of the progress curve (over the first 2-3 min). One unit of tyrosinase was defined as the amount of the enzyme catalyzing the oxidation of 1 µmol L-DOPA to dopachrome per minute at 30ºC. The extinction coefficient of dopachrome at 475 nm was 3600 M^{-1} cm^{-1}.

Determination of anti-tyrosinase activity

Kojic acid was used as the positive standard. A 4.0 mg/mL solution of the standard/sample was prepared by dissolving 40 mg of the solid in 10 mL of 10% DMSO in 50 mM phosphate buffer solution from which a serial dilution was done to make a series of the following concentrations (4.0, 2.0, 1.0, 0.5, 0.25) mg mL^{-1}.

1.40 mL volume of each different concentration was added to test tubes followed by 0.60 mL of tyrosinase enzyme. Similarly, a negative control was prepared by using 1.40 mL of phosphate buffer in place of the standard/sample. The solution mixtures were incubated at 30ºC for 5 minutes after which 2.20 mL of L-DOPA was added. The mixtures were incubated at room temperature for 30 minutes and the amount of Dopachrome produced was measured by obtaining the absorbance at 475 nm wavelength using the microplate reader (Biotek, USA) (Koodkaew and Sukonkhajorn, 2019).

Percentage inhibition of tyrosinase enzyme was calculated by using the equation 1. Using the plot of % inhibition vs log concentration, IC_{50} value was calculated.

Determination of sun protection factor (SPF) values of both standard and sample

All fractions were dissolved in water to prepare a solution of 2.0 mg mL^{-1}. Reference standard (a commercial sunscreen protection cream (SPF 30+) was also dissolved in water to obtain the same concentration solution. Water was used as the blank solution. The absorption data of sample/standard were obtained in the range of 290 nm to 320 nm at every 5 nm, and the determinations were made at each point, followed by the application of Mansur equation given below (Mbanga *et al.,* 2014).

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad \ldots (2)$$

Where, CF – correction factor (= 10)
EE – erythemal effect spectrum
I – solar intensity spectrum
Abs – absorbance of sunscreen product

The values of EE x I are constants and obtained using Table 1.

Statistical analysis

All analyses were carried out in triplicates and values were presented as mean ± standard error of the mean (SEM). GraphPad Prism Data Editor for Windows, Version 10.0 (GraphPad software Inc., San Diego, CA) was used for the calculations of IC_{50} values of standards and extracted samples by plotting inhibition % vs log concentration.

Table 1: Normalized product function used in the calculation of SPF (Mbanga *et al.,* 2014)

Wavelength (λ/nm)	EE x 1 (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

RESULTS AND DISCUSSION

The percentage of extractive yield of the fresh fruit of *Garcinia quaesita* was found to be 15%. The preliminary phytochemical analysis of the extract indicated the presence of alkaloids, polyphenols, flavonoids, glycosides, coumarins, tannins, anthraquinones, steroids, terpenoids, saponin, and xanthoproteins while anthocyanins were found to be absent in the fresh fruit extract. The TPC in the extract was found to be 3053.25 ± 30.07 µg GAE g^{-1}. The total flavonoid content in the extract was found to be 443.19 ± 4.76 µg CE g^{-1}. The antioxidant activity of the extract by DPPH radical scavenging ability, was found to be moderate (Table 2) with IC_{50} value of 238.14 ± 0.68 µg mL^{-1} compared to the standard ascorbic acid.

The aqueous ethanol extract of fresh fruit rind of *G. quaesita* exhibited notable anti-inflammatory activity as assessed through the egg albumin denaturation method, which serves as an indirect measure of inflammation. The results revealed a dose-dependent relationship, with percentage inhibitions increasing in proportion to extract concentrations (Table 3). Across all concentrations tested, ranging from 200 to 1000 µg mL^{-1}, the fruit extract of *G. quaesita* at 1000 µg mL^{-1} demonstrated a higher inhibition activity at 57.80 ±1.13%, surpassing that of the standard sample, *O*-acetyl salicylic acid, which showed inhibition activity at 51.72 ±1.03%. The calculated IC_{50} value for the *G. quaesita* sample was notably lower (762.49 ±1.70 µg mL^{-1}), compared to the standard which exhibited IC_{50} value of 966.74 ±2.32 µg mL^{-1}.

According to the anti-inflammatory activity by heat induced haemolysis assay, the results revealed a dose-dependent relationship, with percentage inhibitions increasing in proportion to extract concentrations (Table 4). Across all concentrations tested, ranging from 200 to 1000 µg mL^{-1}, the fruit extract of *G. quaesita* at 1000 µg mL^{-1} demonstrated a superior inhibition activity at 70.62 ± 2.14%, compared to the standard sample, *O*-acetyl salicylic acid, which showed inhibition activity at 53.66 ± 3.36%. The calculated IC_{50} value for the *G. quaesita* sample was notably lower (617.66 ± 1.75 µg mL^{-1}), whereas the standard exhibited IC_{50} value of 931.79 ±2.88 µg mL^{-1}.

Table 2: Percentage inhibition by the standard and the *Garcinia quaesita* Pierre sample vs concentration (µg mL^{-1}) in DPPH assay

Concentration (µg/mL)	Percentage inhibition of the standard	Inhibition % of the *Garcinia quaesita* Pierre sample
500	96.05 (±0.39)	61.59 (±0.43)
250	93.80 (±0.42)	52.32 (±0.96)
125	93.10 (±0.15)	43.95 (±0.41)
62.50	91.38 (±0.20)	38.44 (±0.41)
31.25	91.00 (±0.05)	35.13 (±0.92)
15.625	89.52 (±0.03)	33.56 (±0.92)

Table 3: Percentage inhibition by the standard and the *Garcinia quaesita* Pierre sample vs concentration (µg mL^{-1}) in egg albumin denaturation assay

Concentration (µg/mL)	Percentage inhibition of the standard	Inhibition % of the *Garcinia quaesita* Pierre sample
200	18.68 (±4.35)	37.19 (±2.40)
400	28.39 (±3.47)	45.32 (±2.09)
600	38.80 (±1.44)	48.80 (±1.12)
800	45.51 (±1.33)	52.46 (±1.76)
1000	51.72 (±1.03)	57.80 (±1.13)

Table 4: Percentage inhibition by the standard and the *Garcinia quaesita* Pierre sample vs concentration (µg mL^{-1}) in heat induced haemolysis

Concentration (µg/mL)	Percentage inhibition of the standard	Inhibition % of the *Garcinia quaesita* Pierre sample
200	25.11 (±4.33)	41.16 (±2.81)
400	31.49 (±2.43)	42.96 (±2.22)
600	37.16 (±1.18)	48.57 (±1.47)
800	49.71 (±3.13)	59.65 (±0.48)
1000	53.66 (±3.36)	70.62 (±2.14)

Table 5: Pearson's Correlation Coefficient (r) among TPC, TFC with DPPH antioxidant activity and anti-inflammatory activity assays

	1/ IC_{50} value for DPPH	1/IC_{50} value of heat induced haemolysis inhibition	1/ IC_{50} value of egg albumin denaturation
TPC (μg mL^{-1})	0.801	0.725	0.605
TFC (μg mL^{-1})	0.316	0.237	0.313

The relationship between TPC and TFC with various bioactivities of *G. quaesita* fruit extract was examined. The Pearson's correlation coefficient (r) was used to measure the strength of correlation between variables and ranges between -1.00 and +1.00. Table 5 provides the Pearson's correlation coefficients among TPC, TFC and DPPH scavenging activity and anti -inflammatory activity assay.

It was found that there was a positive correlation between TPC and the antioxidant activity as measured by the DPPH radical scavenging assay (r=0.801). Similarly, there was a positive correlation between TPC and anti-inflammatory activity as assessed through heat-induced hemolysis (r= 0.725) and egg albumin denaturation (r= 0.605). These findings suggest that the observed antioxidant and anti-inflammatory activities of the *G. quaesita* fruit extract are primarily attributed to its TPC rather than its TFC.

According to the tyrosinase inhibitory assay, at a concentration of 4 mg mL^{-1}, the *G. quaesita* sample exhibited a lower percentage inhibitory activity (78.54 ± 0.11%) compared to the standard kojic acid sample (88.36 ± 0.06%) (Table 6). The IC_{50} value for the extract was found to be 3.31 ± 0.04 mg mL^{-1}, whereas for kojic acid, it was 0.86 ± 0.22 mg mL^{-1}.

In terms of the SPF value determination for the fresh fruit extract of *G. quaesita*, it displayed an SPF value of 15 at a concentration of 2 mg mL^{-1}. Consequently, it can be concluded that fresh fruit extract of *G. quaesita* possesses a moderate level of UV protection capability.

Table 6: Percentage inhibition of tyrosinase enzyme by the standard and the *Garcinia quaesita* Pierre sample vs concentration (μg mL^{-1})

Concentration (μg/mL)	Percentage inhibition of the standard	Inhibition % of the *Garcinia quaesita* Pierre sample
4.00	88.36 (±0.06)	78.54 (±0.11)
2.00	69.21 (±0.17)	49.36 (±0.27)
1.00	58.27 (±0.22)	41.21 (±0.31)
0.50	42.99 (±0.30)	25.79 (±0.59)
0.25	31.78 (±0.36)	12.85 (±0.66)

CONCLUSION

In this study, we conducted a comprehensive analysis of the fresh fruit extract of *G. quaesita*, assessing its phytochemical composition and bioactivities such as antioxidant, anti-inflammatory, anti-tyrosinase and sun protection ability. The investigation revealed a significantly higher total phenolic content (TPC) in the extract, compared to the total flavonoid content (TFC). The antioxidant activity, assessed through DPPH radical scavenging, displayed a moderate level compared to ascorbic acid. The investigation into anti-inflammatory activity of the fruit extract through egg albumin denaturation and heat-induced hemolysis assays revealed a dose-dependent activity. Notably, the *G. quaesita* fruit extract exhibited significant inhibition activity, even surpassing that of the standard, *O*-acetyl salicylic acid, with lower IC_{50} values, highlighting its potential to be used as a potent anti-inflammatory agent. Moreover, correlation analysis revealed a positive relationship between TPC and antioxidant, as well as anti-inflammatory activities, emphasizing the pivotal role of TPC in these effects. However, compared to kojic acid, *G. quaesita* exhibited lower inhibitory activity at 4 mg mL^{-1}, suggesting reduced efficacy as a skin-lightening agent. In sun protection, the extract demonstrated an SPF value of 15 at 2 mg mL^{-1}, indicating moderate UV protection ability.

These findings indicate promising possibilities for utilizing the fresh fruit extract of *G. quaesita* in medicine and skincare products. Further investigation is necessary for identification of bioactive compounds, elucidation of their mechanisms of action, and exploration of potential therapeutic applications.

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Conflict of interest

The authors declare that there are no competing interests.

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Research Article

Formulation of muskmelon seed and flaxseed multi-nuts spread and their characterization

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ABSTRACT

Nuts are a good source of nutrients that are a decent well spring of energy, protein, omega unsaturated fats, calcium, and phosphorus which are vital for the advancement of developing kids and provide essential nutrients that are substantially useful for the human body and reduce the possibility of heart-related problems. The present research on nutrient evaluations of multi-nut spread was carried out 2022 to standardize simple, nutritional blends by utilizing peanut, almond cashew, walnut, chironji, muskmelon seeds, and flaxseed. Honey and chocolate flavors were used for sweetness and taste. The formulation of five samples from peanuts, almonds, and cashew nuts were prepared in various concentrations in the ratios of A: (5:4:3), B: (4:3:5), C: (3:5:4), D: (1:1:1), and E: (2.5:4:4), respectively. In sample E, milk was added but the rest of the samples were made in the absence of milk. In all samples, walnut, chironji, flaxseed, muskmelon seed, and honey are used in equal quantities. Samples D and E were selected based on organoleptic evaluation. The composition of sample D is peanut, almond, cashew are added in the ratio (1:1:1) while walnut and chironji are added ¼th (one-fourth) part of the almond/peanut/cashew. Apart from that, flaxseed and muskmelon seeds were added to half the quantity of chironji or walnut. In sample E all ingredients are added in equal ratios similar to sample D except peanut (less amount) and milk. Sample D and E have the highest acceptability after the analysis of the product. The major results of Samples D and E contain protein content of 16.32% and 15.89%, fat content of 46% and 49%, and moisture content of 11.8% and 16% respectively. Therefore, the formulations can be utilized for the population and development of such functional food that would be beneficial to improve the general health population and health-conscious people.

Keywords: Nutrition, seed, flavor, energy, health benefits

INTRODUCTION

The multi-nut spread created was wealthy in proteins, omega fats, calcium, and phosphorus. The proportion of omega 6 and omega 3 unsaturated fats was extremely near the fitting proportion of 4: 25: 1. They are usually eaten whole, either raw, roasted, or salted, or as components of

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many processed meals, including spreads, baked goods, and confections. Additionally, tree nut oils are utilized in cooking, salad dressings, and as flavoring agents, among other things. Moreover, certain skin moisturizers and cosmetic items contain tree nut oils. (Kaur and Maruf, 2018). Ghosal *et al*. (2022) examined that spreads derived from nuts and seeds have been utilized in place of dairy butter. Cozentino *et al.* (2022) reported the development of the potential function of the chocolate spread containing prebiotics and structural triglycerides. It has been noted that after 180 days of storage, there were no discernible variations between the samples at room temperature (Cevoli *et al*., 2022).

The healthy spread contains antioxidant properties. Sweet potato, beetroot, peanut, guava, and sugar variations were used to optimize the healthful and nutritious spread. Healthy and nutrient-rich spreads have a two-day shelf life at room temperature and a fourteen-day shelf life in the refrigerator. Various nuts and oil seeds were combined to create a multi-nut salad. The production of a nut spread can increase the consumer's interest in a healthier breakfast or snack food and expanding the dietary usage of peanuts, almonds, and cashew nuts (Kumar *et al.,* 2023).

A large portion of individuals have a preference for better food things, sweet spreads like jams, preserves, and cocoa spreads which are undesirable on account of their high satisfaction with starches (for the most part sugars) and fats and low happiness of proteins and micronutrients. Items like peanut butter are accessible on the lookout, which goes under the classification of sweet spreads, however, individuals have less knowledge about the medical advantages it has contrasted with other sweet spreads. Nuts and seeds store polyphenolic flavonoids, and cell reinforcements such as carotenes, resveratrol, cryptoxanthin, and other health-promoting compounds (Fischer *et al.*, 2013). Nuts also reduce many diseases like cardiovascular, and neurodegenerative diseases. Raw materials such as almonds, peanuts, walnuts, cashew, chironji, resins, muskmelon seeds, flaxseed, honey, oil, and milk. Peanuts contain protein (14%), fat (22%), fiber (10%), carbohydrates (2%) respectively, and some minor nutrients like vitamin B2, vitamin B6, vitamin E, magnesium, etc. (Bonku and Yu, 2020). Almonds serve as an excellent source of essential nutrients and contain significant amounts of magnesium, manganese, copper, phosphorus, vitamin E, vitamin B2, and vitamin B6, all of which are essential for good health. They are an excellent supplement to any diet because of their high quantities of mono- and polyunsaturated fats (22%), protein (12%), and dietary fiber (14%) (Barreca *et al*., 2020).

Almonds are a nutrient-rich food that offers a number of health benefits, including a reduced risk of chronic illnesses including coronary heart disease (CHD) and type 2 diabetes, as well as maintaining weight (Lindgärde, 2000). Almonds can be used in a variety of food products and recipes, including spreads like almond butter and whole (fresh or roasted) almonds for consumption (Richardson *et al.,* 2009). Especially, when consumed as low diet, there are considerable benefits for the cardiovascular system's health and the lowering of blood cholesterol (Jenkins *et al*., 2006). Walnuts contain energy (190 calories), fat (23%), carbohydrate (1%), Dietary fiber (7%), protein (1%) and some micronutrients are present like calcium (2%), Iron (4%), potassium (2%,), magnesium and some vitamin (Hayes *et al.*, 2016). Walnuts have been utilized as a very healthy food source for a very long time in various parts of the world. Additionally, they are a vital component of the Mediterranean diet. It has been asserted that their health-promoting characteristics lower the risk of illnesses associated with lifestyle (Sánchez *et al.,* 2017). Peanuts are the most cultivated food crops in the world and have a high lipid content ranges between 47% and 50% (Carrín and Carelli, 2010).

Cashew nuts are a wonderful origin of protein (20%), fats (45%), sugars (23%), and carbs. Dietary fiber, as well as some micronutrients like calcium, potassium, magnesium, copper, and phosphorus, as well as vitamins A, and C, and their derivatives (Rico *et al*., 2016). Chironji is a low-fat substance, it has fiber content and vitamins B1, B2, and C (Phogat *et al*., 2020). In resins gum acids ($C_2OH_3OO_2$), unsaturated fats, esters of these fats, sterols, alcohols, waxes, and resins are all included (Sulaiman *et al*., 2020). The muskmelon seed contains a high level of lipid 30.6% and protein 14.9, rough fiber 23.3%, Debris 2.4% carbohydrate19.8%, etc. (Deepa and Krishnaprabha, 2016). Flaxseed has long been considered a vital edible ingredient due to its abundance of proteins, lignans, linolenic acid (ALA), and dietary fibers (Tang *et al*., 2021). In honey comprehensive list of more than 180 ingredients