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Metroxylon sagu Rottb. Fruit Flour as Potential Prebiotics for Selected Probiotics and Phytochemical Profiling of its Methanolic Extract by LC-MS/MS

(Tepung Buah *Metroxylon sagu* Rottb. sebagai Potensi Prebiotik untuk Probiotik Terpilih dan Pemprofilan Fitokimia Ekstrak Metanolnya dengan LC-MS/MS)

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ABSTRACT

The plant of *Metroxylon sago* Rottb. which is found abundantly in Indonesia, Malaysia, Philippines, and Papua New Guinea, is famous as carbohydrate producing plants. The sago fruit has not been yet extensively used, although they are rich in oligosaccharides which is potential as prebiotic. Sago fruit flour was studied as growth substrate for five selected probiotics and phytochemical profile of its methanol extracts were determined. All bacteria grew well up to 24 h on the growth medium containing sago fruit flour as carbohydrate substitute. However, *Lacticaseibacillus rhamnosus* FNCC 0099 and *Lactobacillus acidophilus* FNCC 0051 showed viability stability up to 72 h, while the others were slightly decreased. The sago fruit flour contained 20.4 mg/g total sugars, 14.8 mg QE/g flavonoids, and 36.7 mg GAE/g phenolics. After extraction of the fruit flour with 80% methanol using an ultrasonicator at 55 °C at 40 kHz for 40 min, the extract was analysed for its phytochemical profiles using *untargeted* LC-MS/MS screening with negative ionization mode. Seven compounds categorised in three distinct groups i.e., sugar alcohol, plant glycosides and fatty acids, have been identified as having possible prebiotic activity. These include *((1xi)-1,5-anhydro-2,3,6-tris-O-(carboxymethyl)-1-methyl-4-O-methyl-D-glucitol)*, *quercitrin*, *(15Z)-9,12,13-trihydroxy-15-octadecenoic acid*, *corchorifatty acid F*, *3,5-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl-hexopyranoside*, *gynocardin*, and *(4-methylumbelliferone)-β-D-glucopyranoside*. As the sago fruit flour has been proved as potential prebiotic in this study, its extensive prebiotics activity, prebiotic index, and stability under digestive condition will be subjected for further study.

Keywords: *Lactobacillus*; *Metroxylon sagu* Rottb.; phytochemical profile; prebiotic; sago fruit

ABSTRAK

Tumbuhan *Metroxylon sagu* Rottb. yang banyak terdapat di Indonesia, Malaysia, Filipina dan Papua New Guinea, terkenal sebagai tumbuhan penghasil karbohidrat. Buah sagu masih belum digunakan secara meluas, walaupun ia kaya dengan oligosakarida yang berpotensi sebagai prebiotik. Tepung buah sagu dikaji sebagai substrat pertumbuhan untuk lima probiotik terpilih dan profil fitokimia ekstrak metanolnya telah ditentukan. Semua bakteria tumbuh dengan baik sehingga 24 jam pada medium pertumbuhan yang mengandungi tepung buah sagu sebagai pengganti karbohidrat. Walau bagaimanapun, *Lacticaseibacillus rhamnosus* FNCC 0099 dan *Lactobacillus acidophilus* FNCC 0051 menunjukkan kestabilan kebolehhidupan sehingga 72 jam, manakala yang lain menurun sedikit. Tepung buah sagu mengandungi 20.4 mg/g jumlah gula, 14.8 mg QE/g flavonoid dan 36.7 mg GAE/g fenol. Selepas pengekstrakan tepung buah dengan 80% metanol menggunakan ultrasonik pada 55 °C pada 40 kHz selama 40 minit, ekstrak dianalisis untuk profil fitokimianya menggunakan saringan LC-MS/MS yang tidak disasarkan dengan mod pengionan negatif. Tujuh sebatian yang dikategorikan dalam tiga kumpulan berbeza iaitu gula alkohol, glikosida tumbuhan dan asid lemak, telah dikenal pasti mempunyai kemungkinan aktiviti prebiotik. Ini termasuk *((1xi)-1,5-anhydro-2,3,6-tris-O-(carboxymethyl)-1-methyl-4-O-methyl-D-glucitol)*, *quercitrin*, *(15Z)-9,12,13-trihydroxy-15-octadecenoic acid*, *corchorifatty acid F*, *3,5-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl-hexopyranoside*, *gynocardin* dan *(4-methylumbelliferone)-β-D-glucopyranoside*. Oleh kerana tepung buah sagu telah dibuktikan sebagai potensi prebiotik dalam kajian ini, aktiviti prebiotik yang meluas, indeks prebiotik dan kestabilan dalam keadaan penghadaman akan tertakluk untuk kajian lebih jauh.

Kata kunci: Buah sagu; *Lactobacillus*; *Metroxylon sagu* Rottb.; prebiotik; profil fitokimia

Introduction

The sago plant (*Metroxylon sago* Rottb.) grows widely and is spread throughout Southeast Asia, especially Indonesia, Malaysia, the Philippines, and Papua New Guinea. This palm species is tolerant in a wet swamp-to-peat soil environment (Mohamad Naim, Yaakub & Awang Hamdan 2016). In Aceh, Indonesia, local people usually use this fruit as an essential ingredient for various traditional foods. For example, *rujak buah, kuah sayur pliek u, kuah beulangong, sambal ganja, tasak telu, gulai kambing*, and *sie reuboh*. Daud et al. (2009) reported that the sago fruit flour, extracted using ethanol as solvent, showed a potential oligosaccharides content, i.e., raffinose (3.95%), sucrose (7.12%), and stachyose (4.56%). This high concentration of oligosaccharides is suitable for further development as prebiotic compounds.

The International Scientific Association for Probiotics and Prebiotics (ISAPP) claims that the bacteria living in the host organism selectively metabolize prebiotics as substrates, leading to potential beneficial effects on the body's general health. The term 'host' embraces humans and animals within this conceptual framework. Studies indicate that polyphenols can selectively modulate the composition of microflora selectively, hence broadening the scope of prebiotics to include non-carbohydrate compounds as well (Gibson et al. 2017). Polyphenols are a major class of secondary metabolites in many plant species. The identification of these compounds is based on the presence of aromatic rings and hydroxyl groups within their chemical structure. Considering the context of the natural environment, there is a presence of both simple phenolics and complex polymers that are distinguished by their considerable molecular mass. Studies commonly classify polyphenols into flavonoids and non-flavonoids (Brglez Mojzer et al. 2016). However, it is essential to acknowledge that only a small percentage, specifically 5-10%, of polyphenols are absorbed by the human body. Most of the remaining polyphenols interact with the gastrointestinal microbiota as prebiotics, producing antimicrobial compounds (Singla et al. 2019).

Bacterial strains from the genera *Lactobacillus*, *Lactiplantibacillus*, *Lacticaseibacillus*, and *Bifidobacteria* grow well in medium to high concentrations of polyphenols in culture media. Polyphenols serve as a carbon substrate for bacteria after the deglycosylation regulations. During metabolism, certain compounds can function as electron acceptors, such as hydroxycinnamic acid, or as sources of protons, such as gallic acid. Microbes use several processes to utilize substrates for generating energy in the form of high-energy molecules such as ATP. Microbes use oxygen (aerobic) or alternative substrates (anaerobic) as terminal electron acceptors during respiration to produce ATP. Lactic acid bacteria (LAB) can carry out anaerobic respiration by utilizing external electron acceptors, a mechanism known as extracellular electron transfer (EET). The production of ATP during respiration occurs by oxidative phosphorylation. During anaerobic metabolism, energy acquisition processes involve fermentation, which generates ATP by using organic substances as electron donors and acceptors (Kim & Gadd 2019).

When polyphenol chemicals are present, they directly affect the microbiota bacteria in the gut by turning on genes that code for enzymes that break down polyphenols. Enzymes include tannase, phenolic acid decarboxylases, gallate decarboxylase, esterase, and quercetinase. *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* grow quickly in media that are high in polyphenols because they have enzymes that break down tannin compounds (de las Rivas et al. 2019; Fritsch et al. 2016). The metabolic system of *L. plantarum*, concerning polyphenols, has received significant attention in academic studies due to its remarkable capacity to thrive on various kinds of fermented vegetables. Plaza-Vinuesa et al. (2019) reported the presence of genes encoding enzymes involved in polyphenol degradation. These enzymes are aryl glycosidase, phenolic acid decarboxylase (hcrB, lp_3665), rhamnosidase (rhaB1, rhaB2), gallate decarboxylase (lpdB, lpdC), phenolic acid reductase (lp_3125), and tanBLP (Rodríguez-Daza et al. 2021).

This study aimed to evaluate the potential of sago fruit flour as a prebiotic and to determine the phytochemical profile of its methanolic extract using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The data obtained in this research will provide new information and future considerations in the use of sago fruit as a prebiotic.

Materials and Methods

Sampel preparation

The sago fruits (*Metroxylon sago* Rottb.) were obtained from the Aceh Besar Regency market in the Aceh Province of Indonesia. The fruit used in this study was at its optimal ripeness. The initial step in processing the sago fruit was to meticulously examine its physical

condition to identify any signs of damage. Subsequently, altogether remove and cleanse the fruit's outer skin using clean water. The subsequent phase involves cutting and chopping to expedite the evaporation of the water content. The dried process involves the utilization of a dehydrator cabinet set at a temperature of 70 ºC for 3-4 h. Subsequently, the desiccated sago fruit underwent the process of grinding and filtration utilizing a 200-mesh screen, as described by Daud et al. (2009).

EXTRACT PREPARATION

The extraction procedure for sago fruit flour involved the utilization of 80% methanol (1:5 w/v) as the solvent. An ultrasonicator (Ultrasonic Bath Grant XUB25) was employed for this purpose. The extraction process was conducted at a temperature of 55 ºC, a frequency of 40 kHz, and 40 min. The pure extract was acquired using centrifugation at a velocity of 3000 × g for 15 min. Subsequently, the supernatant was isolated and dehydrated in a water bath maintained at 50 ºC. The dried extract was diluted again in an adequate amount of methanol and subsequently filtered using a 0.45 µm Whatman filter paper, as described by Jovanovic-Malinovska, Kuzmanova and Winkelhausen (2015). The formula for determining percent yield was calculated according to the following equation:

$$Percent\ yield = \frac{Extract\ weight\ (g)}{Sample\ weight\ (g)}\ x\ 100\%$$

EVALUATION OF PROBIOTICS GROWTH IN MODIFIED MEDIUM

The growth of probiotics was observed *in vitro*. The strains used in this study were *L. lactis* FNCC 0086, *L. casei* FNCC 0090, *L. plantarum* FNCC 0027, *L. rhamnosus* FNCC 0099, and *L. acidophilus* FNCC 0051. These strains were cultured in De Man, Rogosa, and Sharpe broth (MRS; Merck Millipore, Darmstadt, Germany), and modified De Man, Rogosa, and Sharpe broth (m-MRSB) media, in which the carbon source was substituted by 1 g of sago fruit flour. After growing for 24 h in MRSB medium, 1 µL of each fresh culture was inoculated into 10 mL media and incubated at 37 °C. Cell viability was determined regularly at 24, 48, and 72 h on the MRS agar media (MRS; Merck Millipore, Darmstadt, Germany).

PROXIMATE ANALYSIS

The proximate analysis included determining water, fat, protein, ash, and carbohydrate content, which was conducted using the guidelines outlined in the official reference (AOAC 2023).

TOTAL SUGAR ANALYSIS

The determination of the total sugar concentration was carried out using the phenol sulphate method (Zebua, Silalahi & Julianti 2018). The sample was dissolved in a solution consisting of 2.5 mL of sulfuric acid and 0.5 mL of a phenol solution with a concentration of 5% (w/v), resulting in a total volume of 0.5 mL. The solution was then homogenized for 10 min using a vortex at room temperature. Subsequently, the homogenous solution was heated using a water bath set at 40 °C for 20 min. After that, the solution was diluted by adding 12 mL of distilled water. The total sugar concentration was determined using an Ultraviolet-visible (UV-Vis) spectrophotometer (Infinite M200 PRO, Tecan) set at a specific wavelength of λ 490 nm.

TOTAL FLAVONOID ANALYSIS

The quantification of total flavonoids was performed utilizing the colorimetric method (Mansoori et al. 2022). The sample was dissolved by mixing 0.5 mL with a mixture of 2.8 mL of distilled water, 1.5 mL of ethanol, and 0.1 mL of $AlCl_3$ (10%). Following this, a 0.1 mL aliquot of a 1 M solution of CH_3COOK was introduced, and subsequently, an incubation period of 30 min was observed. Following that, the absorbance of the solution was determined using a UV-Vis spectrophotometer that was mainly set to a wavelength of 417 nm. The quantification of total flavonoids (ΔF) was measured in mg of quercetin (QE) per mL of extract, utilizing the following equation:

$$\Delta F = \frac{Total\ flavonoids\ from\ standard\ curve\ (mg\ quercetine\ quivalent^{\ L\text{-}1})}{Concentration\ of\ sample\ (mL\ L^{-1})\ x\ dilution\ factor}$$

TOTAL PHENOLIC COMPOUND ANALYSIS

Total phenolics were quantified using the Folin-Ciocateu method, wherein gallic acid was used as the reference standard (Calvindi, Syukur & Nurcholis 2020). The 50-µL supernatant was reconstituted by dissolving it in an

equal volume of distilled water, namely 50-µL. Then, a 50-µL volume of a 10% foline solution and a 50-µL volume of a bicarbonate solution with a concentration of 60 g/L were added. Subsequently, the resultant mixture underwent incubation at room temperature for 60 min. The spectrophotometer utilized measurements at the set wavelength of 725 nm. The following formula could have been used to find the total phenolic equivalents (ΔP), and the result was given as mg GAE/g;

$$\Delta P = \frac{Standard\ total\ phenolic\ concentration\ (mg\ L^{-1})\ x\ Sample\ Volume\ (L)}{Extract\ sample\ weight\ (g)\ x\ dilution\ factor}$$

PHYTOCHEMICAL PROFILING USING LC-MS/MS

The phytochemical profiles were assessed using the methods described by Faraone et al. (2019). The LC-MS/MS analysis was performed utilizing the Xevo G2-XS QTof equipment, using an Atlantis T3 C18 column (2.10 × 100 mm; particle size 3.00 µm). The electrospray ionization (ESI) method was used in the negative ionization mode, running at 40 ºC. The mass range that was taken into consideration for analysis ranged from 50 to 1200 *m/z*. Mobile phase A comprised a solvent solution containing 0.10% formic acid dissolved in water, whereas mobile phase B used a solvent solution consisting of 0.10% formic acid dissolved in acetonitrile. The gradient program involves the subsequent conditions: during the time interval from 0 to 1 min, a 5% concentration of B is used; from 1 to 8 min, a 40% concentration of B is used; from 8 to 11 min, a 100% concentration of B is used; from 11 to 13 min, a 100% concentration of B is maintained; and from 13 to 16 min, a 95% concentration of B is used. The previous requirements were observed at a 0.30 mL/min flow rate. The samples were prepared by dissolving 0.01 g of the substance in 1 µL of 100% methanol, followed by a further tenfold dilution. The identified compound profiles with mass error < ± 5 ppm were verified by cross-referencing them with the UNIFI Scientific Library and ChemSpider databases.

STATISTICAL ANALYSIS

The statistical software SPSS v.26 (IBM Corporation, New York, US) was utilized to analyse the *in vitro* fermentation results using a one-way ANOVA. The statistical significance was assessed at a predetermined threshold of $p<0.05$, and subsequent analysis was performed utilizing the Duncan test.

RESULTS AND DISCUSSION

THE GROWTH OF SELECTED PROBIOTICS IN MEDIUM CONTAINING SAGO FRUIT FLOUR

Figure 1 shows the growth of five selected probiotics in medium containing sago fruit flour. After 24 h of incubation, a significant increase in the accumulated quantity of probiotics was observed. All bacteria were able to grow above 6 log CFU/mL, which is the minimum requirement for probiotics. In particular, *L. lactis* FNCC 0086 and *L. casei* FNCC 0090 showed significant growth, obtaining 7.09 and 6.65 log CFU/mL, respectively.

After 24 h, *L. rhamnosus* FNCC 0099 and *L. acidophilus* FNCC 0051 showed viability stability up to 72 h, while the others were decreased. The decrease percentage, from the highest to the lowest order, was found in *L. casei* FNCC 0090 at 45.95%, followed by *L. plantarum* FNCC 0027 at 41.63%, and *L. lactis* FNCC 0086 at 38.42%. The initial pH of the m-MRSB medium (which had been modified by replacing the sugar source with sago fruit flour) was 3.12 (Figure 2). The growth test medium has a significantly low acidity, which likely due to the presence of phenolic and flavonoid components in the fruit flour (Table 1). The acidity of most flavonoids is attributed to the presence of phenolic hydroxyl groups. The acidity is determined by the quantity and arrangement of phenolic hydroxyl groups in each aromatic ring (Fuguet et al. 2023; Musialik et al. 2009;). In addition, the rumbia fruit is a variety of fleshy fruit whose degree of acidity is also affected by the existence of organic acids, specifically malic acid and citric acid (Etienne et al. 2013). Moreover, based on the phytochemical LC-MS/MS data, the quinic acid (Table 2), which was found in sago fruit, likely affected the pH of the starting medium. After 72 hours, the highest increase of pH was observed in medium with *L. lactis* FNCC 0086 at 3.26, and the lowest was in medium with *L. acidophilus* FNCC 0051 at 3.24 (Figure 2).

Probiotics are living microorganisms that, when given in sufficient quantities, provide beneficial effects on the health of the host. These bacteria have several health advantages, including directly protecting against pathogenic microorganisms and being involved in the regulation of the microbiome. For probiotics to have beneficial effects on the host, they need to be able to survive the acidic conditions of the stomach and make it to the large intestine in sufficient amounts to colonize and proliferate (Naissinger da Silva et al. 2021). According to the guidelines and recommendations of the FAO/WHO,

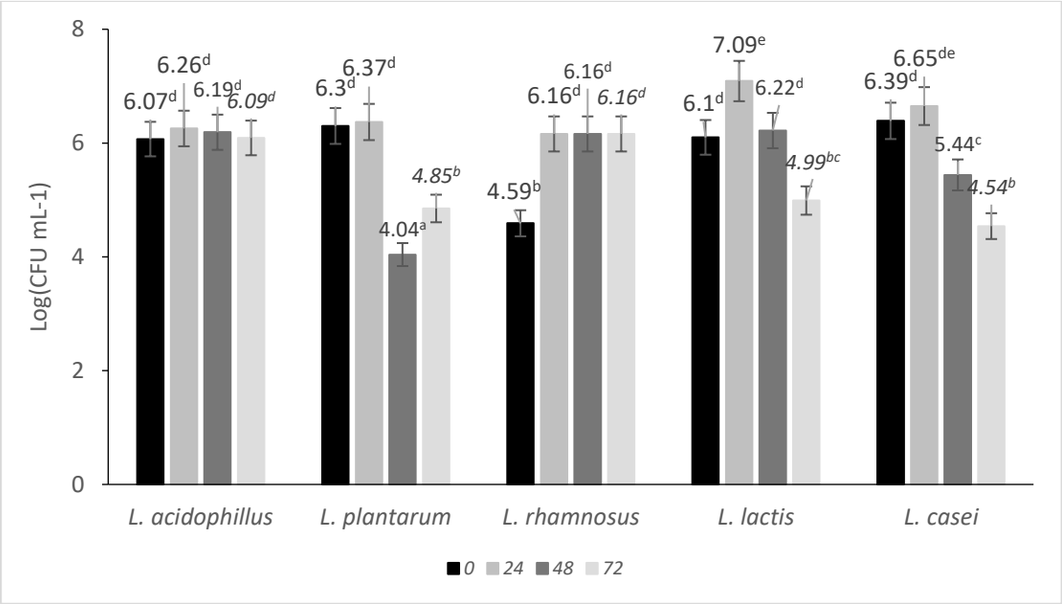

FIGURE 1. The growth of probiotics for 24, 48 and 72 h in modified De Man, Rogosa, and Sharpe broth (m-MRSB) media, in which the carbon source was substituted by 1 g of sago fruit flour. The different letters show that the results are significantly different ($p < 0.05$)

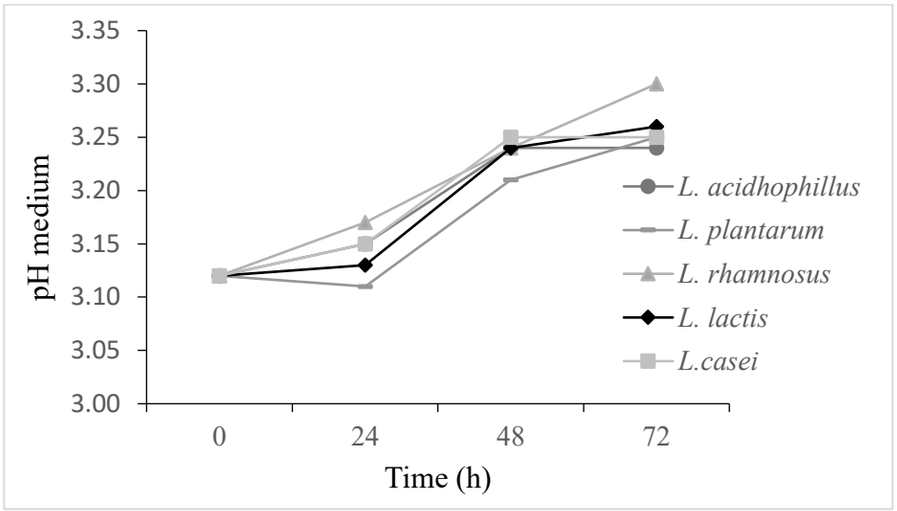

FIGURE 2. Changes in pH of in modified De Man, Rogosa, and Sharpe broth (m-MRSB) media, in which the carbon source was substituted by 1 g of sago fruit flour

probiotic products must have a minimum cell count of at least 6 log (CFU/g) or 6 log (CFU/mL), and some researchers suggest that it should be increased to 7 log (CFU/g) (Alfaro-Galarza et al. 2020). For food products with claims to health benefits, the addition of probiotics must contain viable cells of at least 6 – 7 CFU/g or in the portion to be consumed (FAO/WHO 2006).

Gao et al. (2022) identified multiple resistance mechanisms applied by LAB to survive in low pH conditions. The resistance to low pH in LAB includes membrane fluidity adjustments, safeguarding and restoration of macromolecules, reinforcement of proton pumps, synthesis of enzymes, metabolic regulation, and proton consumption. The cytoplasmic membrane is the primary barrier for solutes and maintains pH homeostasis. In a study by Huang et al. (2016), the membranes of cells that had been treated with acid had a higher ratio of cyclic fatty acids (CFA) to saturated fatty acids (SFA) than the membranes of cells that had not been treated with acid. Increased membrane disruption in an acidic environment leads to alterations in membrane fluidity, eventually causing cellular destruction. In this particular circumstance, there is an attempt to prevent the influx of protons into BAL cells by inducing alterations in membrane fluidity and modifying the composition of fatty acids. It enhances membrane hardness and density (Gao et al. 2022).

CHARACTERISTICS AND PHYTOCHEMICALS PROFILE OF METHANOLIC EXTRACT OF SAGO FRUIT FLOUR

The proximate and phytochemical contents of the methanolic extract of sago fruit flour are presented in Table 1. The ultrasonication during extraction resulted in a percentage yield of 50.5%. Previous studies reported that ultrasonication has shown yield increase and extraction efficiency (Jovanovic-Malinovska, Kuzmanova & Winkelhausen 2015). During ultrasonication, air bubbles are generated by sound waves. These air bubbles directly affect the cell wall's structure, reduce particle size and facilitate the transfer of cell components into the solvent for dissolution. Consequently, ultrasonication has several benefits, such as enhanced the extraction efficiency and speed, decreased the extraction duration, minimized solvent usage, and lowered the extraction temperature (Jovanovic-Malinovska, Kuzmanova & Winkelhausen 2015).

The proximate values obtained in this study are similar to those published by Daud et al. (2009), who reported that sago fruit contains 5.73% protein, 3.79% ash, 0.13% fat, 94.11% carbohydrates, and crude fiber 5.90%. Furthermore, Daud et al. (2009) also showed the presence of tannin and triterpenoid components in sago fruit, as well as oligosaccharides such as raffinose (3.95%), sucrose (7.12%), and stachyose (4.56%).

As shown in Table 1, the fruit flour contains 36.7 mg GAE/g phenolic compounds and 14.80 mg QE/g of flavonoids. Polyphenols are a class of secondary plant metabolites that exhibit various biological actions, including prebiotic, antioxidant, antibacterial, anticancer, and anti-inflammatory properties. It is estimated that only a small portion (<5-10%) of polyphenols is absorbed directly in the intestine, while the rest is further metabolized by the intestinal microbiota (Santos-Buelga et al. 2019). Due to its limited absorption, scientists are investigating its impact on the gut microbiota as it has the potential to stimulate the growth of beneficial microorganisms, therefore serving as a potential source of novel prebiotic (Thilakarathna, Langille & Rupasinghe 2018).

TABLE 1. Proximate of flour and phytochemicals of methanolic extract of sago fruit

Proximate composition	
Ash content	3.20%
Water content	8.65%
Carbohydrates (by difference)	83.27%
Fat content	0.22%
Protein content	4.66%
Phytochemical analysis	
Yield extract	50.5%
Total sugars	20.4 mg/g
Total flavonoid compounds	14.8 mg QE/g
Total phenolic compounds	36.7 mg GAE/g

The present study also focused on identifying chemical compounds derived from the methanolic extract of sago fruit flour. Using *untargeted* LC-MS/MS screening approach, in the negative ionization mode, 41 compounds were obtained (data not shown). Figure 3 shows the chromatogram appointing some important compounds.

Furthermore, Table 2 shows the 14 compounds, including seven potential compounds as prebiotics and seven compounds as probiotics growth factors. Seven potential prebiotics are categorized into four groups, i.e., sugar alcohol, flavonoid, plant glycosides (chromone), and fatty acids. The sugar alcohol category consists of a compound known as *(1xi)-1,5-Anhydro-2,3,6-tris-O-(carboxymethyl)-1-methyl-4-O-methyl-D-glucitol*, *Quercitrin, 3,5-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl hexopyranoside, gynocardin* are flavonoid categories. The plant glycoside (chromone) category includes *(4-Methylumbelliferone)-β-D-glucopyranoside*. Lastly, the fatty acid category encompasses compounds like *(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid* and *corchorifatty acid F*. Seven additional chemical compounds have been identified as bacterial growth factors. As shown in Table 2, these compounds are all carbohydrates.

Sugar alcohols are naturally occurring compounds found in various fruits and vegetables, such as grapes, bananas, strawberries, yellow plums, cauliflower, and lettuce. Studies have reported that certain sugar alcohols, such as xylitol, possess prebiotic properties. Sugar undergoes transit via the upper gastrointestinal system until it enters the cecum. Saccharolytic bacteria participate in the metabolic process of fermentation, leading to the synthesis of diverse by-products, including ethanol, gases such as methane, hydrogen, and carbon dioxide, as well as short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate (Lugani & Sooch 2017). Specifically, SCFA possesses several health benefits, including antimicrobial, anti-inflammatory, immunoregulatory, anti-obesity, anti-diabetes, anti-cancer, cardiovascular protective, hepatoprotective, and neuroprotective properties (Lamas et al. 2019; Xiong et al. 2022).

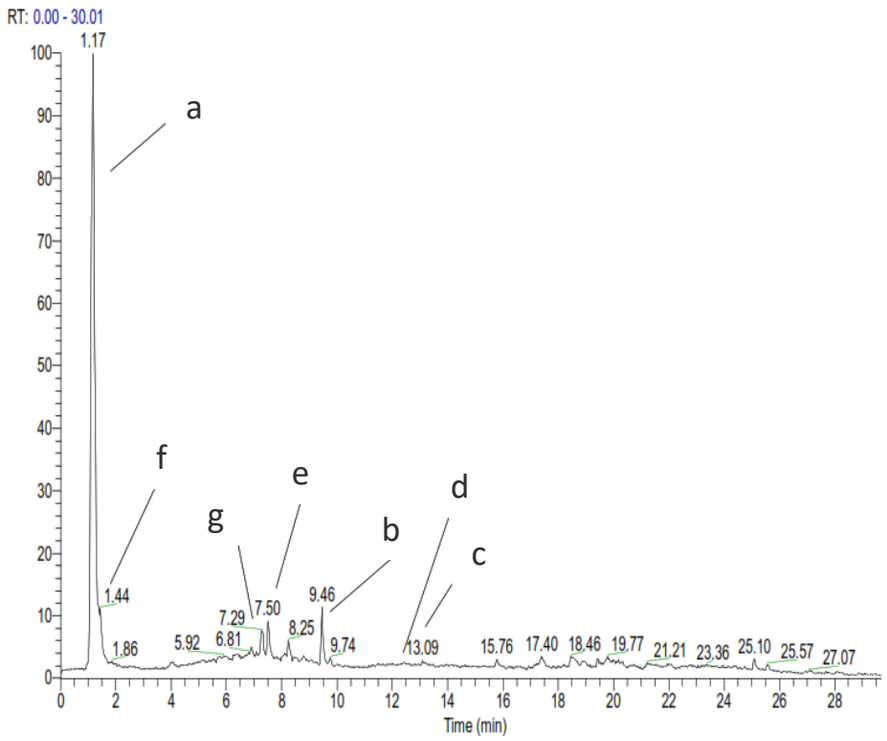

FIGURE 3. LC-MS/MS chromatogram profile negative ionization mode methanol extract of sago fruit flour with prebiotic potential: (a) *(1xi)-1,5-Anhydro-2,3,6-tris-O-(carboxymethyl)-1-methyl-4-O-methyl-D-glucitol*; (b) *quercitrin*; (c) *(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid*; (d) *corchorifatty acid F*; (e) *3,5-Dihydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl hexopyranoside*; (f) *gynocardin;* and (g) *(4-Methylumbelliferone)-β-D-glucopyranoside*

TABLE 2. LC-MS/MS phytochemical profile negative ionization mode methanol extract of sago fruit flour with prebiotic potential

No	Component name	Chemical formula	m/z	Retention Time (min)	Percent Area (%)	Categories	Reference
1	(1xi)-1,5-Anhydro-2,3,6-tris-O-(carboxymethyl)-1-methyl-4-O-methyl-D-glucitol	$C_{14}H_{22}O_{11}$	365.1084	1.175	3.89	Sugar alcohol	- Lugani et al. (2017) - Klewicka et al. (2009) - Zubrycka et al. (2020)
2	Quercitrin	$C_{21}H_{20}O_{11}$	447.0929	9.458	3,36	Plant glycoside	- Kawabata et al. (2013) - Bokkenheuser et al. (1987) - Uyanga et al. (2021) - Theilmann et al. (2017) - Landete et al. (2014)
3	3,5-Dihydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl hexopyranoside	$C_{21}H_{22}O_{11}$	449.1089	7.433	0.17		
4	Gynocardin	$C_{12}H_{17}NO_8$	302.0879	1.438	0.16		
5	(4-Methylumbelliferone)-β-D-glucopyranoside	$C_{16}H_{18}O_8$	337.0927	7.295	0.16		
6	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	$C_{18}H_{34}O_5$	329.2332	13.086	0.43	MUFA/PUFA	- Hirata et al. (2015) - Takeuchi et al. (2015)
7	Corchorifatty acid F	$C_{18}H_{32}O_5$	327.2176	12.441	0.23		
8	D-(-)-Quinic acid	$C_7H_{12}O_6$	191.0552	1.175	27.92	Sugar acid	
9	β-D-Mannopyranose, 1,3,4,6-tetraacetate	$C_{14}H_{20}O_{10}$	347.0980	1.239	6.13		
10	1-O-acetyl-a-maltose	$C_{14}H_{24}O_{12}$	383.1191	1.175	10.15	Disaccharide	- Whiting et al. (1971) - Stead (1994) - Filannino et al. (2015) - Bel-Rhlid et al. (2013)
11	6-O-Heptopyranosylglucopyranose	$C_{13}H_{24}O_{12}$	371.1191	1.175	0.80		
12	D-(+)-Galactose	$C_6H_{12}O_6$	225.0610	1.050	0.27		
13	Hept-2-ulose	$C_7H_{14}O_7$	209.0659	1.175	0.22	Monosaccharide	
14	3-Deoxy-D-manno-octulosonic acid	$C_8H_{14}O_8$	237.0610	1.175	0.20		

Polyol compounds can stimulate the proliferation of LAB and can be converted into lactic and acetic acids. The final results of metabolic processes depend on the carbon substrate utilized and the LAB culture conditions. In a study conducted by Klewicka and Klewicki (2009), it was observed that the metabolism of sorbitol by *L. casei* 0908 and *L. paracasei* 0919 resulted in the production of lactic acid at concentrations of 3.8 g/L and 4.8 g/L, respectively. Additionally, the growth of *L. casei* 0908 was directly linked to using glucose as a carbon substrate, specifically going up to 9.5 log units instead of 9.7 log units. When the *L. acidophilus* 0933 strain is present, it can grow and break down lactitol, sorbitol, and galactosylopolyols. It can also make lactic acid at concentrations between 2.5 and 4.8 g/L. Lipinska-Zubrycka et al. (2020) reported that galactosyl polyols enhance the functionality of glycolytic enzymes such as α-galactosidase, β-galactosidase, and α-glucosidase. It is also thought that galactosyl polyols help the *Bifidobacterium* and *Lactobacillus* genera grow in the cecum, which produces lactic acid metabolites and SCFA.

In addition, *quercitrin* is a flavonoid that has glycoside, *3,5-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl hexopyranoside* and *gynocardin* are a member of flavonoids attached to a glycoside and (4-Methylumbelliferone)-β-D-glucopyranoside is chromone derivative that linked with sugar. Unabsorbed dietary flavonoids and their metabolites may affect the intestinal environment by influencing the intestinal microbiota's microbial richness, variety, and composition. Flavonoids have a prebiotic-like activity by promoting the growth of beneficial microbes and inhibiting harmful bacteria, hence regulating the composition of intestinal flora (Xiong et al. 2023). Chromones constitute the core structure for the fundamental structure of flavonoids, flavone, and isoflavone (Lauridsen et al. 2022). Furthermore, chromones have primary biological and pharmacological activities such as anticancer, anti-inflammatory, antioxidant, and anti-microbial properties (Nazhand et al. 2020).

According to Uyanga et al. (2021), certain types of gut microbiota, like *Bacteroides*, *Streptococcus*, *Bifidobacterium*, *Lactobacillus*, and *Pediococcus*, can change quercetin into different phenolic acids, such as 3-hydroxybenzoic acid, 3,4-dihydroxybenzoate, and 3,4-dihydroxyphenylacetic acid. Furthermore, most probiotics can hydrolyse O-glycosylated polyphenols into glucose and aglycone groups during the growth phase for their utilization as a carbon source. As a way to get energy, *Lactobacillus* strains can break down ferulic, gallic, protocatechuic, coumaric, and caffeic acids (Wu et al. 2021). The results of phytochemical profiles done by LC-MS/MS show that plant glycoside chemicals have been found. Bacteria can use these compounds to make energy. Theilmann et al. (2017) reported the ability of *L. acidophilus* NCFM to grow on plant glycosides such as the monoglucosylated compounds salicin, esculin, and diglucosylated amygdalin. However, other strains originating from the digestive tract do not grow on this type of phytoglucoside.

L. rhamnosus FNCC 0099 was able to grow, as shown by the colonies that were still there after 72 h, with a count of 6.17 log CFU/mL. This growth was likely because of the enzyme activity of β-glucosidase, which let glycosides be used as a carbon substrate for proliferation. β -glucosidase is an important enzyme that hydrolyses flavonoid glycosides into their aglycone forms. Most *Lactobacillus* can synthesize the enzyme β-glucosidase in their cell membranes but with different activities ranging from 0.08 to 2.18 U/mL. When grown on Esculin-MRS agar media, *L. rhamnosus* L08 could make the enzyme β-glucosidase and form black colonies. Because β-glucosidase hydrolyses esculin into its aglycone, it reacts with Fe^{3+} to make it black. The activity of the β-glucosidase enzyme was measured to have the highest enzyme activity of 2.74 U/mL (Liu et al. 2021).

The methanol extract from the sago fruit also contained the phytochemicals corchorifatty acid F and (15Z)-9,12,13-trihydroxy-15-octadecenoic acid. These compounds can be classified as polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). The gut *Bifidobacterium* and *Lactobacillus* have been proposed for their ability to hydrolyse fatty acids. When *L. plantarum* breaks down linoleic acid, conjugated oxo fatty acids and hydroxy fatty acids are simpler. Another study found that an enzyme known as fatty acid hydratase 1 (FA-HY1) can help change linoleic acid into 13-hydroxy-cis-9-octadecenoic acid in the *L. acidophilus* NTV001 strain. This enzyme shows the capacity to catalyse the hydrolysis of both polyunsaturated fatty acid (PUFA) C18 and PUFA C20 and C22. Also, the bacterial strains *L. acidophilus* and *L. plantarum* can break down linoleic acid into 10-hydroxy-cis-12-octadecenoic acid. Hirata et al. (2015) assert that the cytotoxicity of LAB is responsible for the hydrolysis of unsaturated fatty acids.

The LC-MS/MS phytochemical profile analysis showed that the compound with the highest abundance

was 27% quinic acid of the total composition. Quinic acid is accumulated during the initial stages of fruit growth. Quinate dehydrogenase catalyzes the conversion of dehydroquinic acid, which is the cyclized result of dehydroquinate synthase, into quinic acid (Marsh et al. 2009). Two quinate metabolic pathways in *L. plantarum* co-occur under anaerobic conditions, namely the oxidative and reductive pathways. The oxidative pathway produces catechol as the final product, and no further metabolism was observed. *L. plantarum* even showed the ability to metabolize quinate after removing fructose because it uses lactic acid as a hydrogen donor (Whiting & Coggins 1971).

Two types of *L. collinoides* (L3 and L5) grew faster when quinic acid was added, leading to more cells being present during incubation. Hence, it can be asserted that *L. collinoides* can catalyse the reduction of quinic acid to dihydroshimic acid. Most *Lactobacilli* possess this capability due to their significant role in fructose and glucose energy metabolism and their ability to enhance CO_2 production in anaerobic environments (Stead 1994). According to a study by Filannino et al. (2015), lower levels of quinic acid showed that it could be used instead of fructose and pyruvate because it can take hydrogen in heterofermentative bacteria. This observation was made during experiments conducted on a medium of low-fructose broccoli puree. This effect was seen in *L. reuteri* FUA3168 and *L. fermentum* FUA3165, which were able to break down chlorogenic acid into caffeic and quinic acids by taking electrons from them (Bel-Rhlid et al. 2013).

Conclusions

Five selected probiotics grew well up to 24 h on the growth medium containing sago fruit flour as carbohydrate substitute. *Lacticaseibacillus rhamnosus* FNCC 0099 and *Lactobacillus acidophilus* FNCC 0051 showed viability stability up to 72 hours. The sago fruit flour contained 20.4 mg/g total sugars, 14.8 mg QE/g flavonoids, and 36.7 mg GAE/g phenolics. The identification procedure uses LC-MS/MS in an *untargeted* screening approach showed seven compounds were identified as having possible prebiotic properties. Furthermore, seven other molecules were also identified which can serve as growth factors. This study convinced that the sago fruit flour can be used as prebiotics source. Analysis of the active phytochemicals present in water extracts with prebiotic potential utilizing an LCMS-MS-based metabolomics approach are interesting objects in the future study.

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