RESEARCH

Phenolic-rich lees from Philippine rice wine (*tapuy*) increases *Caenorhabditis elegans* lifespan in spite of having low antioxidant activity

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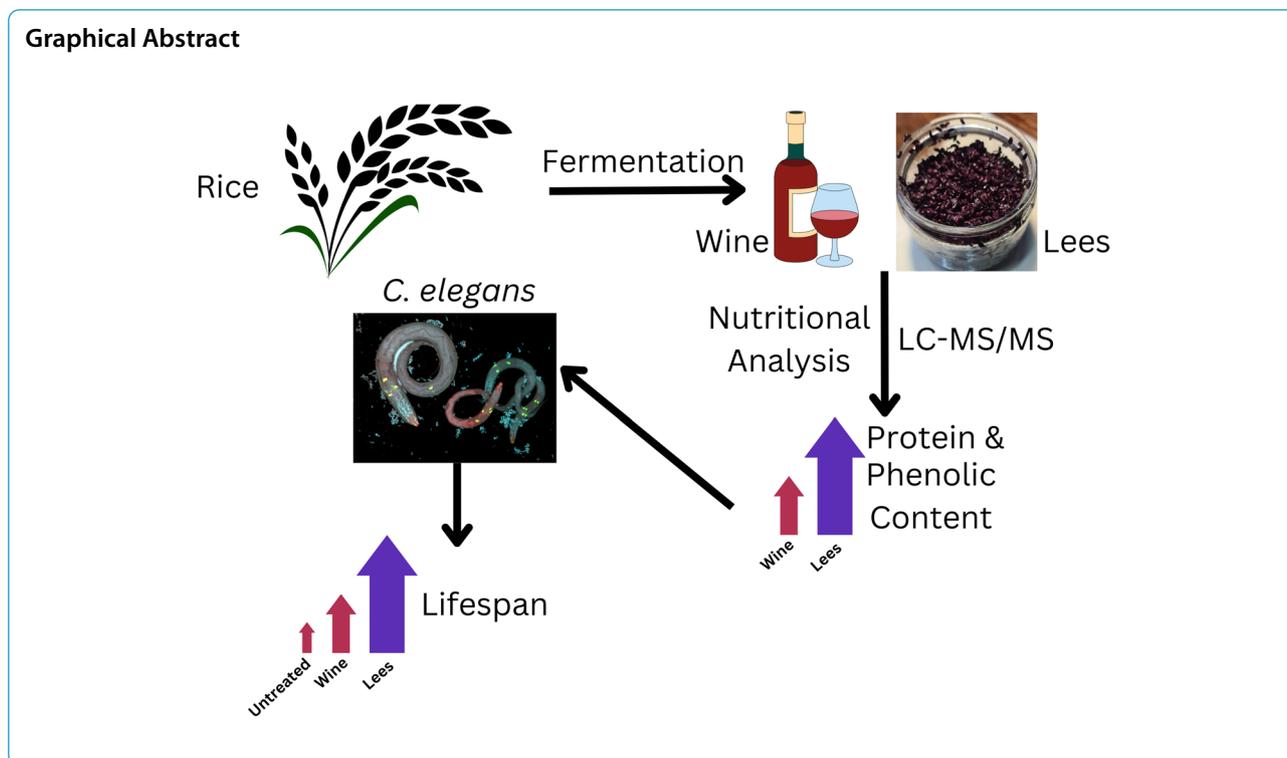
Abstract

Tapuy is an indigenous wine produced in the Philippines. Rice wine fermentation produces a by-product of rice leftover and microbial biomass, collectively called lees, which usually is discarded as a waste product. However, studies have shown the potential value of lees as a healthy food source. We wanted to determine the nutritional profile of *tapuy* wine and its lees and their abilities to extend lifespan in *Caenorhabditis elegans*. *Tapuy* lees (7.65 g/100 g dry weight) was 18.5-fold greater in protein content compared to *tapuy* wine. Its sugar content (27.66 g/100 g dry weight) is similar to the *tapuy* wine's sugar content (23.465 g/100 g dry weight). Through LC–MS/MS, 18.75% and 12.39% of spectral peaks in *tapuy* wine and lees were matched, respectively, to specific compounds, and several of them are associated with beneficial health effects. Furthermore, the phenolic content in *tapuy* lees (19,475.526 Gallic Acid Equivalent) is 6.5-fold greater compared to *tapuy* wine. Surprisingly, the DPPH and FRAP antioxidant assays show that *tapuy* lees had a lesser antioxidant ability compared to *tapuy* wine. However, *C. elegans* fed with supplementation of *tapuy* lees extract showed a 16.24% increase in mean lifespan, which is higher compared to the 6.10% increase in mean lifespan when supplemented with *tapuy* wine. Taking all these findings together, our study revealed that *tapuy* lees has a greater nutritional value than *tapuy* wine, and this underutilized and wastefully discarded product can be repurposed as a potential functional food.

Keywords Rice wine, *Tapuy*, Lees, Lifespan, *Caenorhabditis elegans*, Fermented food

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Graphical Abstract

Introduction

Fermentation is a process in which complex macromolecules are converted into simpler molecules (Sharma et al. 2020). It has long been used in human civilizations to change food characteristics such as flavor and aroma, preserve food, and remove unwanted compounds (Allwood et al. 2021; Şanlier et al. 2017; Sharma et al. 2020). A surge in interest in fermented food sources has increased in recent years due to numerous studies pointing to their benefits as functional food or food that have biofunctional properties leading to the improved health of the consumer (Dimidi et al. 2019). Bioactive molecules produced as a by-product of fermentation have been shown to display a wide range of health benefits such as being antihypertensive, prebiotic, antimicrobial, anticarcinogenic, antioxidant, antiallergenic, and an opioid antagonist (Şanlier et al. 2017; Sharma et al. 2020). One such fermentation by-product is whey from the cheese-making process, which has many nutritional benefits such as a source of protein and antioxidants, among others (Mazorra-Manzano et al. 2020). Another fermentation by-product often used is kimchi juice, the liquid by-product of kimchi fermentation. This juice is frequently used in kitchens.

Rice wines such as *sake* (Japan), *cheongju* (Korea), and *shaoxing* (China) are a group of fermented alcoholic beverages that are usually made from a mixture of different cereals, mainly rice, and are grown in different countries in Asia (Kwon et al. 2014). Rice wines have been discovered to have properties that lead to better health. Chinese rice wine has been demonstrated to have antifatigue and antiaging effects (Zhao et al. 2018). Korean *makgeolli* has also been suggested to be a source of antioxidants, dietary fibers, vitamins, bioactive compounds, and probiotics (Shimoga & Kim 2021). A common by-product of rice wine fermentation is wine lees, the rice leftover after wine fermentation. The wine lees has been proposed to have potential health benefits. *Sake* lees are frequently used in Japanese cooking after the rice wine fermentation process. *Sake* lees are a potent source of proteins, fiber, vitamins, and have been shown to decrease serum triacylglycerol concentrations (Tsutsui et al. 1998). It has also been shown to decrease lipid accumulation in adipocytes (Motono et al. 2021).

Tapuy is an indigenous rice wine produced in the northern provinces of the Philippines, particularly in the Cordillera regions. The native tribes produce this alcoholic beverage during special occasions such as weddings, fiestas, harvesting ceremonies, baptism, and as an offering to their deities or *anitos.* However, appreciation for *tapuy* and its production has been decreasing due to intense competition from commercially produced rice wines such as *sake* and *soju. Tapuy* wine is produced

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mainly from white and/or black glutinous rice mixed with a starter culture called *bubod*, a disc made of hardened starch powder packed with various fermenting microorganisms.

Tapuy lees is a product of the *tapuy* wine fermentation process, and it may contain the same health benefits as the rice wine itself. About 27% of the rice weight used in *tapuy* fermentation forms wine lees and it is typically discarded (Manaois & Morales 2014; Morales & Manaois 2011). Lees have been shown to have health benefits, as seen in the use of *sake* lees in Japanese cuisine (Motono et al. 2021; Tsutsui et al. 1998). This study revealed the potential of *tapuy* lees as a healthy food source by determining its nutritional profile and its effect on lifespan in *Caenorhabditis elegans*.

Methodology

Nutritional profile analysis of *tapuy* wine and lees
Mean total sugar content was determined via the Munson-Walker Method at the Intertek Testing and Service Center, Makati, Metro Manila. Mean total fat content was determined via Acid Hydrolysis at the Intertek Testing and Service Center, Makati, Metro Manila. Mean total protein content was determined via the Kjeldahl Method at the Intertek Testing and Service Center, Makati, Metro Manila.

Preparation of *tapuy* wine and lees extracts
Tapuy wine extracts were prepared by dehydrating the samples at 35 °C. The dried extract was then placed in a screw-cap glass vial. *Tapuy* lees extract was prepared by first dried and then powdered. The powdered lees were then soaked in 95% ethanol for 96 h in a 1:2 ratio (v/v) and separated from the ethanol via filtration and the filtrate was concentrated using a vacuum concentrator (SpeedVac) for 24 h. The dried lees extract were then resuspended in 0.1% DMSO. High concentrations of *tapuy* wine or lees were prepared as 100,000 µg/mL. Low concentrations of *tapuy* wine or lees were prepared as 10,000 µg/mL.

Total phenolic content and antioxidant activity of *tapuy* wine and lees
Mean total phenolic content was determined via the Folin-Ciocalteu Assay at the Natural Products Laboratory, Department of Molecular Biology and Biochemistry. Total phenolic content was determined using a modified Folin-Ciocalteu Assay from Benabdellah et al. (2016). In brief, 15.4µL of sample and gallic acid standard (at different concentration) were mixed with 61.54µL of Folin-Ciocalteu reagent (in a 1:10 dilution with deionized water). The mixture was neutralized with 1234µL of 7.5% sodium carbonate. The mixtures were left to stand at room temperature for 30 min. Absorbance was then read at 765 nm.

DPPH and FRAP antioxidant assays were performed at the Natural Products Laboratory, Department of Molecular Biology and Biochemistry. Antioxidant potentials were determined using the ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays. For the DPPH assay, 10µL of ascorbic acid was used as a standard. 10 µL of *tapuy* samples were loaded into a 96-well microplate. 140µL of 6.85×10^{-5} M DPPH was added into each well and left to incubate at room temperature and in the dark for 30 min. The solvents used were methanol for DPPH and 0.1% DMSO for the *tapuy* samples. Absorbance was then read at 517 nm. The absorbance values were plotted against the concentration of the sample. The equation of the line was then used to obtain the actual IC_{50} value. For the FRAP assay, 70µL of butylated hydroxytoluene (BHT) was used as a standard. Samples were mixed with 176.5µL of 0.2 M, pH 7.4 sodium phosphate buffer and 176.5µL of 1% $(K_3Fe(CN)_6)$. The resulting mixture was incubated at 50 °C for 20 min. Following this, the mixtures were acidified with 176.5µL of 10% trichloroacetic acid and centrifuged at 650xg for 10 min. An aliquot of 273µL of the supernatant was added to 273µL of deionized water. 55µL of 0.1% $FeCl_3$ was added to this solution. The solvent used was distilled water. Absorbance was read at 700 nm (Bueno et al. 2013). The absorbance values were plotted against the concentration of the sample. The equation of the line was then used to obtain the actual EC_{50} value.

Liquid chromatography tandem mass spectrometry (LC–MS/MS) was performed at the Molecular Diagnostics and Multi-omics Laboratory, Department of Molecular Biology and Biochemistry, UP Manila.

Lifespan assay
Three plates of thirty age-synchronized (via bleaching) wild-type *Caenorhabditis elegans* at the L4-stage each were worm-picked and placed in a fresh nematode growth media (NGM) petri dish. All treatment plates had an equal volume of heat-killed *Escherichia coli* (OP50) solution and either *tapuy* wine or lees extract solution. The final concentration of the extracts after mixture with OP50 were 50,000 µg/mL for the high concentration and 5,000 µg/mL for the low concentration. The worms were transferred to freshly prepared NGM petri dishes every other day. The proportion of dead, surviving, and missing worms were counted every 12 h until no surviving worm was present on the petri dish. Worms that provided a response to a gentle touch with a platinum wire pick were categorized as alive. Worms that provided no response to a gentle touch with a platinum wire pick were categorized as dead. A control group was set up and fed with *E. coli*

(OP50 strain). Lifespan assays were carried out in three trials and pooled prior to statistical analyses ($n = 90$). Worms that went missing during the lifespan assay were subtracted from the original sample size.

Statistical analyses

The means and standard deviations were calculated for the total protein, sugar, fat, and phenolic contents. Means and standard deviations were also calculated for the IC_{50} and EC_{50} for the antioxidant activities and for the lifespan of *C. elegans*. A one-way analysis of variance (ANOVA) and Tukey's honest significance test were conducted using R 4.2.2 and RStudio 2022.02.0 to determine significant differences between the means. A Kaplan–Meier survival plot was generated using the ggplot2 package on R. Finally, all bar graphs were generated using the ggplot2 package on R.

Results and discussion

Tapuy lees has a significantly higher mean total protein content compared to *tapuy* wine

The difference between the mean protein content of *tapuy* wines and lees is significant ($p < 0.05$) as shown in Table 1. There is an 18.5-fold greater protein content in *tapuy* lees (7.65 g/100 g dry weight) than of *tapuy* wine (0.41 g/100 g dry weight). This is similar with the nutritional analysis observed in *sake* lees, which also has a high protein content (Ito et al. 2022; Tsutsui et al. 1998). Black rice, one of the main ingredients used in the production of *tapuy* wine, has been shown to have increased levels of protein compared to other rice varieties (Peng 2021; Rathna Priya et al. 2019). The mean protein content of *tapuy* lees is lower than the average protein content of black rice (11.5 g/100 g dry weight) reported by Peng

et al. (2021). This may be due to protein breakdown during fermentation, which has been linked to an increased metabolite pool and more available amino acids (Diether & Willing 2019). Furthermore, the fermentation process can increase the digestibility of proteins (Alrosan et al. 2021; Çabuk et al. 2018). The lower mean protein content of *tapuy* lees compared to plain black rice may be due to some of the rice proteins dissolving into the wine.

The difference between the mean sugar content of *tapuy* wines and lees is not significant ($p > 0.05$) as shown in Table 1. However, the *tapuy* lees (27.66 g/100 g dry weight) has a slightly higher sugar content compared to the *tapuy* wine (23.47 g/100 g dry weight), which may be due to the higher content of the mono- and disaccharides after starch breakdown during fermentation. The metabolism of starch by amylolytic organisms involves two steps, the saccharification step in which starch is broken down into constituent mono- and disaccharides, and the fermentation step, in which the sugars are converted into ethanol (Toksoy Oner et al. 2005). We postulate that increasing the time of fermentation would lead to lower sugar content and higher ethanol content. Increasing the time of fermentation would give the microorganisms more time to convert the sugars within the substrate into ethanol. The similar sugar content would lead to a very similar level of sweetness between the *tapuy* wine and the lees.

Although the difference between the mean sugar content of *tapuy* wine and lees is not significant, the assay used to determine the sugar content only detects reducing sugars (Jackson & Mcdonald 1941). It may be possible that a large concentration of starch will remain in the *tapuy* lees and will be broken down inside the body upon consumption. This would effectively increase the total sugars consumed when eating *tapuy* lees and one should exercise caution in concluding that the total sugar content of the *tapuy* wine and lees is comparable in this sense. Alternatively, a large percent of the undetected starch is undigestible and may serve a prebiotic function, thus, benefitting the gut microbiota. The small disparity between the mean sugar content between the *tapuy* wine and lees may be due to the sugars being more diluted in the supernate (liquid wine) than in the precipitate (solid lees). Each grain of rice will have a smaller volume than the entire vat of liquid and thus, would have a more concentrated sugar content in spite of an overall lower amount of sugars inside the grain.

The traditional method of *tapuy* fermentation is done over a period of one month, which leads to the production of a relatively sweet wine. The soluble mono- and disaccharides from starch breakdown during fermentation give the wine its characteristic sweet taste. The *tapuy* lees, having both a higher mean sugar content and

Table 1 Summary of the nutritional profile of *Tapuy* wine and lees

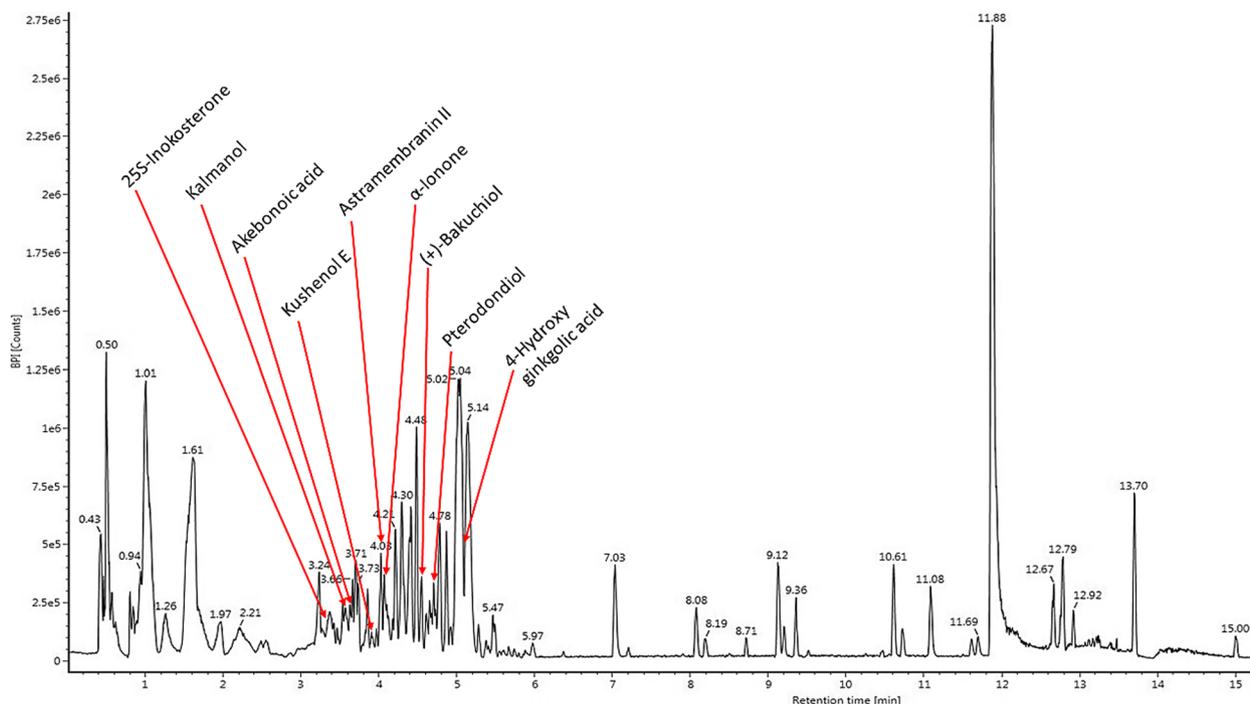| Sample	Trial 1	Trial 2	Mean	
Protein Content				
Tapuy Wine	0.41	0.41	0.41	
Tapuy Lees	7.67	7.63	7.65	
Sugar Content				
Tapuy Wine	23.75	23.18	23.47	
Tapuy Lees	26.28	29.04	27.66	
Fat Content				
Tapuy Wine	0.15	0.13	0.14	
Tapuy Lees	1.24	1.29	1.26	
Total Composition				
Sample	Proteins (%)	Sugars (%)	Fats (%)	Others (%)
Tapuy Wine	0.41	23.47	0.14	75.98
Tapuy Lees	7.65	27.66	1.26	63.43

Fig. 1 Chromatogram of the base peak intensity vs. retention time of *tapuy* wine sample. Identified analytes are shown labelled. There are 9 identified analytes from 43 spectral peaks

an overall higher concentration of sugars (by virtue of its smaller and denser size compared to the liquid wine), would be more palatable and appealing to people. The palatability of *tapuy* lees would make it easier to repurpose it as a functional food. However, *tapuy* can also be fermented for up to two months, which is enjoyed by the few who prefer a dryer taste with a higher alcohol content.

The difference between the mean fat content of *tapuy* wines and lees is significant ($p < 0.05$) as shown in Table 1. However, despite this significant difference, the overall fat content of both *tapuy* wine and lees is small (0.14 g/100 g dry weight and 1.27 g/100 g dry weight, respectively). Thus, the low-fat content of the *tapuy* wine and lees contributes minimally to their nutritional value.

Tapuy lees composition is more complex and diverse than *tapuy* wine

As shown in Figs. 1 and 2, the *tapuy* lees has 113 spectral peaks while *tapuy* wine has 43 spectral peaks. The more varied compound composition in the *tapuy* lees increases the likelihood of it containing compounds that may confer health benefits. Table 2 shows the summarized phenolic compounds identified using LC–MS/MS and screened using the Waters Traditional Chinese Medicine Library. *Tapuy* wine has 9 compounds identified from 43 spectral peaks (21%) while *tapuy* lees

has 14 compounds identified from 113 spectral peaks (12.4%). There is only one common compound between the two samples (kalmanol), which suggests a clear difference in the phenolic compositions of the *tapuy* wine and lees. The more varied phenolic compounds in the lees suggests a wider spectrum of antioxidant activities. Of the compounds with identified functions, bakuchiol (Bluemke et al. 2022; Ma et al. 2020), 25S-inokosterone (Liu et al. 2022), and 4-hydroxy ginkgolic acid (Noor-E-Tabassum et al. 2022) are present in the *tapuy* wine and have antioxidative properties. In the *tapuy* lees, 6-hydroxykaempferol (Chen & Chen 2014; Ren et al. 2019; Silva dos Santos et al. 2021), cholestenone (Ramalingam & Rajaram 2018), flazin (Fuda et al. 2019; Wu et al. 2022), and ganoderic acid B (Cör et al. 2018; Sheikha 2022) are present and have antioxidative properties. Furthermore, the total number of spectral peaks matched to the database represent only a fraction of the total number of spectral peaks of the two samples. The use of a more exhaustive library or multiple libraries may identify more of the peaks can be identified as to what compounds they are.

The antioxidant assays only measure a fraction of the potential antioxidant activity of *tapuy* lees

There is a significant difference between the mean phenolic content of *tapuy* wine and lees as shown in
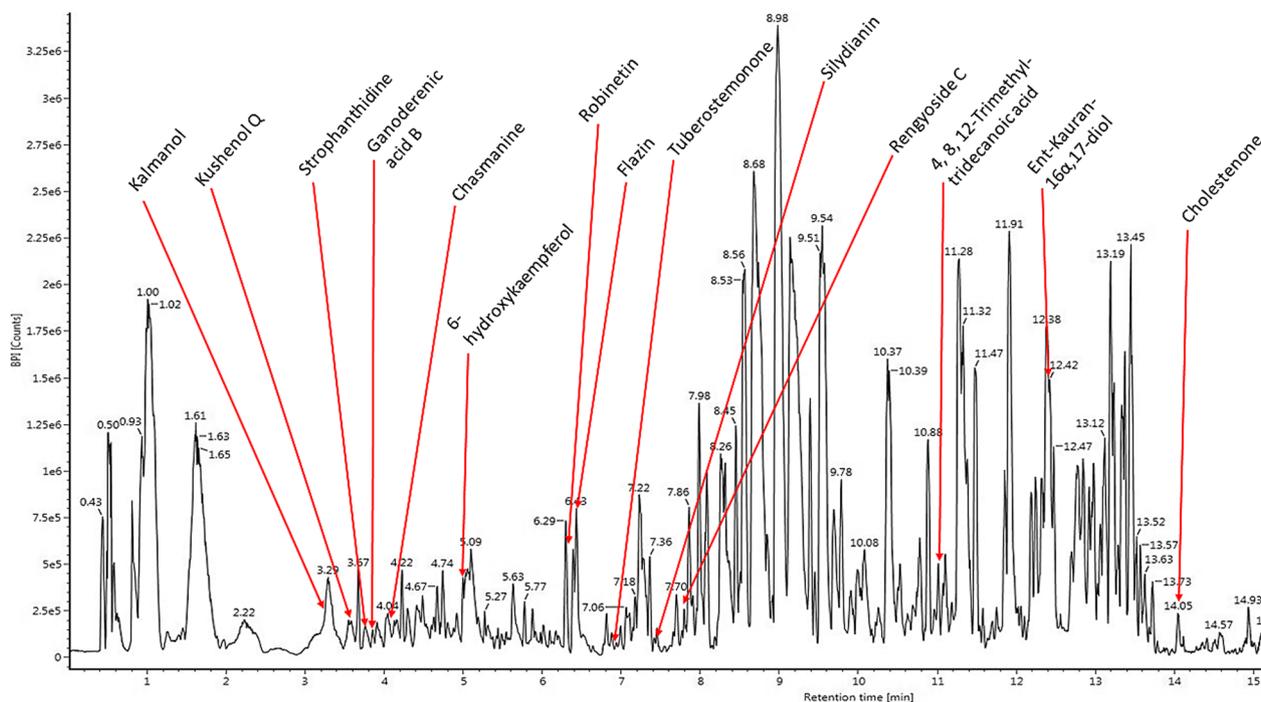
Fig. 2 Chromatogram of the base peak intensity vs. retention time of *tapuy* lees sample. Identified analytes are shown labelled. There are 14 identified analytes from 113 spectral peaks

Fig. 3A. The *tapuy* lees has a 6.5-fold greater mean phenolic content than the *tapuy* wine. This could be attributed to a higher occurrence of non-polar phenolic compounds (as reported in previous studies) remaining in the *tapuy* lees, because they have a low dissolution in wine. Phenolic compounds such as triterpenoids (Vrancheva et al. 2021), terpenes (Jiang et al. 2016), and steroids (Hartonen & Riekkola 2017) are non-polar. The high abundance of these compounds in *tapuy* lees has been shown to have many health benefits such as anti-inflammation activity (Karimi et al. 2012; Kumar et al. 2019), anti-cancer activity (Badhani et al. 2015; Karimi et al. 2012; Kumar et al. 2019; Mori et al. 1999; Rocha et al. 2012), antioxidant activity (Badhani et al. 2015; Karimi et al. 2012; Medina et al. 2007; Middleton et al. 2002), antifungal activity (Badhani et al. 2015), antibacterial activity (Badhani et al. 2015; Bhattacharya et al. 2010; Bodini et al. 2009), antiviral activity (Badhani et al. 2015).

Figure 3B and C show that according to the positive controls of the DPPH (ascorbic acid) and FRAP (butylated hydroxytoluene) assays, the *tapuy* lees had higher EC_{50} and IC_{50} values than the *tapuy* wine. Interestingly, higher phenolic content and greater phenolic compound variety in the *tapuy* lees than the *tapuy* wine did not translate to better *tapuy* lees antioxidant activity as measured by the DPPH and FRAP antioxidant assays employed. Phenolic compounds found in the *tapuy* lees are generally non-polar and are not easily miscible in an aqueous solution. In contrast, the phenolic contents of wine are generally polar and easily mix in aqueous solutions. Thus, the DPPH and FRAP assays measured only the phenolic compounds present in the aqueous extract of the *tapuy* lees, which may not be indicative of the total phenolic content as most of these would be trapped within the physical *tapuy* lees. This is in contrast to the *tapuy* wine in which all of its phenolic contents will already be suspended in solution. An extraction process that will give a phenolic amount in the extracts that is more proportionate of the actual phenolic content of the source may reflect the total antioxidant activities of the *tapuy* lees more accurately.

Alternatively, it is also possible that the two assays employed only measure the antioxidant activities of a limited class of phenolic compounds. Therefore, the use of other antioxidant assays such as the HORAC, ORAC, TEAC, TAC, TRAP, CUPRAC, TOSC, and ABTS, which measure other classes of phenolic compounds, may yield a more complete and accurate measure of the total antioxidant activity of the

Table 2 Summary of detected compounds in *Tapuy* wine and lees using LC–MS/MS

	Tapuy Wine	*Tapuy* Lees	Known Functions	Compound Class	Reference(s)
(+)-bakuchiol	✔		Anti-aging Antioxidative properties Protective effects against diabetic cardiomyopathy	Phenol	Ma et al. 2020; Bluemke et al. 2022; Chaudhuri & Bojanowski 2014; Dhaliwal et al. 2019)
25S-inokosterone	✔		Enhances the lifespan of yeast models and mammalian cells Antioxidative properties	Steroid	Liu et al. 2022)
4, 8, 12-trimethyltridecanoic acid		✔		Fatty acid	Shahrajabian et al. 2021)
4-hydroxy ginkgolic acid	✔		Antibacterial Neuroprotective effects Antioxidative properties Antiaging Antiobesity Antidiabetic Antidementia	Phenol	Noor-E-Tabassum et al. 2022; Mango et al. 2016; Hua et al. 2017)
6-hydroxykaempferol		✔	Antioxidant Reduces risk of chronic diseases Anticancer effects Neuroprotective effects Anti-inflammatory properties	Flavonoid	Chen & Chen 2014; Ren et al. 2019; Silva dos Santos et al. 2021)
Akebonoic acid	✔			Terpenoid	Maciąg et al. 2021; Huang et al. 2022)
Astramembranin II	✔			Glycoside	
Chasmanin		✔		Alkaloid	
Cholestenone		✔	Antibacterial Antioxidant Antidiabetes effects	Steroid	Ramalingam & Rajaram 2018; Neuvonen et al. 2014; Kobayashi et al. 2021; Nagao et al. 2022)
ent-Kauran-16a, 17-diol		✔	Protects against neuroinflammation	Terpene	Dutra et al. 2014; Nhiem et al. 2014; Kim et al. 2016)
Flazin		✔	Anti-HIV Antioxidant Immunomodulatory activity Lipid droplet regulator	Alkaloid	Fuda et al. 2019; Wu et al. 2022; Tang et al. 2008; Kong et al. 2021)
Ganoderic acid B		✔	Antimicrobial Antioxidant Anti-HIV	Triterpenoid	Cör et al. 2018; Sheikha 2022; Chen 2020)
Kalmanol	✔	✔		Terpenoid	
Kushenol E	✔		Autophagy regulation	Flavonoid	Kwon et al. 2020)
Kushenol Q		✔		Flavonoid	
Pterodondiol	✔			Terpenoid	
Rengyoside C		✔			
Robinetin		✔		Flavonoid	
Silydianin		✔			
Strophanthidine		✔	Anticancer Antiviral	Glycoside	Reddy et al. 2020)
Tuberostemonone		✔		Alkaloid	
α-ionone	✔			Terpene	

samples derived from phenolic compounds. Each antioxidant assay targets a specific method of antioxidant activity such as hydrogen atom transfer (ORAC, TRAP, and HORAC), the transfer of one electron (CUPRAC and FRAP), and mixed tests (ABTS and DPPH) (Munteanu & Apetrei 2021). Each of these methods has its own pros and cons and has the capability to detect different classes of antioxidants based on their mechanism of action. Thus, the usage of only FRAP and DPPH may be a limitation in the determination of the total antioxidant activity of *tapuy* wine and lees.
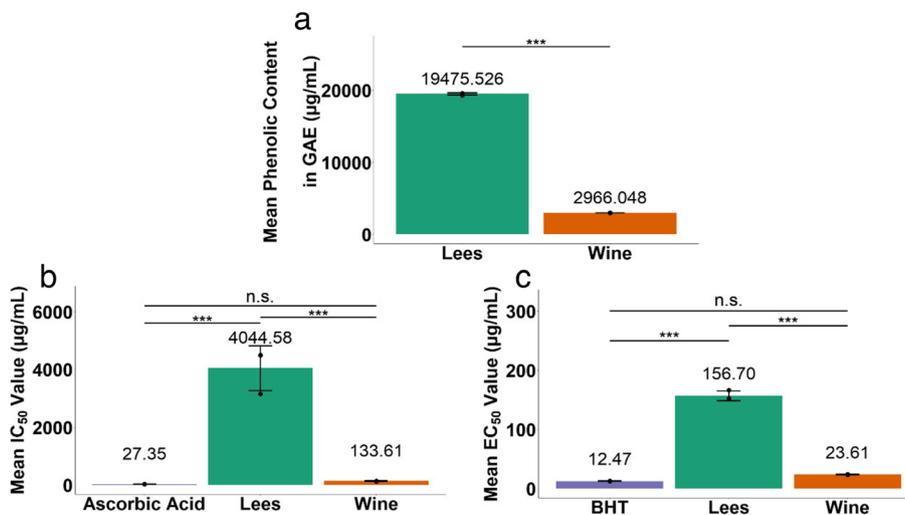
Fig. 3 Phenolic content of *tapuy* wine and lees and their antioxidant activities. A *** indicates a *p*-value less than 0.001 and an n.s. indicates no significant difference. **a** Mean phenolic content of *tapuy* wine and lees. The phenolic content was determined using the Folin-Ciocalteu assay. **b** DPPH assay of *tapuy* wine and lees (IC_{50}). IC_{50} values were determined using a DPPH assay. The IC_{50} values indicate the minimum concentration of the wine or lees sample that elicits a 50% inhibition of decolorization of DPPH from a purple to a clear solution. **c** FRAP assay of *tapuy* wine and lees (EC_{50}). EC_{50} values were determined using a FRAP assay. The EC_{50} values indicate the minimum concentration of the wine or lees sample that elicits a 50% change from clear to an intense, blue-colored solution

Tapuy lees supplementation can moderately increase the mean lifespan of *C. elegans*

Antioxidants are tightly associated with an increased lifespan (Ding & Zhao 2022; Duangjan et al. 2019; Lin et al. 2019; Rangsinth et al. 2019). We have shown that *tapuy* lees has high phenolic content and thus, will have a high potential for antioxidant activity. With this, we sought to determine the effect of *tapuy* supplementation on the lifespan of *C. elegans*. Since the *tapuy* wine and lees are rich in potential antioxidants
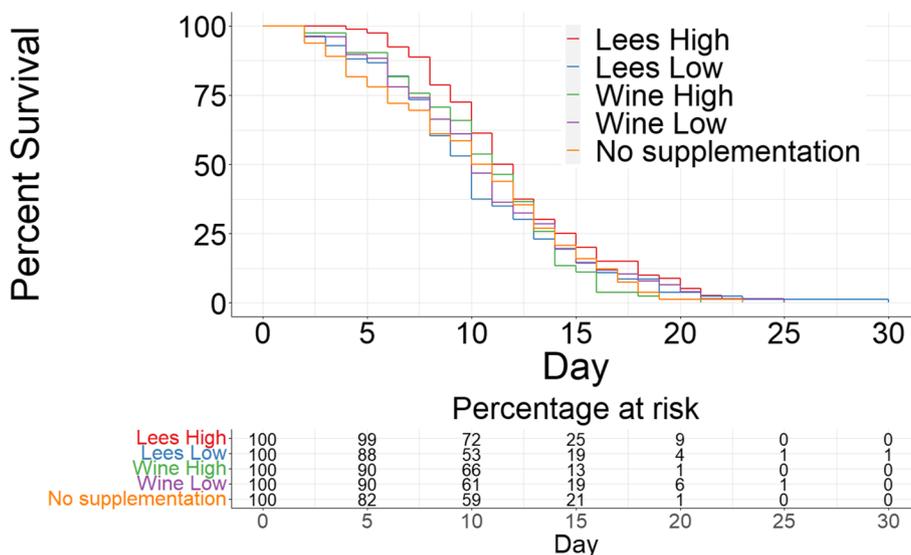

Fig. 4 Kaplan–Meier survival curves for *Caenorhabditis elegans* fed with varying amounts of *tapuy* wine or lees or fed without *tapuy* supplementation. A risk table showing the percentage of the population at risk is included below the Kaplan–Meier survival curves. Results of lifespan assays of three trials were pooled together to form one survival curve per experimental group. Sample sizes are $n = 80$ for the *tapuy* lees high, $n = 83$ for the *tapuy* lees low, $n = 82$ for the *tapuy* wine high, $n = 77$ for the *tapuy* wine low, and $n = 82$ for the no supplementation group. The concentrations of *tapuy* wine and lees high are 50,000 µg/mL. The concentrations of *tapuy* wine and lees low are 5,000 µg/mL

due to its high total phenolic content, we wanted to determine if supplementation with *tapuy* wine and lees can lead to increased lifespan in *C. elegans*. Figure 4 shows the Kaplan–Meier survival curves of various experimental groups. Overall, there is no significant difference between high and low concentrations of *tapuy* wine, lees, and with the no supplementation group (negative control). However, a slight increase in survival is seen in low concentration supplementation of *Tapuy* wine or lees. Supplementation with high concentration *Tapuy* wine and lees has slightly worse percent survival compared to the negative control. This could be attributed to two possible reasons. First, the high concentration of antioxidant compounds converts them into functional prooxidants (Sotler et al. 2019). This could explain why the higher concentration of *Tapuy* wine and lees extracts results in a decreased lifespan compared to the low concentration and the negative control. Second, the high consumption of either *tapuy* wine or lees may lead to an excessive accumulation of sugars. A high-sugar diet is associated with a decrease in the lifespan of *C. elegans* (Alcántar-Fernández et al. 2018; Lee et al. 2009). A high-sugar diet in *Drosophila melanogaster* is also associated with decreased lifespan (Baenas & Wagner 2022). Musselman et al. (2013) report that while triacylglyceride storage in high-sugar diets is a protective mechanism, the synthesis of more triacylglycerides over the capacity of the fly's fat body would lead to dysregulated lipogenesis. A high-sugar diet is, in general, adverse to the

health of mammalians (Kelm et al. 2011). An increase in sugar consumption and thus, lipogenesis, translates into an increased risk for diseases that involve high-fat concentrations, such as cardiovascular diseases and obesity (Simons et al. 2022; Stanhope 2016). Similarities in lipogenesis dysregulation due to high-sugar diets show the translatability of diet-based studies from animal models to humans.

Our experiments demonstrate that high *tapuy* lees supplementation increases the mean lifespan of *C. elegans* by 16.24% compared to no supplementation, albeit below statistical significance. However, the trend suggests that if we increase the *tapuy* lees dosage in the *C. elegans* diet, we will eventually reach statistical significance (Fig. 5). This is consistent with the observed lifespan extension seen in *Drosophila melanogaster* (Dela Cruz, in preparation) fed with *tapuy* lees. Though high sugar consumption is associated with high supplementation of either *tapuy* wine or lees, there were no significant adverse effects on the mean lifespan of *C. elegans*. The other metabolites present in the *tapuy* lees (especially the high mean phenolic content) may contribute enough health benefits to counteract the potential lifespan-shortening effects of a high-sugar content with *tapuy* lees. The high sugar content may even be desirable due to the increase in palatability conferred by a sweeter taste. Since both *C. elegans* and humans follow the same trend of decreased lifespan induced by a high-sugar diet, *C. elegans* is a good model for lifespan-affecting diet assays. Therefore, it is likely that the moderate increase
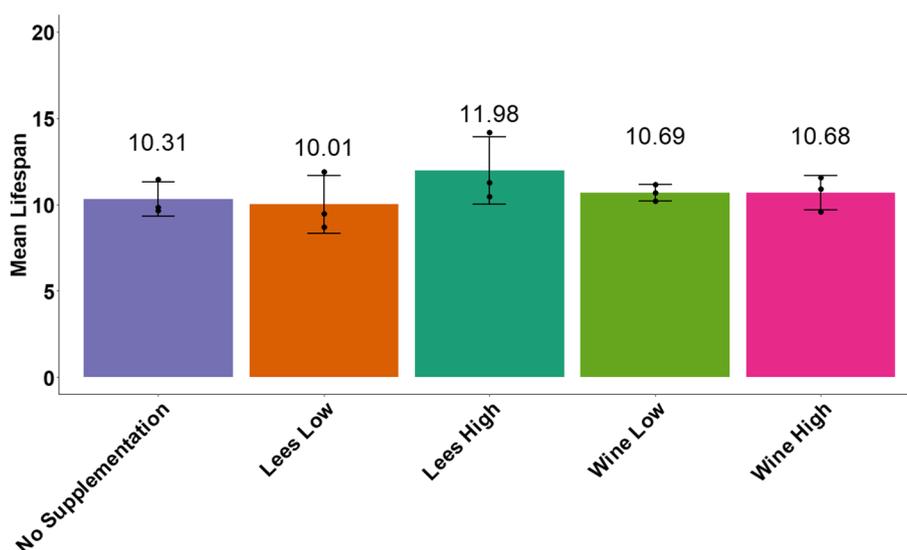

Fig. 5 Mean lifespan of *Caenorhabditis elegans* supplemented with varying amounts of either *tapuy* wine or lees extracts. Only high supplementation of *tapuy* lees led to a moderate increase in mean lifespan over other worms. The concentrations of *tapuy* wine and lees high are 50,000 µg/mL. The concentrations of *tapuy* wine and lees low are 5,000 µg/mL

in the lifespan of *C. elegans* induced by high *tapuy* lees supplementation may also be observed in humans. It can be observed that in the Kaplan–Meier survival curve, a few *C. elegans* were able to survive when supplemented with low dosage of *tapuy* wine or lees as compared to *C. elegans* supplemented with high dosage of *tapuy* wine or lees. The few individuals that did survive longer in the Kaplan–Meier survival curve may have been outliers. However, when determining the overall effect on lifespan of *tapuy* supplementation on all the *C. elegans* in each treatment group by taking the mean lifespan, it was revealed that the high dosage of *tapuy* lees slightly increases the lifespan the most compared to other treatment groups and the negative control. The mean lifespan of the *C. elegans* supplemented with a high dosage of *tapuy* lees had a 16.24% increase in lifespan compared to *C. elegans* not fed with either *tapuy* wine or lees (i.e. negative control).

Conclusion

Through fermentation technology, we were able to enhance the quality of the nutrients of a substrate (rice) in the form of *tapuy* wine and lees. Our experiments have revealed the nutritional value of *tapuy* lees, with a high protein content, low-fat content, moderate sugar content, and high phenolic content (Table 1 and Fig. 3). We found that *tapuy* lees has a wider variety of compounds identified via LC–MS/MS than *tapuy* wine and many of these compounds have been linked to beneficial health effects. We recommend that further analysis be done to identify unidentified peaks and conduct functional screening of these putative compounds to identify their biofunctionalities. The two antioxidant assays employed in the study may not be indicative of the actual antioxidant activity of *tapuy* lees. There are two options to address this limitation. First, by employing a more comprehensive analysis of its antioxidant activities through a wider range of antioxidant assays. Second, to utilize a more efficient extraction technique for *tapuy* lees that would also efficiently extract the nonpolar phenolic components from the substrate. Interestingly, even though the measured antioxidant activity of the *tapuy* lees is lower than the wine, albeit having a higher phenolic content than the *tapuy* wine, functional lifespan assays performed on *C. elegans* show that *tapuy* lees supplementation led to a higher increase in lifespan than *tapuy* wine supplementation. Furthermore, worms supplemented with high amounts of *tapuy* lees had a slightly higher mean lifespan than both *tapuy* wine supplementation and with no supplementation. Establishing dose dependency in increasing percent survival and mean lifespan may be of great value in recommending

an ideal amount for consumption. Overall, we show that *tapuy* lees, although heavily underutilized and wasted, is a healthy food source. Repurposing the *tapuy* lees into a food product and its consumption may nutritionally enhance consumer diet.

Supplementary Information

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> **Additional file 1.**
>
> **Additional file 2.**

Compliance with ethical standards
All authors comply with BMC's ethical policies regarding conflicts of interest, informed consent, and ethical treatment of humans and animals in research.

Authors' contributions
Paul Mark B. Medina contributed the following: Conceptualized the research project. Managed the administration of the research project. Implemented the research project. Analyzed the data. Wrote the manuscript. Sean Philippe L. Chua contributed the following: Analyzed the data. Wrote the manuscript. Lesley Dale Umayat contributed the following: Conceptualized the research project. Managed the administration of the research project. Implemented the research project.

Availability of data and materials
Data is available upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have no relevant financial or non-financial interests to disclose. The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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