

Phytochemical composition and health-enhancing properties of *Oryza sativa* L. leaf tea

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Abstract

Phytochemical composition and potential health-enhancing properties of young organic jasmine rice (*Oryza sativa* L.) leaf tea, cv. Khao dok mali 105 (KM) and Homnil (HN), were investigated. Sequential extraction using organic solvents (acetone, ethanol, water) revealed that phenolic acids were the dominant compounds of all extracts, with chlorogenic acid being the major contributor. The highest level of total phenolics (113.8 mg GA E/g DW) and antioxidant capacity [FRAP: 1484.2 $\mu\text{mol Fe}^{2+}$ /g DW, DPPH: EC_{50} 1.31 mg/mL and ORAC: 4093.1 $\mu\text{mol T E/g DW}$] were identified for KM acetone-based extract. This extract exhibited pronounced α -amylase (IC_{50} = 73.96 \pm 2.7 $\mu\text{g/mL}$) and pancreatic lipase (IC_{50} = 65.7 \pm 5.8 $\mu\text{g/mL}$) inhibitory activities. Both rice leaf teas contained volatiles (tridecane, caryophyllene, and dihydroactinolide) responsible for their attractive and unique aroma. This study suggests that young organic leaf of jasmine rice can be utilized for the production of a novel herbal tea that promotes good health (Figure 1).

Introduction

Herbal teas are a rich source of plant-derived phytochemicals that contribute towards lowering the incidences of chronic diseases [1]. Green tea leaf (*Camelia sinensis* (L.) Kuntze) is the classic example of a plant-based beverage that, due to its health properties, become extensively utilized in nutraceutical and functional foods industries [2]. Catechins - the dominant phenolic compounds of green tea, are responsible for its distinctive sensory [2] and health promoting properties, including antioxidant and antimicrobial [3], alleviation of clinical features of metabolic syndrome, memory disorders [4], reduction of pathological angiogenesis [5] and cancer prevention [6].

Locally grown botanicals are utilized for the production of herbal teas that display comparable antioxidant capacities to *C. sinensis* leaf tea [7]. In agreement, herbal teas obtained from rooibos, rosemary, lemongrass, mulberry, bamboo, lotus, peppermint, persimmon and mate are rich sources of antioxidant and antimicrobial compounds [3]. Among numerous water lily cultivars, the 'Conqueror' and 'Virginia' produced teas with an outstanding fragrance, the highest total polyphenols content and superior antioxidant capacities [8].

Recently the Thai government initiated the "One District One Product" program. This initiative represents an important strategy to support local communities through the promotion of their products and sustainable utilization of the local flora. Under this program new plant sources for production of functional tea are evaluated. Rice (*Oryza sativa* L.) is the staple food crop of Thailand [9] with the largest areas of rice cultivation being the central plains and Northeastern Region [10]. Due to a high market demand for jasmine rice its cultivation in Northeastern Region is growing. The unique scent of jasmine rice can be captured in young leaf tea. The present project aimed at the development of a functional tea obtained from young jasmine rice leaf characterized by unique sensory and health-promoting properties.

Materials and methods

Plant materials

Seeds of two Jasmine rice cultivars, white long grain Khao dok mali 105 (KM) and black long grain Homnil (HN) purchased at organic market (Surin province, Thailand) were soaked overnight in water and placed in trays (40 \times 50 \times 15 cm) containing soil mixture. They were kept at room temperature under daylight and watered every second day. On the 10th day the leaves were collected, washed in MilliQ water and cut into 3 cm long segments that were air-dried in the dark (room temperature). Subsequently they were roasted at 70°C, placed in aluminum foiled plastic bags and kept at room temperature until analysis (<2 months). These samples represented herbal teas obtained from the respective rice cultivars. Commercially available green tea (GT; Fujian Lung Ching green tea, China Imex) was included as a reference sample.

Chemicals

All solvents and chemicals used were of analytical grade. Chlorogenic acid, 2',7'-dichlorofluorescein diacetate (DCFH), D-(+) glucose solution (45%), 4-(dimethylamino) cinnamaldehyde (DMAC), acetic acid, aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) and Folin-

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Key words: functional drink, jasmine rice tea, polyphenols, volatiles, α -amylase, pancreatic lipase, antioxidant activity

Received: October 09, 2018; **Accepted:** October 31, 2018; **Published:** November 05, 2018

Ciocalteu reagent were obtained from Merck (Darmstadt, Germany) and 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ) was obtained from Fluka (Madrid, Spain). Hydrochloric acid (HCl), (+)-catechin hydrate, iron chloride (FeCl_3), iron sulphate (FeSO_4), ethanol, methanol, sodium hydroxide (NaOH) and sodium nitrite (NaNO_2) were purchased from QReC (New Zealand). Acetone, 2,2'-azobis (2-amidinopropane (AAPH), DPPH, intestinal acetone powders from rat, sucrose, formic acid, maleic acid, 4-methylumbelliferyl oleate, porcine pancreatic lipase (Sigma type II), trifluoroacetic acid and fluorescein (120 nM) were obtained from Sigma-Aldrich Inc. (Darmstadt, Germany). Acarbose was obtained from Glucobay (Bayer Healthcare Pharmaceutical, Leverkusen, Germany).

Preparation of lyophilized extract of rice leaf tea

Lyophilized extracts of teas (KM, HN, GT) were prepared according to Dalar and collaborators [11]. Briefly, tea samples were ground, mixed with a 20-fold volume of acidified acetone (80% acetone, 19% MilliQ water and 1% of 0.1% trifluoroacetic acid, v/v/v), shaken for 2 h at room temperature and centrifuged for 20 min at 10,000 rpm and 4°C (Centurion, K2R, Centurion Scientific, UK) with supernatant collected. Next, the pellet was extracted using acidified ethanol (80% ethanol, 19% MilliQ water and 1% of 0.1% trifluoroacetic acid, v/v/v) and then MilliQ water (Synergy[®], Ultrapure Type I, Merck, France) with supernatants collected each time. The supernatants obtained from three organic solvents extractions were evaporated individually under reduced pressure at 37°C using a rotary evaporator (Model N-1N, Eyela, Tokyo Rikakikai, Chuo-ku, Tokyo, Japan). The derived concentrated extracts were dissolved in a minimum amount of MilliQ water, pre-frozen (Eyela PFR1000, Tokyo Rikakikai, Chuo-ku, Tokyo, Japan) and freeze-dried (Eyela FDU2100, Tokyo Rikakikai, Chuo-ku, Tokyo, Japan) under a vacuum at -51°C to obtain fine lyophilized powders.

Antioxidant capacity

Total phenolic content (Folin – Ciocalteu assay) and ferric reducing antioxidant power (FRAP) assay: The levels of total phenolics (TP) and total reducing capacities of extracts were assayed as described previously [12].

Free radical scavenging activity (DPPH assay): Free radical scavenging activity was assayed using the 2,2-diphenyl-1-picrylhydrazyl

(DPPH) method according to Sharma and Bhat [13]. Briefly, lyophilized tea extracts were dissolved in 80% acetone (concentration range 0.01 to 10.0 mg/mL) and 0.06 mM of DPPH was dissolved in 100% methanol. The DPPH radical solution in methanol (3.5 mL, 0.06 mM) was mixed well with 100 μL of tea extract solutions. The mixture was then incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using a spectrophotometer (Libra S22, Holliston, MA, USA). The half effective concentration required for 50% decrease in absorbance of DPPH radicals (EC_{50}) was calculated and expressed as mg DW of lyophilized tea extract per mL of the solution (mg/mL).

Oxygen radical scavenging capacity (ORAC) assay: The ORAC assay was performed as previously described [11]. The antioxidant capacity of the samples was expressed as μmol trolox equivalent per gram of dry weight of lyophilized extract ($\mu\text{mol TE/g DW}$) based on a trolox standard curve.

Sugar determination

Reducing sugars and total sugars were measured as reported by AOAC [14]. All results were expressed as mg of glucose equivalent per gram dry weight (mg G E/g DW) of lyophilized extract.

Identification and quantification of phenolic compounds

Identification of phenolic compounds by liquid chromatography–diode array–mass spectrometry (LC-DAD-MS/MS): Phenolic compounds were identified with the help of a Quantum triple stage quadrupole (TSQ) mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) as described by Dalar and Konczak [15] with the exception that the gradient elution was modified as follows: 0% solvent B for 2 min, then 40% B for 6 min, following 60% of B for 8 min and 100% B for 4 min.

Quantification of phenolic compounds by high performance liquid chromatography–diode array detector (HPLC-DAD): Phenolic compounds were quantified according to Dalar and Konczak [16]. A modified linear gradient elution was applied of solvent B from 0 to 20% over 3 min, to 40% over 5 min, to 60% over 5 min, to 100% over 5 min and the elution was held at the rate of 100% B for another 5 min.

Identification of volatile compounds

Head space solid phase microextraction (HS-SPME) procedure: Two grams of lyophilized plant extracts were weighed into 40 mL vials to undergo a solid-phase microextraction (SPME) procedure. The procedure was carried out using divinyl benzene/carboxen/polydimethylsiloxane fiber, with 50/30 mL film thickness, preconditioned before analysis. The vials were equipped with “mininert” valves and kept at 35°C with permanent internal stirring. The samples were left to equilibrate for 30 min. Next, the SPME fiber was placed in the headspace for 40 min. Then the fiber was introduced into GC injector and left for 3 min to thermal desorption to increase the volatility of compounds. The microextraction procedure was optimized according to Verzera and collaborators [17].

Gas chromatography mass spectrometry (GC-MS) analysis

The analysis was carried out by gas chromatography mass spectroscopy as described by Dool and Kratz [18]. Fatty acids and volatile compounds were analyzed on Varian 3800 gas chromatograph as described by Uzun and collaborators [19].

Enzyme inhibitory activities

Alpha-amylase inhibitory activity: Inhibition of α -amylase was conducted using the Caraway–Somogyi iodine/potassium iodide (IKI) method as described by Dalar and collaborators [11]. The α -amylase inhibitory activity was expressed as micromoles of acarbose equivalents per gram dry weight of lyophilized extract ($\mu\text{M A E/g DW}$).

Lipase inhibitory activity: The lipase inhibitory activity assay was performed according to Dalar and collaborators [11] using 4-methylumbelliferyl oleate (0.1 mM) as a substrate. The lipase inhibition was reported in micromoles of orlistate equivalents (μM orlistate E/g DW of lyophilized tea extract).

Statistical analysis

The mean of results and standard deviations (SD) were calculated based on at least three independent evaluations ($n=3$). The half effective concentration (EC_{50}) value was calculated from the corresponding dose inhibition curve according to their best fit shapes based on at least four reaction points using Microsoft Excel. Statistical correlation analyses were performed using Graphpad Prism 5 (Graphpad Software, CA, USA).

Results and discussion

Yield, sugar content and antioxidant capacities of lyophilized tea extracts

Samples of dry tea (KM, HN and GT) were extracted sequentially using solvents with increasing polarity level: 1) acidified acetone, 2) acidified ethanol and 3) acidified MilliQ water. This approach has been suggested to achieve the maximum recovery of phytochemicals and good compounds differentiation during extraction from unknown plant sources [20].

The highest product yield (28.9%) was recorded for GT acetone-based extract (Table 1). The yields of water-based extracts obtained from KM and HN rice leaf teas were 2.5- to 2.0-fold that of GT water extract yield indicating comparatively higher levels of polar compounds in rice teas. All three extracts of KM rice tea contained the highest level of total sugar for each category of extract (acetone-, ethanol, or water-based) and were followed by GT extracts. The lowest total sugars contained water- and acetone-based HN tea extracts (Table 1). The water-based GT extract and ethanol- and acetone-based KM extracts contained the highest level of reducing sugars for each extract category (Table 1). These levels of reducing sugars are comparable or higher than those of *Eryngium bornmuelleri* leaf tea extracts (acetone-based extract: 71.1±0.6 mg G E/g DW, ethanol-based: 49.0±0.9 mg G E/g DW, water-based: 37.0±1.1 mg G E/g DW [11]).

The antioxidant potential of sequential leaf tea extracts was evaluated using four reagent-based assays, including total phenolics (TP, Folin-Ciocalteu method), 1,1-diphenyl-2-picrylhydrazyl (DPPH•) free radical assay, total reducing capacity (ferric reducing capacity power, FRAP) and oxygen radical absorbance capacity (ORAC).

The highest levels of total phenolics were found in acetone-based extracts of all teas (Table 1). These levels were approximately 3-times higher than those of the respective ethanol-based extracts. The least amount of phenolic compounds contained water-based extracts, with the exception of KM. In summary, the highest TP contained green tea, followed by KM and HN tea (Table 1).

The DPPH free radical assay allows to measure the amount of lyophilized extract required to obtain 50% decrease in absorbance of DPPH free radicals in reaction solution (expressed as EC₅₀). The highest DPPH radical scavenging capacity exhibited the acetone-based extracts

of all teas (Table 1). This result correlates well with the highest levels of total phenolic compounds detected in the same extracts (Table 1). The lowest DPPH radical scavenging ability within their extract category exhibited HM-acetone-, KM ethanol- and KM water-based extract. The HN acetone-based and ethanol-based extracts had comparable DPPH free radical scavenging ability and were slightly more potent than HN water extract (Table 1). Similar tendency was observed for the FRAP and ORAC values; among all the extracts acetone-based exhibited the highest values and were followed by ethanol- and water-based extracts (Table 1). Superior FRAP and ORAC values in each category of extracts were obtained for green tea (Table 1). Among the acetone-based extracts KM performed better than HN, however among the water-based extracts opposite result was observed (Table 1). Both acetone-based jasmine rice leaf tea extracts (KM and HN) and the reference sample of green tea (GT) exhibited higher ORAC and FRAP values than those of *Eryngium bornmuelleri* leaf tea extracts obtained through the same extraction process. However, the ORAC and FRAP values of the ethanol- and water-based extracts evaluated in this study were comparable to those of the respective ethanol- and water-based *Eryngium bornmuelleri* leaf tea extracts [11]. These results suggest that with regards to antioxidant capacity jasmine rice leaf teas are inferior to green tea, and they are comparable or superior to other traditionally utilized ethnic teas prepared from locally grown botanicals.

Identification and quantification of phenolic compounds present in tea extracts

Identification of phenolic compounds in sequential extracts obtained from rice leaf tea was carried out by high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC-MS) analyses (Table 2). The HPLC profiles of acetone- and ethanol-based extracts of both jasmine rice leaf teas were very similar with the exception that the concentration of phenolic compounds in acetone-based extract was higher (Figures 2 and 3). The majority of compounds present in KM and HN teas were visible on HPLC chromatogram at 326 nm wavelength (Figure 2). The spectral characteristics of HPLC peaks revealed that chlorogenic acids (4- and 5-chlorogenic acid) are the dominating phenolic compounds of both jasmine rice teas. The majority of compounds present in green tea extracts were found at 280 nm with gallic acid, catechin and epigallocatechin identified (Figure 3C). Gallic acid was also the dominating phenolic compound in KM water extract (Figure 4A)

Table 1. Extraction yield, reducing and total sugars, total phenolics, and antioxidant capacity of lyophilized fractions obtained from jasmine rice young leaf tea, cultivars Khao Dok Mali 105 and Homnil, and reference sample green tea

Extraction solvent	Tea Sample	Yield (%)	Reducing sugar (mg G E/g DW) ^a	Total Sugar (mg G E/g DW) ^a	TP (mg GA E/g DW) ^b	DPPH (EC ₅₀ ; mg/mL)	FRAP (μmol Fe ²⁺ /g DW)	ORAC (μmol TE/g DW)
Acetone (A)								
	KM	6.0	58.8±1.8 ^a	112.5±1.6 ^a	113.8±2.7 ^b	1.31 ^b	1484.2±43.4 ^b	4093.1±177.0 ^b
	HN	3.6	12.01±1.2 ^b	32.2±2.1 ^c	107.5±5.5 ^c	3.67 ^a	1285.9±50.2 ^c	3452.8±146.4 ^c
	GT	28.9	4.8±0.3 ^c	87.2±0.7 ^b	346.7±5.1 ^a	1.25 ^c	9596.4±70.5 ^a	5115.1±170.7 ^a
Ethanol (E)								
	KM	5.1	61.6±5.8 ^a	125.3±2.8 ^a	31.3±1.0 ^b	6.41 ^a	747.8±41.4 ^b	2320.8±123.5 ^c
	HN	2.3	42.0±2.1 ^c	98.4±1.4 ^b	28.9±1.6 ^c	3.79 ^c	543.8±32.0 ^c	2906.0±76.9 ^b
	GT	6.4	44.6±1.8 ^b	92.1±2.1 ^c	142.7±2.2 ^a	4.73 ^b	4577.3±61.4 ^a	3981.0±79.5 ^a
Water (W)								
	KM	7.6	65.2±2.6 ^b	128.2±1.6 ^a	36.6±1.6 ^a	4.76 ^a	215.3±13.1 ^c	1762.1±68.5 ^c
	HN	6.3	6.24±2.1 ^c	77.5±2.8 ^c	16.4±1.1 ^c	4.52 ^b	441.9±13.1 ^b	1784.2±41.0 ^b
	GT	3.0	71.0±1.3 ^a	105.3±3.0 ^b	32.8±2.2 ^b	3.57 ^c	685.5±21.7 ^a	2024.9±72.0 ^a

Data represents the mean ±standard deviation of at least three independent experiments. For each parameter investigated values with no letters in common are significantly different at $p < 0.05$. ^a: mg GE/gDW; milligram glucose equivalent per gram dry weight; ^b: mg GA E/gDW milligram gallic acid equivalent per gram dry weight; **FRAP**: Ferric reducing antioxidant power; **ORAC**: Oxygen radical absorbance capacity; **μmol Fe²⁺/g DW**: μmol of ferrous equivalents per g dry weight of lyophilized extract; **EC₅₀**: Half of the concentration which reduced a response halfway; **KM**: Jasmine rice Khao Dok Mali 105; **HN**: Jasmine rice Homnil; **GT**: Green tea

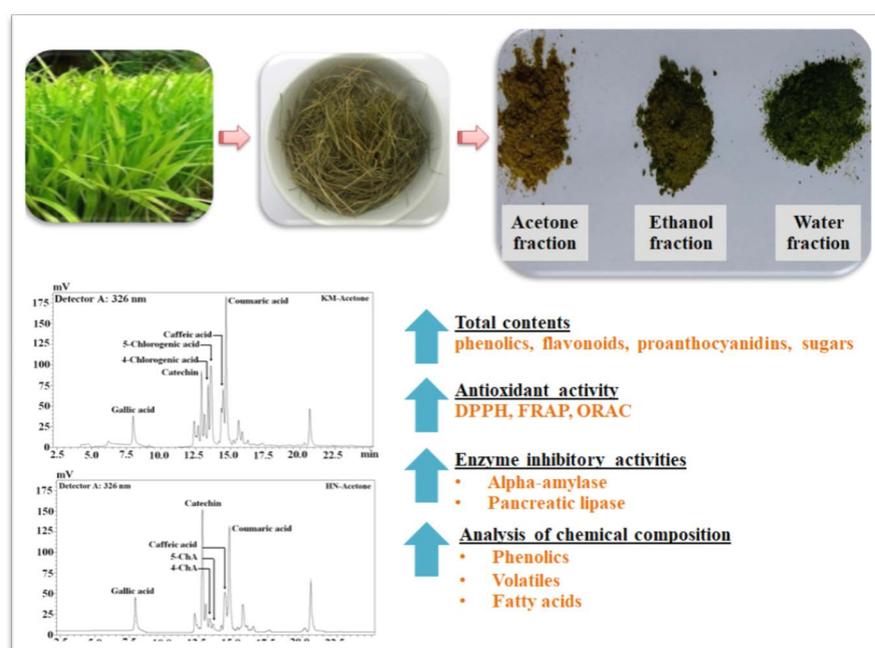


Figure 1. Graphical abstract

Table 2. Phenolic compounds identified in jasmine rice young leaf tea, cultivars Khao Dok Mali 105 and Homnil, and reference sample green tea

Phenolic compound	MS/MS		Concentration (mg/g extract)		
	[M+1] ⁺ /[M-1] ⁻	Fragments (m/z) (+/-)	Acetone	Ethanol	Water
KM					
<i>p</i> -Coumaric acid	-/163	-/119	52±4.2	11.2±0.6	T
Gallic acid	-/169	-/125	7.2±0.1 ^c	13.0±0.6 ^b	32.1±1.4 ^a
Caffeic acid	-/179	-/135	19.2±1.8 ^a	9.6±0.2 ^b	ND
Catechin	-/289	-/245	17.1±0.5 ^a	4.4±0.0 ^b	T
Epigallocatechin	-/305	-/225	ND	ND	ND
Chlorogenic acid	-/353	-/191	54.7±6.4 ^a	25.3±1.1 ^b	T
HN					
<i>p</i> -Coumaric acid	-/163	-/119	36.8±2.5 ^a	16.3±0.9 ^b	3.3±0.0 ^c
Gallic acid	-/169	-/125	8.1±0.4 ^b	12.6±0.0 ^a	13.8±0.4 ^a
Caffeic acid	-/179	-/135	15.1±0.6 ^a	12.6±1.0 ^b	4.2±0.2 ^c
Catechin	-/289	-/245	28.4±1.6 ^a	3.6±0.1 ^b	1.6±0.0 ^c
Epigallocatechin	-/305	-/225	ND	ND	ND
Chlorogenic acid	-/353	-/191	8.2±0.4 ^c	65.3±1.7 ^a	26.6±1.8 ^b
GT					
<i>p</i> -Coumaric acid	-/163	-/119	ND	ND	ND
Gallic acid	-/169	-/125	ND	20.1±0.9	24.6±1.2
Caffeic acid	-/179	-/135	ND	ND	ND
Catechin	-/289	-/245	32.5±1.4 ^b	55.4±3.8 ^a	8.0±0.0 ^c
Epigallocatechin	-/305	-/225	52.5±3.9 ^b	98.2±6.5 ^a	T
Chlorogenic acid	-/353	-/191	ND	ND	ND

T: Traces; ND: not detected; KM: Jasmine rice Khao Dok Mali 105; HN: Jasmine rice Homnil; GT: green tea

while chlorogenic acid was the dominating compound in HN water extract (Figure 4B). In agreement with the HPLC chromatograms the mass spectroscopy data confirmed that hydroxycinnamic acids were the major phenolic compounds identified in both jasmine rice leaf teas (Table 2). Chlorogenic acid and *p*-coumaric acid were the major phenolic compounds of KM acetone and ethanol extracts representing, respectively, 36% and 34% of total phenolics (Table 2). Chlorogenic acid identified in HN ethanol and water extracts accounted for, respectively, 59% and 54% of total phenolics. Significant amounts of gallic acid and

catechin were also detected (Table 2). The dominating compounds of GT extracts were catechin and epigallocatechin gallate (Figures 2-4). Dai and Mumper [20] reported that ethanol is an efficient extraction solvent of low molecular weight polyphenols, while aqueous acetone is more suitable for the extraction of high molecular weight flavanols. This study identified similar phenolic composition of both, ethanol and acetone jasmine rice leaf tea extracts (Table 2). These results suggest that in the evaluated jasmine rice leaf teas high molecular weight compounds are present at very low levels or are absent. Similar results were reported for *Eryngium borrmuelleri* leaf tea [11].

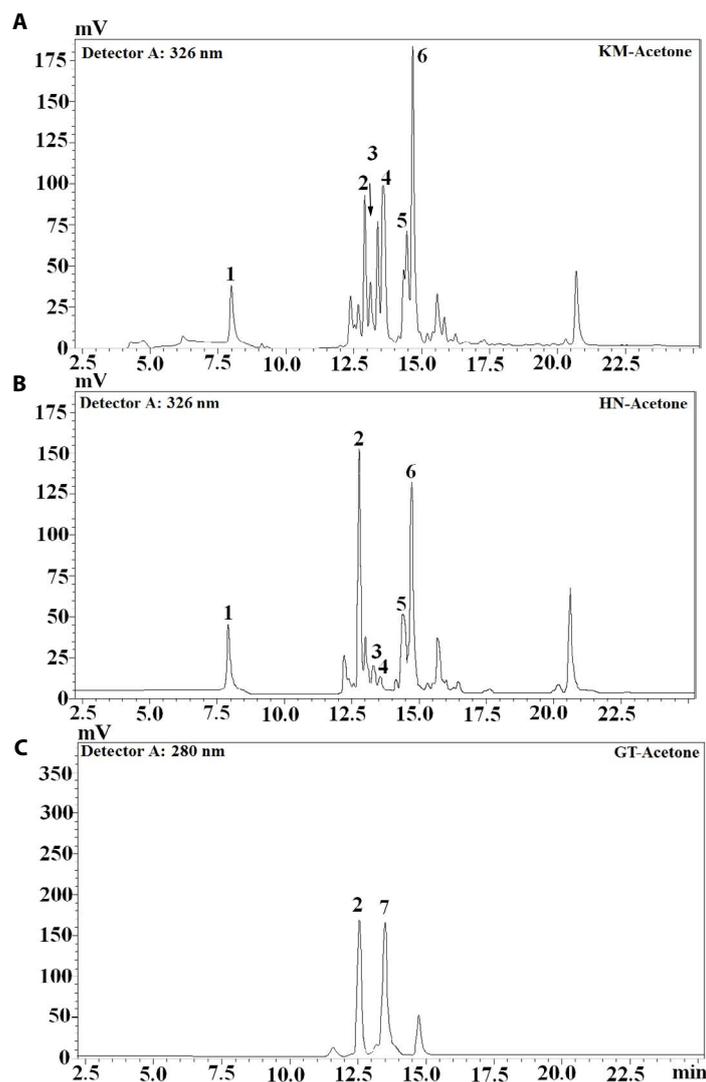


Figure 2. Chromatograms of acetone extracts obtained from jasmine rice leaf and green tea (A: Khao Dok Mali 105 (KM); B: Homnil (HN); C: green tea (GT); 1: Gallic acid; 2: Catechin; 3: 4-Chlorogenic acid; 4: 5-Chlorogenic acid; 5: Caffeic acid; 6: Coumaric acid; 7: Epigallocatechin gallate)

Identification of volatile compounds by gas chromatography mass spectroscopy (GCMS)

Volatile compounds are required in food development as they provide a pleasant scent. Moreover, they exhibit pharmacological activities through their antioxidant, anti-inflammatory, anti-cancer and anti-obesity properties [21,22]. Jasmine rice is known for its attractive aroma, therefore the presence of volatile compounds in jasmine rice leaf teas was investigated. The GC-MS profiles of KM, HN and GT volatiles revealed the presence of similar compounds in all teas (Figures 5-7) (Tables 3 and 4). Tridecane was the predominant volatile of KM acetone extract, followed by 2,6-Di-tert-butylphenol and pentadecane. In the KM ethanol extract dominated caryophyllene and 2,6-Di-tert-butylphenol. Five compounds were identified in KM water extract: eicosane, 3-ethyl-5-(2-ethylbutyl) octadecane, etricosane, heneicosane 11-(1-ethylpropyl) and dodecane. Traces of the same compounds were also found in acetone- and ethanol-based extracts (Table 3). Dihydroactinoliolide was found in HN acetone extract, while dodecane isomer and dodecane were detected in ethanol- and water-based extract, respectively (Table 3). Three volatile compounds (tridecane,

dodecane and 2,6-Di-tert-butylphenol) were found in HN and KM water-based extracts. Five volatile compounds, including tridecane, 1-dodecene, dodecane, 1-tetradecene and 2,6-di-tert-butylphenol were identified in GT acetone extract. Green tea ethanol- and water-based extracts comprised, respectively, 3 compounds (tridecane, dodecane, α -fenchylacetate) and 4 compounds (dodecane, 1-tetradecene, 2,6-di-tert-butylphenol, eicosane) (Table 4). The results revealed that similar volatile compounds are present in KM and HN water-based extracts. This finding suggests that these volatile compounds will be released into tea during the infusion process and may influence its sensory properties and consumers' preferences.

Identification of fatty acid composition

In a life cell the fatty (carboxylic) acids from foods are utilized in conversion of biochemical energy from nutrients into adenosine triphosphate (ATP). The GCMS analysis of fatty acids revealed the presence of similar fatty acids in all evaluated teas (Table 5). Moreover, similar compounds were detected in all extracts categories (acetone, methanol and water). Acetone extract of KM comprised α -linolenic

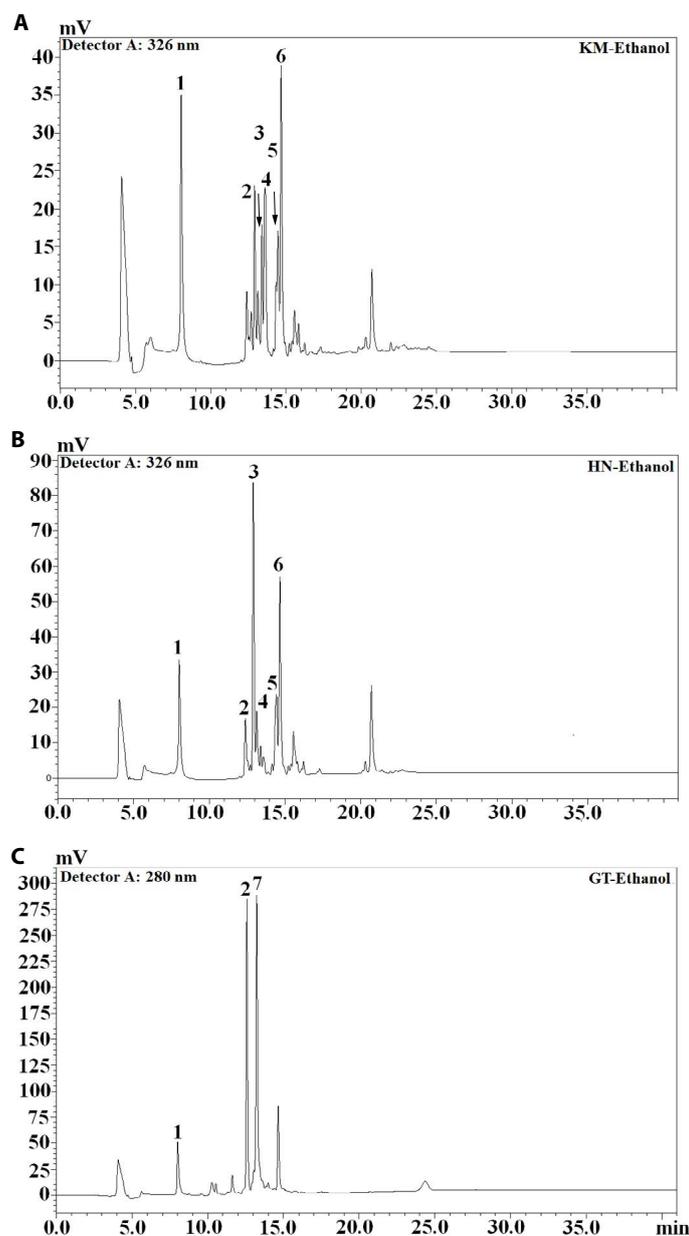


Figure 3. Chromatograms of ethanol extracts obtained from jasmine rice leaf and green tea (A: Khao Dok Mali 105 (KM); B: Homnil (HN); C: green tea (GT); 1: Gallic acid; 2: Catechin; 3: 4-Chlorogenic acid; 4: 5-Chlorogenic acid; 5: Caffeic acid; 6: Coumaric acid; 7: Epigallocatechin gallate)

Table 3. Volatile compounds identified in Khao Dok Mali 105 jasmine rice young leaf tea

No.	Retention time	Name of compound	Molecular formula	Molecular mass	Fragment ions	Relative concentration (%)		
						Acetone	Ethanol	Water
1	15.494	Tridecane	C ₁₃ H ₂₈	184	57, 71, 85, 99, 127, 155	35.91	ND	ND
2	20.169	Dodecane	C ₁₂ H ₂₆	170	57, 71, 85, 99, 127, 170	ND	ND	5.19
3	21.909	Pentadecane	C ₁₅ H ₃₂	212	57, 71, 85, 99, 141, 212	3.97	T	T
5	29.181	Caryophyllene	C ₁₅ H ₂₄	204	55, 69, 79, 93, 133, 147, 189	ND	36.48	ND
6	31.727	2,6-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206	57, 74, 91, 163, 191, 206	9.12	18.14	T
7	37.553	3-Ethyl-5-(2-Ethylbutyl) Octadecane	C ₂₆ H ₅₄	366	57, 71, 85, 99, 183, 281	ND	ND	17.30
8	40.219	Tetracosane	C ₂₄ H ₅₀	338	43, 57, 71, 85, 99, 113, 141, 155	ND	ND	10.93
9	40.356	Nonadecane	C ₁₉ H ₄₀	268	43, 57, 71, 85, 99, 113, 154	T	ND	T
10	42.628	Eicosane	C ₂₀ H ₄₂	282	43, 57, 71, 85, 99, 113, 127	ND	ND	17.68
11	45.100	Heneicosane 11-(1-ethylpropyl)	C ₂₆ H ₅₄	366	57, 71, 85, 99, 295	ND	ND	6.70

T: Traces; ND: Not detected

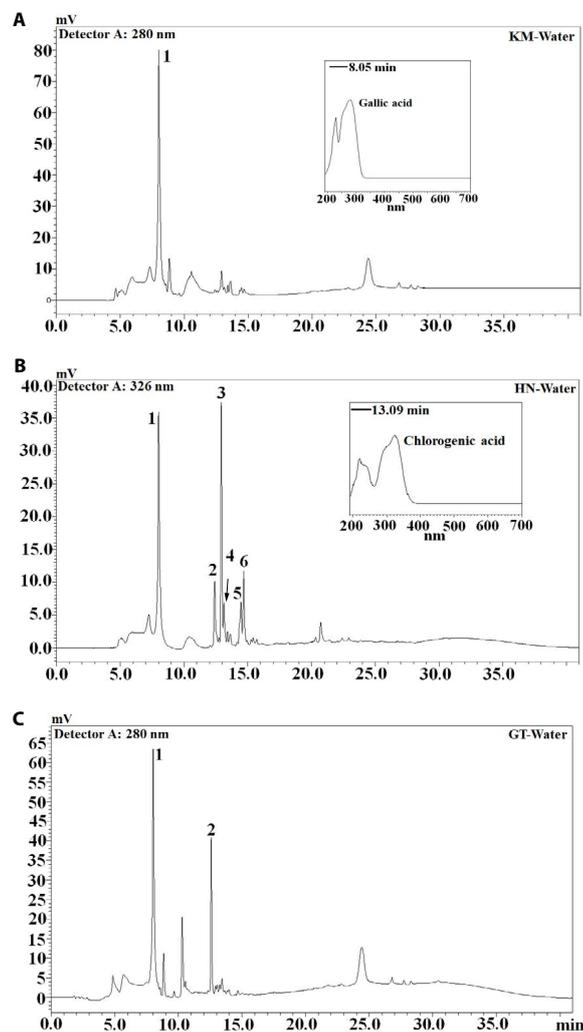


Figure 4. Chromatograms of water extracts obtained from jasmine rice leaf and green tea (A: Khao Dok Mali 105 (KM); B: Homnil (HN); C: green tea (GT); 1: Gallic acid; 2: Catechin; 3: 4-Chlorogenic acid; 4: 5-Chlorogenic acid; 5: Caffeic acid; 6: Coumaric acid)

Table 4. Volatile compounds identified in Homnil jasmine rice leaf tea and reference sample green tea

No.	Retention time	Name of compound	Molecular formula	Molecular mass	Fragment ions	Relative concentration (%)		
						Acetone	Ethanol	Water
Jasmine rice Homnil								
1	15.495, 19.883	Tridecane	C ₁₃ H ₂₈	184	57, 71, 85, 99, 127, 155	ND	ND	11.17
2	19.883	Cyclohexasiloxane, dodacamethyl-	C ₁₄ H ₃₆ O ₆ Si ₆	444	73, 117, 147, 207, 341, 429	ND	ND	3.02
3	20.175	Dodecane	C ₁₂ H ₂₆	170	57, 71, 85, 99, 127, 170	ND	ND	23.12
4	20.478	Cyclohexanone, 4-hydroxy-4-methyl	C ₇ H ₁₂ O ₂	128	71, 85, 95, 110, 128	28.47	ND	ND
5	21.897	Dodecane isomer	C ₁₂ H ₂₆	170	57, 71, 85, 99, 127, 170	T	21.21	8.90
6	24.163	Cycloheptasiloxane, tetradecamethyl	C ₁₄ H ₃₂ O ₇ Si ₇	518	73, 147, 221, 281, 327, 415, 503	ND	ND	3.37
7	26.034	Tetradecane	C ₁₄ H ₃₀	198	57, 71, 85, 99, 198	ND	ND	11.35
8	31.722	2,6-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206	57, 74, 91, 163, 191, 206	10.82	T	T
9	36.546	Dihydroactinoliide	C ₁₁ H ₁₆ O ₂	180	67, 111, 137, 180	47.01	ND	ND
Green Tea								
1	15.483	Tridecane	C ₁₃ H ₂₈	184	57, 71, 85, 99, 127, 155	10.82	29.15	ND
2	20.238	1-Dodecene	C ₁₂ H ₂₄	168	55, 69, 83, 97, 168	21.25	ND	ND
3	21.909	Dodecane	C ₁₂ H ₂₆	170	57, 71, 85, 99, 127, 170	2.61	7.78	7.01
4	26.109	1-Tetradecene	C ₁₄ H ₂₈	196	55, 69, 97, 196	20.53	ND	9.88
5	28.249	Alpha-Fenethylacetate	C ₁₂ H ₂₀ O ₂	196	69, 80, 95, 121, 136, 154, 196	ND	5.60	ND
6	31.716	2,6-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206	57, 74, 91, 163, 191, 206	12.56	ND	3.10
7	42.714	Eicosane	C ₂₀ H ₄₂	282	43, 57, 71, 85, 99, 113, 127	ND	ND	17.89

T: Traces; ND: Not detected

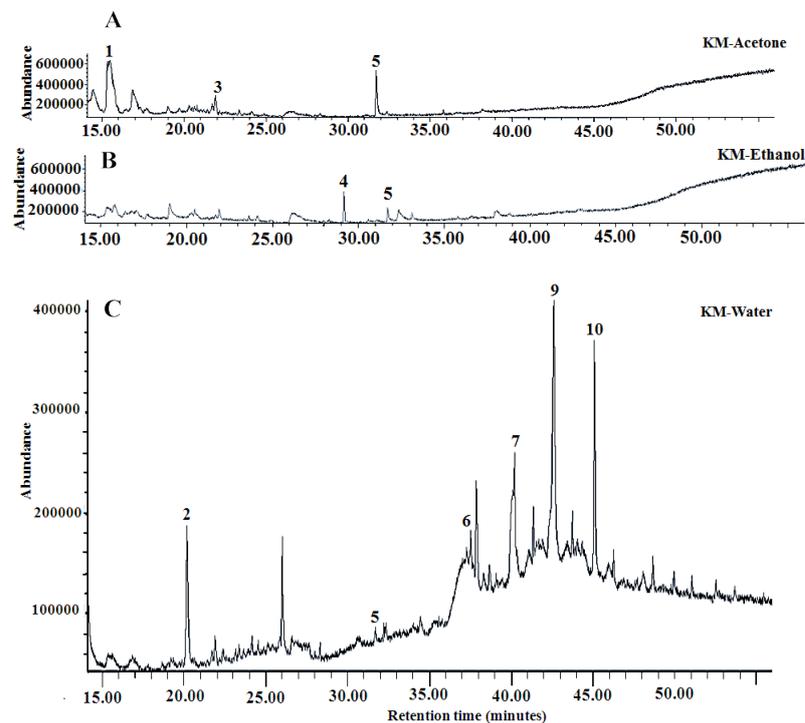


Figure 5. Total ion profile of volatile compounds present in lyophilized sequential extracts obtained from jasmine rice Khao Dok Mali 105 (KM) (A: Acetone; B: Ethanol; C: Water; 1: Tridecane; 2: Dodecane; 3: Pentadecane; 4: Caryophyllene; 5: 2,6-Di-tert-butylphenol; 6: 3-Ethyl-5-(2-Ethylbutyl) Octadecane; 7: Tetracosane; 8: Nonadecane I; 9: Eicosane; 10: Heneicosane 11-(1-ethylpropyl))

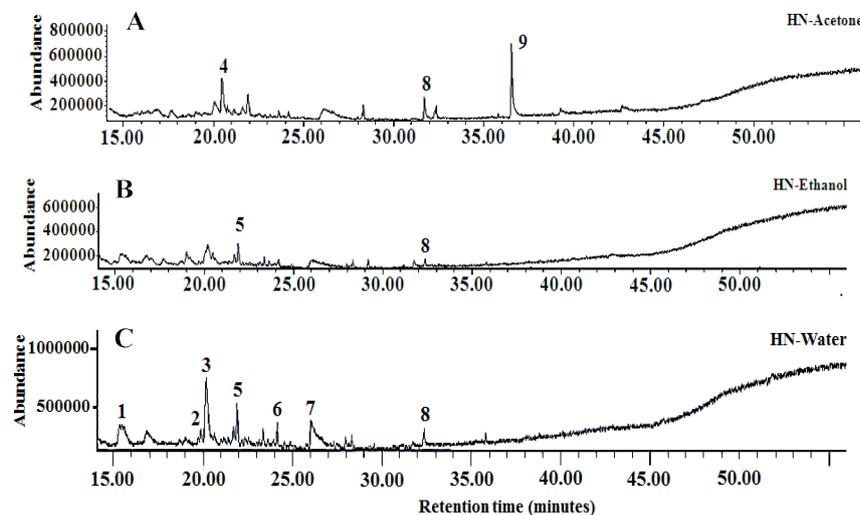


Figure 6. Total ion profile of volatile compounds detected in lyophilized sequential extracts obtained from jasmine rice Homnil (HN) (A: Acetone; B: Ethanol; C: Water; 1: Tridecane; 2: Cyclohexasiloxane; dodacemethyl-; 3: Dodecane; 4: Cyclohexanone; 4-hydroxy-4-methyl; 5: Dodecane isomer; 6: Cycloheptasiloxane; tetradecamethyl; 7: Tetradecane; 8: 2,6-Di-tert-butylphenol I; 9: Dihydroactinoliolide)

acid, palmitic acid and linoleic acid. The fatty acids compositions of ethanol- and water-based extracts of KM and HN were comparable (Table 3). The ethanol-based KM and HN extracts also contained α -linolenic acid, palmitic acid and linoleic acid. Traces of stearic acid were also present. The water extracts contained α -linolenic acid, palmitic acid and linoleic acid. Similar fatty acids composition and content was found in GT extracts with the exception of vaccenic acid which was detected in acetone and ethanol extracts and stearic acid detected in water extract. According to Kobayashi and collaborators

[23], presence of the major fatty acid derivatives: C6 and C9 alcohols and aldehydes in green tea is the key contributor to its fresh odor.

Enzyme inhibitory activities

Obesity - one of the greatest threats to global health is currently treated with synthetic inhibitors of digestive enzymes, such as acarbose and orlistat. Because an uptake of synthetic inhibitors may lead to unwanted side effects, the attention of researchers is directed to natural compounds from plant sources that provide safer alternative. Within

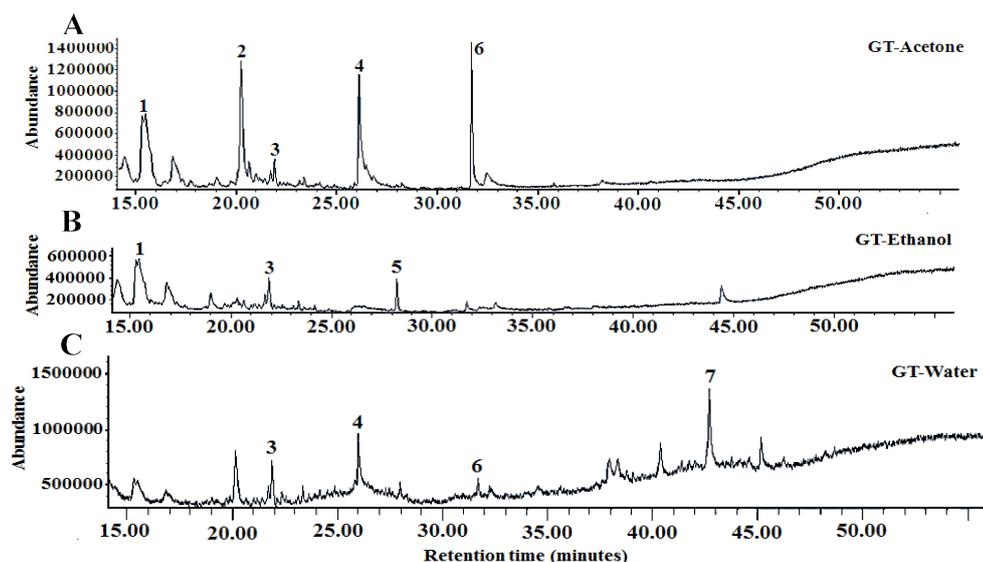


Figure 7. Total ion profile of volatile compounds presents in lyophilized sequential extracts obtained from green tea (GT) (A: Acetone; B: Ethanol; C: Water; 1: Tridecane; 2: 1-Dodecene; 3: Odecan; 4: 1-Tetradecene; 5: Alpha-Fenchylacetate; 6: 2;6-Di-tert-butylphenol; 7: Eicosane)

Table 5. Fatty acid composition identified acetone, ethanol and water-based extracts of jasmine rice young leaf tea, cultivars Khao Dok Mali 105 (KM) and Homnil (HN) and reference sample green tea (GT)

Compounds	Acetone (A)	Ethanol (E)	Water (W)
KM			
Palmitic acid	21.10	25.53	27.17
Stearic acid	T	2.52	T
Vaccenic acid	ND	ND	ND
Linoleic acid	16.6	21.33	22.66
α -Linolenic acid	56.74	47.62	44.73
HN			
Palmitic acid	21.81	25.39	26.90
Stearic acid	T	T	4.02
Vaccenic acid	ND	ND	ND
Linoleic acid	15.97	19.70	19.43
α -Linolenic acid	57.79	49.37	46.11
GT			
Palmitic acid	24.11	29.63	33.13
Stearic acid	T	T	23.92
Vaccenic acid	6.72	9.38	T
Linoleic acid	17.76	21.97	7.64
α -Linolenic acid	45.12	33.99	20.12

this study enzyme-inhibitory activities of jasmine rice leaf teas were evaluated.

Alpha-amylase and pancreatic lipase inhibitory activities

The extracts of KM and HN tea inhibited the activity of α -amylase in a dose response manner (data not shown). The inhibitory activity of KM acetone extract was 2.3-fold that of KM ethanol- and 9.8-fold that of KM water-based extract. The acetone and ethanol extracts of HN exhibited comparable α -amylase inhibitory activities and were 6.6-fold those of HN water extract. Alpha-amylase inhibitory activities of water extracts obtained from all evaluated samples were comparable (Table 6).

Acetone extract of KM as well as acetone extract of GT exhibited the strongest and similar pancreatic lipase inhibition. The inhibitory activity of HN acetone extract was 3.5-fold lower (Table 6). Similar low inhibitory activities towards pancreatic lipase showed ethanol and water extracts of KN and HN. Lipase inhibitory activities of jasmine rice leaf

teas were lower than those of *Eremochloa ophiuroides* (centipede grass) leaf methanolic extracts (IC_{50} of $33.6 \pm 2.0 \mu\text{M}$ equal to $0.019 \pm 0.001 \text{ mg/mL}$) [24].

Chlorogenic acid possesses pronounced α -amylase inhibitory properties [24]. The presence of chlorogenic acid at high levels in ethanol- and acetone-based extracts of jasmine rice leaf teas may be responsible for the enzyme inhibition observed in this study. Lower levels of chlorogenic acid in KM and HN water-based extracts correlate well with the lower IC_{50} values (Table 6). Structure of dietary polyphenols influences their α -amylase inhibitory effect (Xiao *et al.*, 2013). This effect increases with the presence of hydroxylation of flavonoids and/or presence of an unsaturated 2,3-bond in conjugation with a 4-carbonyl group. Glycosylation of flavonoids as well as methylation and methoxylation weakens the inhibitory effect [25,26]. In agreement, in the present study the chlorogenic acid rich extracts obtained from KM and HN rice teas exhibited pronounced enzyme-inhibitory activities.

Table 6. Enzyme α -amylase and pancreatic lipase inhibitory activities of acetone, ethanol and water-based extracts of jasmine rice young leaf tea, cultivars Khao Dok Mali 105 (KM) and Homnil (HN) and reference sample green tea (GT)

Tea sample inhibitory activity samples	Acetone (A)	Ethanol (E)	Water (W)
KM			
α -amylase (IC ₅₀ ; μ g/mL)	73.96 \pm 2.7 ^a	168.65 \pm 19.2 ^b	724.99 \pm 21.3 ^c
Acarbose E. (μ M/g extract)	656.22 \pm 48.0 ^a	288.87 \pm 32.8 ^b	66.85 \pm 2.03 ^c
Pancreatic lipase (IC ₅₀) μ g/mL)	65.7 \pm 5.8 ^a	329.6 \pm 0.7 ^b	870.9 \pm 33.6 ^c
Orlistat E. (μ M/g extract)	42.2 \pm 3.9 ^a	8.4 \pm 0.0 ^b	3.2 \pm 0.1 ^c
HN			
α -amylase (IC ₅₀ ; μ g/mL)	113.6 \pm 7.07 ^a	116.2 \pm 0.05 ^a	752.48 \pm 0.67 ^b
Acarbose E. (μ M/g extract)	426.92 \pm 26.6 ^a	415.15 \pm 2.01 ^a	64.32 \pm 0.06 ^b
Pancreatic lipase (IC ₅₀) μ g/mL)	227.0 \pm 1.4 ^a	353.8 \pm 5.1 ^b	821.0 \pm 14.9 ^c
Orlistat E. (μ M/g extract)	12.2 \pm 0.1 ^a	7.8 \pm 0.1 ^b	3.4 \pm 0.1 ^c
GT			
α -amylase (IC ₅₀ ; μ g/ml)	93.86 \pm 2.54 ^a	102.4 \pm 0.79 ^b	700.02 \pm 5.18 ^c
Acarbose E. (μ M/g extract)	515.92 \pm 13.95 ^a	472.48 \pm 3.59 ^b	69.51 \pm 0.02 ^c
Pancreatic lipase (IC ₅₀) μ g/mL)	63.3 \pm 1.2 ^c	147.4 \pm 3.3 ^b	788.9 \pm 6.9 ^a
Orlistat E. (μ M/g extract)	43.6 \pm 0.8 ^a	18.7 \pm 0.4 ^b	3.5 \pm 0.0 ^c

These activities are comparable to those of commonly used medicinal plant teas utilized in Turkey [11].

Conclusions

Acetone- and ethanol-based extracts of jasmine rice leaf contained predominantly chlorogenic acid, accompanied by other hydroxycinnamic (*p*-coumaric acid, caffeic acid) and phenolic acids (gallic acid). The acetone-based extract of KM and ethanol-based extract of HN contained predominantly chlorogenic acid and exhibited comparable α -amylase inhibitory activities. This study indicates that jasmine rice leaf tea extract may modulate the metabolism of sugars and fats through inhibition of the relevant digestive enzymes. Young jasmine rice leaf can be utilized as an alternate plant source for the production of a novel functional tea.

Acknowledgements

Authors acknowledged the Faculty of Agriculture and Technology, Rajamangala University of Technology Isan (RMUTI), Surin campus, Thailand and Faculty of Pharmacy, Van Yuzuncu Yil University, Turkey, for providing necessary facilities and financial support to carry out this research.

Conflicts of interest

The Authors declare no conflict of interest in carrying out this research or preparing this article for publication.

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