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Phytochemistry and antifatigue activities of *Carica papaya* leaf from geothermal, coastal and urban areas, Indonesia

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Abstract

Fatigue, a condition of lack of energy and motivation resulting in the feeling of extreme tiredness or exhaustion, is usually prevented and treated with ergogenic aids, such as in the form of nutritional supplements. Papaya (*Carica papaya)* may be a potential candidate for ergogenic aids, considering its healthy secondary metabolite properties and number of metabolite compounds that could be affected by the location where the plant growing. The aim of this study was to identify the phytochemicals of papaya leaves from three different locations: geothermal, coastal, and urban areas in Aceh province, Indonesia. Concentrations of papaya leaf with the highest number of secondary metabolite compounds were tested in rats to measure blood lactate acid concentrations after strenuous exercise. The number of chemical compounds identified from the three locations was 24 compounds; 23 compounds and 17 compounds, respectively. The highest concentration of chemical compounds that have antifatigue activity contained in all papaya leaf samples were neophytadiene, linolenic acid, gamma tocopherol, hexadecanoic acid, vitamin E, carpaine, octadecatrienoic acid, nor lean-12-ene, squalene, and phytol. Furthermore, most of the compounds' highest concentrations were found in papaya leaves from the coastal area and, therefore, tested on the animal model. Treatment was provided in 12 male rats with different doses of papaya powder supplements for 15 days. The results showed that lactic acid levels of rats received a dose of 400 mg/kg of papaya leaf extract reduced the lactic acid concentration (p=0.014) compared with the control group. This study highlights that papaya leaves from the coastal area have the most potential activities as ergogenic herbal aid and were able to reduce lactic acid levels in rats after strenuous exercise.

Keywords: *Carica papaya,* phytochemistry, GC-MS, anti-inflammation, ergogenic aid

Introduction

Fatigue is simply defined as the lack of energy and motivation resulting in the feeling of extreme tiredness or exhaustion. Fatigue can be a result of the body's response to activities carried out continuously with moderate and heavy intensity, causing the inability of muscles to contract strongly and for a long time due to reduced available energy sources in the form of muscle glycogen [1-4]. Fatigue can be overcome by utilizing ergogenic aids, which are chemical substances with certain nutrients that increase energy production and body metabolism and can

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accelerate the healing process from muscle injuries and infections during exercise [5,6]. Exercise programs, sports equipment, nutrition, various medications derived from chemicals or natural plant compounds, and psychology are all examples of ergogenic aids which can alter the physiological characteristics of bodily organs directly [7,8].

It has been reported that about 50% of athletes generally use supplements to increase stamina [9]. Ergogenic aids in the form of nutritional supplements can be used as an alternative to prevent muscle damage due to infection and improve exercise performance if consumed within a certain period of time that has been programmed [10]. A nutritional supplement is included as an ergogenic aid if it can produce muscular hypertrophy, inhibit inflammatory muscle breakdown, increase strength, and improve performance during physical exercise [5]. Several studies have shown that plants' secondary and essential metabolites preserve health in many different ways, such as anti-inflammatory, antioxidant, antiallergy, antimicrobials, antivirus, antibacterial, anticancer, and others [11]. Furthermore, studies stated that the efficacy of herbal supplements varies greatly depending on the geographic location of the plant taken, the method of extraction, and the type of plant [10,11]. *Carica papaya*, also known as papaya, is a plant with antibacterial, antioxidant, anti-inflammatory, and anticancer properties, posing its potential as an excellent ergogenic nutraceutical [12-15]. In Asia, apart from being a garden plant, it is also used as a commercial medicinal ingredient, and all its parts can be used as herbal medicine to cure various diseases [16,17].

The aim of this study was to analyze the phytochemical compounds of *C. papaya* leaf and its known biological activities from three different locations (geothermal area and coastal area in Aceh-Besar district, and the urban area in Banda Aceh, Indonesia) and to determine the effect of papaya leaf extract on lactic acid concentration as one of indicator of fatigue in animal model.

Methods

The samples of *C. papaya* leaves were collected from three separate locations: the geothermal area (Ie Suum village), the coastal area (Kajhu village) in Aceh Besar District, and the urban area (Lambhuk) in Banda Aceh, Indonesia. All leaves were subjected to identification in the Biology Laboratory of the Faculty of Mathematical and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia. The processing of simplicia consisted of collecting raw materials, wet sorting, washing, counting, drying, dry sorting, milling, packaging, and storage. In this study, the samples used as samples were papaya leaves that were still green which were harvested from the 4^{th}, 5^{th} and 6^{th} stalks of the shoots, had no defects and were not affected by pests.

Extraction methods

Dried simplicia of *C. papaya* leaves were air dried for five days, blended, and sieved to obtain the powder. Two kilograms of simplicia powder was mixed with 96% ethanol for 72 hours. The result of maceration in the form of a thick liquid extract was then concentrated using a rotary evaporator to obtain ethanol extract of *C. papaya* leaves.

Phytochemical screening

Phytochemical screening was conducted to determine the presence of different secondary metabolites in *C. papaya* leaves. Secondary metabolites from the flavonoid group were examined using the cyanidin reagent (magnesium powder and concentrated hydrochloric acid); phenolic group with the iron (III) chloride reagent; saponin group with foam and water testing; and steroid, alkaloid, and triterpenoid groups with the Lieberman Burchard reagent (anhydrous acetic acid and concentrated sulphuric acid).

Gas chromatography-mass spectrophotometry analysis

Gas chromatography-mass spectrophotometry (GC-MS) of *C. papaya* leaves was conducted in the Laboratory of the Special Regional Health Office of Jakarta, Indonesia, using an Intelligent Technology 7890 Gas Chromatograph (California, USA) with an automatic sampler. The electron ionization model with an energy of 70 eV, column hp ultra 2, and an initial temperature of 80°C. The carrier gas was helium. The constant flow column mode has a column flow of 1.2 mL/min and an injection volume of 5 L.

Animal model and powder supplement administration protocol

An experimental study with post-test only with control group design was conducted using male *Rattus norvegicus* rats aged 2–3 months weighing 120–250 grams. The rats were adapted to the environment for 1 week; provided with food and drink according to their needs. A total of 12 rats were used and divided into three groups. Group 1, a negative control group (NC), was provided feed and drink only. Group 2 (CP200) and group 3 (CP400) were supplemented with oral 200 mg/body weight and 400 mg/body weight of papaya leaf extract powder supplements, respectively, for 15 consecutive days with a frequency of once daily. On day 16, all rats were subjected to a heavy-intensity physical activity test in the form of running on an automatic treadmill at a speed of 30 m/min. The heavy intensity speed was expected for the rats to experience fatigue, which is characterized by inflammation, the release of lactic acid, and free radicals [18,19].

Examination of blood lactic acid concentration

The lactic acid concentration was examined using the Accutrend Plus system kit (Roche Diagnostics, Germany). The examination was carried out immediately after the exercise. Briefly, 0.2 ml of the orbital blood was collected using a 1 mL syringe and collected in ethylenediaminetetraacetic acid (EDTA) tubes and the lactic acid level was conducted no more than five minutes.

The statistical analysis

To determine the differences in fatigue assessment parameters for the three experimental groups, one-way ANOVA and Duncan tests were used [20]. Data analysis in this study was conducted using SPSS software version 16.0 (IBM, New York, USA). The level of significance was tested at $\alpha=0.05$.

Results

Species confirmation

Identification of papaya leaves was performed in the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia, and confirmed that the three samples submitted were *C. papaya* leaves.

Phytochemical screening of papaya leaves

Phytochemical analysis of papaya leaf samples from three different locations showed different results. Papaya leaves from coastal and urban areas possessed five metabolic contents (alkaloids, steroids, saponins, flavonoids, and phenolics), while the geothermal area had only four metabolic contents (alkaloids, steroids, flavonoids, and phenolics) (**Table 1**).

Table 1. Phytochemical constituent of *Carica papaya* leaves from three different locations

Metabolite content	Reagent	*Carica papaya*		
		Geothermal area	Coastal area	Urban area
Alkaloid	Mayer	+	+	+
	Wagner	+	+	+
	Dragendorff	+	+	+
Steroid	Liebermann Burchard test	+	+	+
Terpenoid	Liebermann Burchard test	-	-	-
Saponin	Shuffle	-	+	+
Flavonoid	HCl and Metal Mg	+	+	+
Phenolic	$FeCl_3$	+	+	+
Tannin	Gelatin+H_2SO_4	-	-	-

Gas chromatography-mass spectrophotometry analysis of papaya leaves

The GC-MS analysis showed that papaya leaf samples from coastal, geothermal, and urban areas contained 24, 23, and 17 putative compounds, respectively (**Table 2**). The following is an order of the highest compounds contained in all samples from three locations that had anti-inflammatory and antioxidant effects as ergogenic candidates for overcoming muscle fatigue:

neophytadiene, gamma-tocopherol, hexadecanoic acid, vitamin E, octadecatrienoic acid, squalene, and phytol (**Table 3**).

Lactic acid levels

The average lactic acid levels in the control group with the administration of papaya leaves compared to the negative control group are presented in **Table 4**. The mean of lactic acid from negative control, *C. papaya* 200 mg/kg and *C. papaya* 400 mg/kg group were 6.45±2.64 mmol/L, 5.23±1.61 mmol/L, and 3.75±0.58 mmol/L, respectively. The negative control group showed the highest lactic acid level as expected, while *C. papaya* 400 mg/kg had the lowest concentration. Statistical analysis of the concentration of lactic acid showed that there was a significant difference among groups ($p<0.05$) (**Table 4**).

Table 4. Effect of Mean of lactic acid after exercise

Group	Number of animals	Lactic acid (mmol/L)	*p*-value
		Mean±SD	
Negative control	4	6.45±2.64 b	<0.05
Carica papaya 200 mg/kg	4	5.22±1.61 ab	
Carica papaya 400 mg/kg	4	3.75±0.58 a	

Discussion

All phytochemical compounds found in the papaya leaf are known for their wide biological and pharmacological properties such as pesticide, anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, and preventive medicines [21-24]. Phytochemical compounds produced by a plant are influenced by external factors such as temperature, humidity, light, pH, altitude, and nutrients contained in the soil [25]. A study proved that pH and soil minerals are different from place to place and found that coastal areas have the most alkaline pH and the highest essential soil mineral concentration compared to pH and soil minerals of geothermal and urban areas [26]. In addition, this study also found that the chemical compounds of *C. papaya* that grew in the coastal area had the highest number of secondary metabolites compared to the other locations.

Alkaloids are known for their role in preventing the production of cytotoxic, analgesic, antispasmodic, antibacterial, immunosuppressive T lymphocytes and lower macrophage populations. Flavonoids have properties of anti-inflammatory, antioxidant, antibacterial, analgesic, antiviral, immunostimulant, and antifungal. Flavonoids suppress the release of lysozyme and arachidonic acid, interfering with the phospholipase A2, lipoxygenase, and cyclooxygenase processes by raising interleukin-2 (IL-2) and lymphocyte proliferation, which causes the cluster of differentiation 4 (CD4) cells to activate type 1 T helper (Th1) cells and alter specific macrophage activating factor (SMAF). Natural steroids in plants are expected to provide anti-inflammatory effects, and muscle and bone hypertrophy compared to synthetic and anabolic steroids [12].

One of the advantages of the GC-MS method is that it can analyze bioactive compounds in papaya leaves. This method can identify, separate, and determine various kinds of chemical compound structures and their concentration in plants [12]. The method has the advantage of measuring retention time (RT) and peak areas which are parameters for confirming the presence of various chemical compounds, over the phytochemical screening that can only qualitatively assess the content possessed [32].

C. papaya is one of the most popular plants in the world of medicine [16,33]. This plant is known to treat several diseases, such as dyspepsia, skin disorders, diarrhea, influenza, cervical cancer, prostate cancer, breast cancer, and can even be used as a contraceptive for men [17,34]. Papaya leaves are believed to have many elements of bioactive compounds, including methanol extract of papaya leaves containing alkaloids, flavonoids, and n-hexane extract containing steroid compounds that act as anti-inflammatories. Flavonoids in inflammation play a role in inhibiting cyclooxygenase (COX), lipoxygenase, prostaglandins, and thromboxane. The anti-inflammatory properties of *C. papaya* bioactive compounds play an important role in inhibiting fatigue because muscle damage triggered by activity, excessive exercise, and glycogen deficit will cause muscle damage, which is characterized by inflammation [12,35].

Table 2. Gas chromatography-mass spectrophotometry results of ethanol extract of *Carica papaya* leaves from different areas

C. papaya from geothermal area				*C. papaya* from from coastal area				*C. papaya* from L urban area			
Compound	RT	Quality	%	Compound	RT	Quality	%	Compound	RT	Quality	%
Methylsuccinic anhydride	3.856	72	2.43	Neophytadiene	27.169	94	6.17	Neophytadiene	27.155	94	9.95
Diethyl malate	10.710	91	6.44	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	27.262	22	1.83	Neophytadiene	27.410	95	2.49
Diethyl 2,3-dihydroxysuccinate	14.833	91	34.32	Neophytadiene	27.410	99	1.88	Neophytadiene	27.596	95	3.62
Butanedioic acid, methoxy-, dimethyl ester	26.865	27	3.82	Neophytadiene	27.596	93	2.69	Hexadecanoic acid, ethyl ester	28.569	99	1.01
Neophytadiene	27.341	99	5.81	Hexadecanoic acid, ethyl ester	28.568	99	1.88	Phytol	29.403	91	2.31
Neophytadiene	27.596	44	1.32	Hexadecanoic acid	28.886	99	7.58	Ethyl (9Z,12Z,15Z)-9,12,15-Octadecadienoate	29.665	99	3.96
Neophytadiene	27.776	86	1.48	N-Hexadecanoic acid	29.210	94	1.47	Ethyl 15-Methylheptadecanoate	29.775	95	2.01
Hexadecanoic acid, ethyl ester	28.748	99	2.12	Phytol	29.417	91	2.24	2-dodecen-1-Yl (-) succinic Anhydride	30.665	90	1.18
Hexadecanoic acid	29.051	99	2.95	9,12,15- Octadecatrienoic acid, ethyl ester	29.692	99	6.03	3,1'-Dihydroxy Preussomering G	31.492	72	1.46
Ethyl 9,12,15-Octadecatrienoate	29.844	99	4.55	Linolenic acid	29.913	99	10.19	Squalene	32.954	99	11.41
Linolenic acid	30.058	98	4.04	Tridecanedial	30.499	83	1.41	Delta-tocopherol	34.112	98	2.76
Squalene	33.237	99	10.42	D-Gluconic acid,1,2;3,4;5,6-tri-o-ethaneboronate-	30.672	43	1.32	Gamma-tocopherol	35.099	97	6.09
Delta-tocopherol	34.567	95	1.07	Bicyclo (4,3,1) decan-10-one	31.271	45	2.16	Stigmastan-3,5-diene	35.560	83	1.17
Gamma-tocopherol	35.643	95	1.44	Ethyl (9Z,12Z)-9,12-octadecadienoate	32.230	93	1.77	Vitamin E	36.016	99	10.34
Vitamin E	36.657	99	5.27	Squalene	32.954	99	3.33	2,6,10,14-Hexadecatetraen-1-Ol,2,6,10,14-tetramethyl-16-(phenylthio)-,(all-E)-	38.305	90	1.11
Gamma-sitosterol	39.987	99	1.48	(+)-(P,1r,3s)-5-(4,5-) dimethoxy-2-methyl-1-naphthyl)-6,8-dimethoxy-1,2,3-trimethyl-1,2,3,4-tetrahydroisoquinoline 1(+)-O-Methylancistroline)	33.374	74	1.05	Norolean-12-Ene	39.008	95	6.90
6,6,10-Trimethyl 1-phenylthiospiro (3,6) dec-1-ene	41.125	70	4.63	Gamma-tocopherol	34.154	99	3.70	6,6,10-Trimethyl-1-phenylthiospiro (3,6) dec-1-Ene	39.911	70	6.68

C. papaya from geothermal area				*C. papaya* from from coastal area				*C. papaya* from L urban area			
Compound	RT	Quality	%	Compound	RT	Quality	%	Compound	RT	Quality	%
				Gamma-tocopherol	35.160	97	5.62	3-O-Acethyl-delta24-cycloartenol	40.601	53	4.30
				Vitamin E	36.112	99	9.01	Longipinane,(E)-	40.704	50	1.97
				Norolean-12-ene	39.104	93	4.84	Pyridine-3-carboxamide,oxime,N-(2-trifluoromethylphenyl)-	41.028	91	1.20
				Methyl commate B	40.049	84	1.57	3-Cyclohexene-1-carboxaldehyde,4-methyl-	41.525	64	2.30
				5-(5-(Hydroxymethyl)-5,8a-dimethyl-2-methylenedecahydro-1-naphthalenyl)-3-methyl-1-Penten-3-Ol	40.759	93	1.11	Carpaine	44.000	58	4.93
				1-Cyclohexene-4-carboxaldehyde,1-methyl-	41.745	92	2.41	Cycloartenol	45.890	90	1.04
				Carpaine	44.517	74	7.92				

RT: retention time (minutes)

Table 3. Major metabolite compounds of *Carica papaya* leaves from three locations and their known biological activities

Compound	*Carica papaya* leaf									Biological activities
	Geothermal area			Coastal area			Urban area			
	RT	Quality	Content (%)	RT	Quality	Content (%)	RT	Quality	Content (%)	
Neophytadiene	27.341	99	5.81	27.169	94	6.17	27.155	94	9.95	Anti-inflammatory, antibacterial, analgesic, antipyretic [10,17,27]
	27.596	44	1.32	27.410	99	1.88	27.410	95	2.49	
	27.776	86	1.48	27.596	93	2.69	27.596	95	3.62	
Hexadecanoic acid	28.748	99	2.12	28.568	99	1.88	28.569	99	1.01	Anti-inflammatory, antioxidant, antibacterial, antifungal, pesticide, antiviral, prevention, and therapy for many diseases [4,27,28]
	29.051	99	2.95	28.886	99	7.58	-	-	-	
Phytol	-	-	-	29.417	91	2.24	29.403	91	2.31	Anti-inflammatory, antioxidant, antimicrobial, anticancer [27,29]
9,12,15-Octadecatrienoate acid	29.844	99	4.55	29.692	99	6.03	29.665	99	3.96	Antioxidant, anti-inflammatory, anti-oxidant, anti-cancer, anti-radiation, anti-histamine [14,27,30]
	-	-	-	32.230	93	1.77	-	-	-	
Linolenic acid	30.054	98	4.04	29.913	99	10.19	-	-	-	Anti-malaria [17,27,31]
Squalene	33.237	99	10.42	32.954	99	3.33	32.954	99	11.41	Antioxidant, anticancer, antimicrobial, hepatoprotective [16,23]
Gamma-tocopherol	35.643	95	1.44	34.154	99	3.70	35.099	97	6.09	Antioxidant, antimicrobial and anticancer [17,26]
	-	-		35.160	97	5.62	-	-	-	
Vitamin E	36.657	99	5.27	36.112	99	9.01	36.016	99	10.34	Antioxidant [15,31]
Carpaine	-	-	-	44.517	74	7.92	44.000	58	4.93	Reduce cardiovascular problems [27,31]

RT: retention time (minutes)

Various studies showed that *C. papaya* contains compounds that act as anti-inflammatories and antioxidants such as neophytadiene, vitamin E, 9,12,15 octadecatrienoic acid, squalene, and phytol [14-17,29]. A study was conducted to test neophytadiene compounds anti-inflammatory effect in lipopolysaccharide(LPS)-induced inflammation rats both in vitro and in vivo conditions and showed that neophytadiene (12.25, 50 mg/kg) administered for seven days prior to induced inflammation significantly inhibited the production of nitric oxide (NO) and the inflammatory cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) [36].

9,12,15-octadecatrienoic acid is grouped into fatty acids and esters which are useful as the main energy provider during cell growth and this compound plays a role in anti-melanogenic, antioxidant, anti-fungal and anti-inflammatory activities [37,38]. Squalene is a compound found in papaya and moringa leaves which functions as an antioxidant [23,39]. Vitamin E is known to have antioxidant effects so that it can protect muscle tissue from various damages caused by ROS and increase glucose absorption as an energy source from carbohydrate metabolism, optimize pancreatic function with effective insulin work, and the adipogenesis process [40,41]. Phytol is grouped under terpenes and terpenoids and is known as phytol (acyclic monounsaturated alcohol), which plays an important role in various biological properties as anti-inflammatory, anticancer, antioxidant, diuretic, anti-allergic, immunostimulant, anti-trypanosomal, antimicrobial activity as well as cholesterol-lowering effects. Phytol acts as an anti-inflammatory that inhibits hyperalgesia, reduces myeloperoxidase (MPO), releases pro-inflammatory cytokines, reduces the production of IL-6, COX-2, significantly downregulates p38MAPK expression, and increases NFκB activity [21,42].

The occurrence of muscle fatigue caused by a buildup of lactic acid has long been suspected. The opinion that the accumulation of lactic acid accompanies the fatigue process is further strengthened by the fact that there are two physiological mechanisms by which lactic acid inhibits muscle function. Both mechanisms depend on the effect of lactic acid on intracellular pH or hydrogen ion (H) concentration. As lactic acid increases, the H concentration increases, and the pH decreases. On the other hand, increasing the concentration of H ions hinders the process of the excitation circuit, by decreasing the amount of Ca released from the sarcoplasmic reticulum and disrupting the binding capacity of troponin. Increasing the concentration of H ions also inhibits the activity of phosphofructokinase, a key enzyme involved in anaerobic glycolysis. Thus slowing down glycolysis, reduces the supply of ATP for energy [43]. *C. papaya* leaves are believed to contain many bioactive compounds, namely the methanol extract of papaya leaves contains alkaloids, flavonoids, and n-hexane extract contains steroid compounds which act as anti-inflammatory. Flavonoids in inflammation play a role in inhibiting COX, lipoxygenase, prostaglandins and thromboxane. The ability of bioactive compounds from *C. papaya* as anti-inflammatory plays an important role in preventing fatigue, because muscle damage triggered by activity, excessive exercise, and glycogen deficit will cause muscle damage characterized by inflammation [12].

Conclusion

Higher concentrations of alkaloids, steroids, saponins, flavonoids, and phenolics were found in the coastal and urban areas. The GC-MS analysis on papaya leaf samples revealed that papaya leaf extract from coastal areas contained the highest number of secondary metabolites that have potential antifatigue activities. Administration of papaya leaf extract at a concentration of 400 mg/kg of body weight in rats was able to maintain lactic acid concentration at normal levels after strenuous exercise.

Ethics approval

The study was approved by the Ethical Committee of Faculty of Veterinary, Universitas Syiah Kuala, Banda Aceh, Indonesia (no 166/KEPH/IX/2022).

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Competing interests

All authors declare that there are no conflicts of interest.

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Underlying data

The underlying data can be requested to corresponding author.

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