The Third International Conference
on Innovations in Social Sciences Education and Engineering (ICoISSEE)-3
Bandung, Indonesia, July 08th, 2023

Soil Analysis Test, Phytochemistry, and Anti-Inflammatory Ability of Papaya Leaf as an Ergogenic Antifatigue Candidate in Wistar Rats

[1]**Aditya Candra**,[2]**Yudha Fahrimal**,[3]**Yusni**
[*4]**Azwar**,[5]**Tahara Dilla Santi**

[1]Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
[1]Department of Physiology, Faculty of Medicine, Universitas Abulyatama, Aceh Besar 23372, Indonesia
[2]Faculty of Veterinary Medicine, University of Syiah Kuala, Banda Aceh 23111, Indonesia
[3]Department of Physiology, Faculty of Medicine, University of Syiah Kuala, Banda Aceh 23111, Indonesia
[4]Department of Chemical Engineering, Faculty of Engineering, University of Syiah Kuala, Banda Aceh 23111, Indonesia
[5]Faculty of Public Health, Univerity Muhammadiyah Aceh, Banda Aceh 23245, Indonesia

Author's email:
[1]aditya_c@mhs.unsyiah.ac.id;[2]yudhafahrimal@unsyiah.ac.id;[3]yusni@unsyiah.ac.id
[4] azwar.yahya@unsyiah.ac.id;[5]tahara.dilla@unmuha.ac.id

**Corresponding author: [4]azwar.yahya@unsyiah.ac.id*

Abstract. *The main objective of this study was to identify differences in soil nutrient composition, biological activity, and anti-inflammatory properties of papaya leaves at three different locations above sea level. The three locations are in Ie Seum (geothermal area), Kajhu (coastal), and Lambhuk (lowland). The chemical composition of the soil on papaya leaves was analyzed using GC-MS, AAS, a Flamephotometer, and a Spectrophotometer. The highest soil macro and micronutrients were found in the Kajhu area with a pH (H2O) value of 8.05; pH (KCl) 7.15; P 51.35 mg kg-1; Ca++ 13.20 cmol kg-1; K+ 0.99 cmol kg-1; Na+ 0.32 cmol kg-1; cation exchange capacity 21.20 cmol kg-1; base saturation 71.04%. The number of compounds identified from biological activity in Kajhu District was 24 compounds; 23 compounds in Lambhuk District; and 17 compounds in Ie Seum District. The highest compounds contained in all samples were neophytadiene, linolenic acid, gamma, tocopherol, hexadecanoic acid, vitamin e, carpaine, 9,12, and 15 octadecatrienoic acid, norolean-12-ene, squalene, and phytol. From the three locations, it can be concluded that the coastal area of Kajhu contains higher levels of nutrient compounds, biological activity, and anti-inflammatory and antioxidant properties than the other two locations.*

Keywords: *Carica papaya, phytochemistry, anti-inflammation, ergogenic, fatigue*

1. INTRODUCTION

Fatigue is a response of the body to activities carried out continuously with moderate and heavy intensity. Fatigue can also be interpreted as the inability of muscles to contract strongly and for a long time due to reduced available energy sources in the form of muscle glycogen (Lee et al., 2021). Fatigue is defined as a physiological condition in which the body is unable to respond to the same stimulus repeatedly (Phillips 2015; Candra et al., 2016; Ng et al., 2021). Ergogenic can be defined as a tool, substance, or certain nutrients that can function to increase energy production and body metabolism and can accelerate the healing process from muscle injuries and infections during exercise (Kerksick et al., 2018; Andersen et al., 2021). Exercise programs, sports equipment, nutrition, various medications derived from chemicals or natural plant compounds, and psychology are all examples of ergogenics, which can alter the physiological characteristics of bodily organs directly (Porrini and Del Bo 2016; Li and Liu, 2022).

Carica papaya herbal supplement is the best solution to overcome the above problems because, in addition to having strong antioxidant properties, it is also known as a secondary metabolic compound that acts as an ergogenic antifatigue. Another benefit of *Carica papaya* leaves is that they can lower blood sugar, protect insulin-producing cells in the pancreas, reduce tissue damage due to inflammation and can reduce oxidative stress due to excessive physical activity. Based on the problems above, the preliminary study of this work was to analyze soil nutrient composition, biological activity, and anti-inflammatory properties of papaya leaves as an ergogenic anti-fatique candidate. To obtain the best research results, sampling will be conducted in three different locations, namely in Ie Seum (geothermal area), Kajhu (coastal), and Lambhuk (lowland), Aceh-Besar, Indonesia.

2. LITERATURE REVIEW

1.1 Ergogenic

Various ways are done to inhibit and overcome fatigue due to infection and muscle damage, such as nutrition and dietary supplements (Egan and Zierath 2013; Ryan et al., 2020). It has been reported from various studies that about 50% of athletes generally use supplements to increase stamina (Hoffman 2015; Muchlisin et al., 2020; Yarar et al., 2022). Ergogenics 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 (Hurst et al., 2020; Cervantes-Luna et al., 2021). Most athletics interested in maintaining muscle strength, increasing endurance, burning fat, or increasing muscle strength activity use herbal supplements as ergogenic (Several previous studies have shown that the clinical and secondary metabolic results of a plant vary depending on the extraction method, the type and part of the plant, the location of the soil, and its geographical location (Schnyder and Handschin 2015; Ju 2016; Trivedi et al., 2022).

2.2 Carica papaya Leaf

Various therapies have been used to inhibit muscle infections due to fatigue after moderate and strenuous activities, such as the herbal supplement *Carica papaya* (Al-Seadi et al., 2021; Sekti et al., 2022). These herbal supplements contain phytochemicals such as antibacterial, antioxidant, anti-inflammatory, and anticancer so that they can be used as excellent ergogenic antifatigue ingredients (Paikra et al., 2017; Nwidu et al., 2018; Bangun et al., 2021). Some herbal supplements can function as ergogenics because, after consumption, they can increase muscle and physical strength during exercise (Kerksick et al., 2018; Sharma et al., 2022). According to ISSN (International Society of Sports Nutrition), nutritional supplements are an ergogenic aid in a broad sense because they can increase muscle strength and performance and can inhibit inflammation, muscle damage, and muscle hypertrophy. Several studies have examined whether *Carica papaya* contains important secondary and essential metabolites because they can function as ergogenics. *Carica papaya* is very beneficial for the health of the body, such as anti-inflammatory, antioxidant, anti-allergic, antimicrobial, antiviral, antibacterial, anticarcinogenic, and others (Sellami et al., 2018; Enenya et al., 2022).

Reduction of inflammation and oxidative stress can maintain effective use of muscle ATP, prompt muscle recovery, and inhibit fatigue during physical activity. However, the effectiveness of these supplements varies greatly depending on the geographic location of the plant taken, the method of extraction, and the type of plant (Sellami et al., 2018; Munir et al., 2022).

3. RESEARCH METHODS/METHODOLOGY

Plants that will be used as research samples are identified (determination test) first in the biology laboratory of FMIPA to determine the type and taxonomy of the plants to be studied. The sample will be used as a reference for taking leaves for further research. *Carica papaya* leaf samples were taken from three different locations. The samples used in this study were selected in three different locations, namely: Ie Seum (geothermal area), Kajhu (coastal), and Lambhuk (lowland) areas using purposive sampling. In general, the manufacture of simplicia consists of collecting raw materials, wet sorting, washing, chopping, drying, dry sorting, milling, packaging, and storage. In this study, leaves that were still green and not too old were used as samples.

3.1 Soil Physical and Chemical Properties

Soil is one of the components of land that plays a vital part in plant growth and production because it serves as a source of nutrients for plants as well as a place or medium for plant growth and keeping and delivering water to plants. The formation of the soil layer is influenced by various factors such as climate, parent material, topography, relief, organisms, and time. The elements and qualities of the soil will be determined by the interaction of these many factors. Soil and plant fertility are strongly influenced by the physical, chemical, and biological properties of the soil. Therefore, generalization of soil fertility status in geothermal, coastal, and lowland areas with different plant compounds is irrelevant (Bünemann et al., 2018).

3.2 Extracted by Using the Maceration Methods

Maceration is a technique used to obtain compounds from a solution through an immersion process. Organic solvents are used to soak the refined simplicia for several days. This process causes the rupture of cell walls and membranes so that the active compound will be dissolved in the organic solvent. As a result, the recovery of the active chemical is inextricably linked to the choice of the appropriate solvent (Ibrahim 2013; Koirewoa 2012). The solvent used in this papaya leaf simplicia powder sample is 96% ethanol. The papaya leaf samples (*Carica papaya*) used in this study originated from three separate areas: Kajhu, Ie Seum, and Lambhuk. Dried simplicia was selected from three different places, then blended and filtered to obtain the desired fine powder size. For each sample from a different location, 2 kg of simplicia powder was prepared, then mixed into 96% ethanol for 72 hours. The result of maceration in the form of a thick liquid extract was then concentrated using a rotary evaporator so that the ethanol extract of *Carica papaya* leaves was obtained as much as 18 g. Plants that have the most active compounds, secondary metabolites, and macro and micro elements that have the most nutrients will be sampled to be tested on Wistar rats. Figure 1 shows the morphological part of the papaya plant. This plant is characterized by large oval leaves with a diameter of 20–28 inches, the flowers have pale white petals where male and female flowers are fused on the petals, with a height of up to 10 m, and all parts contain latex (Nugroho et al., 2017; Wadekar et al., 2021).

Figure 1. Morphology of Herbarium *Carica papaya* samples

3.3 Phytochemical Screening

Phytochemical screening is a technique for determining the presence of different secondary metabolites in Carica 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 sulfuric acid).

3.4 GC-MS Analysis

The combination method of gas chromatography and mass spectrometry is known as gas chromatography-mass spectrometry (GC-MS). This method aims to analyze various compounds in a sample. The compounds in papaya leaves were identified using gas chromatography and mass spectrometry in this investigation (Sumarno, 2001). The extracted papaya leaves are put into the GC-MS instrument. The material is then fed into the GC-MS system. Each sample was dried for at least three days in an oven to verify that it was fully dry and sterile. The next step is to combine until smooth, then macerate for 5 days with 96 percent ethanol. After becoming the extract, it was then taken using a pipette as much as 10 ml and dried at a temperature of 600 C for 1 hour. The was dissolved in the remaining extract (up to 200 liters) and injected into the GC-MS instrument after drying. The balitro, m model was used for GC-MS analysis. The gas chromatograph employed was an intelligent technology 7890 with an automatic sampler and a mass of 5975. The electron ionization model with an energy of 70 eV, column hp ultra 2, and an initial temperature of 80 0 C was then increased from 3 0 C to 150 0 C, which was maintained for 1 minute, and finally increased from 20 0 C to 280 0 C, which was maintained for 26 minutes. The carrier gas is helium. The constant flow column mode with a column flow of 1.2 mL/min, an injection volume of 5 L, and the balitro file method.

4. RESULTS AND DISCUSSION

4.1 Determination of Sample

Table 1 shows the findings of the identification of papaya leaf herbarium samples performed in the biology department of Shia University, Kuala Banda Aceh, in the laboratory of the Faculty of Mathematics and Natural Sciences. This identification is done to ensure that the leaves being investigated are correct.

Tabel 1. Identification of papaya leaf herbarium samples

Regnum/Kingdom	Plantae
Sub Regnum	Tracheobionta
Super Divisio	Spermatophyta
Divisio	Magnoliophyta
Classis	Magnoliopsida
Sub Classis	Dilleniidae
Ordo	Brassicales
Familia	Caricaceae
Genus	Carica L.
Species	*Carica papaya* L.

4.2 Analysis of Soil

Soil samples were taken from three locations, namely Kajhu, Lambhuk, and Ie Seum, to assess macro and micro nutrients. Based on the results of the soil analysis, it will be possible to determine which papaya leaves will be utilized as study samples for experimental animals. Table 2 shows the results of a papaya leaf soil study conducted at the Faculty of Agriculture's soil and plant research laboratory in Kuala Banda Aceh.

Table 2. Soil analysis results

No	Character	Lambhuk	Kajhu	Ie Seum
1	pH H_2O	7.1	8.05	7.63
2	pH KCL	6.10	7.15	6.43
2	C-organic (%)	0.79	0.88	0.89
3	N total (%)	0.11	0.11	0.13
4	P available (mg kg^{-1})	27.65	51.35	3.70
5	Base cations can be exchanged			
	Ca^{++} (cmol kg^{-1})	11.57	13.2	7.23
	Mg^{++} (cmol kg^{-1})	0.54	0.55	0.56
	K^{+} (cmol kg^{-1})	0.26	0.99	0.54
	Na^{+} (cmol kg^{-1})	0.24	0.32	0.30

6	Cation exchange capacity (cmol kg^{-1})	18.4	21.20	19.6
7	Base saturation (%)	68.53	71.04	44.03

From table 2, soil chemical analysis on *Carica papaya* leaves shows the results of sample testing in the Kajhu area have the highest macro and micro nutrients with values; pH (KCl) 7.15; pH (H2O) 8.05; Ca++ 13.20 cmol kg-1; P 51.35 mg kg-1; K+ 0.99 cmol kg-1; Na+ 0.32 cmol kg-1; cation exchange capacity 21.20 cmol kg-1; base saturation 71.04%. These results show that the kajhu sample (coastal area) has the highest value, which plays an important role in producing the best secondary metabolic content and compounds for herbal plants. Soil texture is important to know because it will determine the physical, chemical, and biological properties of the soil. The results of laboratory analysis always show that the soil contains particles of various sizes. Comparison of different particles will cause differences in each soil compared to one another (Jaconiet al., 2019).

Lambhuk is an area adjacent to the Krueng Aceh river that has the potential for the development of various plants. Ie Seum is a geothermal area located at the foot of the mountain and has hot water flowing directly from the mountain. Kajhu is a coastal area that has the potential for plant development with high levels of salinity. Soil formation is influenced by various factors such as climate, parent material, topography, organisms, and time. The different influences of the various soil-forming factors will produce soil characteristics, both physical, chemical, and biological characteristics, which ultimately affect the fertility of the soil and the plants concerned. Therefore, generalization of soil fertility status on land with bioactive compounds contained in a plant is very irrelevant (Bünemann et al., 2018).

The pH of papaya leaf soil samples from Kajhu and ie Seum is alkaline, with the soil from Kajhu (coastal area) being more alkaline than the soil from Ie Seum and Lambhuk. Soil with an alkaline pH greatly affects macronutrients, nutrient availability, and papaya productivity. These results are in accordance with research conducted by Liza Nuriati Lim choo that showed significant pH, availability of nitrate, ammonium, P, and K in soil for optimal absorption by plants can increase yield, quality, and growth of papaya (Choo 2020).

4.3.1 Phytochemical Screening of Papaya Leaf

Phytochemical examination to assess secondary metabolism from three locations of papaya leaf sampling, namely kajhu, ie seum, and lambhuk, showed different results. Papaya leaves from kajhu and lambhuk exhibited the highest metabolic content, including alkaloids, steroids, saponins, flavonoids, and phenolics, according to secondary metabolic results from three sample locations. Meanwhile, alkaloids, steroids, flavonoids, and phenolics were found in the sample's site, ie seum, as shown in table 3.

Table 3. The content of secondary metabolites of papaya leaves from phytochemical examination

Metabolite content	**Reagen**	**C. Papaya (ie seum)**	**C. Papaya (Kajhu)**	**C. Papaya (Lambhuk)**
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	-	-	-

Phytochemical examination will produce secondary metabolic compounds from a plant, where the content of the leaves or parts of the research sample is influenced by external factors such as temperature, humidity, light, pH, altitude, and nutrients contained in the soil. while genes are internal factors (Katuuk et al., 2019). Lambhuk is an area adjacent to the Krueng Aceh river that has the potential for the development of various crops. Ie Seum is a geothermal area located at the foot of the mountain and has hot water flowing directly from the mountain. Kajhu is a coastal area that has the potential for plant development with high levels of salinity.

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-inflammatory. Flavonoids in inflammation play a role in inhibiting COX, lipoxygenase, prostaglandins, and thromboxanes. The ability of bioactive compounds from *Carica papaya* to be anti-inflammatory plays 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 (Candra and Santi 2017).

Table 3 shows that papaya leaves contain secondary metabolic compounds such as alkaloids, steroids, saponins, flavonoids, and phenolics. The three sampling sites revealed that kajhu and lambhuk had all of these chemicals, however the sample ie seum did not. Alkaloids found in *Carica papaya* prevent the production of cytotoxic, analgesic, antispasmodic, antibacterial, immunosuppressive T lymphocytes and lower macrophage populations. One of the properties of flavonoids in papaya plants has many advantages, such as being anti-inflammatory, antioxidant, antibacterial, analgesic, antiviral, immunostimulant and antifungal. Flavonoids suppress the release of lysozyme enzymes and arachidonic acid, interfering with the phospholipase A2, lipoxygenase, and cyclooxygenase processes by raising IL-2 and lymphocyte proliferation, which causes CD4 to activate Th1 cells and alter SMAF (specific macrophage activating factor). Natural steroids in plants are expected to provide anti-inflammatory effects and muscle and bone hypertrophy compared to synthetic and anabolic steroids (Candra and Santi 2017).

3.5 Phytochemical Screening with GC-MS Analysis

One of the advantages of the GS-MS tool is that it is able to analyze bioactive compounds in papaya leaves. This tool identifies chemical compounds such as alcohols, esters, long chains, acids, and more. Retention time and peak area are parameters in confirming the presence of chemical compounds. Chemical compounds such as flavonoids, terpenoids, saponins, tannins, and alkaloids are components that have therapeutic effects. The GC-MS tool is able to identify, separate, and determine various kinds of chemical compound structures in plants (Santi 2015; Candra and Santi 2017). *Carica papaya* leaves contain secondary metabolic and bioactive compounds consisting of hundreds of components in plant cells. Phytochemical screening can qualitatively assess the content possessed, while GC-MS provides quantitative information on compound yields (Doughari et al., 2011).

From the results of research on papaya leaves carried out at the chemical and doping laboratory of the special regional health office of Jakarta with GC-MS examination, it can be seen that the compounds contained in the research samples are presented in Figure 2 and Table 4.

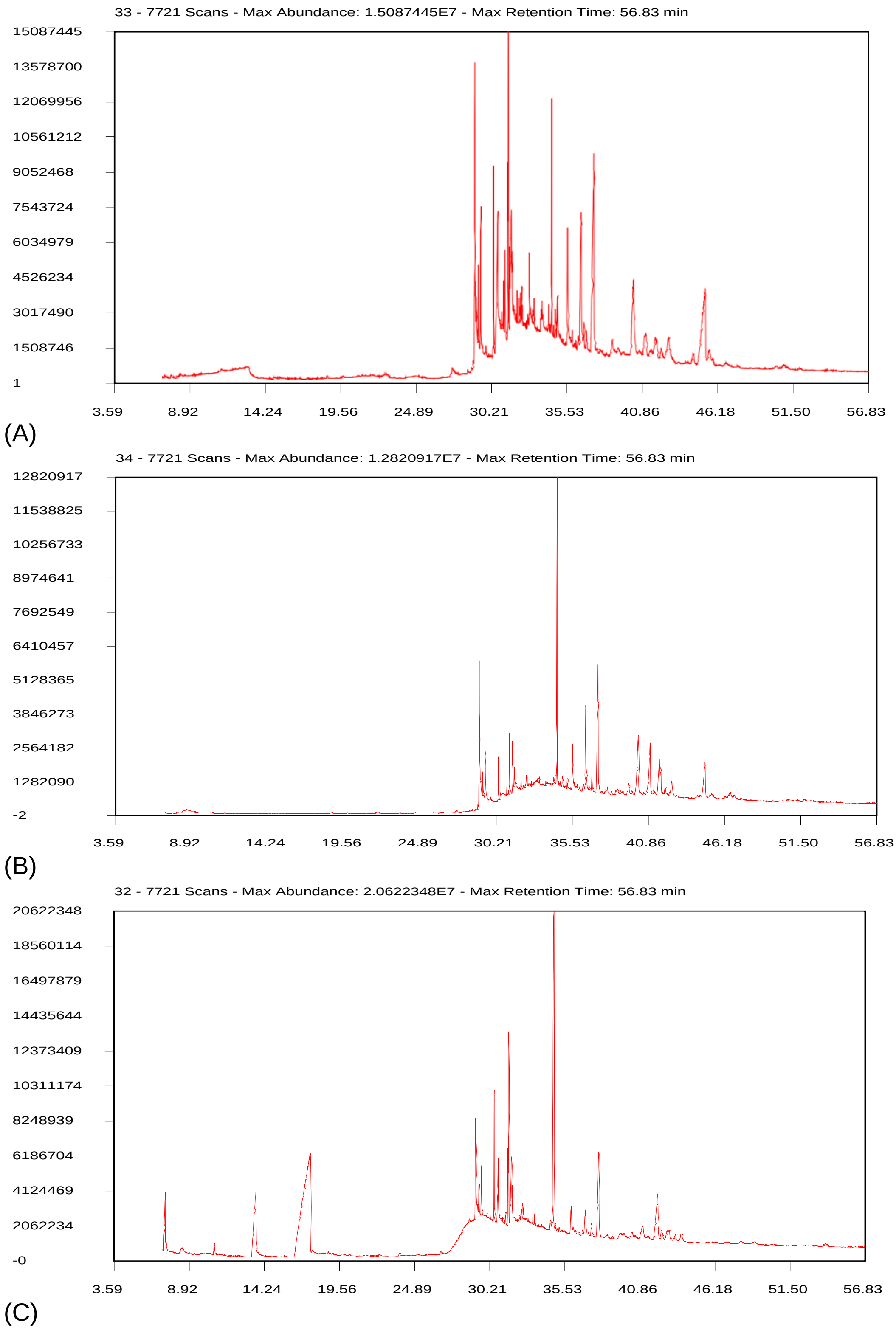

Figure 2. The GC-MS chromatogram above shows Figure A with 24 compounds of papaya leaf samples kajhu (coastal area), Figure B with 23 compounds of papaya leaf samples lambhuk, and Figure C with 17 compounds of papaya leaf samples ie seum (gheothrmal). The percentages of metabolic content and function of compounds in this image from the three sampling locations can be seen in table 4.

Table 4. Papaya leaf compounds through GC-MS samples at three locations

Compound	*Carica papaya* Leaf									BIOLOGICAL ACTIVITIES
	Ie Seum			Kajhu			Lambhuk			
	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 (Al-Seadi et al., 2021).
	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	
Linolenic acid	30,054	98	4,04	29.913	99	10,19	-	-	-	Anti-malaria (Sankarganesh 2018).
Gamma -Tocopherol	35,643	95	1,44	34.154	99	3.70	35,099	97	6,09	Antioxidant, antimicrobial and anticancer (Al-Seadi et al., 2021).
	-	-		35,160	97	5,62	-	-	-	
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 (Taiwo et al., 2000).
	29,051	99	2,95	28.886	99	7,58	-	-	-	
Vitamin E	36,657	99	5,27	36,112	99	9,01	36,016	99	10,34	Antioxidant (Joy Ugo et al., 2019).
carpaine	-	-	-	44.517	74	7,92	44.000	58	4,93	Reduce cardiovascular problems (Sankarganesh 2018).
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 (de Alencar et al., 2019).
	-	-	-	32.230	93	1,77	-	-	-	
Squalene	33,237	99	10,42	32,954	99	3,33	32,954	99	11,41	Antioxidant, anticancer, antimicrobial, hepatoprotective Abdel-Halim et al., 2020).
phytol	-	-	-	29.417	91	2,24	29.403	91	2,31	Anti-inflammatory, antioxidant, antimicrobial, anticancer Pejin et al., 2014).

The biological activity that produced the most compounds was detected in the kajhu sample selection region with 24 compounds, lambhuk with 23 compounds, and the lowest ie seum area with 17 compounds, according to GC-MS analysis of papaya leaves. Table 4 describes all the compounds found in all papayas in three sampling locations, namely kajhu, which represents the coastal area, lambhuk represents the lowlands, and ie seum, which represents the sample of the geothermal area. The following is an order of the highest compounds contained in all samples from three locations that have anti-inflammatory and antioxidant effects as ergogenic candidates in overcoming muscle fatigue. These compounds are neophytadiene, gamma-tocopherol, hexadecanoic acid, vitamin E, 9,12, and 15 octadecatrienoic acid, squalene, and phytol. Assessment of RT, quality and content of compounds in table 4 with GC-MS concluded that from the three sampling locations, papaya leaves had the highest macro and micro nutrients and active compounds as anti-inflammatory and antioxidant were kajhu samples compared to samples ie seum and lambhuk.

Research on herbal supplements continues to be developed, including *Carica papaya* leaves. The biological activity of its compounds has activity as antibacterial, antioxidant, anti-inflammatory, and anticancer so that it can be used as an excellent antifatigue ergogenic material (Nwidu et al., 2018; Sekti et al., 2022). For example, to treat carrageenan-induced inflammation in mice, herbs were given to inhibit the expression of cyclooxygenase-2 (COX-2) and induced nitric oxide (iNOS), which are key factors in the inflammatory process (Jaja-Chimedza et al., 2017; Kou et al., 2018).

Carica papaya is one of the most popular plants in the world of medicine (Nugroho et al., 2017; Enenya et al., 2022). Some diseases can be treated by this plant, such as dyspepsia, skin disorders, diarrhea, influenza, cervical cancer, prostate cancer, breast cancer, and can even be used as a contraceptive for men (Hossain et al., 2020; Wadekar et al., 2021). 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-inflammatory. Flavonoids in inflammation play a role in inhibiting COX, lipoxygenase, prostaglandins, and thromboxane. The ability of bioactive compounds from *Carica papaya* to be anti-inflammatory plays 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 (Candra and Santi, 2017; Santi 2019).

Research conducted by Seadi, Ugo, De, Alencar, Halim, and Pejin showed that *Carica papaya* contains compounds that act as anti-inflammatory and antioxidants such as neophytadiene, vitamin E, 9,12,15 octadecatrienoic acid, squalene, and phyto 1 (Al-Seadi et al., 2021). Tests of neophytadiene compounds to assess the level of LPS-induced inflammation in both in vitro and in vivo conditions conducted by Bhardwaj showed that neophytadiene (12.25, 50 mg/kg) administered for 7 days in experimental animals was followed by LPS (10 mg/kg). The results concluded that this compound significantly inhibited the production of NO and the inflammatory cytokines TNF-, IL-6, and IL-10 both in vitro and in vivo conditions (Bhardwaj et al., 2020). However, research to assess the effect of *Carica papaya* leaves as an antifatique in inhibiting inflammation and the emergence of free radicals has not been widely studied, so further research is needed that is specific to muscle fatigue.

CONCLUSION

In conclusion, papaya leaf is one of the herbal plants with secondary metabolism which can prevent and treat inflammation due to fatigue. The active compounds in papaya leaves contain the most, namely alkaloids, steroids, saponins, flavonoids, and phenolics. The use of drugs and other substances to treat infections caused by fatigue can cause hormonal cycle irregularities, health problems, and so on. Herbal plants are effective, natural, and have a high level of influence, so they can be utilized as an alternative treatment. Papaya leaf samples were taken, researched, and evaluated individually from three places in Indonesia: Kajhu, or Ie Seum, and Lambhuk Aceh Besar. The largest concentrations of alkaloids, steroids, saponins, flavonoids, and phenolics were found in the Kajhu and Ie Seum. From the GC-MS analysis on papaya leaf samples, it was revealed that the biological activity that produced the most chemicals was found in the kajhu area. Meanwhile, chemical compounds such as neophytadiene, linolenic acid, gamma-tocopherol, hexadecanoic acid, and vitamin E were found in abundance in the three sampling areas. The Kajhu area (coastal location) is the best area, according to the study's findings, because it has the most anti-inflammatory and antioxidant biological activity compared to the Ie Seum and Lambhuk locations.

REFERENCES

Aadil, RM. (2022). Delving into the Therapeutic Potential of *Carica papaya* Leaf against Thrombocytopenia. *Molecules,* 27, 2760. https://doi.org/10.3390/molecules27092760

Abdel-Halim SA, Ibrahim MT, Mohsen MMA, Abou-Setta LM, Sleem AA, Morsy FA, & El-Missiry MM. (2020). Phytochemical and biological investigation of *Carica papaya* Linn. Leaves cultivated in Egypt (Family Caricaceae). *J Pharm Phytochem*, 9(5), 47–54. https://doi.org/10.22271/phyto.2020.v9.i5a.12421.

Al-Seadi HL, Sabti MZ, & Taain DA. (2021). GC-MS Analysis of Papaya Leaf Extract (*Carica papaya* L.). IOP Conf Series: Earth Env Sci 910(1): 012011. https://doi.org/10.1088/1755-1315/910/1/012011.

Andersen LK, Mette Aadahl M, & Vissing J. (2021). Fatigue, physical activity and associated factors in 779 patients with myasthenia gravis. *Neuromuscular Disorders*, 31, 716–725. https://doi.org/10.1016/j.nmd.2021.05.007.

Bangun PPA, Rahman AP, & Syaifiyatul H. (2021). Analysis of total flavonoid content in papaya leaves and seeds (Carica papaya L.) using UV-vis spectrophotometer method. *J Ilmiah Farmasi ATTAMRU*, 22(02), 1-5.

Bhardwaj M, Sali VK, Mani S, & Vasanthi HR. (2020). Neophytadiene from *Turbinaria ornata* Suppresses LPS-Induced Inflammatory Response in RAW 264.7 *Macrophages and Sprague Dawley Rats Inflam,* 43(3), 937–950. https://doi.org/10.1007/s10753-020-01179-z

Bünemann EK, Bongiorno G, Bai Z, Creamer RE, De Deyn G, de Goede R, Fleskens L, Geissen V, Kuype TW, Mäder P, Pulleman MM, Sukkel W, van Groenigen JW, & Brussaard L. (2018). Soil quality – A critical review, Soil Biol In Biochem 120: 105–125.

Candra A, Rusip G, & Machrina Y. (2016). Effect of Light and Moderate Intensity Aerobic Exercise on Muscle Fatique in Aceh Football Athletes. *J Kedok Kes*, 3, 333–339. http://ejournal3.undip.ac.id/index.php/jkm.

Candra A, & Santi TD. (2017). The Effectiveness of Papaya Leaf Extract (*Carica papaya* L) As Anti-Inflammatory. *J. Aceh Med,*1(2), 63–66.

Cervantes-Luna BS, Ponce de León MdCE, & Ruíz EJC. (2021). Ergogenic substances and drive for muscularity among gym

Choo L. (2020). Clinoptilolite zeolite on tropical peat soils nutrient, growth, fruit quality, and yield of *Carica papaya* L. CV. sekaki. *Agronomy*,10(9), 10091320. https://doi.org/10.3390/agronomy10091320.

de Alencar MVOB, Islam MT, de Lima RMT, Paz MFCJ, dos Reis AC, da Mata AMOF, &Melo-Cavalcante AAdeC. (2019). Phytol as an anticarcinogenic and antitumoral agent: An in vivo study in swiss mice with DMBA-Induced breast cancer. *IUBMB Life*, 71(2), 200–212. https://doi.org/10.1002/iub.1952.

Doughari JH, Obi C, & Onyema OM. (2011). Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. *African J Biotechn*, 10(66), 15020–15024. https://doi.org/10.5897/AJB11.1948.

Egan B, & Zierath JR. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metabolism,*17(2), 162–184. https://doi.org/10.1016/j.cmet.2012.12.012.

Enenya IG, Agbonghae OW, Nwokoro SO, Onyeaka H, & Agbugba IK. (2022). Some phytochemical and functional properties of Pawpaw (*Carica papaya* L.) leaf protein concentrates obtained from three locations in Benin City, Edo State, Nigeria. Vegetos: 1-6. https://doi.org/10.1007/s42535-022-00386-3.

Hoffman NJ, Parker BL, Chaudhuri R, Fisher-Wellman HKK, Humphrey MSJ, & James DE. (2015). Global Phosphoproteomic Analysis of Human Skeletal Muscle Reveals a Network of Exercise-Regulated Kinases and AMPK. Substrates: 922–935. https://doi.org/http://dx.doi.org/10.1016/j.cmet.2015.09.001.

Hossain MA, Hitam S, & Ahmed SHI. (2020). Pharmacological and toxicological activities of the extracts of papaya leaves used traditionally for the treadment of diarrhea. oman *J King Saud Univ*: 962–969.

Hursta P, Ring C, & Kavussanu M. (2020). Athletes using ergogenic and medical sport supplements report morefavourable attitudes to doping than non-users. *J Sci Med Sport*, 24(3): 307-311. https://doi.org/10.1016/j.jsams.2020.09.012.

Ibrahim S. (2013). Organic Chemistry Laboratory Engineering. Yogyakarta: Graha Ilmu.

Jaconi A, Vos C, & Don A. (2019). Near infrared spectroscopy as an easy and precise method to estimate soil texture. *Geoderma*, 337, 906–913. https://doi.org/10.1016/j.geoderma.2018.10.038.

Jaja-Chimedza A, Graf BL, Simmler C, Kim Y, Kuhn P, Pauli GF, & Raskin I. (2017). Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (*Moringa oleifera*) seed extract. *PLoS ONE*, 12(8), 1–21. https://doi.org/10.1371/journal.pone.0182658.

Joy Ugo N, Raymond Ade A, & Tochi Joy A. (2019). Nutrient Composition of *Carica papaya* Leaves Extracts. *Journal of Food Science and Nutrition Research*,2(3), 274–282. https://doi.org/10.26502/jfsnr.2642-11000026.

Ju J, Jeon S, Park J, Lee J, Seong-cheol, Jeong KC, & Jong-moon. (2016). Autophagy plays a role in skeletal muscle mitochondrial biogenesis in an endurance exercise-trained condition. *Japan springer*: 417–430.

Katuuk RHH, Sesilia AW, & Pemmy T. (2019). The effect of differences in site height on the content of secondary metabolites of babadotan weeds (*Ageratum conyzoides* L.). *Biogenesis,1(4), 1-6.*

Kerksick CM, Wilborn CD, Roberts MD, Smith-Ryan A, Kleiner SM, Jäger R, & Kreider RB. (2018). ISSN exercise & sports nutrition review update: Research & recommendations. *J Int Soc Sports Nutr* ,15(1), 1–57. https://doi.org/10.1186/s12970-018-0242-y.

Koirewoa Y. (2012). Isolation and Identification of Flavonoid Compounds in Beluntas Leaves (*Pluchea indica* L.). *Pharmacon*, 1(1), 47-52.

Kou X, Li B, Olayanju JB, Drake JM, & Chen N. (2018). Nutraceutical or pharmacological potential of *Moringa oleifera* Lam. Nutrients 10(3): 343. https://doi.org/10.3390/nu10030343.

Lee JY, Kim EH, Yoon J-H, Eo W, & Yoon SW. (2021). Traditional Herbal Medicine, Sipjeondaebo-Tang, for Cancer-Related Fatigue: A Randomized, Placebo-Controlled, Preliminary Study. *Integrative Cancer Therapies,* 20, 1-9. DOI: 10.1177/15347354211040830.

Li H, & Liu H. (2022). The influence of Chinese herbal medicines on cancer-related pressure ulcer wound, fatigue, constipation, and anorexia: A meta-analysis. *Wiley, Int Wound J*,1 –10. DOI: 10.1111/iwj.13833.

Muchlisin MA, Sumadi FAN, Hidayat AW, Awal NM, & Jamil AS. (2020). Ergogenic Activities of Some Plants of Family Araliaceae on *Mus musculus*. *Adv in Health Sci Research*, 33, 1-6.

Munir S, Liu Z-W, Tariq T, Rabail R, Kowalczewski PL, Lewandowicz J, Blecharczyk A, Abid M, Inam-Ur-Raheem M, Ng JY, Zhang CJ, & Ahmed S. (2021). Dietary and herbal supplements for fatigue: A quality assessment of online consumer health information. *Integrative Medicine*

Research, 10, 100749. https://doi.org/10.1016/j.imr.2021.100749.

Nugroho A, Heryani H, Choi JS, & Park H-J. (2017). Identification and Quantification of Flavonoid in *Carica papaya* leaf and peroxynitrite scavenging activity. *Elsevier Asian Pacific J Trop Biomed*, 7(3), 208–213.

Nwidu L, Elmorsy E, Aprioku J, Siminialayi I, & Carter W. (2018). In Vitro Anti-Cholinesterase and Antioxidant Activity of Extracts of Moringa oleifera Plants from Rivers State, Niger Delta, Nigeria. *Medicines*, 5(3), 71. https://doi.org/10.3390/medicines5030071.

Paikra BK, Dhongade HKJ, & Gidwani B. (2017). Phytochemistry and pharmacology of *Moringa oleifera* Lam. *J Pharmacopuncture,* 20(3), 194–200. https://doi.org/10.3831/KPI.2017.20.022.

Pejin B, Savic A, Sokovic M, Glamoclija J, Ciric A, Nikolic M, & Mojovic M. (2014). Further in vitro evaluation of antiradical and antimicrobial activities of phytol. *Nat Prod Res,* 28(6), 372–376. https://doi.org/10.1080/14786419.2013.869692.

Phillips RO. (2015). A review of definitions of fatigue - And a step towards a whole definition. Transportation Research. *Traffic Psychology and Behaviour,* 29, 48–56. https://doi.org/10.1016/j.trf.2015.01.003

Porrini M, & Del Bo C. (2016). Ergogenic Aids and Supplements. *Front Horm Res*, 47, 128–152. https://doi.org/10.1159/000445176.

Ryan ED, Gerstner GR, Mota JA, Trexler ET, Giuliani HK, Blue MNM, Hirsch KR, & Smith-Ryan AE. (2020). The Acute Effects of a Multi-Ingredient Herbal Supplement on Performance Fatigability: A Double-Blind, Randomized, and Placebo-Controlled Trial. *J Dietary Supplements,* 1-9. https://doi.org/10.1080/19390211.2020.1790709

Sankarganesh P. (2018). Phytomedicinal chemistry and pharmacognostic value of *Carica papaya* L., Leaf. *J. Pure Appl. Microb,* 12(2), 751–756. https://doi.org/10.22207/JPaM.12.2.35.

Santi TD. (2015). Uji Toksisitas Akut dan Efek Antiinflamasi Ekstrak Metanol dan Ekstrak n-Heksana Daun Pepaya (*Carica papaya* L). *Pharm Sci Res*, 2(2), 101–114. https://doi.org/10.7454/psr.v2i2.3341.

Santi. (2019). Effect of Moderate Physical Activity to Muscle Fatigue on Untrained People. IOP Conference Series: Materials Science and Engineering, 506(1). https://doi.org/10.1088/1757-899X/506/1/012028.

Schnyder S, & Handschin C. (2015). Skeletal muscle as an endocrine organ: PGC-1α, myokines and exercise. *Bone*, 80, 115–125. https://doi.org/10.1016/j.bone.2015.02.008.

Sekti BH, Permata A, & Oktadiva NSP. (2022). Formulation and physical stability testing of papaya leaf extract tablets (*Carica papaya*. L). *Farmasi*, 4(1), 53-62.

Sellami M, Slimeni O, Pokrywka A, Kuvačić G, Hayes LD, Milic M, & Padulo J. (2018). Herbal medicine for sports: A review. *J Int Soc Sports Nutrit*, 15(1), 1–14. https://doi.org/10.1186/s12970-018-0218-y.

Sharma A, Sharma R, Sharma M, Kumar M, Barbhai MD, & Lorenzo JM. (2022). *Carica papaya* L. Leaves: Deciphering Its Antioxidant Bioactives, Biological Activities, Innovative Products, and Safety Aspects. *Oxidative Medicine & Cellular Longevity,* 1-20. https://doi.org/10.1155/2022/2451733

Sumarno. (2001). Chromatography Basic Theory, Pharmaceutical Chemistry Section, 30-34, Universitas Gadjah Mada Yogyakarta.

Taiwo OB, Olajide OA, Soyannwo, OO, & Makinde JM. (2000). Anti-inflammatory, antipyretic and antispasmodic: Properties of *Chromolaena odorata*. *Pharmaceutical Biology*, 38(5), 367–370.

https://doi.org/10.1076/phbi.38.5.367.5970.

Trivedi1 K, Hussain MdS, & Mohapatra C. (2022). Role of Glutamine as an Ergogenic Amino Acid during Fatigue. *J, Clinical Medical Reviews and Reports,* 4(2), DOI:10.31579/2690-8794/111

Wadekar AB, Nimbalwar MG, Panchale WA, Gudalwar BR, Manwar JV, & Bakal RL. (2021). Morphology, phytochemistry and Pharmacological aspects of *Carica papaya*, a review. India: *GSC Bio Pharm Sci.* 234–248.

Yarar H, Eskici G, SEVIL Yilmaz SK, Kuca V, & Yazici B. (2022). Evaluation of the Knowledge, Attitudes, and Behaviors of the Students Studying In the Coaching Education Department Regarding the Utilization of Nutritional Ergogenic Supplement. *P J M H S,* 16(1), 587. DOI: https://doi.org/10.53350/pjmhs22161587.