ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Antiproliferative Activity and GC–MS Analysis from the Leaves Extract of Different Cultivars *Carica Papaya*

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Citation:
Maungchanburi, S.; Chaithada, P.; Rattanaburi, S.; Pinsrithong, S.; Raungrut, P.; Mukem, S. and Phetkul, U. Antiproliferative activity and GC–MS analysis from the leaves extract of different cultivars *Carica papaya*. *ASEAN J. Sci. Tech. Report.* **2024**, *27*(6), e254526. https://doi.org/10.55164/ajstr.v27i6.254526

Article history:
Received: June 13, 2024
Revised: September 27, 2024
Accepted: October 8, 2024
Available online: October 25, 2024

Abstract: Papaya is the fruit of the *Carica papaya* plant. Several secondary metabolites from the *Carica* genus have been reported to exhibit interesting biological activities. Its leaves are normally considered discarded. The research aimed to examine the antiproliferative and antioxidant effects of *Carica papaya* leaves from four different cultivars- Cocoa, Holland, Khaek Dam, and Red Lady and evaluate the chemical composition of the extracts through GC–MS analysis. The MTT assay evaluated the antiproliferative activity of all extracts. Red Lady exhibited higher effectiveness against MCF-7, SW620, and Vero cell lines compared to Khaek Dam, with IC_{50} values of 90.88 ± 0.39, 258.45 ± 2.16, and 301.73 ± 0.73 μg/mL, respectively. Cocoa and Holland extracts showed no cytotoxic effects on the mentioned cell lines. Antioxidant activity, measured through DPPH radical scavenging assays, revealed that Red Lady had the highest antioxidant capacity (IC_{50} 163.87 μg/mL), followed by Khaek Dam, Cocoa, and Holland. As a result, the GC–MS analysis concentrated on the extracts of Lady and Khaek Dam. The chromatograms revealed that the extracts from Red Lady displayed 23 components, while those from Khaek Dam contained 22. The primary metabolite produced in Khaek Dam were n-hexadecanoic acid (17.53%), 1-heptadecanecarboxylic acid (6.86%), and loliolide (5.58%), while in Red Lady 9-octadecenamide (20.82%), n-hexadecanoic acid (8.26%), palmitoleamide (5.43%) were produced. This indicates a difference in the chemical composition between the two cultivars. It is clear from this study that the chosen species included a range of potent phytochemicals with antiproliferative characteristics.

Keywords: *Carica papaya*; phytochemical constituents; antiproliferative activity; antioxidant activity

1. Introduction

Plants are excellent resources for the development of medicinal chemicals and drugs. Natural products could eventually serve as human or livestock medications. These goods and their equivalents may serve as bridges in producing

beneficial medications. Plant-derived biochemical compounds, including phenols, flavonoids, anthocyanins, and terpenoids, offer a wide range of potential applications, including anti-inflammatory, anti-tyrosinase, antioxidant, and anti-cancer properties.

Carica papaya, commonly known as papaya, is a member of the *Caricaceae* family with a rich history of medicinal use. Different parts of the plant, including the leaves, fruit, seeds, bark, and roots, have been traditionally used to treat illnesses such as dengue fever, jaundice, sinusitis, eczema, malaria, and digestive disorders [1–4]. In traditional medicine, papaya leaves have been used for generations in folk remedies to treat various health problems, including inflammation, digestive disorders, infectious diseases, malaria, constipation, irregular menstruation, eczema, diabetes, hypertension, and dengue fever [5-6]. Papaya leaf extract has shown significant antiplasmodial, antibacterial, antiviral, antitumor, hypoglycemic, anticancer, antidiabetic, and anti-inflammatory activities [6-8]. Extracts from various parts of the papaya obtained using different extraction methods are continuously being investigated for their biological activity and phytochemistry. Phytochemical analysis from papaya has revealed flavonoids, phenols, alkaloids, steroids, glycosides, and phenols [9–10]. These substances are recognized for their antioxidant, antimicrobial, antiviral, antibacterial, anti-inflammatory, and anticancer activities [11–14].

In Thailand, several papaya varieties are cultivated, each with distinct characteristics such as flesh color, skin color, and size. The fruit is edible, while the leaves are typically considered as waste and usually thrown away. A recent study investigated the total phenolic and flavonoid contents of extracts from the leaves of three papaya varieties, Khaek Dam, Red Lady, and Holland, to identify beneficial bioactive metabolites. The study found that the ethanol extracts from papaya leaves exhibited robust antioxidant activity [15]. Surprisingly, there are minimal findings on papaya varieties' phytochemical analysis and antiproliferative activity: Cocoa, Holland, Khaek Dam, and Red Lady. This work aimed to evaluate the potential antiproliferative and antioxidant activities of the extracts derived from four different cultivars of *C. papaya*: Cocoa, Holland, Khaek Dam, and Red Lady. Specifically, we aimed to identify the extracts that showed significant activity against cancer cells for further analysis of their chemical composition by GC–MS.

2. Materials and Methods

2.1 Plant material

The leaves of four cultivars of *C. papaya* (Cocoa, Holland, Khaek Dam, and Red Lady) were collected from Hua Sai District and Pakphanang District, Nakhon Si Thammarat province.

2.2 Plant extraction

The samples of all four varieties of papaya leaves were cut into small pieces and ground thoroughly, then dried in a hot air oven at 60°C for 17 hours. Four cultivars of papaya (Cocoa, Holland, Khaek Dam, and Red Lady) leaves weighing 10 grams each were extracted with a soxhlet extractor using 200 mL of ethanol for 4 hours. Then, the solvent was evaporated using a rotary evaporator.

2.3 Cell culture conditions and in vitro cytotoxicity testing

The American Type Culture Collection (ATCC, Manassas, VA, USA) produced the breast cancer cell lines (MCF-7). Professor Dr. Surasak Songkhathat (Faculty of Medicine, PSU, Thailand) provided colorectal adenocarcinoma (SW620) cell lines for research. Kidney epithelial cells (Vero line) were provided by Associate Professor Dr. Potchanapond Graidist (Faculty of Medicine, PSU, Thailand). Cells were maintained in RPMI 1640 (Invitrogen) medium supplemented with 10% fetal bovine serum (Invitrogen), 50 µg/mL penicillin (Invitrogen), and 50 µg/mL streptomycin (Invitrogen) at 37 °C, containing 5% CO_2 and 95% of the air humidity. The cytotoxicity of the extracts and fractions was assessed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay as first described. Half maximal inhibitory concentration (IC_{50}) values below 20 µg/mL were considered to have in vitro cytotoxic activity [16]. Cell viability was checked after treatment with the positive control drug doxorubicin against MCF-7, SW620, and normal monkey kidneys. The selectivity index (SI) was utilized to evaluate the selectivity of the extracts, as first described [17]. The SI of the most active fraction was calculated by dividing the IC_{50} value of the extract on a normal cell line by the IC_{50} value of the extract on each cancer cell. An SI value greater than 3 was considered indicative of an anticancer drug.

2.4 DPPH radical scavenging assay

To evaluate the antioxidant activity, the percentage of inhibition and the IC_{50} value of papaya leaf extract are calculated about a Trolox standard solution. Free radical inhibition was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A slightly modified approach was applied, following the methodologies described by Wasman, Mahmood, Chua, Alshawsh, & Hamdan (2011) [18] and Palafox-Carlos et al. (2012) [19]. A 0.2 mM DPPH methanolic solution was combined with varying extract concentrations (ranging from 0.1 to 0.5 mg/mL). After allowing the mixture to sit in a dark environment at room temperature for 30 minutes, the optical density was recorded at 517 nm using a UV-Vis spectrophotometer. Each experiment was performed in triplicate. The results were expressed as the IC_{50} value, representing the sample concentration that leads to 50% inhibition. IC_{50} values were calculated by extrapolating the linear regression equation from a graph plotting percent inhibition against sample concentrations.

$$\% \text{ inhibition} = (A_{control} - A_{sample}) / A_{control} \times 100.$$

2.5 Gas chromatography-mass spectrometry (GC–MS) analysis

GC-MS was used to analyze the composition of the extracts using an Agilent 7890B GC with an Agilent 5977A single quadrupole MS (Agilent Technologies, Santa Clara, CA, USA). A VF-WAXms column (30 m length, 0.25 μm, ID 0.25 mm) was used as the analytical column. The GC was run in splitless injection mode, with the front inlet temperature set at 250°C and an injection volume of 1.0 μL. The oven was set to 60°C for two minutes, then raised to 250°C in twenty minutes at a rate of 5°C per minute. At 70 eV, electron ionization (EI) was employed for MS detection. The interface, MS quad, and MS ion source temperature were set to 250°C, 230°C, and 150°C, respectively. The scan mass range was 30 to 500 m/z, and the solvent delay was 6 minutes. The components were identified by analyzing the mass spectra using NIST14 and Wiley 10 database systems software. All processes were conducted at the Office of Scientific Instruments and Testing, PSU, Thailand.

2.6 Statistical analysis

The student's t-test was performed, and the results were statistically analyzed using the Microsoft Excel program. A t-test was used to compare the means of the two groups. Results were presented as the mean ± standard deviation, and the experiments were carried out in triplicate. A p-value of less than 0.05 was considered statistically significant.

3. Results and Discussion.

This study assessed the antiproliferative activity of ethanol extracts from *C. papaya* leaves, explicitly focusing on four cultivars: Cocoa, Holland, Khaek Dam, and Red Lady. Furthermore, this study provided insight into how the bioactive compound affects the extract's effectiveness using GC-MS analysis. These investigations showed naturally occurring bioactive substances associated with various biological functions. The extraction yields of the four papaya varieties (Cocoa, Holland, Khaek Dam, and Red Lady) provide valuable insights into the potential efficiency of ethanol as a solvent for extracting bioactive compounds from dried leaves (10 g). Of the tested varieties, Red Lady showed the highest extraction yield with 37.83 ± 0.95%, followed by Khaek Dam with 34.93 ± 2.73%, Cocoa with 32.87 ± 2.73% and Holland with 31.10 ± 2.62%. The difference in extraction yield observed among the four papaya varieties could be due to a combination of genetic factors, metabolite profiles, moisture content, tissue structure, and environmental conditions, consistent with previous reports [20]. These factors affect the solubility and availability of compounds during the ethanol extraction process. Red Lady, having the highest yield, may contain more ethanol-soluble metabolites than the other varieties.

3.1 Antiproliferative activity

In this research, we investigated the antiproliferative activity of four plant extracts, Red Lady, Khaek Dam, Cocoa, and Holland, against three different breast cancer cell lines (MCF-7), colorectal adenocarcinoma cell lines (SW620) and Vero cell as shown in **Table 1**. The following criteria were used to rank the cytotoxic:

≤ 20 µg/mL is highly cytotoxic, 21-200 µg/mL moderately cytotoxic, 201-500 µg/mL weakly cytotoxic and > 501 µg/mL cytotoxic not inhibit [16].

Table 1. Cytotoxicity of ethanolic extract of papaya leaves against Vero, MCF-7, and SW620 cell lines in parameters of IC_{50} values (mean±SD) and SI

Sample	Vero	MCF-7		SW620	
	IC_{50}	IC_{50}	SI	IC_{50}	SI
Cocoa	not inhibit	not inhibit	ND	not inhibit	ND
Holland	not inhibit	not inhibit	ND	not inhibit	ND
Red lady	301.73 ± 0.73	90.88 ± 0.39	3.32	258.41 ± 2.16	1.17
Khak Dam	337.04 ± 8.28	328.60 ± 2.24	1.03	not inhibit	ND
Doxorubicin	1.02 ± 0.01	0.47 ± 0.00	2.17	0.35 ± 0.00	2.90

Data represented mean±SD from three independent experiments. SI Vero cells are a selectivity index calculated by dividing the IC50 of Vero by IC_{50} cancer cells. Not determined (ND)

Our research revealed that Red Lady exhibited moderate antiproliferative activity against MCF-7 cells with an IC_{50} value of 90.88 ± 0.39 µg/mL. At the same time, it showed weak activity against SW620 and Vero cells with IC_{50} values of 258.45 ± 2.16 and 301.73 ± 0.73 µg/mL, respectively. In contrast, Khaek Dam displayed weak antiproliferative activity against MCF-7 and Vero cells with IC_{50} values of 328.60 ± 2.24 and 337.04 ± 8.28 µg/mL, respectively, and no inhibition against SW620 cells. These findings offer promise for the potential of Red Lady as a potential treatment option for breast cancer. Additionally, the absence of cytotoxic effects of Cocoa and Holland extracts against MCF-7, SW620, and Vero cells suggests that these extracts may not be toxic to normal cells. In our study, the unrefined extract exhibited limited anticancer potential, as evidenced by weak inhibition of cancer cell lines. This observation is probably due to impurities in the extract, which consists of various constituents.

Upon examination, the extracted compound revealed a wide range of cytotoxic effects against the tested cancer cell types. The variations in cytotoxicity observed among different cells can be attributed to differences in their structure, genetic makeup, and origins, all of which influence their susceptibility to chemotherapy. Consequently, the potent cytotoxicity displayed by the investigated compound can be directly attributed to its high concentration of chemicals obtained from the plant extract. Notably, the Red Lady extract demonstrated significant cytotoxic activity against MCF-7 cancer cell lines with a Silective Index (SI) of 3.32, compared to doxorubicin. Achieving an SI greater than 3 indicates a high level of selectivity. The SI, which measures the differential activity of a sample, becomes higher as the selectivity increases. When the SI value surpasses two, it suggests the sample may pose potential risks [17]. In light of this, the Red Lady extract exhibited no cytotoxic activity on normal cells while exhibiting selectivity towards MCF-7 cancer cell lines.

3.2 Antioxidant activity

The antioxidant properties of the extracts were assessed through the DPPH radical scavenging assay. The data in **Table 2** illustrate the differences in the antioxidant activity of the four papaya leaves, reflected in their IC_{50} values. Red Lady has the lowest IC_{50} value of 163.87 ± 6.81 µg/mL, meaning it has the highest antioxidant capacity. Khaek Dam outperformed Cocoa and Holland with a moderate IC_{50} value of 288.16 ± 7.19 µg/mL, although it was still significantly less potent than Red Lady. In comparison, Holland (428.34 ± 23.30 µg/mL) showed the weakest antioxidant activity, closely followed by Cocoa (405.06 ± 6.89 µg/mL). The higher IC_{50} values for these varieties indicate a lower ability to neutralize free radicals, probably due to the bioactive compounds' lower concentration or activity. The differences in antioxidant activity between cultivars may be due to several factors. Genetic variations between papaya varieties significantly impact the synthesis and accumulation of secondary metabolites, which are directly related to antioxidant capacity. In addition, environmental factors such as soil composition, climate, and cultivation methods may also play a role in differences in secondary metabolite content, which is in agreement with previous reports [21-22].

Table 2. Antioxidant activity of ethanolic extract of papaya leaves in parameters of IC_{50} values (mean ± SD)

Sample	IC_{50} (μg/mL)
Cocoa	405.06 ± 6.89
Holland	428.34 ± 23.30
Red lady	163.87 ± 6.81
Khak Dam	288.16 ± 7.19
Trolox	49.86 ± 1.29

3.3 GC–MS analysis

Following a thorough antiproliferative activity of four extracts, Red Lady, Khaek Dam, Cocoa, and Holland, we have identified the potent extracts for antioxidant and antiproliferative activities. Our findings indicate that the ethanolic extracts of Khaek Dam and Red Lady exhibit the most significant activity, as evidenced by their IC_{50} values. Consequently, plant components of two extracts were continually analyzed using gas chromatography-mass spectrometry (GC–MS), a widely used technique for phytoconstituent separation. These chemicals were determined by their retention time on the fused silica capillary column. They were categorized as bioactive components by comparing their mass spectrum fragmentation patterns with those of recognized chemicals from the NIST library (NIST14). The GC–MS chromatogram of the identified compounds is shown in **Figure 1**.

Many biologically active components were observed. The identified chemical constituents are listed in **Table 3** by retention time, peak area (%), molecular formula and match factor (> 80.00), and % of the total (> 0.5). The GC–MS chromatograms of the Red Lady and Khaek Dam extracts show a total of 23 and 22 components, respectively. 11 chemicals are present in both varieties, including acetic acid, 1,1-cyclohexanedimethanol, 4'-methylacetophenone, neophytadiene, dodecanoic acid, n-hexadecanoic acid, palmitoleic acid, *cis*-13-octadecenoic acid, 1-heptadecanecarboxylic acid, loliolide, and linolenic acid.

In this study, the main constituents of the Red Lady leaf extract have been identified. The 9-octadecenamide was the most abundant, accounting for 20.82% of the total peak area. It was followed by n-hexadecanoic acid at 8.26%, palmitoleamide at 5.43%, hexadecanamide at 5.36%, and *cis*-13-octadecenoic at 1.92%. Other chemical components included neophytadiene, 1,1-cyclohexanedimethanol, acetic acid, and tetradecanoic acid. On the other hand, the papaya leaf extract from the Khaek Dam variety showed a different composition. The n-hexadecanoic acid was the main component, constituting 17.53% of the total peak area. It was followed by 1-heptadecanecarboxylic acid at 6.86%, loliolide at 5.58%, and 1,2,3-propanetriol at 3.04%. Other notable components included acetic acid, 4-oxo-pentanoic acid, and myristic acid. Several phytoconstituents were found in ethanol extracts of *C. papaya* leaves (Khaek Dam and Red Lady). These results suggest that the chemical components present in papaya leaf extracts vary depending on the variety. Both varieties contained a diverse range of compounds, including monoterpenes, monoterpenoid lactones, sesquiterpenes, diterpenes, fatty acids, fatty acid amide, phenolics, esters, alcohols, ketones, and aldehydes. This work shows that the main components of the two varieties are very different.

From GC–MS data), many components of the Khaek Dam variety were significant, including loliolide, 1-heptadecanecarboxylic acid, and n-hexadecanoic acid. Compared to Red Lady, Khaek Dam contains less loliolide, 1-heptadecanecarboxylic acid, and n-hexadecanoic acid. According to this research, Red Lady appears to have a higher phytochemistry component abundance or concentration than Khaek Dam. The results of the present study show that extracts from papaya leaves contain a broad spectrum of important and valuable chemicals, with the availability and content of these compounds varying depending on the cultivars.

Previous research has studied the chemical composition of papaya. It was revealed that in 2018, the significant phytocomponents found in Red Lady leaves were benzylnitrile, pyridine-2d,6-methyl-1,1'-dimethyl-2'-propenylbenzoylformate, osmium, methyl-2-(1,1-dimethylethyl)-4,5-dihydroxy-3-xazolidinecar

boxylato[2-4,O5]dioxobispyridine[OC644]2R(2α,4β,5β), 6-(N,N-diethylaminomethyl)-2,5-dimethylphenol, and benzene, isothio-cyanatomethyl [23]. However, In 2021, the main phytochemicals discovered in papaya leaves extract were butyl-9,12,15-octadecatrienoate, dasycarpidan-1-methanol, acetate (ester), n-hexadecanoic acid, neophytadiene, oleic acid, phytol, sitosterol, tocopherol, tetramethyl-2-hexadecen, campesterol, squalene, octadecenoic acid, stigmasterol, and D-limonene [24]. In 2022, Smrati *et al.*, reported that 9,12,15-otadecatrienoic acid, (Z,Z,Z)-Linolenic acid, 1,2-benzenedimethanol, 4-methyl-benzaldehyde, nonadecanoic acid, sucrose, d-glycero-dgalacto-heptose, rhodopin, benzyl nitrile, and ethyl-9,12,15-octadecatrienoate from the leaves [25]. Previous reports show that papaya has similar ingredients in research results. However, the main chemical constituents exhibit both similarities and differences. The phytochemical composition of an extract is influenced by factors such as the plant species, plant part used, growth conditions, solvents used, and the extraction method. Different subspecies of a plant species may have genetic variations that result in distinct biochemical pathways and the production of unique secondary metabolites.

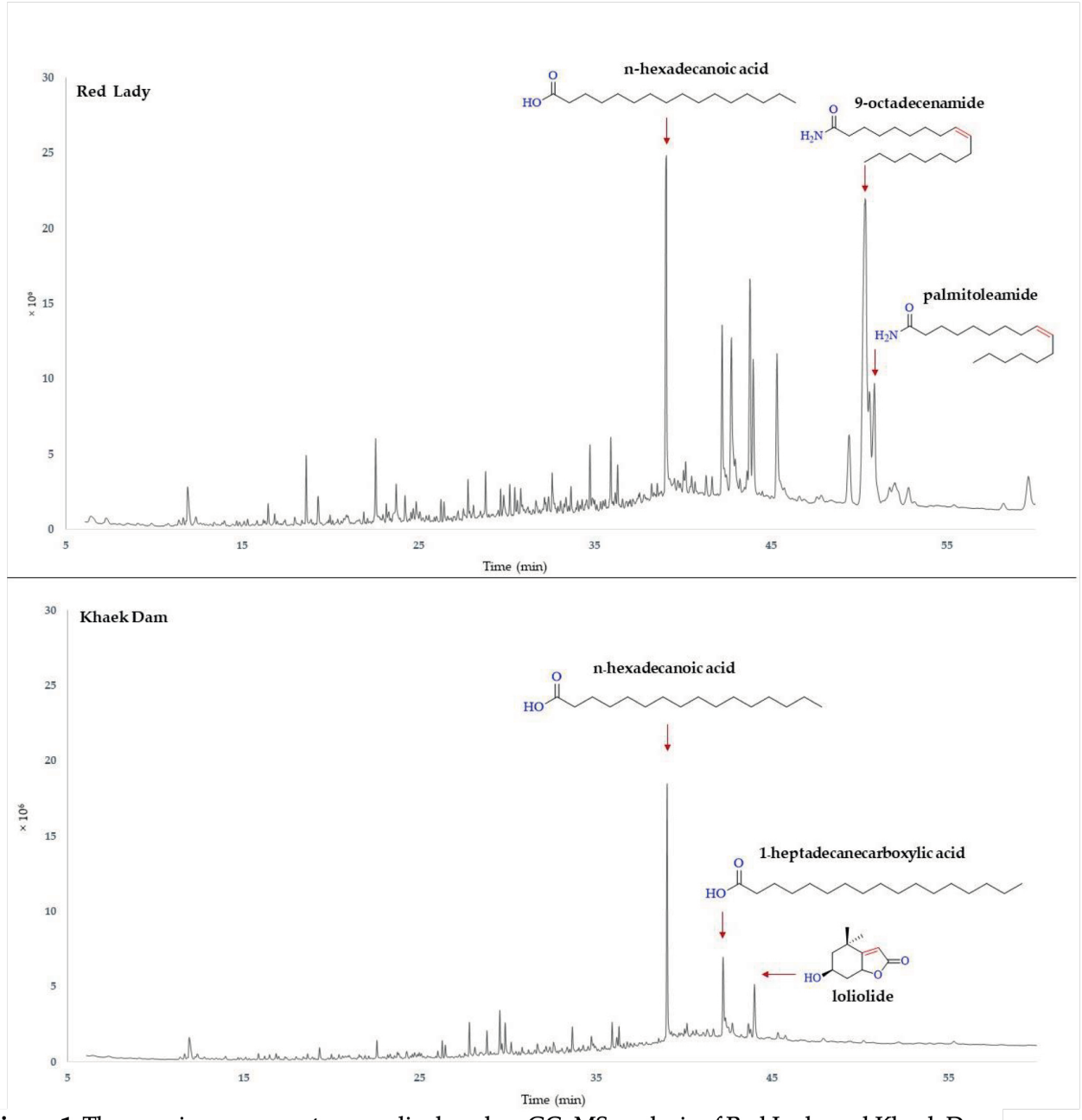

Figure 1. Three main components were displayed on GC–MS analysis of Red Lady and Khaek Dam.

Table 3. GC–MS analysis of ethanol extract from *C. papaya*; Red Lady and Khaek Dam

No.	Red Lady				Khaek Dam			
	RT (min)	Name	Formula	Component Area (% of total)	RT (min)	Name	Formula	Component Area (% of total)
1	11.8997	acetic acid	$C_2H_4O_2$	17091171.6 (1.09%)	11.9268	acetic acid	$C_2H_4O_2$	10880794.1 (2.89%)
2	18.6307	1,1-cyclohexanedimethanol	$C_8H_{16}O_2$	17101098.3 (1.09%)	18.6308	1,1-cyclohexanedimethanol	$C_8H_{16}O_2$	1918112.3 (0.51%)
3	19.3059	4'-methylacetophenone	$C_9H_{10}O$	8555602.5 (0.54%)	19.3060	4'-methylacetophenone	$C_9H_{10}O$	4016117.2 (1.07%)
4	22.5742	neophytadiene	$C_{20}H_{38}$	20291240.2 (1.29%)	22.5634	neophytadiene	$C_{20}H_{38}$	5038236.6 (1.34%)
5	23.7410	phytol	$C_{20}H_{40}O$	8229725.6 (0.52%)	24.2597	11-decyldocosane	$C_{32}H_{66}$	2923372.1 (0.78%)
6	27.8141	phytol isomer	$C_{20}H_{40}O$	9731994.2 (0.62%)	26.2800	1,2-heptanediol	$C_7H_{16}O_2$	3816053 (1.01%)
7	29.8399	levulinic acid	$C_5H_8O_3$	8921515.7 (0.57%)	26.4529	hexahydrofarnesyl acetone	$C_{18}H_{36}O$	3353178.5 (0.89%)
8	30.1802	dihydroactinidiolide	$C_{11}H_{16}O_2$	8135241.2 (0.52%)	27.8196	1-eicosanol	$C_{20}H_{42}O$	8082527.8 (2.14%)
9	32.5841	dodecanoic acid	$C_{12}H_{24}O_2$	12090855.4 (0.77%)	28.1275	methyl palmitate	$C_{17}H_{34}O_2$	2940370.5 (0.78%)
10	34.7341	phytol	$C_{20}H_{40}O$	15738932.4 (1.00%)	29.5483	1,2,3-propanetriol	$C_3H_8O_3$	11449063.1 (3.04%)
11	35.9172	tetradecanoic acid	$C_{14}H_{28}O_2$	16925273.9 (1.08%)	29.6671	2,4-di-tert-butylphenol	$C_{14}H_{22}O$	2304985.4 (0.61%)
12	36.3007	2-tertbutylcyclohexylpropyl-phospho-nofluoridate	$C_{13}H_{26}FO_2P$	10038392 (0.64%)	29.8400	4-oxo-pentanoic acid	$C_5H_8O_3$	8354470.3 (2.22%)
13	39.0720	n-hexadecanoic acid	$C_{16}H_{32}O_2$	129805907.5 (8.26%)	30.1857	dihydroactinidiolide	$C_{11}H_{16}O_2$	3095708.4 (0.82%)
14	40.1632	palmitoleic acid	$C_{16}H_{30}O_2$	9827461.1 (0.63%)	32.5842	dodecanoic acid	$C_{12}H_{24}O_2$	4237708.4 (1.12%)
15	42.7616	*cis*-13-octadecenoic acid	$C_{18}H_{34}O_2$	64966881.5 (4.13%)	33.3621	3-hydroxy-β-damascone	$C_{13}H_{20}O_2$	2275341 (0.60%)
16	42.2268	1-heptadecanecarboxylic acid	$C_{18}H_{36}O_2$	47671291.2 (3.03%)	35.9173	myristic acid	$C_{14}H_{28}O_2$	5955232.8 (1.58%)
17	42.9777	octadec-9-enoic acid	$C_{18}H_{34}O_2$	10140869.1 (0.65%)	39.0397	n-hexadecanoic acid	$C_{16}H_{32}O_2$	66092962.6 (17.53%)
18	43.8042	hexadecanamide	$C_{16}H_{33}NO$	84254217.1 (5.36%)	40.1633	palmitoleic acid	$C_{16}H_{30}O_2$	4512992.1 (1.20%)
19	43.9986	loliolide	$C_{11}H_{16}O_3$	54058360.8 (3.44%)	42.2161	1-heptadecanecarboxylic acid	$C_{18}H_{36}O_2$	25852532.1 (6.86%)
20	45.3437	linolenic acid	$C_{18}H_{30}O_2$	58907641.5 (3.75%)	42.7347	*cis*-13-octadecenoic acid	$C_{18}H_{34}O_2$	3421784.5 (0.91%)
21	49.4385	octadecanamide	$C_{18}H_{37}NO$	48439157.5 (3.08%)	43.9879	loliolide	$C_{11}H_{16}O_3$	21049801.5 (5.58%)
22	50.3406	9-octadecenamide	$C_{18}H_{35}NO$	327220486 (20.82%)	45.3222	linolenic acid	$C_{18}H_{30}O_2$	2799327.2 (0.74%)
23	50.8700	palmitoleamide	$C_{16}H_{31}NO$	85354814.6 (5.43%)				

3.3 Biological activity of major compounds of Red Lady and Khaek Dam variety

In this research on papaya leaf extracts, it has been discovered that the prominent phytochemicals present are n- hexadecanoic acid and 9- octadecenamide. Red Lady extracts rich sources of 9-octadecenamide has proven effective in anti-inflammatory, antimicrobial, and antioxidant activities [26–28]. The 9-octadecenamide, known as oleamide, was isolated from *Moringa oleifera*. It was shown to have anticancer activity on human myelogenous leukemia cells (K562), human squamous cell carcinoma (SCC-15), and breast cancer cells (MDA-MB-231) [29]. Khaek Dam extract enriched n-hexadecanoic acid. It also exhibited against breast cancer and Vero cells, which corresponded with the previous report. The n-hexadecanoic acid-enriched extracts of *Ulva intestinalis*, *Sargassum ilicifolium*, *Halimeda macroloba*, and *Halymenia durvillei* have cytotoxic activity against human breast and hepatocellular carcinoma cells [30-31]. Moreover, it has been demonstrated to be effective against human colorectal carcinoma (HCT-116) cells [32]. It also promoted human leukemia cells [33] and colon cancer cells (HT-29) [34]. Notably, n-hexadecanoic acid showcases its value through its ability as an antibacterial, antioxidant, and anti-inflammatory activity [35–37]. Additionally, other components of the extracts, such as palmitoleamide and *cis*-13-octadecenoic, exhibit anti-inflammatory properties [38], while hexadecanamide has anti-inflammatory, antifungal, and anti-nociceptive [39]. Moreover, 1-heptadecanecarboxylic acid displays antimicrobial properties [40], and loliolide shows antioxidant, antifungal, antibacterial, and anti-cancer effects [41–43].

4. Conclusions

The study assessed the antiproliferative activity of the ethanol extracts from *C. papaya* leaves (Cocoa, Holland, Khaek Dam, and Red Lady). Among these, the Red Lady variety showed the most promising results. It exhibited moderate antiproliferative activity against breast cancer (MCF-7) cells with an IC_{50} value of 90.88 ± 0.39 μg/mL, coupled with a Selectivity Index (SI) of 3.32, indicating a high level of selectivity towards cancer cells without affecting normal cells. Antioxidant analysis revealed that Red Lady again stood out, with the highest antioxidant capacity (IC_{50} = 163.87 μg/mL). The extracts of Khaek Dam and Red Lady show various components. However, further investigations are needed to purify and identify the active compounds to confirm the antiproliferative activity.

5. Acknowledgements

The authors would like to thank the Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Office of Scientific Instrument and Testing, Prince of Songkla University, General Science, Faculty of Education, Nakhon Si Thammarat Rajabhat University, School of Medicine, Walailak University and Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, for providing research facilities.

Author Contributions: Conceptualization, U.P., S.M., P.K., S.R., S.P., P.R., and S.M.; methodology, U.P., S.M., P.K., S.R., S.P., P.R., and S.M.; validation, U.P., and S.M.; formal analysis, U.P., S.M., P.K., S.R., and S.P.; investigation, U.P., S.M., P.K., S.R., S.P., P.R., and S.M.; resources, U.P., S.M., P.K., S.R., S.P., P.R., and S.M.; data curation, U.P., S.M., P.K., and S.R.; writing—original draft preparation, U.P., S.M., P.K., S.R., and S.P.; writing—review and editing, U.P., S.R and S.M.; visualization, U.P., and S.M.; supervision, U.P

Funding: The research was financially supported by the Faculty of Medicine, Prince of Songkla University (REC. 63-544-4-7)

Conflicts of Interest: The authors declare no conflict of interest.

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