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Review

The Future of *Carica papaya* Leaf Extract as Herbal Medicine Products

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Abstract: *Carica papaya* (papaya) leaf extract has been long time used in a traditional medicine to treat fever in some infectious diseases like dengue, malaria and chikungunya. The science and technology development then provide evidences, that this plant is not only beneficial in an informal medication, but scientifically proves its pharmacological and toxicological activities, which leads its usage in a formal - professional health care system. The formulation development in nutraceuticals and cosmeceuticals have enriched this product to be more valuable, nowadays. The good manufacturing practice (GMP) along with the easy facility from national government to register this product, will be absolutely increasing the value of papaya leaf extract as one of vital nutraceutical and cosmeceutical products in the near future ahead. In this article, we review the potential of papaya leaf extract to be a high commodity value in terms of health as well as its industrial benefits.

Keywords: *Carica papaya*, leaf, extract, herbal, medicine, future

1. Introduction

In tropical and sub-tropical regions, there are abundant flora and fauna living in a good climate and circumstance. In flora especially, this is a valuable source in various kind of beneficial products such as dyes, edible tubers, oil crops, furniture, agricultural implements, ornamental plants, pharmaceutical products, rubbers, timbers and cosmetics [1]. Therefore, a preservation of biodiversity is our compulsory task to protect local ecosystems from destructions and to promote healthy conditions for organisms to thrive [2]. There are several ways to promote and to preserve our local biodiversity, including support to local farm, save the bees, plant local flower, fruits and vegetables, take shorter showers, respect local habitats and know the sources [3].

Carica papaya (papaw or papaya) is one of tropical and subtropical trees well known for its utilization in a whole part of the plant. As a tropical species, this plant grows continuedly under distinct winter, growth slows down and fruit set ceases during the colder months [4]. In Indonesia, the export demands of papaya, came from Germany, Hongkong, Japan, Malaysia, Singapore, Taiwan and USA are remained high, however, the export volume was decreasing from 2009 to 2020 [5]. This could indicate the preservation of this crop becoming less intensive leading to a reduction of its productivity.

The productivity of papaya declines along with fronting challenge in the papaya dieback disease, which could jeopardize its future [6]. One of the dieback diseases is *Erwinia mallotivora*, a phytopathogen bacteria, has significant roles to overcome and to limit the effect of this vulnerable crop [7]. It was 4824 Kbp and the G+C content of the genome detected in papaya showing 52-54% homology to that of reference genomes of other Erwinia species [8]. This information is useful for elucidating the infection mechanism of this disease, directing to the pathway inhibition strategy.

Although the whole part of papaya crop has been widely studied, however, the fruit and the leaves are two major parts daily used in various purposes such as food, medicine, pesticide and cosmetics [9].

In food, the fruit has been advantaged as a nutrition [10], appetizer [11], and snack [12], whereas as an herbal medicine, the leaves have been utilized as an antimicrobe [13], antioxidant [14], antivirus [15, 16], haematology disorder [17] and antitumor [18]. Although it is minor, the papaya seeds have been studied for its antidiabetic activity [19]. In non-food and non-medicine purposes, papaya leaf is also used for bioherbicide [20], ectoparasite control [21], larvicide [22], and to control onion pest *Spodoptera exigua* [23]. In cosmetics, a black hair dye and face mask can be made from the papaya seeds [24] and its leaves [25], respectively. In pharmaceutical product, various studies have been conducted to formulate papaya products including oral administration [26], topical [27], and transdermal [28]. To be more specific, most oral route was prepared in capsule and tablet dosage forms [29, 30] instead of liposome delivery system [31] and self-nanoemulsion [32] were also formulated. Various topical dosage forms have been prepared including cream [33], lotion [34], hand sanitizer [35], ointment [36], and emulgel [37].

The phytochemical compounds have been identified in papaya leaf mostly the class of flavonoid such as apigenin, catechin, deoxyquercetin, hesperitin, isorhamnetin, kaempferol, myricetin, naringenin, protocatechuic acid, quercetin, and rutin. Meanwhile, the fruit is enriched by amino acid, protein, carbohydrate, fiber, vitamin C, and other nutrients [38]. Interestingly, the whole part of papaya mainly expresses the white latex highly contains a proteolytic enzyme called papain, which have been studied its crucial role in many pathophysiology of diseases, drug designs, industrial uses such as meat tenderizers and pharmaceutical preparations [39].

One of our local products formulated as herbal extract is papaya leaf extract, prepared in capsule dosage form, manufactured by Sido Muncul Industry in Herbal and Pharmaceutical Product, located in Semarang, Central Java, Indonesia. In this product, the ingredient is claimed as a food supplement which helps to increase the appetite and to gain weight. Most likely, people do not like papaya leaf due to its bitter taste. This product is suggested to be suitable for those who wants to gain weight by natural. This product is composed by 500 mg of leaf extract which is equal to 3 g of its dried leaf, indicated for reducing fever, recovering condition post dengue infection, malaria and chikunguya. This product has advantages to contain a various protein, iron, calcium, vitamin A, B1, C, various alkaloid, enzyme, and ribosomal activating protein (https://www.sidomuncul.co.id/product/detail/85).

The good manufacturing practice (GMP) has been applied to standardize the product by conditioning it in a temperature lower than 60^{o}C to maintain its active ingredient stability. Surely, this product has been registered and licensed by national agency in drug and food control. This product is contraindicated for pregnancy and breast-feeding woman. The regimen dose for health promotion is one capsule in each three times daily, indicated for 12 years old and above. Meanwhile, the dose for fever healing is two capsules in each three times daily. For children 6 – 12 years old, one capsule for a day is sufficient. The sub-chronic toxicity evaluation had resulted the safety of this product to be consumed in a long period (https://www.sidomuncul.co.id/product/detail/85).

This article reviews and gives perspective of some important aspects of papaya leaf extract, to be brought up as one of high commodity in health and industrial businesses. The section starting from the extraction process, capsule preparation, nutrition and phytochemical substances, indication, the GMP, product registration, and its safety will be overviewed. Furthermore, the pharmacological and toxicological properties would be suggested for a new indication, even in its molecular mechanisms.

2. Extraction

The extraction of papaya leaf is carried in a various method, from traditional maceration, percolation, soxhlet, until using more advance instruments such as microwave and ultrasonic cleaner. Table 1 presents the extraction method for papaya leaf along with the solvents being used. The leaves were mostly extracted using maceration method employing 96% ethanol as the solvent. The reason why maceration is the most common method, could be the economically cheaper and simpler than other methods. The leaf have a soft texture; therefore, it is easier for the solvent penetrating the leaf cells while extracting the phytoconstituent. However, this method has a disadvantage about the equilibrium state between out and inside the cell since the solvent does not move. This causes the extraction being stopped, and the residues of filtrate needs to be re-macerated using a new solvent [40, 41]. On the other hand, 96% ethanol and water are used as two most common solvents. The most reason of choosing these solvents is their fewer toxic properties compared to others [42]. Most likely, the flavonoid glycoside as well as alkaloid in a salt form would be easily extracted from the leaf cells due to their suitable polar character with the solvents. Properly, these two solvents are the most recommended by the the national agency of drug and food control.

Table 1. The extraction method for papaya leaves along with the solvents being used.

Extraction Method	Solvents	References
Ultrasonic cleaner	methanol	[43]
	96% ethanol	[44]
Hot presser	water	[45]
Blender	water	[23, 46-48]
Maceration	70% ethanol	[25, 49]
	96% ethanol	[10, 22, 27, 33, 36, 50-53]
	70% methanol	[54, 55]
	80% methanol	[14, 56]
	water	[20]
Mixer	cold water, hot water, cold ethanol 70%	[57, 58]
Microwave	methanol, 70% ethanol and water	[59]
Soxhlet	hexane, acetone, 60% ethanol, 40% ethanol and water	[60]

3. Nutraceuticals/ cosmeceuticals product

Nutraceutical is termed based on the words 'nutrition and 'pharmaceutical'. In general, nutraceuticals, are food or its part having a significant role in modifying and maintaining body in a normal physiological function. Nowadays, nutraceuticals are worldwide growing up in the market and has been becoming a life style in a health promotion. The nutraceutical products can be grouped as dietary fibre, prebiotics, probiotics, polyunsaturated fatty acids, antioxidants and other different types of herbal/ natural foods. Many metabolic disorders such as obesity, cardiovascular diseases, cancer, osteoporosis, arthritis, diabetes, and cholesterol are controlled by supplementing with nutraceuticals. This is supported by the re-orientation of the research, which tends to go to the era of 'nutraceuticals' as the one important sector of a big pharma industry [61].

On the other hand, cosmeceuticals take the chance as a new category of products forming a hybrid between cosmetics and pharmaceuticals. The intentions are to enhance both the health and the skin beauty. The skin care industry is an ever-increasing part, in which cosmeceuticals are formulated from a multitude of ingredients. This attracts physician to consider, then recognize and understand their benefits, limitations, and potential adverse effects, that can ensure patients to use it, whenever they need [62].

The nutraceutical and cosmeceutical products of papaya leaf is mostly manufactured in Asia, especially in India. In this country, there have been at least 58 products of papaya leaf extract in tablet dosage form, manufactured by diverse pharma industries. The tablet of papaya leaf most likely contain 1100 mg extract per tablet. Other minor products such as capsules, oral drops, and tinctures are produced in US and Indonesia. A various products of cosmeceutical are produced in Hungary focusing on the skincare products such as toner, cleanser, peeling gel, eye cream, face cream, body polish, face serum and clarity mask. Table 2 presents a list of nutraceuticals and cosmeceuticals product manufactured by diverse pharma company in a few different countries.

Table 2. The list of nutraceuticals and cosmeceuticals papaya leaf extract products manufactured by diverse pharma company in a few different countries.

Product Form	Manufacturer	Reference
Tablets	Herbo Nutra	https://www.herbonutra.biz/
	Micro Lab Limited	https://www.microlabsltd.com/
	Mesmer Pharmaceuticals	https://www.indiamart.com/mesmer-pharmaceuticals
	Juggat Pharma	
	Meyers Organic PVT LTD	https://www.jagdale.com/divisions/juggat-pharma
	IPCA Laboratories LTD	https://meyer.co.in/
	Intra Life	http://geneticslifescience.com/
	Aden Healthcare LTD	https://www.ipca.com/
	Comed Chemicals	https://intralifeindia.com/
	Tuttsan Pharma	http://www.adenhealthcare.com
	Cubit Healthcare	https://comedchemicals.com/
	Pride healthcare LLP	https://www.tuttsanpharma.com/
	Aero Chem	https://www.cubithealthcare.net/
	Bioceutics	https://www.pridehealthcare.net/
	Intra Labs India PVT LTD	http://www.aerochem-inc.com/
	Bioceutics	https://bioceuticsinc.in/
	Intra Lab	http://www.intralabs.in/m
Capsules	Herbal Goodness	https://www.herbalgoodnessco.com/
	Sido Muncul	https://www.sidomuncul.co.id/
Oral drops	Hawaiian Herbal	https://hawaiian-Herbal
Tinctures	New Way Herbs	https://newwayherbs.com/
Face cleanser, face lotion spf15, peeling gel, clarity mask, crème mask,toner, face serum, peel pad, jelly mask, sunscreen lotion, eye cream, face cream, body polish, shave cream are manufactured by INCIDecoder		https://incidecoder.com/

4. Nutrition and phytochemical substances

The nutraceutical and cosmeceutical products are formulated by considering a few factors, which surely one of them is the nutrition or phytochemical substances in the papaya leaf. A number of articles has reported these stuffs, which is in general, the nutrient of papaya leaves can be categorized in macromolecule, fiber, mineral and vitamin [63]. Table 3 summarizes the nutrition substances are reported to deposit in papaya leaf.

Table 3. The nutrition substances are reported to deposit in papaya leaf.

Nutrients	Quantity	Nutrients	Quantity	Nutrients	Quantity
Proteins	5.8 g	Phosphorous	221.1 mg	Vitamin B3	0.38 mg
Lipids	1.4 g	Magnesium	32.4 mg	Vitamin B2	0.14 mg
Carbohydrates	78.2 g	Iron	6.4 mg	Vitamin B1	0.43 mg
Fiber	13.1 g	Calcium	366.1 mg	Vitamin A	ND

Energy	348.6 kcal	Vitamin C	31.1 mg	Beta-carotene	659.5 IU
sodium	ND	Vitamin B9	ND		
potassium	534 mg	Vitamin B6	ND		

ND = not determined

The phytochemical compounds in papaya leaf have been summarized and reported in some articles including: 2S-sambuningrin, 5,7-dimethoxycoumarin, anthraquinone, apigenin, caffeic acid, caffeoyl alcohol, catechin, deoxykaempferol, deoxyquercetin, dimethoxyphenol, ferulic acid, kaempferol, *p*-coumaric acid, *p*-coumaric alcohol, protocatechuic acid, *R*-prunasin, carpaine, pseudocarpaine, dehydrocarpaine I, dehydrocarpaine II, carposide, emetine, quercetine 3-(2-rhamnosylrutinoside), kaempferol 3-(2-rhamnosylrutinoside), quercetin 3-rutinoside, myricetin 3-rhamnoside, chlorogenic acid, *E*-3-(4-hydroxy-3-(3,4,5-trimethoxybenzyl)phenyl)acrylic acid, galic acid, *o*-coumaric acid [64-73]. Table 4 summarizes and groups the compounds identified in papaya leaf in a different class of natural compounds, whereas Figure 1 depicts the structure of representative compounds deposited in papaya leaf from each class.

Table 4. The phytochemical substances are reported to deposit in papaya leaves.

Class	Compounds
Flavonoids	apigenin, catechin, kaempferol, deoxykaempferol, deoxyquercetin, protocatechuic acid, galic acid
Flavonoid glycosides	quercetin 3-(2-rhamnosylrutinoside), kaempferol 3-(2-rhamnosylrutinoside), quercetin 3-rutinoside, myricetin 3-rhamnoside
Cyanogenic glycosides	2*S*-sambunigrin, *R*-prunasin
Coumarins	5,7-dimethoxycoumarin, *p*-coumaric acid, *o*-coumaric acid, *p*-coumaric alcohol
Quinones	anthraquinone
Cinnamic acids	ferulic acid, chlorogenic acid, *E*-3-(4-hydroxy-3-(3,4,5-trimethoxybenzyl)phenyl)acrylic acid
Phenols	2,6-dimethoxyphenol
Alkaloids	carpaine, pseudocarpaine, dehydrocarpaine I, dehydrocarpaine II, carposide, emetine

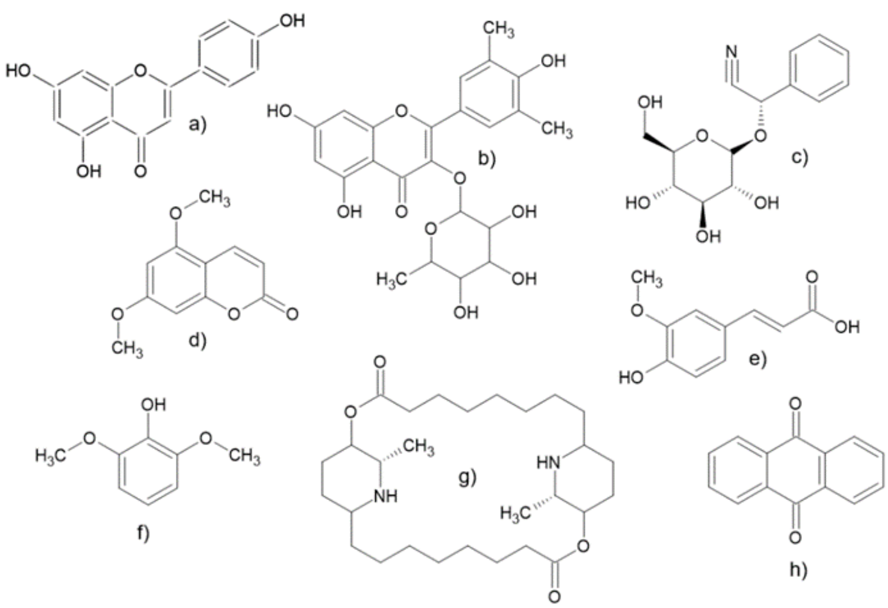

Figure 1. The representative compounds deposited in papaya leaf from each class: a) apigenin, b) myricetin 3-rhanmnoside, c) 2*S*-sambunigrin, d) 5,7-dimethoxycoumarin, e) ferulic acid, f) 2,6-dimethoxyphenol, g) carpaine, and h) anthraquinone.

Talking about papaya, it would not be away from papain (E.C.3.4.22.2), a proteolytic enzyme, which abundantly (80%) presents in its latex instead of other enzymes like chymopapain, caricain, acid phosphatase, amylase, chitinase, endo-1,3-β-glucanase, glutamine cyclotransferase, lysozyme, peroxidase, and lipase are also existing [70]. Papain is a simple and a cysteine protease enzyme composed by 212 amino acid residue chains with 21,000–23,000 g/mol or 23,406 Dalton in molecular weight. This protease activity is optimum at pH 6.0 to 7.0. Papain consists of a single polypeptide chain with tree disulfide bridges and sulfhydryl group for activity of the enzyme. By using casein as the substrate, papain shows a low Michaelis Manten Constance (K_m = 248.68 ppm) along with a high V_{max} (1.514 ppm casein/min) in its Michaelis Manten equation, defining its highly active and fast biocatalyst [74]. The catalytic site is surrounded by amino acid residues such as GLN19, CYS25, HIS158 and HIS159 [75].

The latest 3D structures of papain deposited in protein data bank was coded as 6H8T [76]. The resolved structure shows a homodimer of 2.1 Å resolution structure of the complex obtained by aerated overnight conjugation of [(*Z*6-benzene)Ru(1-{5-[*bis*(pyridin-2-yl)]pentyl}pyrrole-2,5-dione)Cl]Cl with papain (Figure 2). The ligand complexes to the active site interacting with cysteine residue (CYS25) by Michael addition of the thiolate to the double bond of the maleimide ring. This performs hydroxylation to the tyrosine residue, while interacting with GLY66 via H-bond interaction. This result is believed that by modifying tyrosine residues, it would be a good model to understand the mechanism and constraints of reactive oxygen species (ROS)-induced damage to proteins in general.

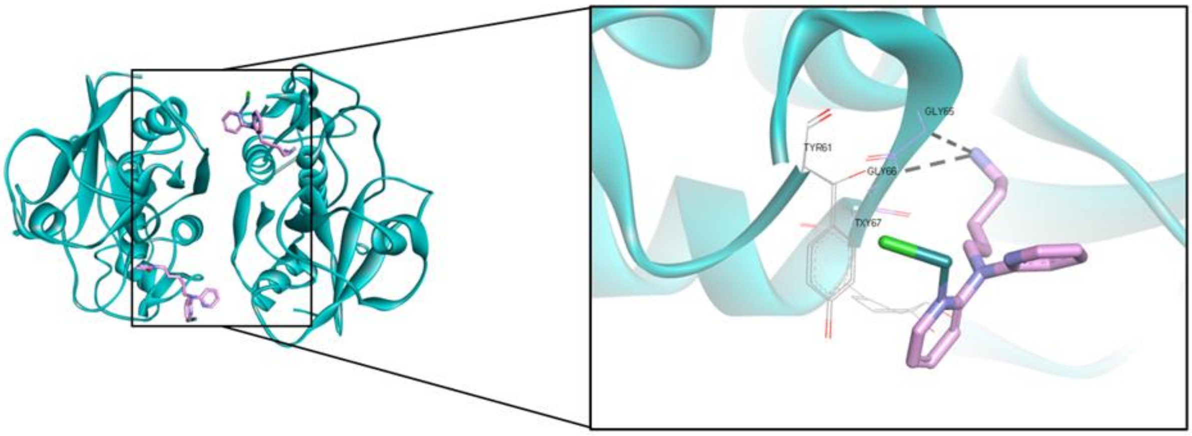

Figure 2. A homodimer of 2.1 Å resolution structure of the complex obtained by aerated overnight conjugation of [(*Z*6-benzene)Ru(1-{5-[*bis*(pyridin-2-yl)]pentyl}pyrrole-2,5-dione)Cl]Cl with papain. The protein is presented in a cyan ribbon and the ligand is in a pink stick model for C, and blue for N.

5. Indications

5.1. Dengue

In Asia, the extract of papaya leaf is well known used in the treatment of fever due to a virus infection such as dengue, malaria, and chikunguya. In dengue, papaya leaf extract had been studied to reduce thrombocytopenia, a condition in which platelet count is less than 150000 per µl of blood. This could be more prevalent because of a decreased platelet production and/or its increased destruction. The active ingredients of papaya up regulate the arachidonate 12-lipooxygenase (ALOX 12) and the platelet-activating factor receptor (PTAFR) gene leading to an increased production of megakaryocytes and its conversion into platelets [77]. Total four trials enrolling 439 subjects were included in the analysis. Of 439 subjects, data of 377 subjects were available for analysis. Clinically, papaya leaf extract was found to increase in the platelet count in 377 subjects after 4th day. However, after 48 h, there was no significant difference between papaya and control group. Interestingly, there was a significant decrease in hospitalization days in the papaya group [78]. This was hypothesized, this blood lysis prevention could be due to the flavonoids and other phenolic compounds effects presenting in the papaya leaf [79]. The alkaloid carpaine showed anti-thrombocytopenic activity in busulfan induced thrombocytopenic Wistar rats. In addition, the papain enzyme has been reported to reverse immune-mediated platelet destruction [80-82].

However, a systematic review and meta-analysis was performed to study the effect of papaya leaf extract to dengue patient in four different countries including Indonesia, Malaysia, Pakistan, and India. Study found that clinically, the platelet count improvement or early discharge was unclear in the absence of more robust indicators of favorable clinical outcome. This made the claim, that the capability of papaya leaf extract in reducing the thrombocytopenia in dengue patient is insufficient. Therefore, it is indispensable for further well-designed clinical trials examining the effect of papaya on platelet counts, plasma leakage, other serious manifestations of dengue, and mortality, with clearly defined outcome measures [83]. Instead of the papaya leaf extract was hypothesized its effect to dengue due to the thrombocytopenia reduction, other hypothesis also had been made. A flavonoid quercetin in papaya leaf extract was able to combat the dengue virus replication through the NS2B/NS3 protease inhibition. This protease is important for cleaving polypeptide-constructing structural protein for a new virion package. Therefore, a compound that either competitively of non-competitively inhibit this protein, could be marked for the dengue antivirus candidate [84-86].

Figure 3 shows the life cycle of dengue virus. The viral replication cycle is initiated by the infection of flavivirus to the host cells such as the monocytes, macrophage and dendritic cells. E protein mediates the penetration of virus via endocytosis process. In the endosomal compartment, the virus is acidified, triggering its fusion to the host cell membrane followed by releasing the nucleocapsid and viral RNA into the cytoplasm. The negative strand RNA is formed and serving as a template for further replication generating positive sense-RNA molecules, that provides RNA packaging as well as the virus assembly. These coordinates produce the new virions allowing the virus maturation in the Golgi apparatus and secrete them through the host secretory pathway. One of the virus serine proteases, NS2B-NS3 supported by host-encoded protease (signalase and furin) processes the translation of the releasing material to generate a polyprotein either co-translationally or post-translationally [87, 88]. NS2B-NS3 can be used as an optimal target in the dengue drug discovery since it is required for post-translation of polyprotein as well as the maturation of the virus. Thus, the inhibition of this enzyme is a promising strategy to combat several cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), pertinently [89].

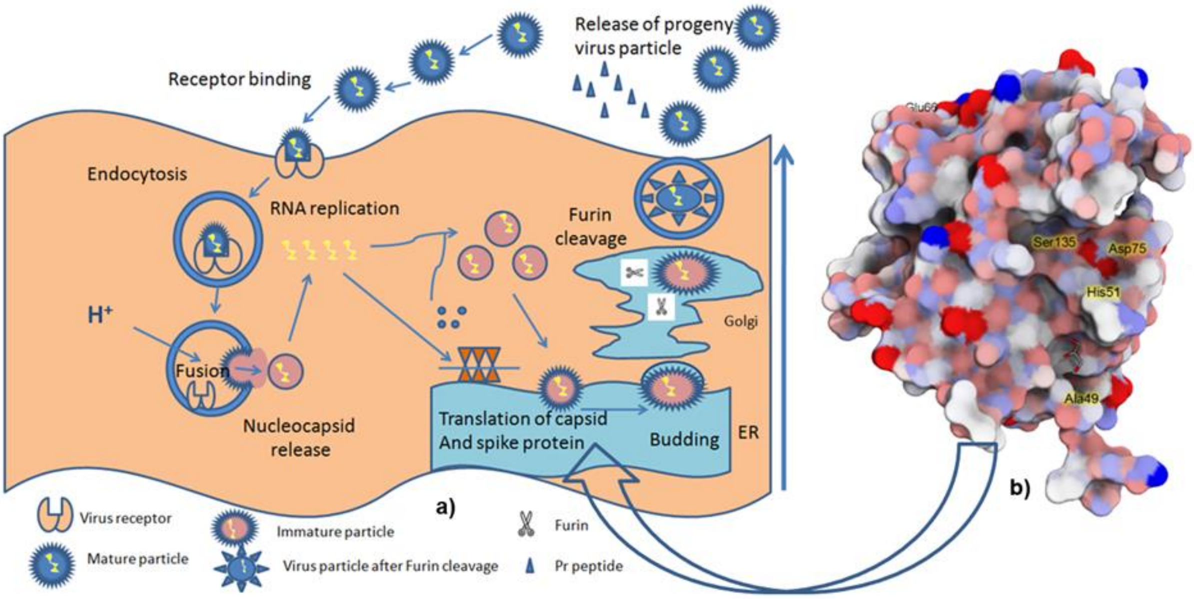

Figure 3. The illustration of a) the life cycle of dengue virus (modified from [90]), and b) the DENV2 NS2B/ NS3 protease retrieved from PDB 2FOM processing polyprotein cleavages either co-translationally or post-translationally [91].

5.2. Malaria

As a tropical disease, although it is deadliest, malaria is often neglected by pharma industries due to the endemic status of this disease [92]. Therefore, there are not so many options of drug to treat this disease, while the resistances are developing and spreading out [93]. The drugs to treat malaria are so far categorized in three groups i.e., aryl aminoalcohol compounds (quinine, quinidine, chloroquine, amodiaquine, mefloquine, halofantrine, lumefantrine, piperaquine, tafenoquine), antifolate compounds (pyrimethamine, proguanil, chlorproguanil, trimethoprim), and artemisinin compounds (artemisinin, dihydroartemisinin, artemether, artesunate) [94].

Alternatively, indigenous people consume papaya leaf aqueous extract to reduce fever of malaria. This empirical medication leads to the pre-clinical trial, in which papaya leaf extract combined with *Vernonia amygdalina* demonstrated synergistic effects in ameliorating plasmodium infection in mice. The results showed that the parasite percentage load between the infected treatment groups and disease control group at day 3 after infection were significantly different ($P<0.05$). This result maintained its difference until the final experiment, in which all treatment groups showed significantly an increase of red blood cell (RBC) and packed cell volume (PVC), compared to the disease control. In contrast, the count of white blood cell (WBC) was reduced indicating the lowering of the infection status. Moreover, the treatment groups showed a significant elevation of their body weight compared to the disease control. Meanwhile, the hepatic cells histological profile indicates the reduction in its cell damage leading to a highlight, that papaya leaf extract is important in the malaria infection remedy [95].

An *in vitro* study of papaya leaf extract against *Plasmodium falciparum* (D10 strain) was performed by preparing the extracts in five different solvents. The activities of the five extracts were expressed in IC_{50} values as follows: 16.4 μg/ mL (petroleum ether), 12.8 μg/ mL (dichloromethane), 2.6 μg/ mL (ethyl acetate), 10.8 μg/ mL (methanol), and > 50.0 μg/ mL (water) [96]. This indicates that the most active compound in papaya leaf extract is deposited in either ethyl acetate or methanol. According to the Pfizer guidance in the using of organic solvent, ethyl acetate and methanol is categorized as the preferred solvents, however, the use of methanol could cause serious effects like acidosis and retinal damage [97]. Further *in vitro* study of papaya leaf methanolic extract against *Plasmodium falciparum* (K1 strain), exhibited 51% inhibition at 4.8 μg/ml of concentration. The isolation work revealed some piperidine alkaloids employing (-)-carpamic acid, (+)-methyl carpamate, (+)-carpaine, along with a (+)-stereoisomer of carpaine and a (+)-derivative of carpaine, which were predicted to be the chemicals responsible for the anti-plasmodium activity. The most potent compound was performed by (+)-carpaine with an IC_{50} of 0.21μM and selectivity index of 98, indicating the potency of this alkaloid to be antiparasite of *P. falcifarum* with a non-toxic dose. However, in the *in vivo* murine model, carpaine (daily dose of 10 mg/kg BW intraperitoneally) did not reduce parasitemia until day 10 after infection [98].

5.3. Chikungunya

The third mosquito-borne disease, which is traditionally treated with papaya leaf extract is chikungunya virus (CHIKV) infection. This arbovirus is often suddenly infecting without any specific diagnosis leading to a severe clinical manifestation [99]. A study was performed to explore the methanolic extract of papaya leaf potency as the CHIKV antiviral agent. This extract showed antiproliferation of infected cell (BHK21) with CC_{50} 15.625 μg/ mL, whereas the aqueous extract showed CC_{50} 62.5 μg/ mL. Surprisingly, the antiproliferative activity of papaya leaf extract was better than ribavirin as the positive control with CC_{50} 125 μg/ mL. From the methanol extract, it was isolated two compounds employing rutin and carpaine. The CC_{50} of these compounds against the virus were 125 μg/ mL and 15.625 μg/ mL, respectively, remarking the potential effect as CHIKV antiviral agent from this plant [100].

There are not too many publications on the effect of papaya leaf extract on CHIKV. However, one *in silico* study was conducted by docking four phytochemicals from papaya leaf i.e., *p*-coumaric acid, caricaxanthin, violaxanthin and zeaxanthin. The docking was applied to chikungunya virus glycoprotein (E3-E2-E1) and chikungunya virus non-structural protein2 (nsp2) protease. The result shows that violaxanthin has the best docking score against the glycoprotein E3-E2-E1 by interacting with ASNB263, but in the docking of nsp2, zeaxanthin showed the best docking score among others. This work gives insight on how papaya leaf extract was active against CHIKV. The chemical structure of violaxanthin and zeaxanthin are presented in Figure 4 [101], which have not included in the classification of compounds in papaya leaves in Table 4.

Further study on papaya leaf extract in the form of silver nanoparticle (AgNPs) was evaluated for its *in vitro* activity against chikungunya virus (CHIKV), demonstrating 125 μg/ml for maximum non-toxic dose (MNTD) and 62.5 μg/ml for its 1/2MNTD. Compared to the virus control, these toxic doses carried about 39% and 52% of CHIKV inhibition. The treatment using AgNPs showed 14% in the infected cell viability leading to a conclusion, that the AgNPs synthesized from papaya leaf showed antiviral activity against CHIKV, when tested on Vero cells [102].

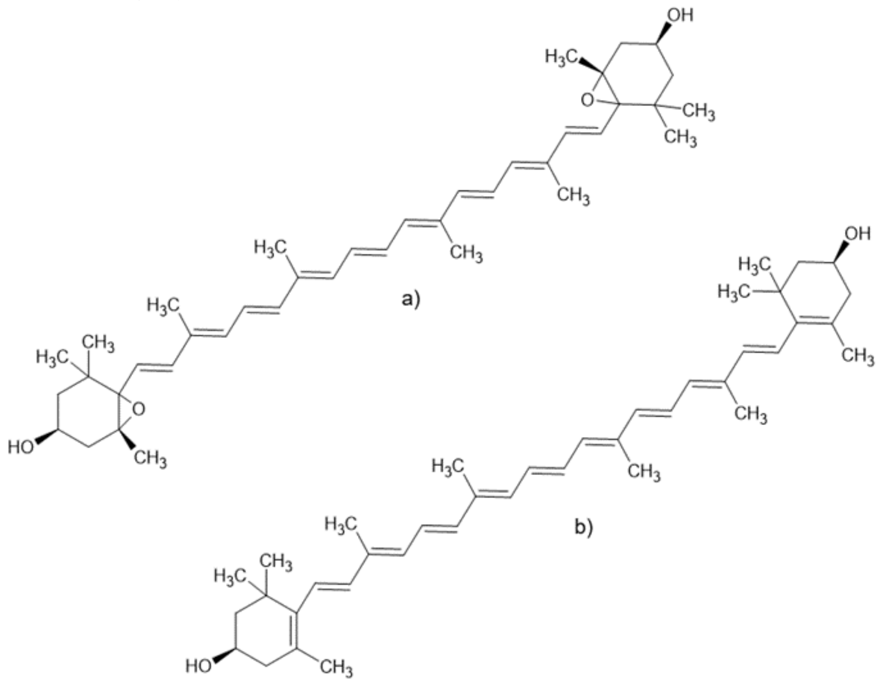

Figure 4. The chemical structures of a) violaxanthin and b) zeaxanthin.

5. Good Manufacturing Practice

As of a common pharmaceutical product, herbals must be ensured its good quality, not only for the consumers, but also for regulators and manufacturers. The regulation and guidelines in each country could be different due to the political, economic, and cultural policy of each country; therefore, a harmonization should be met to standardize the good quality of the herbal products.

A published article in 2015 has described the major GMP regulations for herbal products implemented in five different regions i.e., WHO-GMP, GMP in China, current GMP (cGMP) in the United States (US), Pharmaceutical Inspection Co-operation Scheme (PIC/S) in Singapore and GMP in the European Union (EU), to compare the terms of principles, contents, supervision, and industrial influence. It was found that among regions, they have major differences in the product scope along with the implementation mode. China develops herbal products based on WHO-GMP, whereas EU-GMP reflects the PIC/S, and the cGMP of dietary supplements in the US combines the multiple GMP from all regions. For examples, without any claims of medicinal activity, herbal products in USA are categorized in dietary supplement, while in EU-GMP, WHO-GMP or PIC/S, this is regarded to herbal medicinal product. The components of GMP including personnel, premises and equipment, documentation, production, quality control system, control manufacture and analysis, complaints and product recall, and self-inspection, are almost the same despite occasional differences in expression. In the implementation and supervision of the herbal medicine regulations in different regions and countries, all regions have the same mode of execution, but the agency, supervising organization and the sample inspection method are different. Overall, this study provides consumers, manufacturers, and regulators of the herbal products need to decide or make a strategy in the production of herbal medicine products according to GMP [103].

In Indonesia, the national agency of drug and food control has recently socialized a new regulation about GMP for herbal medicinal products in June 18th, 2021. This new regulation was constructed to adapt the advance of science and technology, and to facilitate the ease and supervision for manufacture, which is gradually categorized in a micro scale herbal medicine product manufacture and a macro scale herbal medicine product manufacture as the pre-requirement for releasing license numbers. A few new points highlighted are following: the amendment of the certification procedure from manual to electronic (e-certificate), the deletion of location agreement requirement by national agency of drug and food control, the trim of service timeline, the prolongation of the GMP for herbal medicine products, and the improvement of GMP for herbal medicine products facility as well as the reduction of the fee for non-government tax down to zero in the IDR currency. The certification of all checked aspects in GMP for herbal medicine products was made gradually starting from a micro scale to a macro scale herbal medicine product manufacture [104].

Sido Muncul as one of the national companies in herbal and pharmaceutical products, which papaya leaf extract is one their marketed products, has implemented the GMP standard for herbal medicine products and certified since 2000. Furthermore, it has been certified by ISO 9001:2015 Quality Management Systems, ISO 14001:2015 Environmental Management Systems, ISO 22000:2009 Food Safety Management Systems, Hazard Analysis Critical Control Point (HACPAPAYA), and Halal Assurance System (SJH). The products have been certified in this following dosage form: oral liquid, capsule, soft capsule, pill, poultice, tablet, effervescent powder, semi solid, powder for external medication and oral powder (https://www.sidomuncul.co.id/en/certification.html).

6. Herbal medicine product registration

In conjunction with GMP, an herbal medicine product should be registered to the national agency of drug and food control by considering these few factors, thereby, it could cross over the globe as follows: 1) the herb must be selected according to the monograph of its own country or could refer to the WHO-monograph. The herbs which have a restriction by the country or WHO should be avoided, 2) the part of the plant should be justified, 3) the solvent, extraction technique, the in-process control, optimization and validation must be developed, 4) the herb safety and traditional usage along with its proposed indication must refer to the solid and reputable literatures, 5) a well-planned GMP must be set up requiring EU GMP/GLP approved manufacturing / R&D laboratories for EU registration or USFDA compliant facility for US registrations, 6) chemical identifications including total ash, ash insoluble in hydrochloric acid, heavy metals, loss on drying, extractable matter, residual solvent etc., must be clearly stated using specified methods such as thin layer chromatography (TLC)/ gas chromatography or other advance instruments, 7) the marker compound must be justified, 8) the impurity profiles including insecticide pesticides, trace metal content, microbial contamination and aflatoxins must be clearly stated, and 8) container closure system with stability studies and storage conditions must be well defined [105].

In Indonesia, an herbal product can be registered according to three categories i.e., "jamu', standardized herbal medicine, and phytomedicine. Jamu is a traditional medicine derives from plants, animals, minerals and or its mixture, which have not been standardized and used in medication basing on experience. The forms could be in steeping powder, steeping slice, etc. On the other hand, standardized herbal medicine is a natural product having a standardized raw material and pre-clinical study result, which proves their efficacy and safety based on *in vivo* pharmacological and toxicological experiments. Meanwhile, phytomedicine is defined as same as standardized herbal medicine, however it must pass the clinical study [106]. Although it is prepared in capsule, but papaya leaf extract produced by Sido Muncul is registered as "jamu" (https://www.sidomuncul.co.id/id/product/sari_daun_pepaya.html).

7. Safety

A full safety study had been conducted to evaluate papaya leaf extract in either pre-clinical or clinical study [107]. Male Wistar rats were given up to 1500 mg/kg of a methanolic papaya leaf extract via gavage, resulting no observed mortality [108]. An aqueous papaya leaf extract with a dose 2000 mg/kg bw, showed LD_{50} greater than those given dose [109], meanwhile, there were no mortalities observed, when a methanolic papaya leaf extract administered to Wistar mice in doses of up to 3200 mg/kg [110]. Further study was carried out by giving Wistar rats with a methanolic papaya leaf extract (400 mg/kg bw/d) via gavage for 28 days, exhibited a reduced activity in aspartate aminotransferase, enhance in blood urea nitrogen levels, and moderate hyperaemia in the kidney and heart muscles [108]. Other study showed, that no extract-related effects were indicated, when green papaya leaf extract (up to 2000 mg/kg/day) was administered to Sprague-Dawley rats for 28 days via gavage [111]). Not so far behind, no adverse effects were shown when Wistar mice were administered by a methanolic papaya leaf extract (up to 3200 mg/kg/day) for 60 days [110]. A safety of aqueous papaya leaf extract was evaluated in pregnant Wistar rats via gavage on days 12-18 of gestation with a dose of 60 or 120 mg/kg [112] causing deformities in morphometry of foetuses, while 100% resorption was noted in rats treated with 120 mg/kg of the extract. Another effect on reproductive system of papaya leaf extract was conducted on male Wistar rats [113] given by 500 mg/kg bw extract orally for 21 days. This exposure results in significant reductions in mean values of sperm count, motility, viability, and serum testosterone concentration, compared to control rats.

A papaya leaf extract in 96% ethanol followed by a partition in hexane, ethyl acetate, and water fractions, were evaluated for their cytotoxicity against T47D, a breast cancer cell line, using MTT assay. The cytotoxicity assay shows that the extract does not interrupt the growth of T47D cells. However, the hexane, ethyl acetate, and water fractions showed a reduced viability of T47D cells with IC_{50} of 2,231.30, 557.33, and 2,112.81 µg/mL, respectively. These results described, that the ethanolic extract of papaya leaves and all partitions have no potential cytotoxicity on T47D cells due to their high IC_{50} values [53].

Recently, a juice and standardized aqueous extract of papaya leaf was reported to be well tolerated by adult humans for short durations (<five days), while one randomised controlled trial reported safe consumption of its use in children (aged 1–12 years). The most commonly side effects were minor uncomfortable gastrointestinal feelings. The hepatotoxicity and reproductive toxicity were concerned in a long-term use, supported by *in vivo* animal studies. There were indicated some unfavourable herb-drug interactions with metformin, glimepiride, digoxin, ciprofloxacin, and artemisinin. In conclusion, papaya leaf consumption by adults is most likely safe for short-term use, but should be carefully managed, when it is given to pregnancy and people with liver impairment. Furthermore, a potential herb-drug interactions could occur with oral hypoglycaemic agents, P-glycoprotein substrates, and antibiotics with cation chelating properties [114]. As introduced, a papaya leaf extract produced by Sido Muncul had shown non-sub chronic toxicity, when it was evaluated using animal study even for a long period of the consumption.

8. Perspective

In a formal medication, traditional medicine was still not recognized as the main pharmacotherapy due to the lack evidence-based data. They were used as an alternative medicine by indigenous people, due to their difficulty to access the formal health care facility or because of the culture. Therefore, the production of traditional medicine was only simply done by micro scale home industry. This industry also seldom detailly estimates, how much fee or budget they have invested for the production as well as the marketing, that leads to the unpredictable income and non-established business.

Papaya is only one of examples in agroindustry, which has been developed to pharma industry. The plant is easily cultivated in both tropical and semitropical climates, in which the whole part of the plant gives benefits to many aspects as mentioned in the previous section. Concerning to the opportunity of papaya as an herbal medicinal product, the leaves' part is most likely discussed either in its folk preparation or already in pharmaceutical dosage form. In a folk medicine, the bitter taste and its flavour are believed containing ingredients that can cure many illnesses. In the pharmaceutical dosage form, scientists have confirmed, what kind of those active ingredients as well as its scientific evidence through pharmacological and toxicological experiments.

As of the science and technology has rapidly developed, traditional medicine began to attract researchers especially to whom it may concern in natural products to provide evidence-based data, that they could not only be traditionally used, but also in advance formal medication. Papaya leaf extract has been identified its nutrition and phytochemical substances, which could be responsible for its pharmacological activity under *in silico*, *in vitro*, pre-clinical *in vivo* and even in human clinical studies. The studies are not only carried out in organism level, but also elucidating the cellular, molecular and atomic mechanisms, on how papaya leaf extract is able to interrupt the pathophysiology of the diseases.

Traditionally, papaya leaf extract is broadly used to treat the infectious diseases caused by virus such as dengue and chikungunya, and also parasite such as malaria. Scientifically, it contains flavonoids, which may exert inhibition to the enzyme NS2B/ NS3 protease of the dengue virus that plays a pivotal role in its life cycle. Furthermore, the contents of alkaloid carpaine had been reported to potently disrupt the growth of cell infected by *P. falcifarum*, which answers the question, why papaya leaf extract was used traditionally in malaria fever. In addition, the active phytoconstituents namely violaxanthin and zeaxanthin may exert the activity of chikungunya virus through the E3-E2-E1 glycoprotein and nsp2 protease enzyme inhibition, respectively.

The world was shocked by the outbreak of SARS-Coronavirus-2 (SARS-CoV-2), which has been lasting for almost two years. Although vaccines have been non-stopped developed and distributed to urgently end up the pandemic, however, there is no specific drug to combat the virus [115, 116], as happened in dengue as well as chikungunya. In a severe SARS-CoV-2 infection, the lungs were full of inflammation mediators such as tumor necrosing factor-α (TNF-α), macrophage, interleukins (ILs), interferons and other factors, which is well known as cytokine storm. This cytokine storm could lead to the cell death, followed by tissue damage and haemorrhages, triggering multiple organ failure. Therefore, by blocking the overproduction of those kind inflammation mediators, the severe of infections could be well controlled. Papaya leaf extract was studied to inhibit TNF-α rather than IL-6 in the inflammation pathway cascade [117]. Further *in vivo* study reported, that papaya leaf extract was able to alleviate the cytokine storm in dengue infection mice model [118]. These two evidences may encourage the further experiments of papaya leaf extract as the possible weapon against Covid-19 pandemic. Other nutrients, protease enzyme and flavonoid contents were also approached through its activity as antioxidant [119], T helper type upregulation and thrombolytic agent [120], as these biochemical reactions occur during Covid-19 pathogenesis had been recently reviewed [121].

The safety of papaya leaf extract was accurately determined, in which either *in vivo* LD_{50} and *in vitro* LC_{50}, much greater than the effective dose/ concentration. However, in a certain dose, the use of papaya leaf extract must be carefully handled in pregnancy because the morphometry of foetus in female rats showed a comparable defect than its negative control. In a reproductive system, to those who still needs a fertility, the use of papaya leaf extract should also be carefully managed since this extract was able to reduce the quality and the quantity of male Wistar sperm. However, to date, there is no study applied in human toxicity except the report, that some human subject feels inconvenient feeling in their stomach (gastrointestinal disturbance). Furthermore, a drug-herb interaction also should be anticipated when a papaya leaf extract was consumed together with the certain drugs to avoid its toxicity or less efficacy.

In term of product form in market, papaya leaf extract is available in many dosages form that are acceptable or ready to use. This makes the consumption of papaya leaf extract practically convenient than drinking the bitter juice extract, which attracts consumers to consume it, not only for medication, but also to prevent and promote their daily health. Furthermore, the cosmeceutical product from papaya leaf extract also enriches the variety of cosmetics with a diverse intention to skin care, which should be a great indicator to bring papaya leaf extract into more formal medicine product from herbal. Properly, a more guided herbal GMP and ISO by the national agency in drug and food control along with the ease of product registration, should make this product having a better future to increase health of consumer as well as the economy of the manufacturer.

9. Concluding remarks

A review of *Carica papaya* leaf extract has been highlighting its high opportunity to be as a potential formal herbal medicine product in the disease prevention, medication as well as health promotion with a high economic value. A good manufacturing process must be kept to maintain its quality and sustainability as a nutraceutical and cosmeceutical product. The ease to get governmental facility in the product registration will boost the spirit and effort to develop this product as one of advance pharmaceutical commodities in the near future ahead.

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