IOP Conf. Series: Materials Science and Engineering **358** (2018) 012036 doi:10.1088/1757-899X/358/1/012036

Potential anti-Dengue Concoction of Carica Papaya (*C. Papaya*) Leaf and G. Mangostana (*G. Mangostana*) Pericarp and Their Bioactivity Enhancement by Fermentation: A Review

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Abstract. This review highlights the anti-dengue potency of *Carica Papaya* leaf (*CPL*) extract which was associated with platelet increase and other medicinal properties such as antiinflammatory and antioxidant. *Garcinia Mangostana'*s pericarp (GMP) extract have much commonalities with *CPL*, in addition to antiviral and immunomodulatory properties of the former. These properties may exhibit, yet unproven, analgesic, hemorrhage prevention and antiviral effects that may facilitate dengue recovery. Nevertheless, the limited bioavailability of native polyphenolic contents of both, as hinted by studies on colonic microbiome metabolism on dietary polyphenols, highlighted fermentation as viable method to enhance the functionality of the compounds. Thus, this review also highlights some relevant parameters in existing fermentation of well known fermented foods that impact their bioactivity, functionality and palatability that may applicable for the development of *CPL* and *GMP* fermentations.

1. Introduction

The dengue disease is affecting an estimated of 50-100 million people worldwide each year [1] mostly in tropical and sub-tropical regions such as Africa, America, Eastern Meditteranean, Southeast Asia and Western Pacific [2]. It is caused by dengue virus (DENV) which is transmitted by *Aedes aegypti* mosquito as its principal vector. Dengue epidemiology Malaysia is a serious problem with more than 50,000 cases resulting 122 deaths as of June 2016 alone [3]. Currently, there is no effective tetravalent antiviral drug that could inhibit antigenically distinct and constantly mutating four DENV serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) [4]. The current dengue management relies on supportive care during hospitalization such as administration of analgesics, rehydration by oral or intravenous fluid intake and constant platelet and hematocrit monitoring [5] as well as preventive measures to control vector through elimination of mosquito's breeding habitat, fumigative spray and public awareness campaign [6].

Wide ranging indigenous plant species used in folk medicines had been recognized by their respective local population for anti dengue potency. Scientific studies revealed their roles in inhibiting viral mechanism, increasing platelet count and alleviating dengue symptoms such as inflammation, fever and bleeding. Sometimes, two plants are used in combination to alleviate dengue symptoms and contain viral progression [2].

2. Anti dengue potency of *CPL* and *GMP*

2.1. CPL

Numerous animal and clinical studies culminate the efficacy of *CPL* in alleviating the dengue symptoms and facilitating the dengue patient's recovery. It was suggested that some medicinal plants, including *CPL* possess bioactive compounds such as *flavonoids*, *quercetin* and natural *chalcone* [1, 2] that inhibit DENV proteases responsible for virus-host attachment as demonstrated by strong affinity between *CPL*'s flavonoid quercetin and NS2B-NS3 proteases of DENV-2 serotype [7]. Clinical studies on serological parameters reported the increase in platelet count, white blood cell and neutrophil as a result of *CPL* juice intake [1, 4]. A thorough study on platelet producing genes, *ALOX12* and *PTAFR* genes confirmed the role of *CPL* in enhancing the expression of these genes among interventional group of 111 dengue patients as compared to control group of 117 dengue patients [5]. Further findings on the increase of platelet among clinical subjects whom administered with *CPL* extract as compared to control provided indisputable evidences of anti-dengue potency of *CPL* with respect to platelet increase to overcome thrombocytopenia [8, 9].

Other well-known medicinal properties of *CPL* such as antiinflammatory [10], antioxidant and immunomodulatory [11] that are attributed to its phytochemical compounds such as alkaloids, saponins, flavanoids, tannins, phenolics and steroids [12] may impart additional anti-dengue activity. Analogous to *Hippophae rhamnoides*, yet requires further proof [2], *CPL*'s antiinflammatory property may reduce TNF-α which responsible for inflammation of T-cell which is the suspected mechanism of vascular permeability and hemorrhagic manifestation [1], whereas its immunomodulatory activity could induce IFN-γ to mount antiviral mechanism comparative to commercial *Rivabirin* antiviral drug [10]. Studies found that normal intake of CPL extract poses no health risk as no abnormality observed on hematological and histopathological results of animal subjects which were administered with *CPL* fourteen times higher than normal dose intake [13, 14] despite the recent debate on the toxicity of *CPL* pertaining to its cyanoglycoside content.

2.2. GMP

The bioactivity specific compounds of *GMP*, most prominently its xanthones such as α-mangostin, β-mangostin and γ-mangostin are better elucidated compared to its *CPL* counterpart. The wide range of *GMP*'s medicinal values includes antiinflammatory [15], antioxidant [16], antiviral [17] and anticancer [18]. Its anti-inflammatory property may exhibit its anti-dengue activity in terms of preventing hemorrhage analogous to its *CPL* counterpart suggested earlier. Antiinflammatory activity may also create analgesic effect to alleviate muscular and joint pain since both activities involve inhibition of cyclooxygenases (COX) and its prostaglandins mediator as well nitric oxide (NO) and superoxide ($\bullet OH$ and $\bullet O^{2-}$) release which responsible for inflammatory and pain mechanisms in many cells including T cell [15, 19]. Additionally, antioxidant activity of *GMP* could act as superoxide scavenger to suppress the inflammation from progressing further [20]. The anti-dengue activity of GMP which may plausibly extended from its γ-mangostin antiviral activity against HIV-1 virus and its well known immunomodulatory activity [18]. The existing commercial antiviral drug such as *ribavirin* and *PegIFN* employ the same protease inhibition mechanism against HIV-1 and HCV (Hepatitis C virus). Since HCV shares strong functionality with its DENV counterpart, the potential of γ-mangostin to inhibit DENV protease similar to HIV-1 protease is intuitive [17, 21].

3. Bioavailability of *CPL* and *GMP*

Unlike its *GMP* counterpart, the bioavailability of *CPL* extract is never debated presumably due to its existing satisfactory effect. However, since polyphenols such as flavonoids and sapponin in *CPL* [11] and xanthones, gartanin, garcinone E in *GMP* [22] are major bioactive compounds of both, studies on dietary polyphenols in other plants and foods implied low bioavailability of these polyphenols compounds. Low bioavailability of around 5-10% from original intake is highlighted by the dependence of enterohepatic circulation on colonic microbiome for polyphenol breakdown [23] thus resulted lower therapeutic effects [24]. Regarding the *GMP*, low presence of total xanthones (around 2%) in serum and urine samples of volunteers who consumed xanthone-rich juice was reported while better bioavailability was achieved when xanthone was consumed with high fat western diet or

ingested in the form of oil suspension gavage. Conjugated form of xanthones, as opposed to free xathone found in most serum and urine samples deliniated time consuming process of phase II metabolism by the liver to facilitate the xanthone absorption into systemic circulation [25, 26].

4. Fermentation of *CPL* and *GMP*

4.1. Enhancement of bioavailability

A few studies highlighted the use of fermentation technique to enhance bioactivity of mangosteen xanthones. Incubation of α-mangostin with fungi *Colletotrichum gloeosporioides* (EYL131) and *Neosartorya spathulata* (EYR042) resulted several phase II metabolites such as *mangostin 3-sulfate, mangostanin 6-sulfate, 17,18-dihydroxymangostanin 6-sulfate* and *isomangostanin 3-sulfate* which probably became more readily-absorbed form at the enterocytes and hepatocytes for systemic distribution [27]. The multi-fold enhancement of α and β-mangostin bioavailability by 11 to 16 times as compared to standard dry mangosteen extract was reported from the fermentation of whole mangosteen fruit using *Saccharomyces boulardii* as a starter culture [28]. Although the fermentation of *CPL* is non-existence, studies on the metabolites of various classes of polyphenol compounds, of which most *CPL* phytochemicals belong to, as a result of colonic microbiota metabolism may shed light on possible bioavailability enhancement if fermentation of *CPL* is carried out [29]. In addition, lactic acid fermentation of *Myrus communis* berries using *L. plantarum* and yeast extract was reportedly enhanced the antioxidant activity of the fruit with respect to DPPH scavenging activity, inhibition of linoleic acid peroxidation and increase of phenolic acid and flavonols [30].

4.2. Industrial fermentation of CPL and GMP

Three-stage fermentation of mangosteen pericarp was described in the production of a local nutraceutical product commencing with three weeks fermentation of rice by *Aspergillus oryzae (A. oryzae)* to produce rice brew, followed by six weeks fermentation of mangosteen pericarp soaked in rice brew and final fermentation stage that matured in nine months [31]. The use of *koji* fermentation is analogous to typical soy sauce production where it serves to provide amylase and protease enzymes for the breakdown of complex saccharide into fermentable sugars and protein respectively as precursor for subsequent lactic acid fermentation [32]. Perhaps for similar reason, yeast extract was used during *Myrus communis* berries fermentation with *L. plantarum* mentioned earlier.

Another related process employed a three-month fermentation of whole mangosteen fruits using *Saccharomyces boulardii (S. boulardi)* as starter culture at 35-55°C. The selection of *S. boulardi* as starter, which is originally the indigenous microorganism that colonises the mangosteen fruit may related to its role in hydrolysing lignin structure into simpler fermentable precursor for subsequent fermentation succession, most probably lactic acid fermentation, and also comparable to the role of *koji* fermentation. As stated earlier, the fermented mangosteen product which was co-administered with standard *GMP* extract exhibited 11 to 16 times higher bioavailability compared to standard dry mangosteen extract products [28]. We surmise the viability of CPL fermentation if appropriate selection of starter culture and saccharide source are made available.

4.3. Fermentation process parameters

Fermentation technique used for *GMP* is non-sterile process akin to other low-tech fermentation method used in many traditional foods such as *kimchi* (Korea) [33], *tempoyak* (Malaysia and Indonesia) [34], *tempe* (Malaysia and Indonesia), *tape* (Malaysia and Indonesia) *doklu* (Cote d'Ivoire) [35], *kishk* (Egypt) [36] and *saeurkraut* (international) [37]. Most of these products were originally produced by spontaneous fermentation using respective indigenous microorganisms. However, technical control parameters evolved through trial and error and iterated use of residue from successful fermentation batch as inoculum for subsequent fermentation batch known as back-slopping, yielded the best adapted strains to be used as starter culture for a better controllability of the fermentation.

The microfloral profile during fermentation, which only came to light in recent time using modern microbiological technique, culminate the prevalence of lactic acid bacteria (LAB) species, shown in table 1 as important parameters of a successful fermentation and believed to impact the palatability, nutritional value and shelf stability of many traditional fermented food products [37].

Table 1. Lactic acid bacteria (LAB) species in traditional fermented food.

Food product	**Main raw material**	**Fermentation process**	**[a]LAB species**	**Ref.**
Tempoyak	Durian (*Durio zabethinus*) pulp	Lactic fermentation	*Lb. plantarum, Lactobacillus sp, Weisella paramesenteroides, P. acidilactici, Lb. fructivorans, L. dextranicum, Lb. collinoides, Lb. paracasei, Lb. Plantarum, F. durionis*	[38], [39]
Tempe	Soybean	Mold fermentation	*E. faecium, L. mesenteroides ssp. Mesenteroides, Lb. delbrueckii ssp. delbrueckii.*	[40]
Tapai or tape	Cassava or glutinous rice	Alcoholic fermentation	*P. pentosaceus, Weisella sp.*	[34]
Soy sauce	Soybean	Mold fermentation (*koji*) followed by high salt (brine) concentration	*Weissella confuse, E. faecium*	[41]

[a]*Abbreviation: Lb (Lactobacillus), P (Pediococcus), L (Leuconostoc), E (Enterococcus), F (Fructobacillus).*

Raw material preparation and selection of ingredients are also critical to promote growth of desirable microorganism and protect the fermentation starter culture from spoilage microorganisms [34]. For example, production of *tempe* involved soaking, de-hulling and cooking of the raw material (soybean) followed by acidic fermentation that inhibits spoilage growth but tolerant to *Rhizopus spp.* mold. Later, *Rhizopus spp.* colonisation of the substrate outgrows other potential undesirable microorganisms and releasing inhibitive substances against spoilage microorganism. Salt addition during *kimchi* and *tempoyak* fermentation or brine fermentation during soy sauce fermentation promoted LAB growth over other spoilage microorganisms [37, 41]. Another process parameter that impact the organoleptic and functionality of fermented foods is physico-chemical succession that results from a predominant microflora that colonise the available food matrix at a particular stage of fermentation. For example, the heterofermentation of *L. fermentum* on glucose and fructose of cocoa bean's pulp during spontaneous fermentation of cocoa bean produced lactic acid and mannitol, the latter was utilised by acetic acid bacteria to produce acetic acid that is essential to modulate optimum pH for endogenous enzyme activity that gives a good sensory quality of chocolate [42].

Recent study on *tempoyak,* correlated the drop in sugar level (sucrose, fructose and glucose) and the rise of organic acids (lactic acid, acetic acid and propionic acid) with the increase in LAB population (mainly *F. durionis* and *Lb. Plantarum)* [39] which indicated substrate utilization and metabolite production respectively. In addition, yeast and bacteria are also known to produce volatile secondary metabolites such as higher alcohols, fatty acids, esters, aldehydes, ketones and thiols by utilizing the primary metabolite (organic acids and ethanol) which were pivotal for the flavor and odour of chocolate [43], soy sauce [41] and tape [36].In this section we discuss how to format the title, authors and affiliations. Please follow these instructions as carefully as possible so all articles within a conference have the same style to the title page. This paragraph follows a section title so it should not be indented.

5. Conclusion

The diverse classes of phytochemicals in *CPL* and *GMP* exhibit potential bioactivities against dengue infection and facilitate patient's recovery. Existing fermentation of GMP and other functional foods suggest that fermentation could surmount the limited bioavailability of polyphenols thus enhancing the functionality and palatability of native *CPL* and *GMP*. Thus, the development of the potential fermentation process of *CPL* and *GMP* should adapt certain artisanal elements of traditional fermented foods in terms of raw material preparation, starter culture and process condition as their process parameters to attain desired functionality and organoleptic attributes.

Acknowledgments
Authors wishing to thank University Teknologi MARA (UiTM) for research funding through 600-IRMI/DANA 5/3/REI (0002/2016) grant.

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