Friedrich Schiller University Jena
Faculty of Biological Sciences
Institute of Nutritional Sciences
Bioactive Plant Products Research Group

FRIEDRICH-SCHILLER-UNIVERSITÄT JENA

Investigations on carotenoids and polyphenols in selected citrus fruits from Aceh, Indonesia, and its *in-vitro* antioxidant, antibacterial, and antidiabetic potencies

Dissertation

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Reviewer

1. Apl. Prof. Dr. Volker Böhm

   Friedrich Schiller University Jena
   Institute of Nutritional Sciences
   Bioactive Plant Products Research Group
   Dornburger Str. 25
   07743 Jena

2. Prof. Dr. Frank Hellwig

   Friedrich Schiller University Jena
   Institute of Ecology and Evaluation
   Philosophenweg 16
   07743 Jena

3. Prof. Dr. Marcus A . Glomb

   Marthin-Luther University Halle-Wittenberg
   Institute for Chemistry – Food Chemistry
   Kurt-Mothes-Str. 2
   06120 Halle (Saale)

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Abbreviations

ABTS	(2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid))
ANOVA	Analysis of Variance
DNSA	3,5-dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazil
DW	Dry Weight
EtOH	Ethanol
FBBB	Fast Blue BB
FW	Fresh Weight
HAT	Hydrogen Atom Transfer
HFD/STZ	High-Fat-Diet/Streptozotocin
HPLC	High Pressure Liquid Chromatography
MeOH	Methanol
MLN	Microplate Laser Nephelometry
ORAC	Oxygen Radical Absorbance Capacity
PG	Prostaglandin
PGG2	Prostaglandin G2
SET	Single Electron Transfer
SNK	Student-Newman-Keuls’s
TEAC	Trolox Equivalent Antioxidant Capacity
THF	Tetrahydrofuran

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1. Introduction

Citrus fruit is one of the major crops in the world and despite some dispute believed originating from the South East Asia, China, India and Malay Archipelago (Liu, Heying, and Tanumihardjo 2012). Indonesia, geographically located in the South East Asia, is believed to have more than 100 species of citrus species (Karsinah et al. 2002).

1.1 Citrus samples general information

1.1.1 Citrus general characteristics

Citrus reproduction is characterized by the ease of inter-hybridization between species and the nucellar embryonic (formation of maternal clonal embryos) (Aleza et al. 2010), thus creating a big genera with more than 300 species. Mabberley (1997) proposed citrus to be originated from three citrus species, which are *Citrus medica* L. (citron), *Citrus maxima* Burm. (Merril) (pomelo), and *Citrus reticulata* Blanco (mandarin). Other citrus species are a result of hybridization between these three citrus species. Citrus genus nevertheless shares some common characteristics: The plants are large shrubs or small trees bearing alternate green leaves. Leaves are consisted of petioles that are variously winged or not and a single terminal leaflet. Flowers are typically aromatic, with fruits being yellow, orange or green in color. The fruits are hesperidium, a modified berry that is globose, with a though leathery external rind and a fleshy interior comprised of several seed-bearing fluid-filled sections called carpels (http://www.britannica.com/plant/Citrus). Citrus fruits used in this study are local citrus fruits from Aceh (Figure 1). The general characteristics and usage of the fruits are described as follows.

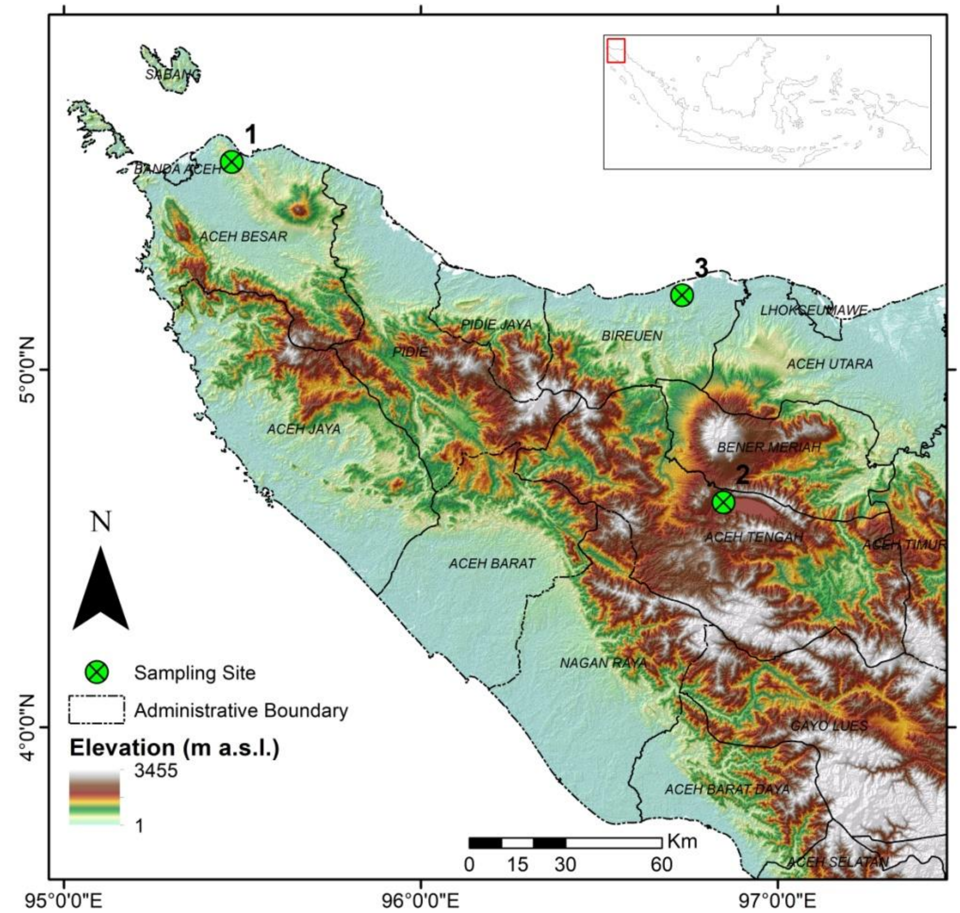

Figure 1 Map of Aceh and area of samples collection: 1 (mentui), 2 (jeruk takengon), 3 (kruet mameh, calung, makin, and jeruk nipis).

1.1.2 *Jeruk takengon* (*Citrus nobilis*)

Jeruk takengon (*C. nobilis*) is also called *jeruk keprok gayo*, literally means Gayo mandarin. Gayo is a highland located 1200 m above sea level in the *Bukit Barisan* mountains. The area is known as Arabica coffee plantation, which produce one of the best coffees in the world. *Jeruk takengon* is until now planted mainly as intercrop in the coffee plantations. According to a report from BPSTPH (*Balai Pengawasan dan Sertifikasi Benih Tanaman Pangan dan Hortikultura/Center for Supervision and Certification of Food Crops and Horticulture)*), *jeruk takengon* was brought to Aceh by a Dutch East India company, and was initially planted in the yard of a coffee plantation administrators house. Afterwards, it was planted in coffee plantations in Pegasing area, Central Aceh, Indonesia (Azharsyah et al., 2015).

Morphological appearances of *jeruk takengon* (Azharsyah et al., 2015) are as follows: the leaf is medium, pointed at the apex, rounded at the base, petiole only narrowly winged, flowers are bell-shaped, petals are white and yellow colored, stigma yellow colored, stamen yellow colored, flowers are 2-4 in racemes. Fruits are flattened globos, peels are 0.5 cm thick, with 11 carpels with 5-7 seeds per carpel. *Jeruk takengon* is mainly consumed as table fruits or as fresh juice. Peel use of jeruk takengon has never been previously reported.

1.1.3 *Jeruk nipis* (C. aurantifolia)

Jeruk nipis, also known as Mexican lime/west Indies lime, is originated and widely cultivated in the Indo-malayan region (Hodgson 1967). The juice from j*eruk nipis* fruits is mainly used in Indonesian households for culinary purposes, such as to reduce fish smells in seafood preparations. The juice is also used to prepare Indonesian style condiment called *sambal* (hot spicy sauce), tea (called lemon tea) or limejuice (juice of this fruit strongly diluted in water, with the addition of sugar or honey). For non-culinary purposes, the fruit is used as traditional medicine, for example, to treat a cough (by adding honey) and to treat various skin disorders.

Jeruk nipis morphologically appears as a small tree, twigs with short, rigid, very sharp spines; leaves small, petioles narrowly winged; inflorescences axillary, short, lax racemes of 2-7 flowers; flowers are small, white, petals 4-5, stamens 20-25, ovary depressed, globose, has 9-12 segments. Fruits are small, oval or sub-globulose, greenish yellow when ripe; the peel is very thin with prominently glandular dots; the seeds are small (Tjitrosoepomo 2001).

1.1.4 *Jeruk purut* (*C. hystrix*)

Jeruk purut is also known as kaffir lime, makrut lime, or Mauritian papeda. This species is believed to be native to tropical Asia, including India, Nepal, Bangladesh, Thailand, Philippines, Malaysia and Indonesia. In Indonesia, *jeruk purut* fruits are used primarily in traditional ceremonies in many Indonesian cultures, as it is required in almost every traditional celebration, from birth to death of a person. *Jeruk purut* is widely cultivated and easily found in the community or the local market. The peel is often used in traditional cakes preparation, while the pulp is not usually consumed. Young leaves are the most utilized part, mainly to prepare vegetables, sauces, and meat-based dishes. In traditional medicine, the fruits are traditionally used to treat skin itchiness, sore throat and cough, and dandruff.

Jeruk purut appears as low tree or shrub, 2-7.5 m high, branch with short, stiff spines with hard brown or orange colored tips. Leaves with winged petiole, which is very similar to the leaves itself. The flowers are terminal or axillary inflorescences, petals 4-5, oblong, yellowish white or tinged with red, stamens 24-30. Fruits pendulous or globose with a rounded base, and a rounded or slightly depressed apex, the peel is very irregularly bumpy, glabrous with many scattered glandular dots. Fruits are yellow or yellowish green when ripe, 5-7 cm diameter, peel thickness 0.2-0.5 cm. The pulp is yellowish green, very sour and slightly bitter, 10-12 segments (Tjitrosoepomo 2001).

1.1.5 *Calung* (*C. aurantium*)

Calung is a locally grown citrus found in Matangglumpangdua area, Bireun, Aceh. The presence of this citrus in other areas is relatively unknown. *Calung*'s juice can be used as a treatment for the post-partum woman by making into a thick paste with ash and kalanchoe. *Calung*, as well as other citrus fruits mentioned here, are used to prepare seafood, mainly fish.

Calung appears as low tree (3-5 m high), branch in dark green color, with no visible spines. *Calung*'s leaves are 9-10 cm in length and 5-6 cm in width, with crenatus edge. Petioles are winged, 2-2.5 cm width and 1-1.5 cm length. Flowers were not found during sampling periods. Fruits are round, with smooth ridges. The fruits have 9-11 segments, with light yellow color when mature.

1.1.6 Makin (*C. aurantium*)

Makin is another locally grown citrus found in Matangglumpang area, Bireuen, Aceh. The fruits have a very pleasant smell, and when available, a favorable use in seafood and *sambal* preparation. Information gathered from local community stated that the fruits are the most used part. Peel favorably is used as *lincah/rujak*, with coconut water and young coconut meat and husk. The peel is also used to clean bronze. Makin's leaves are 6.5-7.5 cm in length, and 3-3.5 cm in width, winged petioles with 2-3 cm in length and 0.5-1 cm in width. Branches are dark green with no visible thorn.

1.1.7 *Kruet mameh* (*C. aurantium*)

Kruet mameh (*jeruk purut manis*), literally translated as sweet kaffir lime, are found in Matangglumpangdua area, Bireuen, Aceh. The fruits have a similar appearance as kaffir lime, except that the peel is easily separated from the pulp and the fruits when ripe taste sweet. According to an interview with the local community, the fruits are sold as fresh fruits and directly eaten. Peel of *kruet mameh* is pounded and applied directly to open wounds to treat an infection.

Kruet mameh is a tree, 5-6 m high, highly branched. Branches are brownish green, with many 1.5-3 cm thorns. Kruet mameh's leaves are 9-10 cm length, 3-4 cm width; winged petioles 2-2.5 cm length and 0.5-1 cm width. Fruits are fairly small, 4 cm in diameter, 10-11 segments.

1.1.8 *Mentui* (*C. aurantium*)

Mentui was harvested in Blang Bintang area, Aceh Besar. As well as another local citrus, information on this fruit is based on the local communication. *Mentui* traditionally is used in food preparation. The large fruits were rich in acidic juice and preferable when preparing a large batch of sea food. Traditionally, the fruits are used to relieve stomach cramp.

Mentui appears as shrub, 2-3 m high. *Mentui* has the largest leaves of all samples, length approximately 14-17 cm, width 5-7 cm; winged petiole, width 1.5-2 cm, and length 4-5 cm. Leaf margins are crenates. Branches are green, and hard thorns are found along the branches, with the length of 1.5-3.5 cm. Fruits are 8-10 cm in diameter, 13-15 segments, light yellow.

1.2 Carotenoids from citrus fruits

Carotenoids are responsible for the color of different flowers and fruits, and food containing carotenoids taken by birds, shrimp or fish also contributes to its coloring (Fraser and Bramley 2004). Carotenoids are usually formed of 8 isoprenoid units joined together, so that the joining of units is reversed at the center of the molecule, to give methyl groups at C-20 and C-20' with a 1,6 positional relationship, whereas the remaining methyl groups are 1,5 (Figure 1.2). The polyene chain in carotenoids can extend from 3 to 15 c.d.b. (conjugated double bond), and the number of c.d.b. is affecting the absorption spectrum (thus, color upon seeing) (Chemistry and Nomenclature 1972).

On the other hand, a long c.d.b. chain is affecting the stability of carotenoids, which leads to isomerization or oxidation upon interaction with O_2, heat or light (Boon et al. 2010). There are approximately 750 carotenoids already identified, and many of them are isomers.

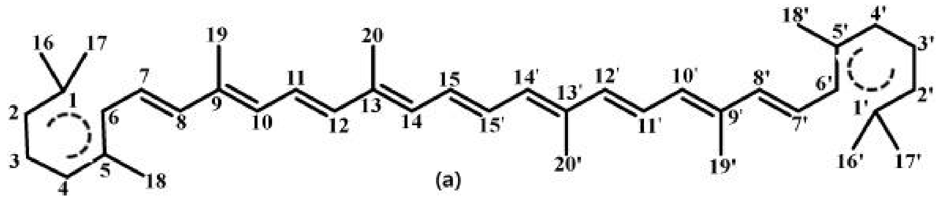

Figure 2 The general structure of carotenoids (Chemistry and Nomenclature 1972)

Carotenoids are C40 hydrocarbons, and depending on the active moiety in the end chain, can be divided into two big groups, namely carotenes and xanthophylls. Carotenoids are reported to be the main pigment in the colored citrus, e.g. in mandarin, orange, clementine, and pomelo, and β-cryptoxanthin and lycopene are found as the main carotenoids in them (Fanciullino et al. 2006). The composition of carotenoids in citrus fruits is influenced by different factors, namely geographical origin, fruit maturity, growing conditions and citrus variety (Mouly et al. 1994), (Lee and Castle 2001), (Dhuique-Mayer et al. 2005). Various methods have been established in order to analyze and quantify carotenoids in different citrus samples. Table 1 summarizes identified carotenoids, extraction solvent, and HPLC conditions for analysis of different citrus samples.

Table 1 Summary of carotenoids identified in citrus, solvent for extractions, and HPLC conditions for carotenoid separation.

No	Citrus samples	Main carotenoids	Source	Solvent extraction	HPLC conditions
1	Ponkan (*Citrus reticulate*)	(9Z)-violaxanthin, (all-E)-violaxanthin, lutein, β-cryptoxanthin, β-carotene, β-carotene isomer, luteoxanthin,phytoene, zeaxanthin, phytofluene	(Zhou et al. 2010)	chloroform/methanol/Tris buffer pH 7.5 containing 1 M NaCl (2:1:1 v/v/v)	YMC reverse-phase C30 (250mm×4.6mm i.d., 5 µm) column, photodiode array detector temperature 25°C, mobile phase gradient elution of methanol, 80% methanol (with 0.2% ammonium acetate), MtBE).
2	Orange peels	(9Z)-violaxanthin, violaxanthin + furanoid, β-citraurin, lutein, β-cryptoxanthin	(Agócs et al. 2007)	methanol, followed by diethyl ether	Licrospher C18 column (250 x 4.6 mm. i.d.), endcapped, photodiode array detector, solvents gradient elution of methanol:water (12% v/v), methanol, dicloromethane in methanol (30% v/v).
	Mandarin peel	β-cryptoxanthin, (9Z)-violaxanthin, β-citraurin, violaxanthin + furanoid, lutein,	Idem		
	Clementine peel	β-citraurin, β-cryptoxanthin, (9Z)-violaxanthin, luteoxanthin, neochrome, lutein,	Idem		
	Kumquat peel	(9Z)-violaxanthin, β-citraurin, β-cryptoxanthin, violaxanthin+furanoid, lutein, luteoxanthin			
	Grapefruit peel	β-cryptoxanthin, β-citraurin, violaxanthin+furanoid, lutein, ξ-carotene, (9Z)-violaxanthin,,			
	Lemon	β-cryptoxanthin, lutein, ξ-carotene, β-citraurin			
3	Orange juice concentrate (different brands sold in Spain)	Mutatoxanthin epimer B, β-cryptoxanthin, mutatoxanthin epimer A, zeaxanthin, auroxanthin A, auroxanthin B, violaxanthin and auroxanthin isomers (mixture), lutein	(Meléndez-Martínez et al. 2008)	methanol/acetone/hexane 825/25/50 v/v/v	YMC C30 column (5µm, 250 x 4.6 mm), photodiode array detector, column temperature 17°C, linear gradient of solvents: methanol, MtBE (with 0.1% and 0.05% trimethylamine, and water
4	Tangerine (*C. reticulate*) from Thailand	β-cryptoxanthin, zeaxanthin, lycopene, lutein	(Stuetz et al. 2010)	ethanol containing pyrogallol (2.5% v7v)	TrentecSpherisorb ODS-2 analytical column (3 µm, 250 x 4.6 mm), UV-vis detector, mobile phase recirculation mode of acetonitrile: dioxine:methanol (82:15:3)
5	Yuza (*C. junos*), Kjool (*C. unshiu*), Dangyooja (*C. grandis*) from Korea	β-cryptoxanthin, zeaxanthin, β-carotene, astaxanthin, canthaxanthin, α-carotene, lycopene.	(Yoo and Moon 2016)	Acetone	µ-Bondapak C18 (3.9 mm x 300 mm, 10 µm) column, column temperature 25°c
6	Grapefruit (*C. paradisi*)	β-carotene, β-cryptoxanthin, zeaxanthin, lutein, lycopene, α-carotene	(Zheng et al. 2016)	hexane:acetone:ethanol (50:25:25, v/v/v)	YMC C30 (250 x 4.6 mm i.d., 5 µm), UV Vis detector, column temperature 25°C, mobile phase mixture of methanol, water and MtBE

1.3 Polyphenols from citrus fruits

Citrus fruits are exceptionally rich in flavanones and polymethoxylated flavones. Citrus fruits are widely consumed worldwide and hence, the interest on its polyphenols contents concerning its beneficial effects is growing (Khan and Dangles 2014). Flavanones are the main flavonoids found in citrus fruits and regarded as the foundation of flavonoid biosynthesis as they act as precursors of all other flavonoid classes (Martens and Mithöfer 2005; Schijlen et al. 2004). Apart from citrus, flavanones are also identified in 42 higher plant families, particularly in Compositae, Leguminosae and Rutaceae (Iwashina 2000). Among all flavanones, the aglycones naringenin and hesperetin and their glycosides are the most important ones due to their high prevalence in food (Khan and Dangles 2014).

Naringin (naringenin-7-neohesperidose) is found mainly in grapefruit and sour orange (Igual et al. 2013), tomato and tomato products (Erlund 2004). Narirutin (naringenin-7-rutinoside) is less common compared to naringin, and the sources are e.g. grapefruit, tangor, sweet orange, tangerine and tangelo (Peterson, Dwyer et al. 2006). Naringenin and its glycosides are relatively heat-sensitive compared to hesperetin and the glycosides (Igual et al. 2013).

Hesperidin (hesperetin-7-rutinoside) is the most widely distributed flavonoid and most studied glycoside of hesperitin. Hesperidin is found mainly in lemons, limes, sweet oranges, tangerine, tangor, clementine and mandarin (Cano, Medina, and Bermejo 2008; Dhuiqe-Mayer et al. 2005). Neohesperidin (hesperidin-7-neohesperidose) is comprised of tangelo and sour orange (Peterson, Beecher et al. 2006; Peterson, Dwyer et al. 2006).

Polymethoxylated flavones (PMF) is a class of flavonoids typically found together in citrus genus, and found in abundance in peels and tissues of some citrus fruits namely tangerine (*C. reticulata*), grapefruit (*C. paradisi*), sweet orange (*C. sinensis*) and sour orange (*C. aurantium*). PMF have a typical benzo-gamma-pyrone skeleton with a carbonyl group at C-3, and are formed by polymethylation of polyhydroxylated flavonoids. This resulted in increased metabolic stability and membrane transport in the intestine, improving its oral bioavailability (Walle et al. 2004).

The potency of nobiletin and tangeretin to manage obesity and insulin intake has been studied. Both PMFs have been reported to increase the secretion of adiponectin (insulin resistance factor) in adipocytes cells. Nobiletin also decreased the secretion of resistin (an insulin resisting factors) (Miyata et al. 2011). Anti-inflammatory activity of PMF from citrus samples has been reported (Huang and Ho 2010; Menichini et al. 2011), as well as inducing apoptosis activity of HeLa carcinoma cells (Kim et al. 2010), and its potency to regulate diabetes and cardioprotective activity related to diabetes (Sundaram, Shanthi, and Sachdanandam 2015).

Biological potencies of flavanones also have been documented. Flavanones have been reported to exhibit antioxidant activity in crochin bleaching assay (Majo et al. 2005), DPPH assay (Bedane et al. 2016), antibacterial activity (Cvetnić and Vladimir- Knežević 2004), antifungal activity (Arcas et al. 2000), apoptosis-inducing and chemo-sensitizing effects on Ramos' lymphoma cells (Nazari et al. 2011).

In addition to flavanones and PMF, phenolic acids such as gallic acid, caffeic acid, syringic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid, synapic acid, chlorogenic acid, as well as the flavonols quercetin, kaempferol, isorhamnetin, tamarixetin and rutin and the flavones apigenin, luteolin, diosmetin, chrysoeriol were detected (Karimi et al. 2012; Xi et al. 2014; Abad-García et al. 2012).

1.4 Bioactivity assays

1.4.1 Antioxidant capacity assays

An antioxidant is defined as a component that is present in the substance which can delay or inhibit the oxidation of the substrates (Halliwell and Gutteridge 1990). Various compounds found in plants are known to exhibit antioxidant activity. Different antioxidant compounds often react differently to varius radical or oxidant sources. Thus, no single assay will correctly reflect all of the radical sources or all antioxidants in a mixed or complex system (Prior, Wu, and Schaich 2005). It is proposed to use more than one assay to test on antioxidant potency compounds.

a. H-TEAC and L-TEAC assays

The TEAC (Trolox equivalent antioxidant capacity assay) assay determines the antioxidant potency of compounds based on SET and HAT mechanisms (Prior, Wu, and Schaich 2005). The test involves the generation of $ABTS^{+\bullet}$ from ABTS by oxidizing agents resulting in a blue, green colored solution. It then reacts with antioxidants source (samples), resulting in a decrease of absorbance at 734 nm (Figure 3).

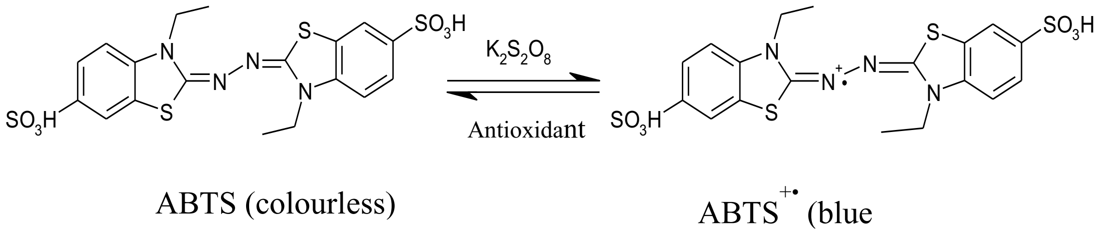

Figure 3 Schematic reactions of ABTS in the presence of antioxidants (Pannala et al. 2001).

The TEAC assay is a simple and robust method to measure antioxidant capacity as the reaction is analysed over a short period (4-6 minutes), adaptable to wide pH range, can be automated and adapted into microplate format and modifiable into hydrophilic (H-TEAC) and lipophilic (L-TEAC) systems (Zulueta, Esteve, and Frígola 2009). Even so, the TEAC assay has some limitations such as narrow range of $ABTS^{+\bullet}$ solution concentration for reproducible optical measurements; different factors donated to reproducible results between laboratories such as oxidizing agent used for $ABTS^{+\bullet}$ generation, the age of $ABTS^{+\bullet}$ solution, etc. (Apak et al. 2013). Nevertheless, TEAC assay is useful to provide a ranking order of antioxidants (Van Den Berg et al. 1999). L-TEAC developed slightly different; lipophilic compounds are dissolved in hexane, and the reaction with the ABTS radical solution is achieved through vigorous agitation followed by gentle phase separation. The resulting decrease in ABTS radical absorbance is monitored precisely at 2 minutes after initial addition of ABTS radical solution (Müller et al. 2010).

b. H-ORAC assay

The oxygen radical absorbance capacity (ORAC) assay is based on HAT mechanism. ORAC antioxidant inhibition of peroxyl radical-induced oxidations thus reflects classical chain-breaking antioxidant activity by H atom transfer. The method was initially developed by Cao, Alessio and Cutler (1993) and modified by Lambert et al. (2001). The advantages of ORAC assay are free radicals of biologically relevant sources, the method has been standardized, and taking into account both degree and time of antioxidant reaction (Zulueta, Esteve, and Frígola 2009). On the other hand, ORAC assay is temperature sensitive, the use of fluorescence markers require specific instruments, and the analysis took a long time to finish (>1 h).

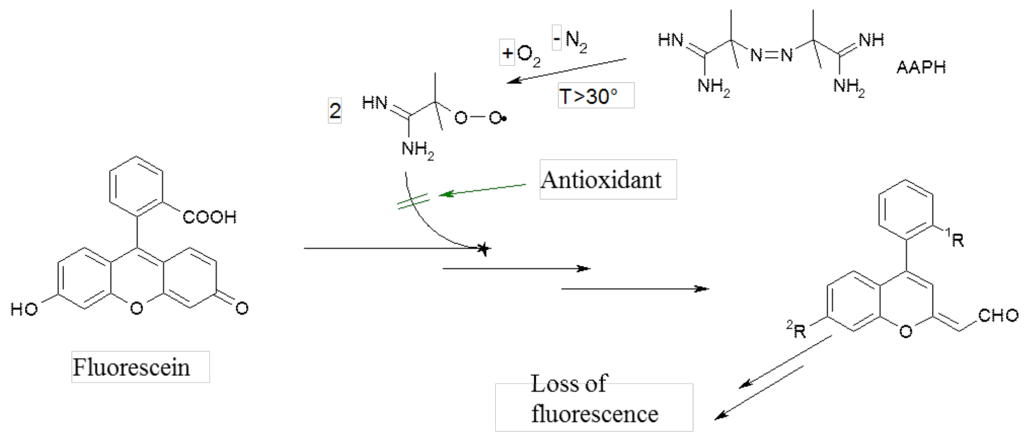

Figure 4 ORAC antioxidant activity schematic reactions

c. Total Phenolic Content according to Folin Ciocalteu method

The Folin-Ciocalteu assay nowadays is also considered as one antioxidant assay due to its ability to measure the reducing capacity of compounds. This test was initially developed for tyrosine analysis, but now has broad application for determination of reducing phenols in different food matrices. In this test, the reagent consists of sodium molybdate, sodium tungstate, and other chemicals which upon reaction with phenols, produce a blue color strongly absorbed at 765 nm (Everette et al. 2010).

1.4.2 Total flavonoid content (TFC) and total phenolic content according to FBBB assay

Determination of TFC is often achieved through colorimetric reaction of aluminum-flavonoid complexation. In this study, complexation reaction was achieved in the presence of $NaNO_2$ in alkali medium. The reaction involves nitration of an aromatic ring bearing a catechol group wherein the three or four positions are neither substituted nor sterically blocked. A complex yellow solution will be formed after addition of $AlCl_3$, which then turns immediately into red upon addition of NaOH. Colour intensity is then monitored at 506 nm (Pekal and Pyrzynska 2014). This method has been used to investigate different samples, i.e. wheat flakes and muesli (Sumczynski et al. 2015), tomatoes (Dewanto et al. 2002), honey (Al et al. 2009), ginger (Ghasemzadeh, Jaafar, and Rahmat 2010).

TPC according to FBBB is an alternative method to quantify phenolic content in food samples. In this method, Fast Blue BB diazonium salt couples with phenolic compounds, resulting in the formation of azo complexes (Figure 1). Coupling occurs mostly in the *para* position to the phenolic group, unless the position is already occupied, then the substitution will occur in the *ortho* position. The reactions occur slightly in an alkaline environment so that they can be converted to the more active phenoxide ions (Medina 2011). This test has been used to determine phenolic contents in some juice and strawberry samples (Lester et al. 2012).

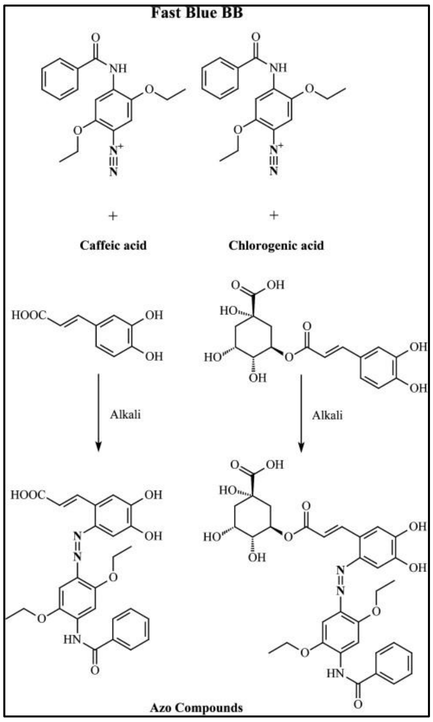

Figure 5 Proposed interactions of phenolic compounds with Fast Blue BB salt in NaOH buffer solution (taken from Medina (2011).

1.4.3 COX-2 inhibitory activity

COX (cyclooxygenase) is an enzyme usually involved in the production of prostaglandin. There are two types of COX found in human, which are COX-1 and COX-2. COX-1 is involved in the production of prostaglandin related to normal body functions such as stomach mucous production and kidney water excretion. COX-2 is produced as inflammatory response, and present in the site of inflammation (Fu et al. 1990; Zarghi and Arfaei 2011). The concentration of COX-2 inside the body thus is inconsistent. COX enzyme exhibits COX and peroxidase activities. COX converts arachidonic acid to a hydroperoxy endoperoxide (PGG2), which is the precursor of prostaglandins (PGs), thromboxanes and prostacyclins. The test conducted was based on manufacturer instruction. The COX inhibitory assay is based on the competition between PGS and PG-acetylcholinesterase (AChE) conjugate (PG tracer) for a limited amount of rabbit PG antiserum. The PG antiserum complex binds to a mouse monoclonal anti-rabbit antibody that has been previously attached to the well. The plate is then washed to remove any unbound reagents and then Ellman's Reagent (which contains substrate to AChE) is added as well. The product of this enzymatic reactions has a distinct yellow colour, which can be spectrophotometrically determined (at 402-412 nm).The intensity of the colour is proportional to the amount of PG tracer bound to the well, which is also inversely proportional to the amount of free PG present in the well during incubation (Anonym, 2017).

1.4.4 α-Amylase inhibitory activity

α-Amylase and α-glucosidase are the first enzymes involved in the metabolism of sugars. These enzymes were studied in relation to the management of diabetes and obesity (Subramanian, Asmawi, and Sadikun 2008). α-Amylase is an enzyme catalyzing the hydrolysis of α-1,4-glucosidic linkages in starch and other polysaccharides to oligosaccharides (Sundarram and Murthy 2014). 3,5-dinitrosalicylic acid (DNSA) assay is the most widely used method to determine α-amylase activity (Visvanathan, Jayathilake, and Liyanage 2016). DNSA tests the presence of free carbonyl group or reducing sugars. The principle of this test is in the alkaline solution. The 3,5-dinitrosalicylic acid reacts with reducing sugars being converted into 3-amino-5-nitrosalicylic acid with orange color (Apostolidis, Kwon, and Shetty 2007), which is then quantified spectrophotometrically at 540 nm.

1.4.5 MLN antibacterial assay

The MLN method is a method to observe bacterial growth over a period utilizing turbidimetry principle. In this method, a red laser diode (633 nm) is used as the source of light (Finger et al. 2012). The MLN method was firstly described in 1927 and employed the calculation of the concentration of suspended particles (Peskett 1927). In MLN method, the intensity of light measured is directly proportional to the particle concentration in a solution (Finger et al. 2013). Laser nephelometry has been described as an efficient technique for monitoring microbial growth, such as bacterial and yeast (Fouda et al. 2006; Joubert et al. 2010).

2. Objectives

Despite the complex carotenoid profiles found in some citrus species, no information can be found on carotenoid profiles of local citrus fruits from Aceh. Moreover, polyphenol profiles of local citrus from Aceh also have never been described previously. Lastly, limited information is available regarding the biological potencies of local citrus fruits from Aceh.

Thus, the objectives of the present thesis can be formulated as follows:

1. The main objective of this study was to investigate carotenoid profiles of local citrus fruits samples from Aceh, Indonesia, namely jeruk takengon, jeruk nipis, jeruk purut, calung, makin, kruet mameh and mentui.
2. Identification and quantification of polyphenol profiles of local citrus fruits samples from Aceh, Indonesia. The investigation on the polyphenol profiles of these local citrus fruits also helps in the classification of the citrus samples.
3. Investigation on the biological potencies of local citrus samples extracts through antioxidant capacity assays, antimicrobial activity test, antidiabetic activity test, and anti-inflammatory activity assay.

3. Schedule of Manuscripts

Manuscript I

Carotenoids of indigenous citrus species from Aceh and its *in vitro* antioxidant, antidiabetic and antibacterial activities

Ernawita, Ruri Agung Wahyuono, Jana Hesse, Uta-Christina Hipler, Peter Elsner and Volker Böhm

European Food Research and Technology 242 (2016) 1869-1881
https://doi.org/10.1007/s00217-016-2686-0
Accepted 2 April 2016; Published 19 April 2016

Manuscript II

***In vitro* lipophilic antioxidant capacity, antidiabetic and antibacterial activity of citrus fruits extracts from Aceh, Indonesia**

Ernawita, Ruri Agung Wahyuono, Jana Hesse, Uta-Christina Hipler, Peter Elsner and Volker Böhm.

Antioxidants, 6 (2017) 2-15.
DOI. 10.3390/antiox6010011.
Accepted 25 January 2017; Published 3 February 2017

Manuscript III

Polyphenols, vitamin C content, *in-vitro* antioxidant capacity, α-amylase and COX-2 inhibitory activities of indigenous citrus samples from Aceh, Indonesia

Ernawita, Constanze Thieme, Anna Westphal, Angelika Malarski and Volker Böhm.

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3.1 Manuscript I

Manuscript I

Carotenoids of indigenous citrus species from Aceh and its *in vitro* antioxidant, antidiabetic and antibacterial activities

Ernawita, Ruri Agung Wahyuono, Jana Hesse, Uta-Christina Hipler, Peter Elsner and Volker Böhm

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Carotenoid contents of peel and pulp of local citrus fruits from Aceh were investigated using HPLC equipped with DAD detector and LC–MS. Most of the citrus samples investigated mainly contained lutein and zeaxanthin. Jeruk takengon (Citrus nobilis) had a more complex carotenoid profile and the highest carotenoid contents compared to other citrus fruits studied here, and peel contained higher carotenoid contents than pulp. (Z)-Violaxanthin and (*all-E*)-violaxanthin were the main carotenoids of the peel, while (*all-E*)-β-cryptoxanthin and (*Z*)-violaxanthin were the main carotenoids of the pulp of jeruk takengon. Determination of the lipophilic antioxidant capacity (L-TEAC) of the carotenoid extracts of jeruk takengon showed promising antioxidant potential. The peel extract showed stronger antioxidant capacity than the pulp extract, and saponification further improved the antioxidant capacity. Investigating the inhibition of α-amylase activity resulted in a moderate α-amylase inhibition by jeruk takengon due to the carotenoid contents and the contribution of some flavonoids. Pulp extracts exhibited better inhibition activity than peel extracts, and saponification led to a reduction of the α-amylase inhibition activity. Antibacterial activity tests showed that jeruk takengon could inhibit the growth of *K. pneumonia* and *S. aureus*, and saponification enhanced the antibacterial activities. In general, the peel extracts showed biological potential due to the relatively strong *in vitro* antioxidant, antibacterial and α-amylase inhibitory activity. In addition, these by-products of food processing may serve as potential carotenoid sources.

Work share

Ernawita : Samples collection, carotenoid extraction. Identification and quantification of carotenoids in citrus samples by HPLC and LC-MS, fractionation of unidentified compounds by HPLC, biological activities tests (*in vitro* antioxidant activity, *in vitro* inhibition of α-amylase activity, *in vitro* antibacterial activity), statistical analysis and preparation of the manuscript.
Total Share: 78%

Ruri A. Wahyuono: FTIR analysis and data interpretation
Total share: 5%

Jana Hesse: *In vitro* antibacterial activity
Total Share: 3%

Uta-Christina Hipler: Correction of the manuscripts
Total share: 2%

Peter Elsner: Correction of the manuscript
Total share: 2%

Volker Böhm: Correction of the manuscript
Total share: 10%

3.2 Manuscript II

Manuscript II

In vitro lipophilic antioxidant capacity, antidiabetic and antibacterial activity of citrus fruits extracts from Aceh, Indonesia

Ernawita, Ruri Agung Wahyuono, Jana Hesse, Uta-Christina Hipler, Peter Elsner and Volker Böhm.

Antioxidants, 6 (2017) 2-15.
DOI. 10.3390/antiox6010011.
Accepted 25 January 2017; Published 3 February 2017

This study reports *in vitro* lipophilic antioxidant, inhibition of α-amylase and antibacterial activities of extracts of peel and pulp of citrus samples from Aceh, Indonesia. HPLC (high-performance liquid chromatography), phytochemical, and FTIR (fourier transform infrared) analysis detected carotenoids, flavonoids, phenolic acids and terpenoids, contributing to the biological potencies. Most peel and pulp extracts contained lutein and lower concentrations of zeaxanthin, α-carotene, β-carotene and β-cryptoxanthin. The extracts also contained flavanone glycosides (hesperidin, naringin and neohesperidin), flavonol (quercetin) and polymethoxylated flavones (sinensetin, tangeretin). L-TEAC (lipophilic trolox equivalent antioxidant capacity) test determined for peel extracts higher antioxidant capacity compared to pulp extracts. All extracts presented α-amylase inhibitory activity, pulp extracts showing stronger inhibitory activity compared to peel extracts. All extracts inhibited the growth of both gram (+) and gram (−) bacteria, with peel and pulp extracts of makin showing the strongest inhibitory activity. Therefore, local citrus species from Aceh are potential sources of beneficial compounds with possible health preventive effects.

Work share

Ernawita : Samples collection, carotenoid extraction. Identification and quantification of carotenoids in citrus samples by HPLC and LC-MS, fractionation of unidentified compounds by HPLC, phytochemical analysis, polyphenol extractions, biological activities tests (*in vitro* antioxidant activity, *in vitro* inhibition of α-amylase activity, *in vitro* antibacterial activity), statistical analysis and preparation of the manuscript.
Total Share: 78%

Ruri A. Wahyuono: FTIR analysis and data interpretation
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Total share: 2%

Peter Elsner: Correction of the manuscript
Total share: 2%

Volker Böhm: Correction of the manuscript
Total share: 10%

3.3 Manuscript III

Manuscript III

Polyphenols, vitamin C content, *in-vitro* antioxidant capacity, α-amylase and COX-2 inhibitory activities of indigenous citrus samples from Aceh, Indonesia

Ernawita, Constanze Thieme, Anna Westphal, Angelika Malarski and Volker Böhm.

International Journal for Vitamin and Nutrition Research. 5-6 (2019) 337-347
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Accepted 17 February 2017; Published 1 April 2019.

This study was conducted to analyse antioxidant potencies, vitamin C contents, polyphenol profiles, antidiabetic and anti-inflammatory potencies of citrus fruits from Indonesia. Total phenolics contents (TPC) of seven citrus fruits from northern Aceh, Indonesia, were measured using Folin-Ciocalteu (FC) and Fast Blue BB (FBBB) methods. Total flavonoid content (TFC) test showed for peel and pulp extracts of calung and jeruk takengon (local mandarin) the highest values. H-TEAC (hydrophilic trolox equivalent antioxidant capacity) and H-ORAC (hydrophilic oxygen reactive absorbance capacity) antioxidant capacity were highest for peel and pulp of jeruk takengon, calung and kruet mameh. Interestingly, peel extracts showed no α-amylase inhibition activity whilst pulp showed weak inhibitory activity. Polyphenol composition was dominated by flavanones, with hesperidin and neohesperidin as main flavanones (hesperidin: 131–5433 mg/100 g DW, neohesperidin: 431–4131 mg/100 g DW). Vitamin C contents were highly correlated with antioxidant capacities in pulp ($R^2 = 0.95$ and $R^2 = 0.94$ at $p < 0.01$ for H-TEAC and H-ORAC, respectively), and TPC and TFC were highly correlated with antioxidant capacities ($R^2 = 0.99$ and $R^2 = 0.98$ for TPC FC in pulp and $R^2 = 0.93$ and $R^2 = 0.84$ in peel for H-TEAC and H-ORAC, respectively; $R^2 = 0.88$ and $R^2 = 0.80$ in pulp, and $R^2 = 0.68$ and $R^2 = 0.75$ for TFC in peel for H-TEAC and H-ORAC at $p < 0.01$). *In-vitro* COX-2 inhibitory activity tests resulted in higher activity for pulp compared to the corresponding peel extracts except for calung. Pulp extract of jeruk takengon showed the highest activity. In general, local citrus fruits from Aceh, Indonesia, are potential sources of polyphenols and vitamin C.

Work share

Ernawita : Polyphenols extraction, determination of water content in citrus fruits, biological assays (antioxidant capacity assays, inhibition of α-amylase, TPC according to FC method, TPC according to FBBB method, total flavonoid content, vitamin C content, *in-vitro* COX-2 inhibitory activity), HPLC analysis

Total Share: 78%

Constanze Thieme: Hydrophilic antioxidant capacity assays,

Total share: 7%

Anna Westphal: *In-vitro* antioxidant capacity assays

Total Share: 3%

Angelika Malarski: HPLC-MS analysis

Total share: 2%

Volker Böhm: Correction of the manuscript

Total share: 10%

4. Discussion

Different phytochemical aspects of various citrus fruits locally found in Aceh, Indonesia, were analysed. Citrus fruit samples used in this study were collected from different species locally named as jeruk takengon, jeruk nipis, jeruk purut, calung, makin, kruet mameh and mentui.

4.1 Carotenoid contents of local citrus samples from Aceh

Carotenoids are a large group of mostly C-40 tetraterpenoid compounds and can be divided into two subgroups, namely xanthophylls (oxygenated carotenoids) and carotenes (non-oxygenated carotenoids). Xanthophylls are often acylated with diverse fatty acids and present in esterified forms in plants (Ma et al. 2017) including in citrus fruits (Agócs et al. 2007). Extraction of carotenoids from citrus fruits samples in this study was done using a mixture of MeOH/THF (1+1 v/v), followed by saponification, which hydrolized esters of xanthophylls. Xanthophyll's esters were not analyzed in this study due to time and methods limitations. Laurate, myristate and palmitate have been reported as main esters found in mandarin, while β-cryptoxanthin has been reported as the main carotenoid (Ma et al. 2017). Carotenoid composition of our samples is presented in manuscript 1, particularly in Table 1. As carotenoids often act as pigments, only jeruk takengon, which is part of mandarin group, exhibited rich carotenoid profiles. Jeruk takengon had the most carotenoids identified as well as quantified. (*all-E*)-β-Cryptoxanthin was the main carotenoid identified in peel of jeruk takengon, while (*9Z*)-violaxanthin was the main carotenoid of pulp of jeruk takengon.

4.2 Polyphenol contents of local citrus samples from Aceh

Citrus fruits consist primarily in abundance of polyphenol groups particularly of flavanones and polymethoxylated flavones (PMF). Meanwhile, main flavonoids identified from citrus fruits are flavanone glycosides, flavone glycosides, and polymethoxylated flavones. Manuscript 3 describes the polyphenols characterization and quantification using ethanolic extracts of citrus fruit samples. Polyphenol contents were analyzed in hydrolized EtOH/H_2O extracts of citrus peel and pulp. Hydrolysis procedure enabled releasing the bound phenolics from the glycosidic form. The resulting aglycones were then subjected to a characterization process (Harborne, 2012). Total flavanone, flavonoid and polyphenol contents quantified by HPLC (manuscript 3) were calculated and are expressed in mmol/100 g and are summarized in Table 2.

Table 2 Contents of total flavanones, flavonoids and total polyphenols (mmol/100 g) of ethanolic citrus extracts. Values are means of samples (n=3). Different letters in one column indicate significant differences within one category (One Way ANOVA, SNK test, $p < 0.05$). Values in bold indicate highest values within one column.

Samples	Total flavanone content (mmol/100 g)		Total flavonoid content (mmol/100 g)		Total polyphenol content (mmol/100 g)	
	Peel	Pulp	Peel	Pulp	Peel	Pulp
Jeruk takengon	**7.9 ± 0.7^{D}**	**9.4 ± 0.5^{D}**	**9.9 ± 0.6^{B}**	**9.5 ± 0.5^{A}**	**11.6 ± 0.8^{B}**	**10.8 ± 0.4^{A}**
Jeruk nipis	$1.8 \pm 0.1^{A,B}$	1.1 ± 0.0^{A}	$1.8 \pm 0.1^{D,E}$	1.1 ± 0.0^{D}	$2.2 \pm 0.1^{D,E}$	1.1 ± 0.0^{D}
Jeruk purut	$1.7 \pm 0.0^{A,B}$	1.4 ± 0.1^{A}	$1.7 \pm 0.0^{D,E}$	1.4 ± 0.1^{D}	$1.9 \pm 0.0^{D,E}$	1.4 ± 0.1^{D}
Calung	**12.7 ± 0.7^{E}**	**5.5 ± 0.1^{C}**	**13.0 ± 0.7^{A}**	**5.6 ± 0.1^{B}**	**14.6 ± 0.8^{A}**	**5.9 ± 0.1^{B}**
Makin	2.4 ± 0.1^{B}	1.3 ± 0.0^{A}	2.5 ± 0.1^{D}	1.3 ± 0.0^{D}	2.8 ± 0.2^{D}	1.4 ± 0.0^{D}
Kruet Mameh	**6.9 ± 0.2^{C}**	**3.5 ± 0.1^{B}**	**7.2 ± 0.2^{C}**	**3.5 ± 0.1^{C}**	**8.1 ± 0.2^{C}**	**3.8 ± 0.2^{C}**
Mentui	1.1 ± 0.1^{A}	1.3 ± 0.0^{A}	1.1 ± 0.1^{E}	1.3 ± 0.0^{D}	1.3 ± 0.1^{E}	1.3 ± 0.0^{D}

In general, mandarin has a higher hesperidin content compared to an orange (Berhow et al., 1998). Even for mandarin groups, some polyphenols, especially from flavanones class, which were quantified in our study, are comparable to results having been reported before (Nogata et al. 2006). Hesperidin content in an orange is reported to vary from 4.3-47.1 mg/100 g FW (Peterson, Dwyer, et al. 2006), while for mandarin, 85-120 mg/100 FW of hesperidin was reported by Abeysinghe and co-workers (Abeysinghe et al., 2007). Xu, Liu, et al. (2008) reported hesperidin contents in the range of 337-451 mg/L. Narirutin, which was also often found in mandarin, showed a content of 0.0-7.7 mg/100 g FW (Johnsen et al. 2003), and Xu, Liu, et al. (2008) which worked on juices reported a much higher value of 169-288 mg/L FW.

For another common citrus, lime (jeruk nipis), the reported hesperidin contents (analysed from juices) were 10.9 mg/100 ml (sample sourced from Mexico) and 16.8 mg/100 ml (sample sourced from Brazil) (Mouly et al. 1994), being lower than our finding (70.8±0.6 mg/100 g FW), while a lower hesperidin value (10.4-19.8 µg/ml) from lime juice was reported by Saeidi et al. (2011).

There is no data available to compare flavanones values of different citrus fruits from Indonesia. High concentrations of flavanones also highlighted the potencies of further investigations on secondary plant products of other citrus fruits, and whenever possible, other fruits in Aceh and Indonesia.

Peel extracts of calung and jeruk takengon had the highest flavonoid and polyphenol contents, and the content was significantly different between the two samples; while for the pulp, the order was reversed. Kruet mameh, as one of the rare local citrus fruits, also showed high flavonoid and polyphenol contents. This is of importance as flavonoids and polyphenols have been linked with various biological potencies.

Polyphenol contents in fruits might be affected by different factors, namely degree of ripeness, harvest time, environmental factors, processing and storage (Manach 2004). Citrus samples used in this study were from different species (*C. nobilis*, *C. hystrix*, *C. aurantiifolia*, *C. aurantium*), and were also at different maturation stages, according to maturation level used in the community. Jeruk takengon showed high flavanone, flavonoid and polyphenol contents despite it is consumed at mature stage, compared to other citrus fruit samples in this study. It is especially of importance as reports have shown that increase in maturity would decrease polyphenol and flavonoid contents (Meinichi et al. 2011; Xu, Ye et al. 2008).

Flavanones composition in citrus samples used in this study was in accordance with previous reports (Peterson, Beecher, et al. 2006; Peterson, Dwyer, et al. 2006), especially with identified (common citrus) samples, which were jeruk takengon (mandarin), jeruk nipis (key lime) and jeruk purut (kaffir lime). Calung (*C. aurantium*) showed the highest flavanone contents, both in peel and in total, followed by jeruk takengon, while mentui, jeruk purut and nipis had the lowest contents (Table 4.1). Calung's flavanones mainly consisted of naringin and neohesperidin (manuscript 3, Table 3).

Previous identification has been done in order to identify citrus species used in this study. Nevertheless, the identification was inconclusive as the flower as the main identification organ was absent in most of the samples. Thus, further identification is needed.

Calung, previously identified as a sour orange (or a bitter orange), fulfilled main characteristics of *C. aurantium* as previously reported, such as main flavanones identified were from neohesperidosyl flavanones, mainly neohesperidin and naringin (manuscript 3). The second characteristic was high flavanone content compared to other citrus species (Berhow et al. 1998); Peterson, Dwyer, et al. 2006), supporting its taxonomical classification as sour orange.

Other rare citruses are believed to be incorrectly identified as *C. aurantium* (makin, kruet mameh and mentui). All three aforementioned citrus fruits had lower flavanones contents compared to other citrus samples. Makin showed similar characteristics to jeruk purut, from its outer appearances (deeply ridged peel) and mainly consisting of neohesperidin and hesperidin in equal amounts both in peel and pulp. Kruet mameh had a high hesperidin content, similar to that of jeruk takengon, but narirutin's presence was undetected. Kruet mameh`s outer appearance resembles jeruk purut (deeply ridged peel) and mandarin characteristics (yellow fleshed, peel easily separated from the pulp, sweet taste). It might be possible that this group of citrus is a hybrid between mandarin and *C. hystrix*. Further identification is needed in order to establish the correct identification of kruet mameh. Mentui had both hesperidin and neohesperidin, but the content of neohesperidin was much higher than that of hesperidin, both in peel and pulp extracts. Mentui showed for characteristics a resemblance to alemow (*C. macrophylla*), such as wide leaves, thorny branches, knobby peel (http://www.citrusvariety). The presence of neohesperidin might suggest that this citrus has been crossbreeding with other local citruses. In conclusion, further identification procedures are needed to ensure taxonomy of these local citruses from Aceh, in order to gain the full benefit of these plants.

4.3 Antioxidant capacities and anti-inflammatory potencies of citrus fruits

4.3.1 Antioxidant capacities

Polyphenols, vitamin C and carotenoids are believed to contribute to antioxidant potencies of citrus fruits. H-TEAC antioxidant capacities of different polyphenol standards and vitamin C were studied and reported in manuscript 3, in order to get some pictures of the antioxidant capacity of identified compounds and their possible contributions to ethanolic citrus extracts antioxidant capacities. Most of the results are in agreement with reports by Rice-Evans and co-workers (1997). The order of the antioxidant capacity of the identified polyphenols, ascorbic acid, and trolox standards was gallic acid>ferulic acid>naringenin>hesperetin>neohesperidin>trolox>caffeic acid>ascorbic acid> hesperidin>narirutin>naringin.

Flavanones have been reported as major components in peel and pulp of citrus fruits, and the finding is of relevance with our ethanolic extracts. Flavanones are not an ideal free radical scavenger due to some structural limitations (Majo et al. 2005), but the high concentrations of flavanones which were quantified in the peel and pulp are compensating the structural limitations.

As flavonoids, phenolic acids, PMF, flavanones and polyphenols contributed to the antioxidant capacities (H-TEAC, H-ORAC, and TPC FC), Table 3 presents correlations between these aforementioned parameters. Contributions of these groups of compounds to biological potencies of citrus ethanolic extracts were observed and will be discussed (Table 3). Spearman correlation tests showed that total flavanones, total phenolic acids, total polymethoxylated flavones, total flavonoids and total polyphenols (in mmol/100 g) calculated from citrus fruits displayed a correlation with H-TEAC, H-ORAC, and TPC FC; and the correlations observed were higher in peel compared to pulp of corresponding citrus ethanolic extracts samples. Higher contents of compounds (identified and unidentified) in peels of citrus fruits samples might have attributed to the correlations observed.

Table 3. Spearman's correlations between total flavanones, total phenolic acids, total PMF, total flavonoids, total polyphenols (in mmol/100 g DW) and antioxidant capacity assays (H-TEAC, H-ORAC, and TPC FC) of ethanolic citrus peel and pulp extracts

		Pulp							
		TF	TPA	TPMF	TFL	TP	H-TEAC	H-ORAC	TPC FC
Peel	TF		-	-	-	-	0.648**	0.616**	0.675**
	TPA	-		-	-	-	0.731**	0.718**	0.742**
	TPMF	-	-	-	-	-	0.794**	0.657**	0.794**
	TFL	-	-	-		-	0.648**	0.616**	0.675**
	TP	-	-	-	-		0.649**	0.614**	0.671**
	H-TEAC	0.769**	0.712**	0.848**	0.827**	0.763**		-	-
	H-ORAC	0.831**	0.777**	0.809**	0.765**	0.822**	-		-
	TPC FC	0.852**	0.775**	0.839**	0.848**	0.845**	-	-	

H-TEAC = hydrophilic trolox equivalent antioxidant capacity, H-ORAC = hydrophilic oxygen radical absorbance capacity, TF = total flavanones, TPA = total phenolic acids, TPC FC = total phenolic content according to Folin Ciocalteu, TPMF = total polymethoxylated flavones, TFL = total flavonoids, TP = total polyphenols, (-) = not calculated.

Total identified flavonoid and polyphenol contents in ethanolic citrus extracts were highly correlated with H-TEAC and H-ORAC antioxidant capacities assays. For H-

TEAC of peel extracts, highest correlations were calculated for total flavonoids content and total polyphenols content (R^2=0.827** and R^2=0.763**, respectively), while for pulp extracts, the correlations were almost identical (R^2=0.648** and R^2=0.649** for total flavonoids and total polyphenols, respectively).

For H-ORAC of peel extracts, highest correlations were observed for total polyphenols followed by total flavonoid contents (R^2=0.822** and R^2=0.765**, respectively). The ORAC activity of pulp extracts showed similar results, as the correlations between total flavonoid contents and total polyphenols contents were R^2=0.616** and R^2=0.614**, respectively.

The reducing potency of ethanolic citrus extracts, as observed through TPC according to FC, also showed higher and significant correlation found in peel extracts rather than in pulp extracts (Table 1). These data suggested that concentrations of flavonoids and polyphenols likely determined the antioxidant capacity of citrus peels, and to a little lesser extent of citrus pulps. Vitamin C has been considered as the main contributor of antioxidant capacity in peel and pulp of citrus fruits, nevertheless our findings report otherwise, as flavanones contributed the highest.

Citrus carotenoid contents have been quantified and characterized in manuscript 1 (Table 1). Carotenoids characterized and quantified in the MeOH/THF extracts contributed to the antioxidant capacity of citrus fruits, as measured by L-TEAC. The results are summarized in Table 4.

Table 4. Summary of total carotenoid contents (µmol/100 g) in peel and pulp (MeOH/THF extracts) of citrus samples and L-TEAC values (µmol-αTE/100 g). Values in bold indicate highest values within one column (LOD=0.02 mg/100 g, LOQ=0.06 mg/100 g).

Samples	Peel (µmol/100 g)			Pulp (µmol/100 g)			L-TEAC (µmol-αTE/100 g)	
	Total carotenoid content	Total xanthophyll content	Total carotene content	Total carotenoid content	Total xanthophyll content	Total carotene content	Peel	Pulp
Jeruk takengon	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	35 ± 5	8 ± 2
Jeruk takengon saponified	**28.8 ± 3.1**	**28.5 ± 3.1**	0.2 ± 0.0	**9.7 ± 1.1**	**9.7 ± 1.1**	**0.02 ± 0.0**	**56 ± 7**	**22 ±2**
Jeruk nipis	0.5 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	n.d	28 ± 14	10 ± 1
Jeruk purut	0.8± 0.0	0.6± 0.0	0.2± 0.0	0.1 ± 0.0	0.1 ± 0.0	n.d.	27 ± 2	5± 1
Calung	0.5 ± 0.0	0.4 ± 0.0	0.1± 0.0	n.d.	n.d.	n.d.	32 ± 7	12 ± 2
Makin	1.3 ± 0.3	0.9 ± 0.2	**0.5± 0.2**	**0.3 ± 0.0**	**0.2 ± 0.0**	0.1 ± 0.0	37 ± 3	**18 ± 2**
Kruet mameh	**2.4 ± 0.2**	**1.8 ± 0.1**	**0.6± 0.1**	0.1± 0.0	0.1± 0.0	n.d.	**43 ± 3**	7 ± 1
Mentui	0.1 ± 0.0	0.1 ± 0.0	n.d.	0.1 ± 0.0	0.1 ± 0.0	n.d.	25 ± 4	7 ± 1

n.d.:not detected, n.q.: not quantified

Spearman's correlation test illustrated the correlations between total carotenoid contents and antioxidant capacity (L-TEAC). Peel extracts showed higher and significant correlation compared to pulp (R^2= 0.702** and 0.374, respectively). Total carotenes content ((*all-E*)-β-carotene, (*all-E*)-α-carotene, (*9Z*)-β-carotene) had high and significant correlation, compared to total xanthophyll content (R^2= 0.752** and 0.376, respectively), suggesting carotenes from pulp had higher contribution to antioxidant capacity of citrus pulp extracts.

(*all-E*)-β-Cryptoxanthin (11 c.d.b) and (*all-E*)-zeaxanthin (11 c.d.b.) were found in saponified peel of jeruk takengon, followed by (*all-E*)-lutein and (*all-E*)-antheraxanthin (10 c.d.b.), and (*all-E*)-violaxanthin (9 c.d.b.). As pointed out before by Müller et al. (2011), the number of c.d.b. highly correlated with the antioxidant activity of carotenoids. β-Cryptoxanthin had comparable L-TEAC antioxidant activity when compared to α-carotene and β-carotene (10 and 11 c.d.b., consecutively). The difference in functional end chains of xanthophylls affected the total contribution to L-TEAC antioxidant capacity due to the presence of hydroxyl groups in each β-ionone ring in zeaxanthin and lutein (Müller, Fröhlich, and Böhm, 2011). The presence of epoxy groups in each ring in (*all-E*)-violaxanthin also greatly reduced its activity compared to corresponding (*all-E*)-antheraxanthin (one epoxy group in the β-ionone ring). At the same time, studies on L-TEAC antioxidant capacity of antheraxanthin and violaxanthin isomers are scarcely found, the previous study on isomers of lycopene, α-carotene and zeaxanthin (Böhm et al. 2002) showed varied results.

4.3.2 Other compounds with antioxidant properties in citrus

In manuscript 3, Figure 1, an attempt to identify the contribution of identified compounds to total antioxidant capacity (measured using H-TEAC) showed unidentified compounds contributing to the total antioxidant capacity of analyzed citrus samples. As a full characterization of polyphenols was not possible, there might be some classes of compounds not fully quantified. Flavonols and flavones, classes of flavonoids with excellent free radical scavenger activity, were not identified in the samples, despite some study report of their presence (Nogata et al., 2006).

Differences between individual phenolic compounds at concentrations found in the fruit and the antioxidant capacity of the entire fruit have been reported before (Rice-Evans, Miller, and Papanga 1997; Zheng and Wang 2003), in which the total antioxidant activity of the entire fruit was higher (Freeman, Eggett, and Parker 2010). The structure of phenolic compounds is greatly affecting its antioxidant activity. In citrus samples from Aceh, compounds identifications were focused mainly on phenolic and carotenoid classes while other classes of compounds were not studied. Furthermore, possible antagonistic interactions between those compounds were not studied. Myricetin, which has been reported to be present in the peel of the citrus fruits, was reported to exhibit antagonistic activity when present together with other phenolic compounds. Moreover, antagonistic activities between compounds present in the mixture of 2,3, 4 or 5 phenolic compounds solution also have been reported (Freeman et al. 2010).

Vitamin E, a strong free radical scavenger, is reported to be present in citrus. For vitamin E, mainly found in the peel and seeds of citrus fruits, the reported contents were up to 5.60 mg/kg, 4.50 mg/kg, and 11.40 mg/kg in orange (*C. sinensis*), tangerine (*C. reticulata*) and lemon (*C. limon*), respectively (Zou et al. 2015). Citrus fruits are also rich in minerals which have been reported to show antioxidant activity, i.e. Fe (iron), Mn (manganese), Cu (copper), Zn (zinc), Se (selenium) (Levander, Ager, and Beck, 1995; Zou et al. 2015). Limonoids, a class of terpenoids, were reported in abundance in citrus fruits. As much as 36 limonoid aglycones and 17 limonoid glycosides have been identified in citrus fruits (Zou et al., 2015). Some limonoids were reported to have higher antioxidant activity than vitamin C (Poulose, Harris, and Patil 2005). Limonoids contents in citrus were in the range of 0-96 mg/100 g (Zou et al. 2015).

4.3.3 Citrus anti-inflammatory activity

Anti-inflammatory activity is one of the bases of many other reactions in the body. Thus, some samples were analyzed for their anti-inflammatory activity, namely ethanolic extracts of peel and pulp of jeruk takengon, makin and calung, as they represented the class of citrus fruits with high hesperidin, neohesperidin and hesperidin, and neohesperidin and naringin contents, respectively (manuscript 3).

The test observed the inhibition of COX-2 enzyme to produce prostaglandin in the presence of ethanolic citrus extracts, polyphenol standards, and indomethacin as a positive control. Anti-inflammatory activities are involved in a large number of reactions, and inhibition of cyclooxygenase (COX) activity is one of them. COX-1 and COX-2 are two forms of cyclooxygenase identified in a human body. While COX-1 is often called as "house-keeping protein," overexpression of COX-2 has been related with the pathogenesis of cancer (O'Leary et al. 2004).

Unlike synthetic medicine to treat inflammation, which has a limitation in structure and pharmacological effects, natural plant compounds have more to offer. Different classes of compounds which have been reported to exert anti-inflammatory activities are summarized, and the lists comprise of polysaccharides, essential oils, alkaloids, flavonoids, terpenoids, polyphenols, quinones and phenylpropanoids (Wang et al. 2013).

Selected citrus sample extracts showed anti-inflammatory properties by inhibiting the production of PGE2 by COX-2. The results are also encouraging as production of PGE2 was lower than with indomethacin (in recommended concentration). The results moreover highlighted the potency of local citrus fruits as probable defense against inflammation. A previous study by Murakami et al. (2000) on the anti-inflammatory potency of various citrus cultivars, highlighted the antioxidative properties of old species of Dancy tangerine, and potent anti-inflammatory activity of nobiletin isolated from the peel of *C. nobilis.*

Alongside citrus fruits, other fruits with reported anti-inflammatory activity such as blackberry and raspberry fruits were investigated. The activity is believed to be related to their anthocyanin contents (Bowen-Forbes, Zhang, and Nair 2010). Intake of vegetables and fruits has been shown to be linked with anti-inflammatory activities and immune-modulatory properties (González-Gallego et al. 2014).

In the *in-vitro* tests, often samples tested were in excessive concentration compared to concentration in plasma. In addition, compounds metabolized inside the body resulted in reasonably different compounds. Thus, better understanding the bioavailability of citrus ingredients will be beneficial.

4.4 Antibacterial potency of citrus fruits

Citrus fruits are regularly used as antibacterial agents in households in Indonesia, notably in the skin. The peel or pulp either is rubbed onto the troubled skin directly or added to some other ingredients such as slaked lime (kapur sirih) (Dalimartha 2006). Juices of jeruk nipis and mentui are used to prepare fish, reducing the fishy smells, and also protecting the fish from bacteria, which would otherwise prefer humid and hot climate of Indonesia.

MeOH/THF extracts of the peel and pulp of citrus samples were used for the determination of antibacterial activity. Extracts were completely dried under a stream of N_2 gas and then weighed for the test. As the extracts contained both hydrophilic and lipophilic compounds, extracts solubility in the broth medium is important. Studies on antibacterial activity of flavonoids also suggested that different methods used were affecting the activity of the tested compounds, especially when methods relied heavily on the solubility of tested compounds in the test system (Cushnie and Lamb 2005). Thus, ensuring total extract solubility is important. In this study, the additions of 9.25% of DMSO and 0.75% of Tween-40 were needed in order to fully dissolve the extract in broth medium. The test showed that this solution did not affect the growth of tested bacteria (*S. aureus* ATCC 6538 and *K. pneumoniae* ATCC 2356).

Citrus fruits antibacterial activity has been reportedly linked with the presence of different compounds such as terpenes, limonoids and polyphenols (Ekwenye and Edeha 2010). Studies were also proposing the action because of the synergistic action of minor and major compounds found in citrus fruits rather than the action of a single compound only. Some bacteria are also susceptible to low pH. Thus, a high concentration of citric acid in citrus fruits also contributed to their antibacterial activity (Tomotake et al. 2006).

Antibacterial activities of MeOH/THF extracts of peel and pulp of citrus samples were presented in manuscripts 1 and 2. For ease of reading, the results are summarized in Table 5.

Table 5. Summarized antibacterial activities of MeOH/THF extracts of peel and pulp of citrus fruits (IC_{50} in mg/ml). Values in bold indicate highest activities within one column.

Sample	Peel				Pulp			
	K. pneumoniae (mg/ml)	Activity	*S. aureus* (mg/ml)	Activity	*K. pneumoniae* (mg/ml)	Activity	*S. aureus* (mg/ml)	Activity
Jeruk takengon	15.3± 2.9	Bacteriostatic	13.4± 0.3	Bacteriostatic	8.2± 2.3	Bacteriostatic	5.5± 0.8	Bacteriostatic
Jeruk takengon saponified	**3.0± 0.8**	Bacteriostatic	**1.6± 0.3**	Bacteriostatic	**2.2± 0.5**	Bacteriostatic	**1.6± 0.5**	Bacteriostatic
Jeruk nipis	4.2 ± 1.8	Bactericide	3.5 ±1.4	Bacteriostatic	4.1 ± 0.4	Bactericide	3.1 ± 0.6	Bactericide
Jeruk purut	4.6 ± 1.2	Bacteriostatic	4.8 ± 1.7	Bactericide	5.5 ± 1.1	Bactericide	3.4 ± 0.9	Bactericide
Calung	5.6 ± 0.7	Bacteriostatic	7.0 ± 1.9	Bacteriostatic	7.5 ± 1.1	Bactericide	6.2 ± 2.0	Bacteriostatic
Makin	**4.1 ± 0.9**	Bacteriostatic	**2.5 ± 0.5**	Bactericide	**3.3 ± 0.3**	Bactericide	**2.6 ± 0.6**	Bactericide
Kruet Mameh	4.3 ± 0.9	Bactericide	4.1 ± 1.0	Bactericide	6.8± 2.5	Bacteriostatic	4.7 ± 1.5	Bacteriostatic
Mentui	11.6 ± 3.5[a]	Bacteriostatic	4.8 ± 1.8	Bacteriostatic	6.3± 1.0	Bacteriostatic	4.1 ± 0.5	Bacteriostatic

Table 5 shows that jeruk takengon after saponification showed the strongest antibacterial activity compared to other citrus fruits, both in peel and pulp, against *S. aureus* and *K. pneumoniae*. Data analysis showed some compounds being responsible for this. Non-esterified xanthophylls in the peel and pulp of jeruk takengon were reported to have antibacterial activity. Hydrophobic substances act by their ability to compromise the structural cell wall and mitochondria of bacteria, prompting the leakage of cell and transport of ions and other cell components, resulting in cell death (Carson, Mee, and Riley 2002; Lambert et al. 2001; Sikkema, de Bont, and Poolman 1995).

Peel and pulp of jeruk takengon is rich in polyphenols. HPLC analysis showed hesperidin as the main flavanone in peel and pulp of jeruk takengon. Hesperidin has been reported to moderately to weakly inhibit the growth of different bacteria (Abuelsaad et al. 2013; Basile et al. 2000; Yi et al. 2008). The saponification step of peel and pulp extracts of jeruk takengon was initially performed to help characterization and quantification of carotenoids. Saponification also resulted in aglycones of glycosylated flavonoids, a class of compounds also present in unsaponified MeOH/THF extracts of peel and pulp of jeruk takengon. A recent study proposed that antibacterial activity of an aglycone is a determinant of compounds activities (Basile et al. 2000); suggesting that aglycones have better antibacterial activity compared to glycosides. Recently, it was reported that treatment of the flavonoid-rich fraction of bergamot peel with pectinase resulted in the conversion of flavonoid glycosides to their aglycones and increase in their antimicrobial activity, as well as proposing synergistic activity of some flavanones (Mandalari et al. 2007). This might be in correspondence with the strong antibacterial activity of saponified extracts of peel and pulp of jeruk takengon.

On the other hand, little is known of antibacterial activity of neohesperidin. Weak antibacterial activity has been reported of neohesperidin in inhibiting the growth of *B. subtilis*, *M. catarrhalis*, *S. aureus* and *P. Aeruginosa* (van der Watt and Pretorius 2001). Naringin showed weak or no antimicrobial activity. Han and You (1989) reported that naringin had antibacterial activity and its aglycone naringenin exhibited even higher antibacterial activity. On the other hand, different studies reported weak inhibition of fungal growth (el-Gammal and Mansour 1986) and no activity against different pathogenic bacteria (Céliz, Daz, and Audisio 2011). Thus, calung peel and pulp, which are rich in neohesperidin and naringin, showed low inhibitory activity.

Tangeretin, sinensetin and nobiletin have been reported to have antibacterial activity against *P. aeruginosa* (Yao et al. 2012), acting by compromising the structural integrity of bacterial cell walls. The extra saponification steps were not present in the preparation of MeOH/THF extracts, except for jeruk takengon. Tangeretin and nobiletin were found in EtOH/H_2O extracts of citrus samples as well as in MeOH/THF extracts. The presence of PMF has been reported in the polar extract (Stuetz et al. 2010) as well as in a less polar solvent (Johann et al 2007). The fact that peel and pulp of citrus fruits have comparable antibacterial activity, suggested that the polymethoxylated flavones might not be the main contributor of citrus antibacterial activity of our citrus samples. Additionally, a report has been resurfaced, contradicting the importance of PMF for the antibacterial potency of citrus fruits (Iranshahi et al. 2015).

4.5 Antidiabetic potency of citrus fruits

Diabetes is a disease characterized by a sharp increase in postprandial blood glucose level. There are two types of diabetes recognized: Type-1 diabetes (pancreas completely lack the ability to produce insulin due to autoimmune/infectious disease). Meanwhile, type-2 diabetes is a metabolic disorder characterized by insulin resistance, followed by the inability of pancreatic cells to compensate for insulin resistance and β-cells dysfunction (Unnikrishnan et al. 2014).

In the year of 2000, it was estimated that 2.2% of the world population was affected by diabetes mellitus, and the number was projected to rise to 4.4% in 2030 (Unnikrishnan et al. 2014). In Indonesia, DM is the third cause of death in adults (WHO, 2015). Currently, diabetes treatment relied on single compounds therapy, which usually addressed only problems in the complex diabetes problems. Thus, the resulting treatments disturbed body physiological balance (Kameswara Rao et al. 2003). Herbal treatments are often chosen as they are reported to have minorside-effects, better cost-effectivity and less possible interactions between compounds (Kameswara Rao et al. 2003).

In Indonesia, mainly fruits or vegetables with bitter taste are believed to have anti-diabetic properties. Citrus fruits, due to some of their bitter moiety, are also believed to be so. Traditionally, citrus fruits have been used as diabetic treatment, especially lime and kaffir lime (Abirami, Nagarani, and Siddhuraju 2014). Evolutionarily, flavonoids effectivity in modulating physiological anomaly is explained as follows: Human favors the food essential for their survival, especially fruits and vegetables, which are rich in flavonoids. In the diabetes front, plant flavonoids such as quercetin, kaempferol, baicalein, luteolin, apigenin, diosmetin, genistein, naringenin, chrysin, hesperidin, hesperetin, epicatechin, epigallocatechin, daidzein, myricetin and shamimin are reported to be promising sources for diabetes management (Kameswara Rao et al. 2003). Other compounds, i.e., terpenoids, phenolic acids and alkaloids are also believed to be beneficial in managing diabetes.

Ethanolic extracts of peel and pulp of citrus samples, which have been analyzed for their antioxidant capacities and total phenolic, flavonoid and ascorbic acid contents, were subjected to α-amylase inhibitory activity assay. For the same reason, the MeOH/THF extracts were also subjected to the α-amylase inhibition assay. The studies are reported in the manuscripts 1-3. In general, the study showed that hydrophilic as well as lipophilic extracts showed weak to moderate α-amylase inhibitory activity. This is a first clue to corroborate the potency of local citrus fruits from Aceh to treat type-2 diabetes. Phenolic acids, characterized in our citrus samples, have been reported to have antidiabetic properties (Jung et al. 2006; Punithavathi et al. 2011; Song et al. 2014). Notwithstanding from the results, studies have highlighted the potencies of citrus fruits in the treatment of diabetes. Naringenin has been demonstrated to inhibit glucose uptake in the intestine and renal brush border in an *in vivo* study in rats. Naringin, however, did not show such activities (Li et al. 2006). Hesperidin and naringin were also important in treating diabetes as they ameliorated hyperglycemia-induced oxidative damage and inflammation in HFD/STZ-induced diabetic rats (Mahmoud et al. 2012). Naringenin also has been proven to lower postprandial glucose intake in experimental rats (Ortiz-Andrade et al. 2008).

Nobiletin also displayed strong antidiabetic potency as it has been reported to improve hyperglycemia and insulin resistance in the obese diabetic mouse by regulating expression of Glut1 and Glut4 in white adipose tissue muscle and expression of adipokines in white adipose tissue (Lee et al. 2010). Tangeretin, together with nobiletin, also positively affected the accumulation of adipose (Miyata et al. 2011).

On the other hand, some reports also emerged on the correlation between carotenoids intake and lower diabetes risk. Sluijs et al. (2015) conducted a cohort study and reported that diets rich in α- and β-carotene were associated with lower risk of diabetes in men and women, while the association was not found for β-cryptoxanthin, lutein, lycopene and zeaxanthin.

Carotenoids antioxidant properties are believed to play an important role in reducing oxidative stress, which plays a vital role in the development of type 2 diabetes.

In conclusion, some citrus samples used in this study are an effective source of compounds to inhibit α-amylase activities. Nevertheless, our study only deals with the initial phase. Problems such as lipophilic extracts solubility and inactivity of hydrophilic extracts have to be addressed. Furthermore, α-glucosidase inhibition assay and *in vivo* anti-diabetic assay will be very important to further understand the antidiabetic potency of these citrus fruits. In conclusion, Table 4.5 describes classes/subclasses of active compounds, samples and their reported activities.

Table 6 Classes/subclasses of active compounds detected in citrus samples from Aceh and their reported activities

<table>
<tr><th colspan="2">Class/subclass of active compounds</th><th>Samples</th><th>Reported activity</th></tr>
<tr><td rowspan="2">Flavonoids</td><td>Flavanones</td><td>Jeruk takengon, Calung, Kruet Mameh</td><td rowspan="3">Total phenolic content (Folin Ciocalteu method), H-TEAC, H-ORAC, COX-2 inhibitory activity</td></tr>
<tr><td>PMF</td><td>Jeruk takengon, Calung, Kruet Mameh</td></tr>
<tr><td colspan="2">Ascorbic acid</td><td>Jeruk Purut, Calung, Jeruk Takengon, Jeruk Nipis</td></tr>
<tr><td colspan="2">Carotenoids</td><td>Jeruk Takengon, Kruet Mameh, Makin</td><td>L-TEAC, antibacterial activity, inhibition of α-amylase activity</td></tr>
</table>

Future reference

Despite the results reported here, there are still many aspects needed to be investigated in this topic. Complete identification of local citrus fruits from Aceh has to be done, either taxonomically or genetically. Regarding the bioactive compounds investigated, various mechanisms of action have to be studied in order to fully understand its biological properties. Further compounds characterization, such as minor flavonoids and carotenoids, also need to be elucidated.

5. Summary

Background

Citrus fruits are commonly found in tropical areas, including Indonesia. Aceh has different local citrus fruits found and the use of these local citrus fruits is limited to seafood preparation and traditional medicine. Limited data is available on biological potencies of these local citruses. Thus, studies are needed to further elucidate the potencies of these local citrus fruits.

Objectives

Objectives of the present thesis are 1) to characterize carotenoid profiles of local citrus fruits from Aceh as well as its biological potencies (lipophilic antioxidant capacity, antibacterial activity, antidiabetic activity), 2) to characterize polyphenol profiles of local citrus fruits from Aceh as well as its biological potencies (hydrophilic antioxidant activity, antidiabetic activity, antibacterial activity and antiinflammatory activity).

Methods

Two main solvents were used as extractants in this study, which were MeOH/THF (1+1; v/v) as lipophilic solvent and MeOH/H_2O (1+1; v/v) as hydrophilic solvent. Carotenoid profiles and quantifications were performed using HPLC-DAD and LC-MS. Lipophilic antioxidant capacity was measured using L-TEAC assay. Hydrophilic antioxidant activities were measured using H-TEAC, H-ORAC and TPC (Folin Ciocalteu and FBBB methods). Total flavonoid contents and vitamin C contents were also determined in correlation with the hydophilic antioxidant potencies. Hydrophilic and lipophilic antibacterial activities were measured against several bacterias using LNM methods. Antidiabetic activities were measured using α-amylase inhibition assay. Anti-inflamatory activity was monitored by COX-2 inhibitory activity.

Results

Xanthophylls were identified as the main carotenoids in citrus samples from Aceh, while lutein and zeaxanthin were the most common xanthophylls identified in all samples analyzed. Jeruk takengon had a more complex carotenoid profile compared to other analyzed citrus samples. (*9Z*)-Violaxanthin was the most abundant xanthophyll identified, followed by (*all-E*)-violaxanthin, in the pulp of jeruk takengon, while (*all-E*)-β-cryptoxanthin and (*9Z*)-violaxanthin were the most abundant carotenoids identified in peel, consecutively.

Flavanone groups were the most abundant flavonoids identified in peel and pulp of citrus samples from Aceh. Hesperidin was identified as the main flavanone in peel and pulp extracts of jeruk takengon, jeruk nipis and kruet mameh. Mixtures of hesperidin and neohesperidin were identified as main flavanones in fruit extracts of peel and pulp of jeruk purut, makin and mentui while a mixture of naringin and neohesperidin was identified in fruits extracts of calung.

Analysis on Total Flavonoid Content showed that calung showed higher flavonoid content, both in peel and pulp compared to other citrus fruits analyzed. Meanwhile, TPC FBBB test showed different results as jeruk nipis fruit extracts showed higher total phenolic content compared to other citrus fruits tested. Quantification of ascorbic acid showed the highest contents for jeruk purut and jeruk takengon in peel, while takengon and kruet mameh showed the highest ascorbic acid contents in pulp. Results on antioxidant capacity assays showed that kruet mameh in general showed high H-TEAC and L-TEAC antioxidant capacities while jeruk takengon extracts showed high antioxidant capacity based on H-TEAC, H-ORAC and TPC-FC.

α-Amylase inhibition assay showed that hydrophilic extract of kruet mameh showed the strongest inhibition activity in peel; while jeruk purut in pulp. For lipophilic extracts, peel of jeruk purut showed the strongest inhibition activity while jeruk nipis and makin showed strongest inhibition in pulp. In antibacterial activity tests, saponified extracts of peel and pulp of jeruk takengon and makin showed the strongest inhibition of *K. pneumonia* and *S. aureus*.

For the COX-2 inhibitory activities, pulp showed higher activity compared to its corresponding peel. Pulp extracts of jeruk takengon, makin and calung showed the highest COX-2 inhibitory activities consecutively.

Conclusion

Analysis of MeOH/THF (1+1; v/v) and EtOH/H_2O (1+1; v/v) extracts of peel and pulp of local citrus fruits from Aceh, Indonesia, showed that these extracts rich in biological compounds might be beneficial for human health. This study to some extend validated the traditional usage of citrus fruits in community. Jeruk takengon as the most famous citrus fruits in Aceh showed notable results with complex carotenoid and flavonoid profiles, high antioxidant capacity, and potent antimicrobial agents while calung and kruet mameh as one of rare citrus fruits showed high ascorbic acid content, inhibition of α-amylase and COX-2 inhibitory activities.

Zusammenfassung

Hintergrund

Zitrusfrüchte kommen häufig in tropischen Gebieten, einschließlich Indonesien, vor. Aceh hat verschiedene lokale Zitrusfrüchte, deren Verwendung auf die Zubereitung von Meeresfrüchten und die traditionelle Medizin beschränkt ist. Bisher liegen nur begrenzte Daten über die biologischen Aktivitäten dieser lokalen Zitrusfrüchte vor. Daher sind Studien erforderlich, um die Wirksamkeit dieser lokalen Zitrusfrüchte weiter zu untersuchen.

Ziele

Ziele der vorliegenden Arbeit sind 1) die Charakterisierung von Carotinoidprofilen lokaler Zitrusfrüchte aus Aceh sowie ihrer biologischen Wirkungen (lipophile antioxidative Kapazität, antibakterielle Aktivität, antidiabetische Aktivität), 2) die Charakterisierung von Polyphenolprofilen lokaler Zitrusfrüchte aus Aceh sowie ihrer biologischen Wirkungen (hydrophile antioxidative Aktivität, antidiabetische Aktivität, antibakterielle Aktivität und entzündungshemmende Aktivität).

Methoden

In dieser Studie wurden zwei Hauptlösungsmittel als Extraktionsmittel verwendet, nämlich MeOH/THF (1+1; v/v) als lipophiles Lösungsmittel und MeOH/H_2O (1+1; v/v) als hydrophiles Lösungsmittel. Carotinoidprofile und Quantifizierungen wurden mit HPLC-DAD und LC-MS durchgeführt. Die lipophile antioxidative Kapazität wurde mit dem L-TEAC-Assay gemessen. Hydrophile antioxidative Aktivitäten wurden mit H-TEAC, H-ORAC und TPC (Folin Ciocalteu und FBBB Methode) gemessen. Der Gesamtflavonoid- und Vitamin-C-Gehalt wurden ebenfalls in Korrelation mit den hydophilen Antioxidantien bestimmt. Hydrophile und lipophile antibakterielle Aktivitäten wurden mit Hilfe von LNM-Methoden gegen mehrere Bakterien gemessen. Die antidiabetische Aktivität wurde mit dem α-Amylase-Inhibitionsassay gemessen. Die antiinflammatorische Aktivität wurde durch COX-2-hemmende Aktivität untersucht.

Ergebnisse

Xanthophylle wurden als die wichtigsten Carotinoide in Zitrusproben aus Aceh identifiziert, wobei Lutein und Zeaxanthin die häufigsten Xanthophylle in allen analysierten Proben waren. Jeruk Takengon hatte ein komplexeres Carotinoidprofil im Vergleich zu den anderen analysierten Zitrusproben. (*9Z*)-Violaxanthin war das am häufigsten identifizierte Xanthophyll, gefolgt von (*all-E*)-Violaxanthin im Fruchtfleisch von Jeruk Takengon, während (*all-E*)-β-Cryptoxanthin und (*9Z*)-Violaxanthin die am häufigsten identifizierten Carotinoide im Fruchtfleisch waren.

Flavanongruppen waren die am häufigsten vorkommenden Flavonoide, die in Schalen und Fruchtfleisch von Zitrusproben aus Aceh nachgewiesen wurden. Hesperidin wurde als Hauptflavanon in Schalen- und Pulpaextrakten von Jeruk Takengon, Jeruk Nipis und Kruet Mameh identifiziert. Mischungen von Hesperidin und Neohesperidin wurden als Hauptflavanone in Fruchtextrakten aus Schalen und Fruchtfleisch aus Jeruk purut, Makin und Mentui identifiziert, während eine Mischung aus Naringin und Neohesperidin in Fruchtextrakten aus Calung identifiziert wurde.

Die Analyse des gesamten Flavonoidgehalts zeigte, dass Calung einen höheren Flavonoidgehalt sowohl in der Schale als auch im Fruchtfleisch zeigte, verglichen mit anderen analysierten Zitrusfrüchten. In der Zwischenzeit zeigte der TPC-FBBB-Test unterschiedliche Ergebnisse, da Jeruk-Nipis-Fruchtextrakte einen höheren Gesamtphenolgehalt an Phenol im Vergleich zu anderen getesteten Zitrusfrüchten aufwiesen. Die Quantifizierung der Ascorbinsäure zeigte die höchsten Gehalte für Jeruk Purut und Jeruk Takengon in der Schale, während Takengon und Kruet Mameh die höchsten Ascorbinsäuregehalte in der Pulpa aufwiesen. Die Ergebnisse der Untersuchungen zeigten, dass Kruet Mameh im Allgemeinen hohe H-TEAC- und L-TEAC-Werteaufwies, während Jeruk takengon-Extrakte eine hohe antioxidative Kapazität auf der Grundlage von H-TEAC, H-ORAC und TPC-FC aufwiesen.

Der α-Amylase-Inhibitionsassay zeigte, dass der hydrophile Extrakt von Kruet Mameh die stärkste Inhibitionsaktivität in der Schale zeigte, während Jeruk Purut in der Pulpa die höchste Aktivität aufwies. Für lipophile Extrakte zeigte die Schale von Jeruk Purut die stärkste Hemmungsaktivität, während Jeruk Nipis und Makin die stärkste Hemmung in der Pulpa zeigten. In antibakteriellen Aktivitätstests zeigten verseifte Extrakte aus Schale und Fruchtfleisch von Jeruk Takengon und Makin die stärkste Hemmung von *K. Pneumonie* und *S. aureus*.

Für die COX-2-hemmenden Aktivitäten zeigte Fruchtfleisch eine höhere Aktivität im Vergleich zu seiner entsprechenden Schale. Pulpa Extrakte von Jeruk Takengon, Makin und Calung zeigten die höchsten COX-2 hemmenden Aktivitäten.

Fazit

Die Analyse von MeOH/THF (1+1; v/v) und EtOH/H_2O (1+1; v/v) Extrakten aus Schalen und Fruchtfleisch lokaler Zitrusfrüchte aus Aceh, Indonesien, ergab, dass diese an biologischen Verbindungen reichen Extrakte für die menschliche Gesundheit von Vorteil sein können. Diese Studie bestätigte in gewissem Umfang die traditionelle Verwendung von Zitrusfrüchten in der Indonesien. Jeruk Takengon als die bekanntesten Zitrusfrüchte in Aceh zeigte bemerkenswerte Ergebnisse mit komplexen Carotinoid- und Flavonoidprofilen, hoher antioxidativer Kapazität, starken antimikrobiellen Wirkstoffen während Calung und Kruet Mameh als eher seltene Zitrusfrüchte einen hohen Ascorbinsäuregehalt zeigten, sowie Hemmung von α-Amylase und COX-2-hemmende Aktivitäten.

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Scientific Publication

Scientific Articles

Ernawita, Westphal, A., Malarski, A., Böhm, V.: Carotenoids, polyphenols, vitamin C and biological activities of citrus fruits from Aceh, Indonesia. *Lebensmittelchemie* 70 (2016) 124-125 (abstract)

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Jena, 20 May 2020

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