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Journal of Functional Food and Nutraceutical (JFFN) is an official journal of **Perhimpunan Penggiat Pangan Fungsional dan Nutrasetikal Indonesia or Indonesian Society for Functional Food and Nutraceutical (P3FNI-ISFFN)** that has been established in collaboration with **Research Center for Food and Health Swiss German University (RC F&H SGU).** JFFN publishes review and research result on frontier research, development, and application in the scope of functional food and nutraceuticals**.** The journal is expected to bring together all stakeholders in relation to the food ingredients and nutraceuticals.

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Journal of Functional Food and Nutraceutical

TINJAUAN MANFAAT BUNGA TELANG (*CLITORIA TERNATEA* L.) BAGI KESEHATAN MANUSIA

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ABSTRACT

All part of the butterfly pea (*Clitoria ternatea*) plant reported having a various positive effect on human health. The blue petal, in particular, shows a wide range of functional activity including as an antioxidant, antidiabetic, antiobesity, anticancer, anti-inflammatory, and antibiotic. The hydrophilic phase of butterfly pea flower extract contains flavonol glycosides, anthocyanins, flavones, flavonols, phenolic acids, and cyclotides. Meanwhile, the terpenoids, alkaloids, and fatty acids were found in the lipophilic phase of butterfly pea flower extract. The proven health benefits and the wide range of the type of bioactive compounds promote butterfly pea flower as the source of functional food and nutraceuticals. However, a series of intensive research, including the clinical trial, is still needed.

Keywords: *Butterfly pea; Clitoria ternatea; functional food; nutraceutical*.

ABSTRAK

Telang (*Clitoria ternatea*) merupakan salah satu dari tanaman yang semua bagiannya memiliki manfaat fungsional bagi tubuh manusia. Bagian kelopak bunganya dilaporkan bermanfaat sebagai antioksidan, antidiabetes, antiobesitas, antikanker, antiinflamasi, antibiotik dan melindungi jaringan hati. Berbagai komponen bioaktif ditemukan pada bunga telang, baik yang bersifat lipofilik maupun hidrofilik. Di antara komponen bioaktif yang dijumpai adalah flavonol glikosida, antosianin, flavon, flavonol, asam fenolat, senyawa-senyawa terpenoid dan alkaloid, serta senyawa-senyawa peptida siklik atau siklotida. Rentang manfaat yang luas menjadikan bunga telang sebagai salah satu bahan potensial baik untuk pangan fungsional maupun nutrasetikal. Sekalipun demikian, serangkaian penelitian hingga ke tahap uji klinis masih diperlukan.

Kata kunci: *Bunga telang; Clitoria ternatea; nutraseutikal; pangan fungsional.*

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PENDAHULUAN

Belakangan ini bunga telang (*Clitoria ternatea* L.) semakin populer di Indonesia sebagai bunga yang memberikan banyak manfaat kesehatan. Sajian minuman bunga telang atau dalam bentuk penganan lain semakin mudah dijumpai di restoran. Bunga telang, segar ataupun kering, kini relatif semakin ramai diperjualbelikan. Semakin banyak pula yang menanam tanaman bunga telang di pekarangan rumah untuk keperluan satu keluarga.

Informasi terkait manfaat atau yang diklaim sebagai manfaat bunga telang tersedia berlimpah di berbagai saluran internet: saluran berita, situs perusahaan atau organisasi, situs pribadi, dan media sosial. Kecondongan masyarakat sekarang untuk saling berbagi pengetahuan, cerita, atau pengalaman pribadi berkontribusi pula kepada semakin berlimpahnya informasi terkait manfaat bunga telang. Persoalannya, tidak semua informasi itu bersandar kepada hasil penelitian ilmiah. Sebagian tak jelas rujukan ilmiahnya. Ada pula yang sekadar pengalaman atau testimoni pribadi. Sebagian lagi berasal dari kepercayaan tradisional. Ada juga informasi ilmiah yang dilebih-lebihkan atau yang diinterpretasikan secara keliru. Telang merupakan tanaman yang seluruh bagiannya memiliki manfaat kesehatan. Sering khasiat bagian tanaman seperti akar, daun, atau biji, diklaim pula sebagai khasiat bunga. Kekeliruan ini bahkan ditemukan pula pada beberapa artikel ilmiah.

Jika demikian, seberapa jauh sesungguhnya manfaat bunga telang bagi kesehatan tubuh kita? Pertanyaan semacam ini tidaklah mudah untuk dijawab, bahkan boleh jadi tidak dapat tuntas terjawab. Akan tetapi, sebuah ulasan dipandang perlu untuk paling tidak memilah dan menelaah sebanyak mungkin data sehingga diperoleh informasi yang benar-benar merujuk kepada manfaat bunga telang yang didukung oleh hasil penelitian ilmiah. Hal inilah yang melatarbelakangi ditulisnya artikel ini. Bagian bunga dari tanaman telang menjadi pokok perhatian pada tulisan ini karena pemanfaatan bagian lain dari telang praktis belum banyak dijumpai di kalangan masyarakat Indonesia.

Ulasan di dalam artikel ini terbagi menjadi empat bagian: (i) bunga telang dalam pengobatan tradisional, (ii) sifat fungsional dan nutrasetikal bunga telang, (iii) komponen bioaktif pada bunga telang, dan (iv) tantangan bunga telang sebagai pangan fungsional dan nutrasetikal.

Bunga Telang Dalam Pengobatan Tradisional

Bunga telang (*Clitoria ternatea* L.), selanjutnya dalam artikel ini disebut sebagai ‘telang’ untuk membedakannya dengan ‘bunga telang’ yang merupakan bagian dari tanaman, merupakan tanaman merambat menahun yang tergolong dalam keluarga *Fabaceae* atau polong-polongan. Pohon, bunga, dan bagian-bagian bunga telang dapat dilihat pada Gambar 1. Tanaman ini tumbuh menyebar di berbagai belahan dunia beriklim tropis dan subtropis di benua Asia dan Pasifik, Amerika dan Karibia, Afrika, dan Australia (Gomez & Kalamani, 2003). Dari mana tanaman ini berasal masih belum dapat dipastikan. Telang tidak berasal dari Pulau Ternate, Maluku Utara meski memiliki nama ilmiah yang berkaitan (Fantz, 1991). Satu versi menyebutkan bahwa telang bersal dari wilayah Asia yang beriklim tropis (Gomez & Kalamani, 2003). Satu versi secara spesifik menyebutkan Asia Tenggara sebagai asal tanaman ini (Manjula *et al.*, 2013). Sementara itu, menurut versi lain telang disebutkan berasal dari Karibia, Amerika Tengah dan Meksiko (Mukherjee et al., 2008) atau Afrika (Poth *et al.*, 2011).

Telang merupakan herbal yang boleh dikata istimewa di dalam pengobatan tradisional. Seluruh bagiannya – mulai dari akar hingga bunga – dipercaya memiliki efek mengobati dan memperkuat kinerja organ (Mukherjee et al., 2008). Khasiat tanaman ini diakui di dalam pengobatan tradisional berbagai peradaban, terutama Asia dan Amerika. Fantz (1991) dan Mukherjee *et al*. (2008) merangkum khasiat seluruh bagian telang untuk mengobati berbagai penyakit dalam pengobatan tradisional Asia (Asia tenggara, Asia selatan, India, Pakistan, Sudan, Filipina, Jawa), Amerika (El Salvador, Kuba, Karibia) dan Afrika (Ghana). Manjula *et al*. (2013) secara khusus mengulas khasiat telang menurut

Gambar 1. (Kiri) Tanaman telang, (kanan atas) bunga telang, (kanan bawah) bagian-bagian bunga telang

tradisi pengobatan India. Di antaranya disebutkan manfaat telang (i) untuk mengobati insomnia, epilepsi, disentri, keputihan, gonorrhea, rematik, bronkhitis, asma, maag, tuberkulosis paru, demam, sakit telinga, penyakit kulit seperti eksim, impetigo, dan prurigo, sendi bengkak, kolik, sembelit, infeksi kandung kemih, asites (akumulasi kelebihan cairan pada rongga perut) (ii) untuk memperlancar menstruasi, melawan bisa ular dan sengatan kalajengking, (iii) sebagai antiperiodik (obat untuk mencegah terulangnya penyakit kambuhan seperti malaria), obat cacing, pencahar, diuretan, pendingin, pemicu mual dan muntah sehingga membantu mengeluarkan dahak bronkitis kronis, dan stimulan seksual. Sebagai tambahan, oleh masyarakat Arab Saudi daun, biji dan bunga telang dimanfaatkan untuk mengobati penyakit *liver* atau hati (Al-Asmari et al., 2014). Di Madagaskar daun telang digunakan untuk meredakan nyeri sendi (Jain et al., 2003). Di Myanmar campuran jus bunga telang dan susu digunakan untuk menyembuhkan sakit mata (DeFilipps & Krupnick, 2018). Sementara itu di Indonesia, khususnya masyarakat Betawi, bunga telang digunakan untuk membuat jernih mata bayi.

Dalam sistem pengobatan kuno India (Ayurveda), telang tergolong herbal yang penting. Hal ini dapat terlihat pada nama yang diberikan kepada telang dalam Bahasa Hindi, yaitu *aparajita* yang berarti 'yang tak terkalahkan'. Tanaman ini sekurang-kurangnya disebutkan pada dua kitab utama Ayurveda, yaitu *Charaka Samhita* dan *Sushruta Samhita* (Kumar et al., 2016). Peran terpenting telang di dalam Ayurveda adalah sebagai salah satu bahan dalam *Medhya Rasayana*, yakni campuran herbal yang dipercaya berkhasiat untuk meremajakan otak, menyembuhkan gangguan neurologis dan meningkatkan atau mempertahankan kecerdasan (Lijon et al., 2017). Tidak semua manfaat tersebut telah dibuktikan secara ilmiah, sehingga hanya dapat dipandang sebagai kearifan masa lalu yang dapat dikembangkan sebagai gagasan penelitian.

Sejak tahun 1950-an tanaman telang telah menjadi obyek penelitian ilmiah, khususnya untuk mengonfirmasi manfaatnya sebagaimana yang diklaim dalam pengobatan tradisional. Publikasi ilmiah pertama adalah pada tahun 1954 yang melaporkan kandungan asam lemak pada biji telang (Oguis et al., 2019). Sementara itu, manfaat fungsional telang yang pertama kali mendapatkan konfirmasi ilmiah adalah efek diuretik dari akar telang pada tahun 1962 (Oguis et al., 2019).

Sebagaimana telah disampaikan, tulisan ini khusus merangkum dan mengulas manfaat bunga telang

yang sudah mendapatkan bukti ilmiah. Sementara itu, bagian-bagian lain dari telang, yang juga banyak memiliki manfaat, berada di luar cakupan artikel ini.

Sifat Fungsional dan Nutraseutikal Bunga Telang

Dunia pengetahuan sudah lama menyadari manfaat produk-produk metabolisme sekunder untuk menopang kesehatan tubuh manusia. Di antara produk metabolisme sekunder itu adalah polifenol. Bunga telang adalah salah satu dari sumber tanaman dengan kadar polifenol relatif tinggi sehingga potensial memberikan manfaat kesehatan bagi manusia (Kamkaen & Wilkinson, 2009; Marpaung et al., 2013; Rabeta & An Nabil, 2013). Pada bab ini dirangkum berbagai penelitian yang mengungkapkan manfaat ekstrak bunga telang sebagai antioksidan, antidiabetes, anti-obesitas, anti-inflamasi, antimikroorganisme, antikanker, hepatoprotektif, dan beberapa manfaat fungsional lainnya.

Antioksidan

Stres oksidatif adalah keadaan yang tak seimbang antara produksi spesies oksigen reaktif dan mekanisme pertahanan antioksidan. Spesies oksigen reaktif (ROS) seperti hidrogen peroksida, anion superoksida, dan radikal hidroksil biasanya dihasilkan melalui jalur metabolisme aerobik dalam tubuh manusia. Jika berlebihan, ROS mengakibatkan kerusakan oksidatif pada biomolekul seluler termasuk DNA, protein, asam nukleat, dan lipida membran. Peningkatan stress oksidatif sangat berperan pada terjadinya berbagai penyakit degeneratif. Asupan antioksidan, menurut sejumlah penelitian, dapat mencegah terjadinya penyakit terkait stres oksidatif.

Aktivitas antioksidan dalam mengelola stres oksidatif pada sistem biologis berlangsung melalui berbagai mekanisme seperti penangkapan radikal bebas, penghambatan enzim oksidatif, sebagai pengkelat ion logam, dan sebagai kofaktor enzim antioksidan (Lakshan et al., 2019). Di antara metode yang umum untuk menguji kemampuan suatu sumber untuk menangkap radikal bebas adalah metode DPPH (*2,2-DiPhenyl 1-PicrylHydrazyl*), ABTS (*2,2'-Azinobis(3-ethylBenzoThiazoline-6-Sulfonate*), ORAC (*Oxygen radical absorbance capacity*), FRAP (*Ferric-Reducing Antioxidant Power*) dan TEAC (Trolox equivalent antioxidant capacity). Metode lain yang juga dikenal adalah HRSA (*Hydroxyl radical scavenging activity*), dan SRSA (*Superoxide radical scavenging activity*). Aktivitas antioksidasi bunga telang yang diekstraksi dengan berbagai prosedur dan pelarut serta diuji melalui berbagai metode telah dilaporkan pada sejumlah penelitian (Tabel 1).

Paling tidak ada tiga cara untuk menakar kemampuan suatu sumber sebagai antioksidan. Cara pertama, adalah mengukur seberapa banyak (dalam %) senyawa radikal yang dinetralkan oleh sumber antioksidan pada konsentrasi tertentu. Cara kedua, cara yang lebih umum dan komparatif, adalah menentukan konsentrasi sumber antioksidan untuk menetralkan 50% senyawa radikal, atau yang biasa dikenal dengan IC_{50}. Cara ketiga adalah dengan menentukan konsentrasi efisien untuk mencapai 50% dari respons maksimum dari suatu sumber atau EC_{50}. Dengan mengetahui IC_{50} atau EC_{50} kinerja suatu sumber antioksidan dapat dibandingkan dengan kinerja sumber antioksidan lain atau dengan kinerja antioksidan standar, biasanya adalah vitamin C (asam askorbat). Semakin kecil IC_{50} atau EC_{50} semakin efektif kerja suatu sumber sebagai antioksidan.

Sebagian besar peneliti menyebutkan bahwa kemampuan bunga telang untuk mereduksi senyawa radikal masih lebih rendah dibandingkan dengan kemampuan vitamin C (Rabeta & An Nabil, 2013; Srichaikul, 2018; Rajamanickam et al., 2015; Chayaratanasin et al., 2015; Phrueksanan et al., 2014). Rajamanickam et al. (2015) melaporkan bahwa IC_{50} ekstrak metanol bunga telang adalah 95,30 mg/ml, sedangkan vitamin C hanya 70,80 mg/ml. Menurut Phrueksenan et al. (2014) IC_{50} ekstrak air bunga telang adalah 0,47 mg/ml atau kira-kira 235 kali lebih tidak efektif dibandingkan vitamin C yang

Tabel 1. Aktivitas antioksidasi ekstrak bunga telang

Jenis Pelarut	Metode	Aktivitas Antioksidan				Referensi
		Konsentrasi (µg/ml)	% Penghambatan	IC_{50} (µg/ml)	EC_{50} (µg/ml)	
Air	DPPH			470		(Chayaratanasin et al., 2015)
				242		(Lakshan et al., 2019)
				1000		(Kamkaen & Wilkinson, 2009)
				84		(Iamsaard et al., 2014)
					0,76	(Siti Azima et al., 2017)
					0,43	(Srichaikul, 2018)
Metanol				95		(Rajamanickam et al., 2015)
Etanol				4000		(Kamkaen & Wilkinson, 2009)
Kloroform				132		(Rajamanickam et al., 2015)
Etil asetat				107		(Rajamanickam et al., 2015)
Air		25	391			(Rabeta & An Nabil, 2013)
		50	401			
		100	449			
		125	491			
		150	507			
Metanol		25	33			
		50	353			
		100	411			
		125	423			
		150	401			
Etanol		600	67			(Madhu, 2013)
Air	SRSA			26310		(Chayaratanasin et al., 2015)
Air	HRSA			19180		(Chayaratanasin et al., 2015)
Air	ABTS			0,1		(Srichaikul, 2018)
Air	ABTS	µM TEAC/g			4,16	(Siti Azima et al., 2017)
Air	FRAP	mmol FeSO4/mg			0,38	(Chayaratanasin et al., 2015)
					0,33	(Iamsaard et al., 2014)
		µg FeSO4/mg			0,78	(Srichaikul, 2018)
		mM TEAC/g			10,91	(Siti Azima et al., 2017)
Air	TEAC	mg TEAC/mg			0,17	(Chayaratanasin et al., 2015)
Air	ORAC	µM TEAC/g			15,76	(Siti Azima et al., 2017)

memiliki IC_{50} 0,002 mg/ml. Menurut (Iamsaard et al., 2014) IC_{50} ekstrak air bunga telang adalah 84,15 µg/ml, sedangkan IC_{50} asam askorbat adalah 5,34 µg/ml. Kontradiktif dengan para peneliti lain, Suganya et al., (2014) menyebutkan bahwa kemampuan ekstrak bunga telang untuk mereduksi senyawa radikal lebih tinggi dibandingkan dengan vitamin C.

Pada penelitian lain dilaporkan bahwa efektivitas bunga telang hanya 10,5% dari efektivitas Trolox untuk menangkap radikal hidroksil dan hanya 2% dari efektivitas Trolox untuk menangkap radikal superoksida. (Chayaratanasin et al., 2019). Uji aktivitas antioksidasi dengan berbagai metode menunjukkan bahwa ekstrak bunga telang memiliki kemampuan yang baik di dalam menangkap berbagai macam radikal bebas, tetapi tergolong sebagai pengkelat logam yang lemah (Chayaratanasin et al., 2015).

Studi terhadap aktivitas antioksidasi 15 jenis bunga menunjukkan bahwa ekstrak bunga telang merupakan salah satu dari bunga yang memiliki aktivitas antioksidasi paling tinggi (Vankar & Srivastava, 2010). Akan tetapi, menurut Siti Azima et al. (2017) aktivitas antioksidasi bunga telang masih lebih rendah dibandingkan dengan aktivitas kulit manggis (*Garcinia mangostana*), buah *Ardisia colorata*, dan buah jamblang (*Syzygium cumini*), baik dengan metode DPPH, ABTS, maupun FRAP. Berdasarkan metode ORAC aktivitas antioksidan bunga telang lebih baik dibadingkan dengan buah *Ardisia colorata* dan buah jamblang. Menurut (Lakshmeesh, 2019) bunga mawar lebih efektif dibandingkan dengan bunga telang sebagai antioksidan.

Ekstrak air bunga telang memiliki aktivitas antioksidasi yang lebih baik dibandingkan dengan ekstrak pelarut organik (Kamkaen & Wilkinson, 2009; Rabeta & An Nabil, 2013). Sementara itu, ekstrak metanol menghambat oksidasi dengan lebih baik dibandingkan dengan ekstrak etil asetat dan ekstrak kloroform (Rajamanickam et al., 2015). Hasil-hasil ini mengindikasikan bahwa fraksi hidrofilik (polar) bunga telang lebih berperan sebagai antioksidan daripada fraksi lipofilik atau nonpolarnya.

Potensi ekstrak bunga telang kemudian dipelajari lebih lanjut untuk melihat efektifitasnya di dalam melindungi sel dari kerusakan akibat oksidasi. Satu penelitian menunjukkan bahwa ekstrak bunga telang melindungi eritrosit anjing dari hemolisis dan kerusakan oksidatif yang disebabkan oleh 2,20 – azobis – 2 – metil - propanimidamide dihydrochloride (AAPH) (Phrueksanan et al., 2014). Ekstrak bunga telang juga efektif melindungi sel-sel kulit dari tekanan oksidatif yang diinduksi oleh hidrogen peroksida dan sinar ultraviolet, yang membuatnya potensial sebagai kosmetika untuk memperlambat kulit keriput (Zakaria et al., 2018). Dalam studi lain sifat antioksidan dalam ekstrak bunga telang memfasilitasi produksi nanopartikel magnesium oksida, bahan yang semakin banyak digunakan untuk aplikasi biomedis (Sushma et al. 2015).

Antidiabetes

Diabetes Mellitus (DM) merupakan gangguan metabolik yang ditandai oleh terjadinya hiperglikemia (gula darah tinggi), dislipidemia (gangguan metabolism lipoprotein), dan metabolism protein abnormal akibat terganggunya sekresi dan atau kerja insulin. Prosedur yang paling umum untuk menguji potensi antidiabetes suatu bahan adalah dengan mengukur efek hipoglikemia atau antihiperglikimia (menurunkan gula darah) bahan tersebut pada hewan percobaan, biasanya adalah tikus yang dibuat mengalami diabetes dengan cara diinduksi alloxan. Alloxan menyebabkan penurunan ekskresi insulin secara drastis akibat kerusakan sel-β pulau Langerhans pada pankreas, sehingga menginduksi terjadinya hiperglikemia.

Efek hipoglikemia ekstrak bunga telang telah dibuktikan melalui beberapa penelitian (Daisy et al., 2009; Rajamanickam et al., 2015; Chusak et al., 2018). Pemberian ekstrak air bunga telang secara oral (400 mg/kg berat badan) kepada tikus percobaan menurunkan glukosa serum dan glikosilasi hemoglobin, serta meningkatkan insulin serum, glikogen otot hati dan tulang (Daisy et al., 2009). Pemberian ekstrak metanol, etil asetat, atau kloroform sebanyak 300 mg/kg berat badan menunjukkan aktivitas hipoglikemia pada tikus albino yang lebih efektif daripada obat diabetes komersial glibencamide (10 mg/kg) (Rajamanickam et al., 2015). Ekstrak kloroform bekerja lebih baik dibandingkan dengan ekstrak etil asetat dan methanol yang mengindikasikan bahwa aktivitas hipoglikemia lebih dikontribusikan oleh komponen bioaktif non-polar. Aktivitas antihiperglikemia ekstrak bunga telang telah pula diamati pada 15 pria sehat berusia rata-rata 22,53 tahun dengan indeks massa tubuh rata-rata 21,57

kg/m^2 yang diberi diet minuman yang mengandung 50 g sukrosa. Setelah 30 menit konsumsi, subjek yang minum minuman mengandung sukrosa bersama dengan ekstrak bunga telang (2 g/400 ml air atau setara dengan 2,16 mg delfinidin 3-glukosida) memiliki kadar glukosa plasma dan insulin postprandial yang lebih rendah (Chusak et al., 2018). Selain itu, konsumsi ekstrak bunga telang juga meningkatkan kapasitas antioksidan plasma dan menurunkan kadar malondialdehida (MDA) yang merupakan penanda stress oksidatif (Chusak et al., 2018).

Mekanisme hipoglikemia ekstrak bunga telang diperkirakan melalui peningkatan sekresi insulin sebagaimana cara kerja glibencamide (Rajamanickam et al., 2015) yang ditandai dengan meningkatnya insulin serum dan kadar glikogen (Daisy et al., 2009).

Aktivitas antidiabetes suatu komponen aktif dapat pula melalui penghambatan pembentukan produk akhir glikasi lanjut (*advanced glycation end products* - AGEs). Ekstrak bunga telang pada konsentrasi 0,25-1,00 mg/ml dilaporkan secara signifikan menghambat pembentukan AGE, serta mengurangi kadar fruktosamin dan oksidasi protein dengan mengurangi kandungan karbonil protein dan mencegah penipisan tiol bebas. (Chayaratanasin et al., 2015).

Keadaan hiperglikemia dapat pula dihadang melalui penghambatan kerja enzim-enzim yang berhubungan dengan produksi glukosa dalam tubuh. Mukherjee et al. (2008) menyebutkan bahwa ekstrak etanol bunga telang menurunkan gula pada serum tikus diabetes melalui penghambatan aktivitas enzim β-galactosidase dan β-glucosidase, tetapi tak ada penghambatan terhadap aktivitas enzim β-d-fructosidase. Daisy et al. (2009) melaporkan bahwa ekstrak bunga telang menghambat aktivitas enzim glukoneogenik, glukosa-6- fosfatase, dan sebaliknya meningkatkan aktivitas enzim glukokinase. Glukokinase adalah enzim yang bertanggungjawab untuk mengubah glukosa menjadi glukosa 6-fosfat yang merupakan langkah pertama untuk membatasi metabolisme glukosa. Potensi ekstrak bunga telang untuk menghambat enzim α-amilase pankreas dan α-glukosidase usus besar telah pula dibuktikan (Adisakwattana et al., 2012). Penelitian yang lebih baru membuktikan bahwa 1% dan 2% (b/v) ekstrak bunga telang menghambat aktivitas enzim α-amilase pankreas dengan substrat zat pati yang berasal dari tepung kentang, singkong, beras, jagung, gandum, dan beras ketan sehingga diusulkan untuk digunakan sebagai bahan untuk mengurangi indeks glikemik berbagai jenis tepung (Chusak et al., 2018). Kemampuan ekstrak air bunga telang untuk menghambat kerja enzim α-amilase secara in vitro juga dilaporkan pada penelitian sebelumnya (Chu et al., 2017).

Upaya untuk meningkatkan efek hipoglikemia ekstrak bunga telang dengan cara mengombinasikannya dengan ekstrak buah lain menunjukkan hasil yang positif (Adisakwattana et al., 2012; Borikar et al., 2018). Kombinasi ekstrak bunga telang dengan rosela meningkatkan aktivitas penghambatan enzim α-amilase, sedangkan kombinasi ekstrak bunga telang dengan mulberi meningkatkan aktivitas penghambatan enzim α-glukosidase (Adisakwattana et al., 2012). Kombinasi bunga telang dan buah delima buah delima (*Punica gratum*) menghasilkan aktivitas hipoglikemik yang setara dengan obat diabetes metformin (Borikar et al., 2018).

Antiobesitas, Antihiperlipidemik dan Regulasi Kolesterol

Obesitas terkait dengan pembentukan jaringan lemak. Oleh karena itu potensi suatu bahan aktif sebagai antiobesitas seringkali dipelajari melalui kemampuannya menghambat adipogenesis (pembentukan jaringan lemak) pada preadiposit 3T3-L1 (lini sel yang diisolasi dari jaringan embrio tikus Swiss albino). Aktivitas anti adipogenesis ekstrak bunga telang baru-baru ini dilaporkan oleh Chayaratanasin, et al (2019).

Sementara itu, dalam rangkaian kajian terhadap aktivitas ekstrak bunga telang melawan diabetes pada tikus percobaan, peran bunga telang untuk menurunkan trigliserida dan total kolesterol darah dan meningkatkan kadar kolesterol-HDL telah pula dibuktikan (Daisy et al., 2009; Suganya et al., 2014; Rajamanickam et al., 2015).

Antikanker

Sekurang-kurangnya terdapat empat mekanisme dari suatu komponen zat aktif untuk melawan kanker: aktivitas antiproliferasi (mencegah atau memperlambat penyebaran sel kanker, penghambatan angiogenesis (pembentukan pembuluh darah baru), induksi apoptosis (sel kanker melakukan bunuh diri), pencegahan metastasis.

Aktivitas anti-proliferasi ekstrak bunga telang terhadap enam jenis lini sel kanker diamati oleh Neda et al. (2013). Peneliti ini melaporkan bahwa ekstrak air bunga telang potensial menghambat lini sel kanker payudara MCF-7 dan tidak efektif menghambat lini sel kanker payudara MDA-MB-231, lini sel kanker ovarium (Caov-3), lini sel kanker serviks (Hela), lini sel kanker hati (HepG2) dan lini sel kanker kelamin pria (Hs27). Analisis GC-MS (*Gas Chromatogram-Mass spectrometry*) menunjukkan bahwa dua komponen aktif pada ekstrak air bunga telang adalah mome inositol (38,7%) dan pentanal (14,3%) (Neda et al., 2013). Efektivitas ekstrak bunga telang untuk menghambat MCF-7 (IC_{50} = 1.14 mg/ml) dilaporkan pula oleh Akter et al. (2014). Akan tetapi, tak sejalan dengan Neda et al. (2013), Akter et al. (2014) melaporkan bahwa ekstrak bunga telang sangat efektif menghambat pertumbuhan sel MDA-MB-231 (IC_{50} = 0.11 mg/ml).

Aktivitas anti-proliferatif ekstrak lipofilik dan hidrofilik bunga telang terhadap lini sel kanker laring (Hep-2: human epithelial type 2) dilaporkan oleh (Shen et al., 2016) dengan ekstrak hidrofilik menunjukkan efektifitas yang lebih baik dibandingkan dengan ekstrak lipofilik. Penelitian ini membawa kepada satu perkiraan bahwa fraksi hidrofilik pada bunga telang berperan lebih efektif sebagai antikanker dibandingkan dengan fraksi lipofiliknya.

Efek sitotoksik in vitro dari ekstrak petroleum eter dan etanol bunga telang menggunakan metode *trypan blue exclusion* menunjukkan bahwa 500 mg/ml ekstrak petroleum eter dan metanol membunuh masing-masing 100% dan 80% lini sel DLA (*Dalton's lymphoma ascites*) (Shyam Kumar & Ishwar Bhat, 2011).

Aktivitas antikanker ekstrak metanol bunga telang melalui jalur induksi apoptosis dilaporkan terjadi pada lini sel kanker payudara MCF-7 (Shivaprakash et al., 2015). Terjadinya induksi apoptosis ditandai dengan fragmentasi DNA dan aktivasi enzim Caspase-3.

Angiogenesis adalah pembentukan pembuluh darah baru yang dilakukan oleh sel kanker untuk memperlancar pasokan makanan bagi pertumbuhan sel kanker. Angiogenesis juga memainkan peran penting dalam transisi tumor dari keadaan tak aktif ke stadium ganas. *Vascular endothelial growth factor* (VEGF) adalah protein yang memegang peran kunci di dalam angiogenesis. Ekstrak metanol bunga telang dilaporkan memiliki aktivitas menekan angiogenesis pada lini sel EAC (*Ehrlich ascites carcinoma*) dengan cara meregulasi sekresi VEGF. Ekstrak metanol bunga telang juga terlihat menekan aktivitas HIF-1α (Hypoxia Inducible Factor-1α) yang diperkirakan dapat menjadi satu pendekatan baru dalam penghambatan pertumbuhan sel kanker (Balaji et al., 2016).

Antiinflamasi dan Analgesik

Inflamasi atau peradangan adalah upaya perlindungan tubuh yang bertujuan untuk menghilangkan rangsangan berbahaya, termasuk sel-sel yang rusak, iritasi, atau patogen dan memulai proses penyembuhan. Antiinflamasi adalah karakteristik yang dimiliki oleh suatu zat atau komponen untuk mengurangi peradangan atau peradangan. Bahan antiinflamasi memiliki kemampuan analgesik yang memengaruhi sistem saraf untuk menghambat sinyal nyeri ke otak.

Efek antiinflamasi dan analgesik ekstrak petroleum eter bunga telang (masing-masing dengan kadar 200 mg/kg berat badan dan 400 mg/kg berat badan) pada tikus percobaan dilaporkan oleh Shyam kumar & Ishwar (2012). Sekalipun demikian, efektifitasnya masih jauh lebih rendah dibandingkan dengan obat antiinfilamasi dan analgesik komersial (diclofenac sodium dan pentazocine).

Aktivitas inflamasi secara in vitro (penghambatan denaturasi albumin) dari ekstrak etanol bunga telang dipelajari Suganya et al. (2014) pada

berbagai konsentrasi. Hasilnya menunjukkan bahwa kinerja ekstrak bunga telang setara dengan kinerja aspirin (Suganya et al., 2014).

Efek antiinflamasi ekstrak bunga telang pada peradangan yang diinduksi oleh lipopolisakarida pada lini sel makrofag RAW 264.7 dilaporkan oleh Nair et al. (2015). Hasil ini menunjukkan potensi bunga telang sebagai bahan nutrasetikal untuk perlindungan terhadap penyakit peradangan kronis dengan menekan produksi mediator pro-inflamasi yang berlebihan dari sel makrofag (Nair et al., 2015). Sebagai kompleks dengan sumber antosianin lain, ekstrak juga menunjukkan aktivitas antiinflamasi yang potensial (Priprem et al., 2015; Intuyod et al., 2014)

Antiasma

Salah satu khasiat bunga telang yang dipercaya di dalam pengobatan tradisional India adalah untuk menyembuhkan asma dan meredakan batuk. Asma merupakan gangguan inflamasi kronik pada saluran pernapasan yang dapat menyebabkan penderitanya mengalami batuk dan sesak napas. Serangkaian studi telah dilakukan untuk mengonfirmasi kinerja bunga telang sebagai antiasma dan pereda batuk (Singh et al., 2018). Rangkaian studi itu meliputi aplikasi ekstrak bunga telang dosis tinggi (100, 200, dan 400 mg/kg berat badan hewan percobaan) sebagai antiasma akut dan kronis, meredakan batuk yang diinduksi sulfur dioksida dan asam sitrat, serta aktivitas anti-inflamasi pada tikus yang diinduksi karagenan dan asam asetat. Rangkaian studi tersebut menghasilkan satu kesimpulan bahwa ekstrak bunga telang yang terstandar berpotensi sebagai terapi alternatif dalam penanganan asma yang diinduksi oleh alergi (Singh et al., 2018).

Antimikroorganisme

Bunga telang yang diekstraksi menggunakan berbagai pelarut menunjukkan rentang aktivitas antimikroorganisme yang luas meliputi bakteri gram positif, bakteri gram negatif maupun fungi (Tabel 2). Di antara aktivitas yang perlu digarisbawahi adalah ekstrak bunga telang menghambat pertumbuhan tiga bakteri patogen yang paling banyak ditemukan pada permukaan tanah, yaitu *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Kamilla et al., 2009; Uma et al., 2009; Pratap et al., 2012; Mahmad et al., 2018). Ekstrak bunga telang juga menghambat pertumbuhan beberapa bakteri patogen penghasil enzim extended-spectrum beta-lactamase (ESBL) yaitu *E. coli*, *Enteropathogenic E. coli* (EPEC), *Enterotoxigenic E. coli* (ETEC), *Klebsiella peumoniae* dan *Pseudomonas aeruginosa* (Kamilla et al., 2009; Uma et al., 2009; Pratap et al., 2012). ESBL adalah enzim yang menyebabkan bakteri tahan terhadap berbagai macam antibiotik seperti penisilin dan sefalosporin. Ekstrak bunga telang juga dilaporkan menghambat pertumbuhan tiga bakteri penyebab kerusakan gigi, yaitu *Streptococcus mutans*, *Lactobacillus casei*, dan *Staphylococcus aureus* (Pratap et al., 2012). Penelitian Kamilla et al. (2009) menunjukkan bahwa ekstrak methanol bunga telang menghambat pertumbuhan bakteri *Salmonella typhi*, sementara Uma et al. (2009) melaporkan bahwa ekstrak methanol, kloroform dan air bunga telang tidak dapat menghambat pertumbuhan bakteri *Salmonella typhimirium* dan *S. entritidis*.

Efektivitas antimikroorganisme bunga telang dipengaruhi oleh jenis pelarut yang digunakan dalam ekstraksi. Uma et al. (2009) menyebutkan bahwa ekstrak petroleum eter dan heksana tidak menunjukkan aktivitas antimikroorganisme, sedangkan aktivitas penghambatan mikroorganisme ekstrak methanol lebih tinggi dibandingkan dengan ekstrak kloroform dan air. Sementara itu Mahmad et al. (2018) melaporkan bahwa ekstrak etanol mampu menghambat pertumbuhan beberapa jenis bakteri dan fungi, tetapi ekstrak air tidak menunjukkan efek antimikroorganisme. Secara umum, methanol dan etanol adalah pelarut terbaik untuk ekstraksi komponen bioaktif bunga telang sebagai antimikroorganisme.

Hepatoprotektif

Aktivitas bunga telang untuk mencegah kerusakan hati (efek hepatoprotektif) dilaporkan oleh Nithianantham, et al. (2013). Pada penelitian mereka ekstrak bunga telang diberikan kepada tikus percobaan yang diinduksi asetaminofen secara berlebihan sehingga mengalami kerusakan

hati. Aktivitas hepatoprotektif dievaluasi dengan memantau kadar enzim aspartat aminotransferase dan alanin aminotransferase, serta kadar bilirubin dan glutation melalui analisis hispatologis. Hasil percobaan menunjukkan bahwa tikus yang diberi ekstrak bunga telang (200 mg/kg) mengalami penurunan kadar keempat senyawa indikator kerusakan hati.

Tabel 2. Aktivitas antimikroba pada bunga telang

Mikroorganisme	Pelarut	Konsentrasi (mg/ml)	Zona Hambatan (mm)	Pustaka
Bakteri Gram Positif				
Bacillus cereus	Metanol	100	14±1	Kamilla et al., 2009
	Etanol	50	14,5 ± 2,1	Leong et al., 2017
Bacillus subtilis	Metanol	100	2,7±1,1	Kamilla et al., 2009
	Etanol	50	15,8 ± 1,7	Leong et al., 2017
	Etanol	Tds	10,0 ± 0,3	Mahmad et al., 2018
Bacillus thuringiensis	Metanol	100	15,7±0,6	Kamilla et al., 2009
Staphylococcus aureus	Metanol	100	13±1	Kamilla et al., 2009
	Etanol	50	13,4 ± 1,4	Leong et al., 2017
	Air	50	8	Pratap et al., 2012
	Etanol	Tds	7,0 ± 0,7	Mahmad et al., 2018
Streptococcus faecalis	Metanol	100	12 ± 1	Kamilla et al., 2009
Streptococcus mutans	Air	50	7	Pratap et al., 2012
Bakteri Gram Negatif				
Escherichia coli	Metanol	100	13,3 ± 0,6	Kamilla et al., 2009
	Air	4*	12	Uma et al., 2009
	Metanol	4*	20	Uma et al., 2009
	Kloroform	4*	18	Uma et al., 2009
	Etanol	Tds	8,0 ± 0,5	Mahmad et al., 2018
Enteropathogenic E. coli	Air	4*	12	Uma et al., 2009
	Metanol	4*	16	Uma et al., 2009
	Kloroform	4*	14	Uma et al., 2009
Enterotoxigenic E. coli	Air	4*	12	Uma et al., 2009
	Metanol	4*	16	Uma et al., 2009
	Kloroform	4*	14	Uma et al., 2009
Klebsiella pneumoniae	Metanol	100	12,7 ± 0,6	Kamilla et al., 2009
	Air	4*	12	Uma et al., 2009
	Metanol	4*	26	Uma et al., 2009
	Kloroform	4*	18	Uma et al., 2009
	Etanol	50	12,0 ± 0,4	Leong et al., 2018
Pseudomonas aeruginosa	Metanol	100	11,3 ± 1,5	Kamilla et al., 2009
	Air	4*	12	Uma et al., 2009
	Metanol	4*	26	Uma et al., 2009
	Kloroform	4*	16	Uma et al., 2009
Salmonella typhi	Metanol	100	10,3 ± 1,1	Kamilla et al., 2009
Enterobacter aerogens	Metanol	100	13 ± 1	Kamilla et al., 2009
Proteus mirabilis	Metanol	100	13,7 ± 2,9	Kamilla et al., 2009
	Etanol	50	14,0 ± 1,1	Leong et al., 2017
Herbaspirillum spp	Metanol	100	11,3 ± 2,3	Kamilla et al., 2009
Streptococcus mutans	Air	50	7	Pratap et al., 2012
Lactobacillus casei	Air	50	8	Pratap et al., 2012
Antifungi				
Candida albicans	Metanol	100	19	Kamilla et al., 2009
Rhizopus	Metanol	100	11 ± 1	Kamilla et al., 2009
Penicillium spp	Metanol	100	8,33 ± 0,6	Kamilla et al., 2009
Penicillium expansum	Etanol	50	15,5 ± 1,3	Leong et al., 2017
Fusarium sp.	Etanol	Tds	10,0 ± 0,6	Mahmad et al., 2018
Trichoderma sp.	Etanol	Tds	8,0 ± 0,5	Mahmad et al., 2018

tds = tidak disebutkan; *konsentrasi dalam mg/disc

Komponen Bioaktif Pada Bunga Telang

Kinerja farmakologis bunga telang merupakan kontribusi dari berbagai komponen aktif, baik yang berasal dari metabolism primer maupun sekunder, baik yang bersifat hidrofilik maupun lipofilik. Pada bab ini dibahas komponen aktif pada bunga telang yang telah berhasil diidentifikasi hingga saat ini.

Komponen metabolit primer utama pada bunga telang adalah lemak, yaitu sebanyak 32,9% per berat kering. Berikutnya adalah karbohidrat (29,3%) dan serat kasar (27,6%). Sementara itu, protein dijumpai dalam kadar yang relatif kecil (4,2%) (Neda et al., 2013). Komponen bioaktif pada bunga telang yang diperkirakan memiliki manfaat fungsional berasal dari berbagai kelompok senyawa fitokimia, yaitu fenol (flavonoid, asam fenolat, tanin, dan antrakuinon), terpenoid (triterpenoid, saponin tokoferol, fitosterol), dan alkaloid.

Komponen bioaktif lipofilik terdapat dalam jumlah lebih banyak dibandingkan dengan komponen hidrofilik, masing-masing sebesar 27,67 dan 11,08 mg/100 g bunga segar (Shen et al., 2016). Pada fraksi lipofilik, yang paling banyak adalah kelompok fitosterol dan asam lemak. Sementara itu pada fraksi hidrofilik yang terbanyak adalah antosianin dan flavonol glikosida (Kazuma et al., 2003; Shen et al., 2016).

Menurut sumber lain kandungan total senyawa fenol pada bunga telang berkisar antara 53-460 mg ekuivalen asam galat per gram ekstrak kering (Adisakwattana et al., 2012; Chayaratanasin et al., 2015; Singh et al., 2018). Senyawa-senyawa fenol tersebut terdiri dari flavonoid, asam fenolat dan tanin. Komponen bioaktif bukan fenol yang telah diidentifikasi pada bunga telang adalah kelompok senyawa fitosterol, terpena, gula alkohol, alkil aldehida dan peptida.

Flavonoid

Satu gram ekstrak kering bunga telang mengandung flavonoid rata-rata 11.2 mg ekuivalen katekin (Chayaratanasin et al., 2015). Flavonoid 25,8 mg setara kuersetin per gram ekstrak (Singh et al., 2018). Komponen flavonoid pada bunga telang adalah flavonol, antosianidin, flavanol, dan flavon (Gambar 2).

Flavonol glikosida

Di dalam bunga telang flavonol dijumpai dalam bentuk glikonnya, yaitu flavonol glikosida, yang terdiri dari kaempferol 3-glukosida (kaempferol 3-(2-rhamnosilrutinosida), kaempferol 3-neohesperidosida, kaempferol 3-(2-rhamnosil-6-malonil) glukosida, kaempferol 3-rutinosida), kuersetin 3-glukosida (kuersetin 3(2-rhamnosilrutinosida), kuersetin 3-neohesperidosida, kuersetin 3-rutinosida, kuersetin 3-glucosida) dan mirisetin 3-glikosida (mirisetin 3-(2-rhamnosilrutinosida)) (Kazuma et al., 2003).

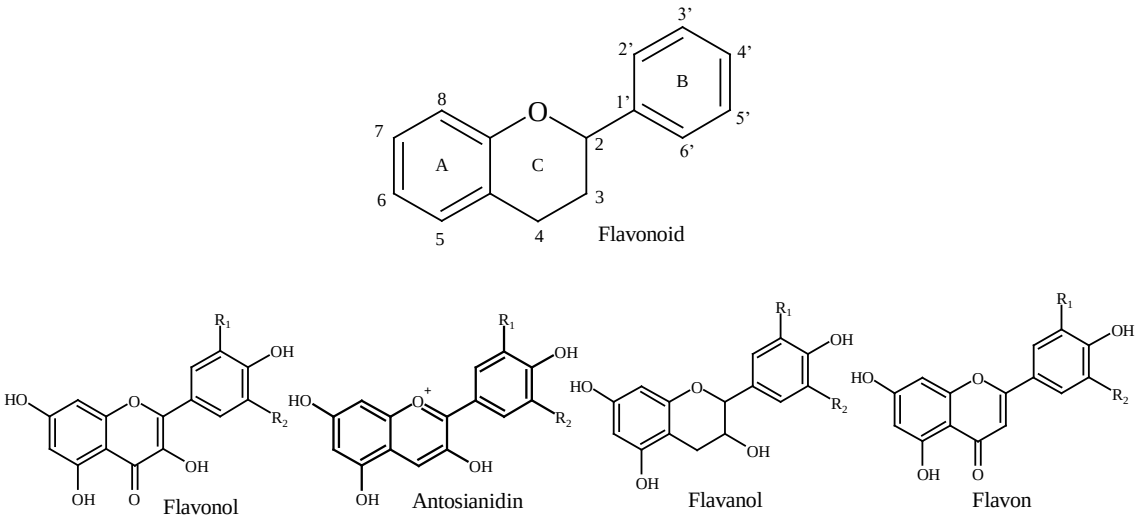
Flavonol glikosida merupakan flavonoid yang paling banyak dijumpai pada bunga telang, dan yang paling utama adalah kaempferol 3-glikosida yang kandungannya sekitar 87% total flavonol glikosida (Kazuma et al., 2003).

Kaempferol, kuersetin, dan mirisetin merupakan komponen bioaktif yang mudah diperoleh di berbagai jenis tanaman. Senyawa-senyawa flavonol ini memiliki manfaat kesehatan yang luas. Kuersetin dikenal sebagai antioksidan diet yang paling menonjol (Boots et al., 2008).

Antosianin

Sama dengan flavonol, antosianidin dalam bunga telang dijumpai dalam bentuk glikonnya, antosianin. Karakteristik bunga yang paling menonjol secara telang secara visual adalah warnanya yang biru pekat yang disebabkan oleh antosianin yang dikandungnya. Sekalipun demikian, antosianin bukanlah flavonoid yang paling banyak di dalam bunga telang. Fraksi antosianin hanya sekitar 27% dari total flavonoid dalam bunga telang (Kazuma et al., 2003).

Antosianin bunga telang merupakan antosianin terpoliasilasi (memiliki lebih dari dua gugus asil) dengan delfinidin sebagai aglikonnya. Antosianin terpoliasilasi memiliki kestabilan lebih tinggi dibandingkan dengan jenis antosianin yang tak memiliki gugus asil.

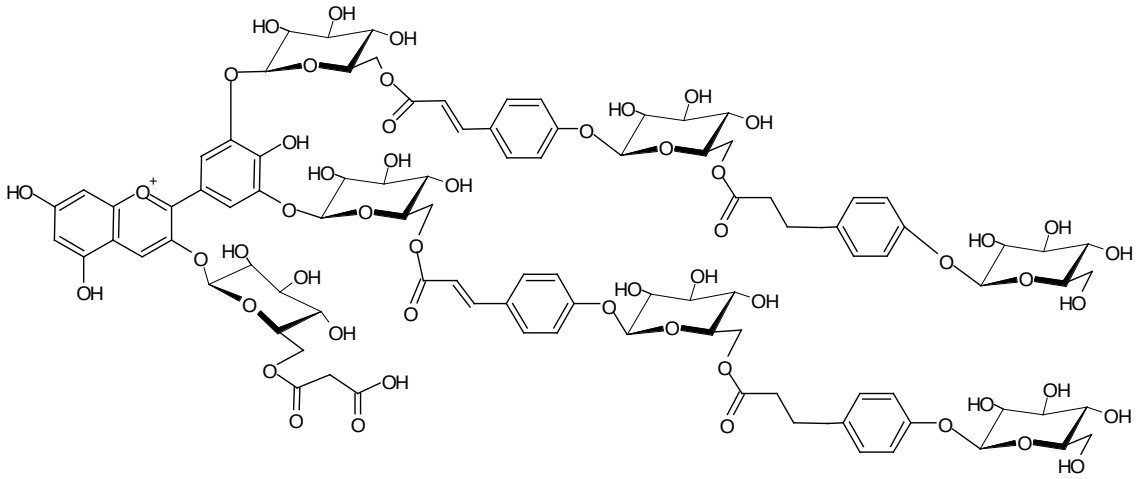


Gambar 2. Struktur dasar flavonoid dan turunannya: flavonol, antosianidin, flavanol, dan flavon

Isolasi antosianin bunga telang dilakukan oleh Kondo et al. pada tahun 1985 yang kemudian dilanjutkan oleh beberapa peneliti Jepang hingga tahun 2003. Oleh karena struktur molekulnya yang khas, antosianin pada bunga telang diberi nama khusus, yaitu ternatin. Hingga saat ini telah berhasil diidentifikasi 9 jenis ternatin pada bunga telang yang telah mekar sempurna (ternatin A1, A2, A3, B1, B2, B3, B4, D1, dan D2). Sementara itu ada tambahan 6 jenis antosianin lagi pada bunga yang masih kuncup (ternatin C1, C2, C3, C4, C5, D3, Preternatin A3 dan C4) (Terahara et al., 1990; Terahara et al., 1989; Kondo et al., 1990; Terahara et al., 1998; Terahara et al., 1996; Kazuma et al., 2003). Antosianin yang paling kompleks adalah ternatin A1 (Terahara et al., 1990), sedangkan yang paling banyak adalah ternatin B2 dan B1 (Kazuma et al., 2003). Hingga saat ini ternatin A1 (Gambar 3) merupakan antosianin yang diketahui memiliki struktur paling kompleks.

Antosianin secara umum dikenal sebagai kelompok pigmen larut air yang memiliki manfaat fungsional yang luas. Semua antosianin adalah antioksidan dan merupakan anggota keluarga flavonoid dengan aktivitas antioksidan paling tinggi. Aktivitas antioksidan antosianin adalah karena kemampuannya menyumbang hidrogen kepada radikal dan membantu mengakhiri reaksi radikal berantai (Iversen, 1999). Aktivitas antioksidan antosianin yang satu berbeda dengan antosianin yang lain tergantung kepada bergantung kepada jumlah dan susunan gugus hidroksil dan gula terkonjugasi.

Selain itu, antosianin juga menunjukkan sifat anti-virus, antiinflamasi, antioksidan, anti-alergi, dan antimikroba, antikanker, anti-arteri aterosklerosis, anti-hipertensi, mencegah diabetes, melindungi sistem kardiovaskular dari kerusakan dan banyak manfaat kesehatan lainnya (Ghosh & Konishi, 2007; Khoo et al., 2017). Studi klinis telah menunjukkan efek menguntungkan antosianin pada manusia seperti meningkatkan kadar kolesterol HDL dan menurunkan kadar kolesterol LDL pada subyek dislipidemik, mengurangi risiko infark miokard pada wanita muda dan setengah baya, dan mengurangi risiko penyakit kardiovaskuler (Intuyod et al., 2014).



Gambar 3. Struktur molekul ternatin A1 dengan rangka utama delfinidin, tujuh molekul glukosa, empat gugus asil dan satu malonat.

Akan tetapi masih sangat sedikit studi terkait efek fungsional dari antosianin seri ternatin yang khas dimiliki oleh bunga telang. Hingga saat ini baru ada dua laporan yang berhasil dilacak terkait efek fungsional ternate. Pertama, aktivitas ternatin D1 untuk menghambat agregasi platelet secara in vitro (Kshirsagar et al., 2015). Kedua, ternatin menghambat translokasi NF-κB nuklir, ekspresi protein iNOS, dan produksi NO (Nair et al., 2015).

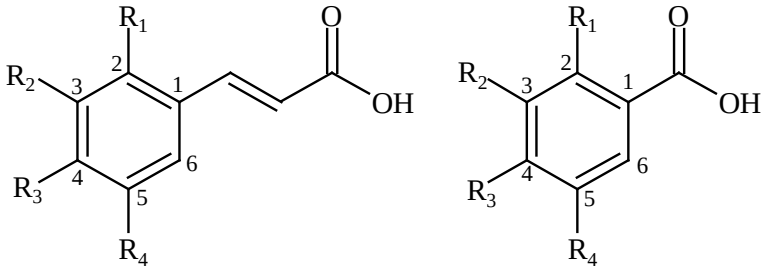
Flavon dan flavanol

Dalam riset mereka untuk memahami bagaimana bunga telang memiliki kemampuan menghambat angiogenesis pada sel kanker, Balaji et al. (2016) mengidentifikasi keberadaan empat senyawa flavon di dalam ekstrak methanol bunga telang. *Scutellarin* adalah yang paling banyak (36,9%), diikuti oleh *baicalein* (12,6%), luteolin (9,3%) dan apigenin (6,3%). Keempat senyawa flavon tersebut diketahui memiliki efek antikanker pada sejumlah sel kanker melalui beberapa mekanisme. Efek farmakologis termasuk sebagai zat antikanker dari *scutellarin*, apigenin, *baicalein* dan luteolin telah dibahas mendalam pada beberapa referensi (Wang & Ma, 2018; Salehi et al., 2019; Liu et al., 2016; Lin et al., 2008)

Satu-satunya senyawa flavanol yang telah dipastikan ada dalam bunga telang adalah epikatekin (Siti Azima et al., 2017). Epikatekin merupakan senyawa polifenol yang banyak dijumpai pada coklat dan teh. Bersama dengan flavon, katekin merupakan flavonoid yang paling tangguh melindungi tubuh dari spesies oksigen reaktif (Tapas et al., 2008).

Asam Fenolat

Asam fenolat terbagi ke dalam dua kelompok senyawa, yaitu asam hidroksisinamat dan asam hidroksibenzoat (Gambar 4). Asam hidroksisinamat lebih banyak dijumpai di alam. daripada asam hidroksibenzoat (Kumar et al., 2014). Para peneliti menunjukkan hasil penelitian yang tidak selalu sejalan dengan jenis asam fenolat apa saja yang terdapat pada bunga telang, hal ini disebabkan oleh perbedaan pelarut untuk ekstraksi dan prosedur isolasi. Secara keseluruhan, asam hidroksisinamat yang dijumpai pada bunga telang adalah asam klorogenat, asam galat, asam p-kumarat, asam kafeat, asam ferulat, sedangkan asam hidroksibenzoat pada bunga telang adalah asam protokatekuat, asam p-hidroksibenzoat, asam siringat dan asam vanilat (Kaisoon et al., 2011; Siti Azima et al., 2017; Pengkumsri et al., 2019). Menurut Siti Azima et al. (2017) urutan asam fenolat yang terdapat paling banyak adalah asam protokatekuat (72 mg/100 g), asam galat (67 mg/100 g) dan asam klorogenat (54 mg/100 g).



Asam hidroksisinamat Asam hidroksibenzoat

Gambar 4. Struktur dasar asam fenolat: asam hidroksisinamat dan asam hidroksibenzoat

Asam fenolat adalah salah satu fitokimia fenol yang paling penting (Dillard & German, 2000). Sama dengan antosianin, semua asam fenolat adalah antioksidan. Asam fenolat memiliki sifat antioksidan karena potensi redoksnya yang tinggi, yang memungkinkan mereka bertindak sebagai agen pereduksi dan mengikat oksigen singlet (Kumar et al., 2014). Di antara keluarga asam fenolat, asam klorogenat adalah yang paling berlimpah di alam sekaligus antioksidan yang paling kuat (Larson, 1988).

Satu catatan menarik, asam protokatekuat dan asam klorogenat merupakan produk akhir dari degradasi antosianin. Oleh karena itu, ketika antosianin terdegradasi menjadi kedua asam fenolat sehingga kehilangan warna secara permanen, aktivitas antioksidannya tetap bertahan.

Terpenoid

Hingga saat ini kelompok senyawa terpenoid yang berhasil ditemukan pada bunga telang adalah triterpenoid (yang kemudian diidentifikasi sebagai tarakserol), fitosterol, dan tokoferol (Gambar 5) (Shyam Kumar & Ishwar Bhat, 2011; Shyam Kumar & Ishwar Bhat, 2012; Suganya et al., 2014; Shen et al., 2016; Singh et al., 2018; Zakaria et al., 2018)

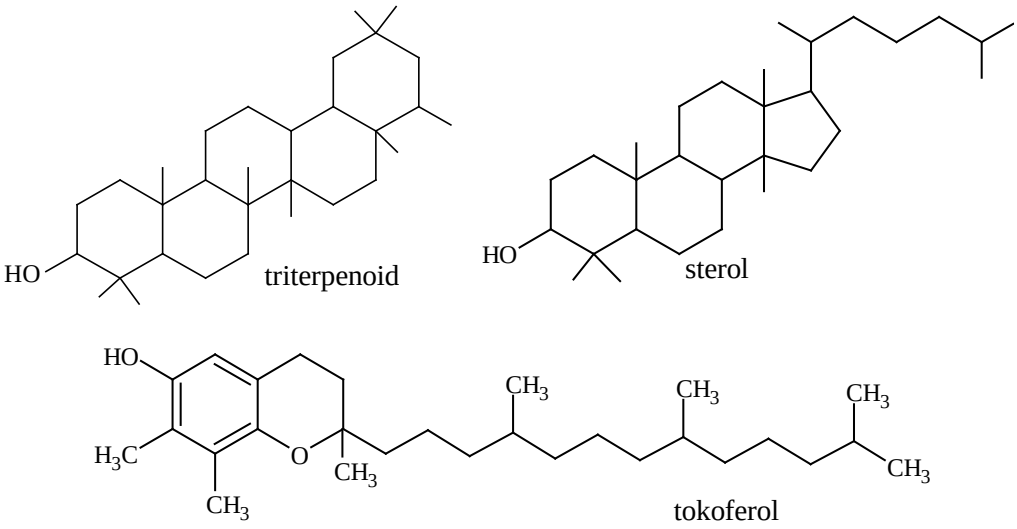
Secara umum triterpenoid memiliki aktivitas inflamasi, sehingga diperkirakan efek antiinflamasi dan analgesik bunga telang dikontribusikan oleh tarakserol (Shyam Kumar & Ishwar Bhat, 2012).

Di dalam 100 g bunga segar terdapat rata-rata 15,91 mg fitosterol dengan komposisi kampesterol 1,24 mg, stigmasterol 6,70 mg, β-Sitosterol 6,77 mg, dan sitostanol 1,20 mg (Shen et al., 2016). 1.24, 76.70, 6.77, 1.20 mg/100 g. Fitosterol dipastikan memiliki fungsi hipokolesterolemik dan mengurangi risiko hiperplasia prostat jinak, penyakit kardiovaskular, perkembangan kanker usus dan payudara, serta efek imunologis pada makrofag. Dari keempat fitosterol, β-sitosterol adalah yang paling efektif dalam menghambatan pertumbuhan sel kanker melalui aktivasi enzim tertentu, yang pada gilirannya menginduksi apoptosis seluler. β-sitosterol dan kampesterol dapat mengurangi perkembangan kanker melalui penghambatan pembentukan senyawa karsinogen dalam metabolisme biologis (Shen et al., 2016).

Terdapat dua senyawa tokoferol pada bunga telang, yaitu α-tokoferol dan γ-tokoferol, masing-masing sebanyak 0,20 dan 0,24 mg per 100 g bunga segar (Shen et al., 2016). Tokoferol telah dibuktikan melindungi membran sel terhadap radikal lipida reaktif, mencegah aterosklerosis dan karsinogenesis (Shen et al., 2016).

Alkaloid

Keberadaan alkaloid di dalam bunga telang secara kualitatif disebutkan pada beberapa referensi (Uma et al., 2009; Manjula et al., 2013; Suganya et al., 2014; Singh et al., 2018). Akan tetapi tidak ada satupun yang berhasil mengungkapkan identitas senyawa alkaloid tersebut.



Gambar 5. Struktur kimia terpenoid yang dijumpai pada bunga telang: triterpenoid, sterol, dan tokoferol

Baru-baru ini satu senyawa alkaloid berhasil diisolasi dari ekstrak kloroform bunga telang dan diidentifikasi sebagai 3-deoxy- 3, 11-epoxy cephalotaxine (Manivannan, 2019). Senyawa alkaloid ini menunjukkan aktivitas antibakteri *Escherichia coli* dan *Staphylococcus aureus* serta antikapang *Aspergillus flavus* dan *Candida albicans*. Selain itu, senyawa ini memiliki aktivitas antiinflamasi pada tikus percobaan yang diinduksi dengan karagenan dengan efektivitas yang sebanding dengan efektivitas *Diclofenac sodium* (Manivannan, 2019).

Peptida: Siklotida

Satu lagi komponen bioaktif bunga telang yang akhir-akhir ini mencuri perhatian para peneliti adalah siklotida. Siklotida adalah keluarga besar protein nabati makrosiklik yang tak lazim dan tersusun oleh 28 hingga 37 asam amino dengan tiga ikatan disulfida intramolekul. Sejak pertama kali berhasil diidentifikasi pada tahun 1971 siklotida mendapat perhatian besar karena sifatnya yang stabil tehadap panas senyawa kimia dan enzim proteolitik serta potensinya untuk memberikan berbagai manfaat terapetik seperti antikanker, anti-HIV, uterotonik, antineurotensin, antimikroba dan aktivitas hemolitik.

Siklotida pada bunga telang pertama kali ditemukan oleh Poth et al. (2011) yang berhasil mengidentifikasi dua belas jenis siklotida pada biji bunga telang. Tidak lama kemudian, pada tahun yang sama, berhasil ditemukan lima belas jenis siklotida dengan tiga di antaranya jenis yang baru pada seluruh bagian bunga telang: daun, batang, akar, biji dan bunga (Nguyen et al., 2011). Penelitian selanjutnya menunjukkan bahwa bunga telang adalah satu-satunya spesies keluarga Fabaceae yang mengandung siklotida. Saat ini telah berhasil diidentifikasi 41 jenis siklotida pada telang, sehingga menjadikannya sebagai salah satu tanaman yang memiliki kandungan siklotida paling kaya (Nguyen et al., 2016).

Komponen lain

Komponen-komponen lain yang juga ditemukan pada bunga telang adalah asam lemak palmitat, stearat, petroselinat, linoleat, arakhidat, behenat dan fitanat (Shen et al., 2016), mome-inositol dan pentanal (Neda et al., 2013). Asam fitanat memiliki peran di dalam mengatur trigliserida atau kolesterol dalam otot rangka (Shen et al., 2016).

Tantangan Bunga Telang Sebagai Pangan Fungsional dan Nutrasetikal

Secara umum, masih diperlukan perjalanan riset yang relatif panjang untuk sampai pada aplikasi komersial bunga telang sebagai pangan fungsional atau nutrasetikal. Hal ini mengingat penelitian yang telah dilakukan hingga saat ini masih sampai pada tahap in vitro (misalkan pada sel kanker untuk menguji aktivitas antikanker) atau in vivo (pada hewan percobaan untuk menguji aktivitas antidiabetes). Akan tetapi, aplikasi bunga telang sebagai minuman pengontrol gula darah dapat dikatakan sudah dekat dengan aplikasi komersial.

Pengontrol Gula Darah

Penelitian Chusak et al. (2018) menunjukkan bahwa bunga telang dapat diolah menjadi minuman pengatur gula darah melalui proses yang relatif sederhana, yakni dengan maserasi atau perendaman dalam air sehingga mencapai kepekatan yang setara dengan 2,16 mg delfinidin 3-glukosida per sajian. Konsentrasi ini dapat diperoleh dengan merendam 10 hingga 15 helai bunga telang di dalam 250 ml air panas selama 15 hingga 30 menit.

Salah satu keunggulan bunga telang, sekaligus juga kekurangannya adalah warnanya yang biru-ungu atau biru pekat pada pH 4-6. Sementara, sebagian besar sumber antosianin lainnya tak berwarna atau memiliki warna merah pucat pada pH tersebut. Warna biru bunga telang ini dapat menjadi daya tarik sensoris, tetapi dapat pula menurunkan nilai sensoris. Survei awal yang dilakukan terhadap lebih dari 400 responden menunjukkan adanya kecenderungan konsumen untuk tidak menyukai warna biru yang terlalu pekat. Kabar baiknya adalah minuman dengan konsentrasi yang efektif mengendalikan gula darah memiliki kepekatan warna biru yang disukai konsumen.

Keunggulan lain adalah bunga telang sangat sedikit memberikan rasa dan aroma yang mungkin dapat menurunkan nilai sensoris. Rasa dan aroma tersebut relatif mudah ditutupi dengan menambahkan perasan jeruk nipis, lemon, nanas, serai, dan lain-lain. Penambahan bahan lain yang bersifat asam dapat menurunkan pH dan mengubah warna biru bunga telang menjadi ungu (Gambar 6).

Keunggulan berikutnya adalah warna bunga telang relatif stabil pada pH pangan (Mohamad et al., 2011; Marpaung et al., 2019) dan relatif stabil pula terhadap aplikasi panas selama pengolahan, seperti sterilisasi (data belum dipublikasi).

Kombinasi Dengan Sumber Antosianin Lain

Beberapa penelitian menunjukkan bahwa aplikasi bunga telang dalam bentuk campuran dengan rosela, mulberi dan delima dapat meningkatkan aktivitas fungsionalnya (Adisakwattana et al., 2012; Borikar et al., 2018). Hasil penelitian ini membuka peluang penelitian untuk mempelajari efek kombinasi ekstrak bunga telang dengan ekstrak sumber antosianin lain.

Sebagaimana telah diketahui, bunga telang menampilkan warna yang pekat pada pH produk pangan, sedangkan kebanyakan sumber antosianin lain hampir tak berwarna. Sifat yang bertolak belakang ini dapat dimanfaatkan untuk menghasilkan produk berkadar antosianin lebih tinggi, dengan warna yang tak terlalu pekat sehingga atraktif secara inderawi.

Kadar antosianin yang lebih tinggi mungkin diperlukan untuk mencapai konsentrasi yang efektif menunjukkan aktivitas fungsional. Sebagai contoh sebagai antidiabetes. Penelitian Daisy et al. (2009) menunjukkan bahwa bunga telang menunjukkan aktivitas antidiabetes yang signifikan pada tikus percobaan dengan jumlah asupan 400 mg ekstrak kering/kg berat badan tikus. Ekstrak kering ini diperoleh melalui perebusan 100 g bunga kering (kira-kira setara dengan 1000 g bunga segar) dalam 1liter air, yang dilanjutkan dengan filtrasi, evaporasi, dan pengeringan. Konsentrasi setinggi ini menghasilkan warna biru yang sangat pekat yang mungkin sulit diterima secara inderawi. Sebagai perbandingan, minuman bunga telang pada Gambar 5 diperoleh melalui maserasi 10 helai bunga telang segar (atau setara sekitar 3 g) dalam 250 ml air.

Gambar 6. Minuman bunga telang

Fokus Pada Ternatin dan Siklotida

Sekalipun sudah banyak komponen bioaktif yang telah berhasil diidentifikasi dalam bunga telang, masih belum jelas komponen mana yang mengambil peran utama pada aktivitas fungsional tertentu. Sebagai tambahan, sebagian besar komponen bioaktif bunga telang dijumpai pula pada banyak sumber tanaman lain, sehingga menjadikannya kurang istimewa. Kecuali ternatin dan siklotida. Oleh karena itu adalah beralasan untuk memberikan fokus kepada penelitian manfaat fungsional ternatin dan siklotida.

Ternatin adalah antosianin yang unik dan hingga sejauh ini diketahui hanya terdapat pada kelopak bunga telang. Keunikan pertama adalah adanya gugus malonil-glukosida pada posisi C3 yang terletak pada cincin C pada kerangka antosianidin. Keunikan kedua, ternatin sekurang-kurangnya memiliki satu gugus asil pada posisi C3' dan C5' yang terletak pada cincin B. Konfigurasi ini membuat ternatin pada pH 4 – 6 berada dalam formasi 3 spesies yang berwarna: kation flavilium yang berwarna merah, basa kuinonoidal yang berwarna ungu, dan basa kuinonoidal anionik yang berwarna biru (Marpaung et al., 2018; Marpaung et al., 2019). Sementara, kebanyakan antosianin lain pada pH tersebut berada dalam bentuk hemiketal yang tak berwarna. Perbedaan formasi ini mungkin berpengaruh kepada efek fungsionalnya. Ketiga, degradasi ternatin selama penyimpanan dapat terjadi melalui proses deasilasi yang menghasilkan residu kumaroil-glukosida yang juga mungkin memiliki aktivitas fungsional tertentu. Hingga saat ini studi terhadap aktivitas fungsional ternatin maupun hasil degradasinya masih sangat jarang atau bahkan tidak ada.

Siklotida menarik perhatian, karena membawa harapan baru bagi komponen antikanker yang stabil terhadap panas, bahan kimia, dan enzim. Bunga telang diketahui sebagai salah satu sumber siklotida yang paling kaya (Nguyen et al., 2016). Kajian terhadap siklotida telang telah menunjukkan aktivitas positifnya untuk melawan sel kanker paru-paru (Sen et al., 2013) serta berperan dalam mengatur sistem imun tubuh (Nguyen et al., 2016).

PENUTUP

Penelitian hingga sejauh ini telah berhasil mengungkapkan manfaat bunga telang untuk menyokong kesehatan manusia melalui berbagai peran dan mekanisme, serta mengisolasi dan mengidentifikasi berbagai komponen bioaktif baik yang bersifat hidrofilik maupun lipofilik. Hasil-hasil penelitian ini memperkuat posisi bunga telang sebagai bahan untuk pangan fungsional dan nutrasetikal. Potensi bunga telang ini didukung pula oleh beberapa keunggulan bunga telang baik

yang meliputi aspek budidaya, kestabilan ekstrak, maupun mutu inderawi.

Serangkaian penelitian, baik yang meliputi identifikasi peran masing-masing komponen bioaktif bunga telang terhadap efek fungsional tertentu, efek sinergestik yang ditimbulkan oleh kombinasi bunga telang dengan bahan lain, maupun uji klinis masih perlu dilakukan sebagai bagian dari persiapan pemanfaatan bunga telang sebagai bahan pangan fungsional dan nutrasetikal.

Merujuk kepada status terkini sesuai dengan penelitian yang telah dilakukan, pengolahan bunga telang sebagai minuman pengontrol gula darah adalah yang paling dekat dengan penerapan komersial.

DAFTAR PUSTAKA

Adisakwattana, S., Ruengsamran, T., Kampa, P. & Sompong, W., 2012. In vitro inhibitory effects of plant-based foods and their combinations on intestinal α-glucosidase and pancreatic α-amylase. *BMC Complementary and Alternative Medicine*, 12(110), pp. 1-8.

Akter, R., Uddin, S. J., Grice, I. D. & Tiralong, E., 2014. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. *Journal of Natural Medicines*, 68(1), pp. 246–252.

Al-Asmari, A. K. et al., 2014. A Review of Hepatoprotective Plants Used in Saudi Traditional Medicine. *Evidence-Based Complementary and Alternative Medicine*, 2014, pp. 22.

Balaji, K. S. et al., 2016. Angio Suppressive Effect of Clitoria ternatea Flower Extract is Mediated by HIF-1α and Down Regulation of VEGF in Murine Carcinoma Model. *Medicinal chemistry*, 6(7), pp. 515-520 .

Boots, A. W., Haenen, G. R. & Bast, A., 2008. Health effects of quercetin: From antioxidant to nutraceutical. *European J. of Pharmacology*, 585, pp. 325-337.

Borikar, S. P., Kallewar, N. G., Mahapatra, D. K. & Dumore, N. G., 2018. Dried flower powder combination of Clitoria ternatea and Punica granatum demonstrated analogous anti-hyperglycemic potential as compared with standard drug metformin: In vivo study in Sprague Dawley rats. *J. of Applied Pharmaceutical Science* , 8(11), pp. 075-078.

Chakraborthy, G. et al., 2018. Phytochemicals and Pharmacological Aspects of Clitoria ternatea - a Review. *J. of Applied Pharmaceutical Sciences and Research*, 1(2), pp. 3-9.

Chayaratanasin, P., Barbieri, M. A., Suanpairintr, N. & Adisakwattana, S., 2015. Inhibitory effect of Clitoria ternatea flower petal extract on fructose-induced protein glycation and oxidation-dependent damages to albumin in vitro. *BMC Complementary and Alternative Medicine*, 15(27), pp. 1-9.

Chayaratanasin, P. et al., 2019. Clitoria ternatea Flower Petal Extract Inhibits Adipogenesis and Lipid Accumulation in 3T3-L1 Preadipocytes by Downregulating Adipogenic Gene Expression. *Molecules*, 24(10), pp. 1894.

Chu, B.-S., Divers, R., Tziboula-Clarke, A. & Lemos, M. A., 2017. Clitoria ternatea L. Flower Extract Inhibits α-amylase During in Vitro Starch Digestion. *American Research Journal of Food and Nutrition*, 1(1), pp. 1-10.

Chusak, C. et al., 2018. Influence of Clitoria ternatea Flower Extract on the In Vitro Enzymatic Digestibility of Starch and Its Application in Bread. *Foods*, 7(7), pp. 102.

Chusak, C., Thilavech, T., Henry, C. J. & Adisakwattana, S., 2018. Acute effect of Clitoria ternatea flower beverage on glycemic response and antioxidant capacity in healthy subjects: a randomized crossover trial. *BMC Complementary and Alternative Medicine*, 18(6), pp. 1-18.

Daisy, P. & Rajathi, M., 2009. Hypoglycemic Effects of Clitoria ternatea Linn. (Fabaceae) in

Alloxan-induced Diabetes in Rats. *Tropical J. of Pharmaceutical Research,* 8(5), pp. 393-398.

Daisy, P., Santosh, K. & Rajathi, M., 2009. Hypoglycemic Effects of Clitoria ternatea Linn. (Fabaceae) in Alloxan-induced Diabetes in Rats. *Tropical Journal of Pharmaceutical Research,* 8(5), pp. 393-398.

DeFilipps, R. A. & Krupnick, G. A., 2018. The medicinal plants of Myanmar. *PhytoKeys* , 102, pp. 1–341.

Deka, M. et al., 2013. Proximate Analysis of Primary Metabolites in Different Parts of Clitoria ternatea L. A Comparative Study. *International Archive of Applied Sciences and Technology,* 4(3), pp. 62-67.

Dillard, C. J. & German, J. B., 2000. Phytochemicals: nutraceuticals and human health. *J. of the Science of Food and Agriculture,* 80, pp. 1744–1756.

Fantz, P. R., 1991. Ethnobotany of Clitoria (Leguminosae). *Economic Botany,* 45(4), pp. 511-520.

Ghosh, D. & Konishi, T., 2007. Anthocyanins and Anthocyanin-Rich Extracts: Role in Diabetes and Eye Function. *Asia Pacific Journal of,* 16(2), pp. 200-208.

Gomez, S. M. & Kalamani, K., 2003. Butterfly Pea (Clitoria ternatea): A Nutritive Multipurpose Forage Legume for the Tropics - An Overview.. *Pakistan Journal of Nutrition,* 2, pp. 374-379.

Gunjan, M. et al., 2010. Pharmacognostic and antidiabetic study of Clitoria ternatea. *International Journal of Phytomedicine,* 2, pp. 373-378.

Gupta, G. K., Chahal, J. & Bhatia, M., 2010. Clitoria ternatea (L.): Old and new aspects. *J. of Pharmacy Research,* 3(11), pp. 2610-2614.

Horbowicz, M., Kosson, R., Grzesiuk, A. & Dbski, H., 2008. Anthocyanins of Fruits and Vegetables - Their Occurrence, Analysis and Role in Human Nutrition. *Vegetable Crops Research Bulletin,* 68, pp. 5-22.

IAMSAARD, S. et al., 2014 . Antioxidant activity and protective effect of Clitoria ternatea flower extract on testicular damage induced by ketoconazole in rats. *Journal of Zhejiang University-SCIENCE B,* 15(6), pp. 548-555.

Iamsaard, S. et al., 2014. Antioxidant activity and protective effect of Clitoria ternatea flower extract on testicular damage induced by ketoconazole in rats. *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology),* 15(6), pp. 548-555.

Intuyod, K. et al., 2014. Anti-inflammatory and anti-periductal fibrosis effects of an anthocyanin complex in Opisthorchis viverrini-infected hamsters. *Food and Chemical Toxicology,* 74, pp. 206–215.

Iversen, C. K., 1999. Black Currant Nectar: Effect of Processing and Storage on Anthocyanin and Ascorbic Acid Content. *Journal of Food Science,* 64, pp. 37-41.

Jain, N. N. et al., 2003. Clitoria ternatea and the CNS. *Pharmacology, Biochemistry and Behavior,* 75, pp. 529–536.

Kaisoon, O., Siriamornpun, S., Weerapreeyakul, N. & Meeso, N., 2011. Phenolic compounds and antioxidant activities of edible flowers from Thailand. *J. of Functional Foods,* 3, pp. 88-99.

Kamilla, L., Mnsor, S., Ramanathan, S. & Sasidharan, S., 2009. Antimicrobial Activity of Clitoria ternatea (L.) Extracts. *Pharmacologyonline,* 1, pp. 731-738.

Kamkaen, N. & Wilkinson, J. M., 2009. The Antioxidant Activity of Clitoria ternatea Flower Petal Extracts and Eye Gel. *Phytotherapy Research,* 23, pp. 1624–1625.

Kazuma, K., Noda, N. & Suzuki, M., 2003. Flavonoid composition related to petal color in

different lines of Clitoria ternatea. *Phytochemistry*, 64, pp. 1133–1139.

Khoo, H. E., Azlan, A. T. T. S. & Meng Lim, S., 2017. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, 61(1), pp. 1361779.

Kondo, T., Ueda, M. & Goto, T., 1990. Structure of Ternatin B1, a Pentaacylated Anthocyanin Substituted on the B-Ring Asymmetrically with Two Long Chains. *Tetrahedron*, 46(13-14), pp. 4749-4756.

Kshirsagar, S., Takur, A. S. & Khsirsagar, J., 2015. Immunomodulatory and antioxidative properties of Clitoria ternatea. *International Journal of Plant Sciences*, 10(2), pp. 158-162.

Kumar, H. et al., 2014. Phenolic compounds and their health benefits: A review. *J. of Food Research and Technology*, 2(2), pp. 46-59.

Kumar, S., Kumar, A. & Singh, A. K., 2016. Scientific Basis for the Therapeutic use of Clitoria ternatea linn. (Aprajita): A Review. *International Journal of Applied and Pure Science and Agriculture*, 2(4), pp. 180-187.

Lakshan, S. A. T., Jayanath, N. Y., Abeysekera, W. P. K. M. & Abeysekera, W. K. S. M., 2019. A Commercial Potential Blue Pea (Clitoria ternatea L.) Flower Extract Incorporated Beverage Having Functional Properties. *Evidence-Based Complementary and Alternative Medicine*.

Lakshmeesh, N. B., 2019. Antioxidant and Anticancer Activity of Edible Flowers. *Journal of Drug Delivery and Therapeutics*, 9(3-s), pp. 290-295.

Larson, R. A., 1988. The Antioxidants of Higher Plants. *Phytochemistry*, 27(4), pp. 969-978.

Leong, C.-R.et al., 2017. Anthocyanins from Clitoria ternatea Attenuate Food-Borne Penicillium expansum and its Potential Application as Food Biopreservative. *Natural Product Sciences*, 23(2), pp. 125-131.

Leong, H. Y. et al., 2018. Natural Red Pigments from Plants and Their Health Benefits – A Review. *Food Reviews International*, 34, pp. 463-482.

Li, D. et al., 2017. Health Benefits of Anthocyanins and Molecular Mechanisms: Update from Recent Decade. *Critical Reviews in Food Science and Nutrition*, 57(8), pp. 1729-1741.

Lijon, M. B. et al., 2017. Phytochemistry and pharmacological activities of Clitoria ternatea. *International Journal of Natural and Social Sciences*, 4(1), pp. 1-10.

Lin, Y., Shi, R., Wang, X. & Shen, H.-M., 2008. Luteolin, a flavonoid with potentials for cancer prevention and therapy. *Current Cancer Drug Targets*, 7(634–646), pp. 8.

Liu, H. et al., 2016. The Fascinating Effects of Baicalein on Cancer: A Review. *International Journal of Molecular Sciences*, 17(1681), pp. 1-18.

Madhu, K., 2013. Phytochemical Screening and Antioxidant Activity of in vitro Grown Plants Clitoria ternatea L., using DPPH Assay. *Asian Journal of Pharmaceutical and Clinical Research*, 6(2), pp. 38-42.

Mahmad, N. et al., 2018. Anthocyanin as potential source for antimicrobial activity in Clitoria ternatea L. and Dioscorea alata L.. *Pigment & Resin Technology*.

Manivannan, R., 2019. Isolation and Characterizations of new alkaloid 3-deoxy- 3, 11-epoxy cephalotaxine from Clitoria ternatea. *Journal of Drug Delivery and Therapeutics*, 9(4-A), pp. 458-462.

Manjula, P. et al., 2013. Phytochemical Analysis of Clitoria ternatea Linn,, A Valuable Medicinal

Plant. *The Journal of Indian Botanical Society*, 92(3&4), pp. 173-178.

Marpaung, A. M., Andarwulan, N., Hariyadi, P. & Faridah, D. N., 2018. *The Wide Variation of Color Stability of Butterfly Pea (Clitoria ternatea L.) Flower Extract at pH 6-8.* Jakarta, Southeast Asian Food & Agricultural Science & Technology, pp. 283-291.

Marpaung, A. M., Andarwulan, N., Hariyadi, P. & Faridah, D. N., 2019. The Difference in Colour Shifting of Clitoria ternatea L. Flower Extract at pH 1, 4, and 7 during storage. *Current Nutrition and Food Science*, 15(7), pp. 694-699.

Marpaung, A. M., Andarwulan, N. & Prangdimurti, E., 2013. The optimization of anthocyanin pigment extraction from butterfly pea (Clitoria ternatea L.) petal using Response Surface Methodology. *Acta Horticulturae*, 1011, pp. 205-211.

Mohamad, M. F., Nasir, S. N. S. & Sarmidi, M. R., 2011. Degradation kinetics and colour of anthocyanins in aqueous extracts of butterfly pea. *Asian Journal of Food and Agro-Industry*, 4(05), pp. 306-315.

Mukherjee, P. K., Kumar, V., Kumar, N. S. & Heinrich, M., 2008. The Ayurvedic medicine Clitoria ternatea – From traditional use to scientific assessment.. *J. of Ethnopharmacology*, 120(3), pp. 291-301.

Nair, V. et al., 2015. Protective Role of Ternatin Anthocyanins and Quercetin Glycosides from Butterfly Pea (Clitoria ternatea Leguminosae) Blue Flower Petals against Lipopolysaccharide (LPS)-Induced Inflammation in Macrophage Cells. *J. of Agricultural and Food Chemistry*, 63, pp. 6355−6365.

Nair, V. et al., 2015. Protective Role of Ternatin Anthocyanins and Quercetin Glycosides from Butterfly Pea (Clitoria ternatea Leguminosae) Blue Flower Petals against Lipopolysaccharide (LPS)-Induced Inflammation in Macrophage Cells. *Journal of Agricultural and Food Chemistry* , 63(28), pp. 6355-6365.

Neda, G. D., Rabeta, M. S. & Ong, M. T., 2013. Chemical composition and anti-proliferative properties of flowers of Clitoria Ternatea. *International Food Research Journal*, 20(3), pp. 1229-1234.

Nguyen, G. K. T. et al., 2011. Discovery and Characterization of Novel Cyclotides Originated from Chimeric Precursors Consisting of Albumin-1 Chain a and Cyclotide Domains in the Fabaceae Family. *The Journal of Biological Chemistry*, 286(27), pp. 24275–24287.

Nguyen, K. N. T. et al., 2016. Immunostimulating and Gram-negative-specific antibacterial cyclotides from the butterfly pea (Clitoria ternatea). *The FEBS Journal*, 283, pp. 2067–2090.

Nithianantham, K. et al., 2013. Evaluation of hepatoprotective effect of methanolic extract of (Linn.) flower against acetaminophen-induced liver damage. *Asian Pacific Journal of Tropical Disease*, 3(4), pp. 314-319.

Oguis, G. K., Gilding, E. K., Jackson, M. A. & Craik, D. J., 2019. Butterfly Pea (Clitoria ternatea), a Cyclotide-Bearing Plant With Applications in Agriculture and Medicine. *Frontiers in Plant Science*, 10, pp. 1-23.

Patel, J. M., 2008. A Review of Potential Health Benefits of Flavonoids .. *Lethbridge Undergraduate Research Journal*, 3(2).

Pengkumsri, N., Kaewdoo, K. & Leeprechanon, W., 2019. Influence of Extraction Methods on Total Phenolic Content and Antioxidant Properties of Some of the Commonly Used Plants in Thailand. *Pakistan Journal of Biological Sciences*, 22(3), pp. 117-126.

Pengkumsri, N., Kaewdoo, K., Leeprechanon, W. & Sivamaruthi, B. S., 2019. Influence of Extraction Methods on Total Phenolic Content

and Antioxidant Properties of Some of the Commonly Used Plants in Thailand. *Pakistan Journal of Biological Sciences,* 22(3), pp. 117-126.

Phrueksanan, W., Yibchok-anun, S. & Adisakwattana, S., 2014. Protection of Clitoria ternatea flower petal extract against free radical induced hemolysis and oxidative damage in canine erythrocytes. *Research in Veterinary Science.*

Poth, A. G. et al., 2011. Discovery of Cyclotides in the Fabaceae Plant Family Provides New Insights into the Cyclization, Evolution, and Distribution of Circular Proteins. *ACS Chemical Biology,* 6(4), pp. 345-355.

Pratap, G. M. J. S. et al., 2012. Evaluation of three medicinal plants for anti-microbial activity. *An International Quarterly Journal of Research of Ayurveda,* 33(3), pp. 423-428.

Priprem, A., Limsitthichaikoon, S. & Thappasarapong, S., 2015. Anti-Inflammatory Activity of Topical Anthocyanins by Complexation and Niosomal Encapsulation. *International Journal of Chemical and Molecular Engineering,* 9(2), pp. 142-146.

Rabeta, M. S. & An Nabil, Z., 2013. Total phenolic compounds and scavenging activity in Clitoria ternatea and Vitex negundo linn. *International Food Research Journal,* 20(1), pp. 495-500.

Rabeta, M. S. & An Nabil, Z., 2013. Total phenolic compounds and scavenging activity in Clitoria ternatea and Vitex negundo linn. *International Food Research Journal,* 20(1), pp. 495-500.

Rajamanickam, M., Kalaivanan, P. & Sivagnanam, I., 2015. Evaluation of Anti-oxidant and Anti-diabetic Activity of Flower Extract of Clitoria ternatea L. *Journal of Applied Pharmaceutical Science,* 08, pp. 131-138.

Salehi, B. et al., 2019. The Therapeutic Potential of Apigenin. *International Journal of Molecular Sciences,* 20(1305), pp. 1-26.

Sen, Z. et al., 2013. Chemosensitizing activities of cyclotides from Clitoria ternatea in paclitaxel-resistant lung cancer cells.. *Oncology Letters,* 5, pp. 641-644.

Shekhawat, N. & Vijayvergia, R., 2010. Evaluation of antimicrobial potential of some medicinal plants against plant and human pathogens. *J. of Pharmaceutical Research,* 3, pp. 700-702.

Shen, Y. et al., 2016. Butterfly pea (Clitoria ternatea) seed and petal extracts decreased HEp-2 carcinoma cell viability. *International Journal of Food Science and Technology,* 51, pp. 1860–1868.

Shivaprakash, P. et al., 2015. Induction of Apoptosis in MCV-7 Cells by Methanolic Extract of Clitoria ternatea L.. *International Journal of Applied Biology and Pharmaceutical Technology*, 6(4), pp. 80-86.

Shyam Kumar, B. & Ishwar Bhat, B., 2012. Antiinflammatory, Analgesic and Phytochemical Studies of Clitoria ternatea Linn Flower Extract. *International Research Journal of Pharmacy*, 3(3), pp. 208-210.

Shyam Kumar, B. & Ishwar Bhat, K., 2011. In-vitro Cytotoxic Activity Studies of Clitoria ternatea Linn Flower Extracts. *International Journal of Pharmaceutical Science Review and Research,* 6(2), pp. 120-121.

Singh, N. K. et al., 2018. Anti-allergy and anti-tussive activity of Clitoria ternatea L. in experimental animals. *Journal of Ethnopharmacology,* 224, pp. 15-26.

Siti Azima, A. M., Noriham, A. & Manshoor, N., 2014. Anthocyanin content in relation to the antioxidant activity and colour properties of Garcinia mangostana peel, Syzygium cumini and Clitoria ternatea extracts. *International Food Research Journal,* 21(6), p. 2369–2375.

Siti Azima, A., Noriham, A. & Manshoor, N., 2017. Phenolics, antioxidants and color properties of aqueous pigmented plant extracts:

Ardisia colorata var. elliptica, Clitoria ternatea, Garcinia. *J. of Functional Foods,* 38, pp. 232–241.

Smeriglio, A., Barreca, D., Bellocco, E. & Trombetta, D., 2016. Chemistry, Pharmacology and Health Benefits of Anthocyanins. *Phytotherapy Research,* 30(8), pp. 1265-1286.

Srichaikul, B., 2018. Ultrasonication Extraction, Bioactivity, Antioxidant Activity, Total Flavonoid, Total Phenolic and Antioxidant of Clitoria Ternatea Linn Flower Extract for Anti-aging Drinks. *Pharmacognosy Magazine* , 14(56), pp. 322-327.

Srichaikul, B., 2018. Ultrasonication Extraction, Bioactivity, Antioxidant Activity, Total Flavonoid, Total Phenolic and Antioxidant of Clitoria Ternatea Linn Flower Extract for Anti-aging Drinks. *Pharmacognosy Magazine*, 14(56), pp. 322-327.

Suganya, G., Sampath Kumar, P., . Dheeba, B. & Sivakumar, R., 2014. In Vitro Antidiabetic, Antioxidant and Anti-inflammatory Activity of Clitoria ternatea L.. *International Journal of Pharmacy and Pharmaceutical Sciences,* 6(7), pp. 342-347.

Tapas, A. R., Sakarkar, D. M. & Kakde, R. B., 2008. Flavonoids as Nutraceuticals: A Review. *Tropical Journal of Pharmaceutical Research,* 7(3), pp. 1089-1099.

Terahara, N. et al., 1996. Five new anthocyanins, ternatins A3, B4, B3, B2, and D2, from Clitoria ternatea flowers. *J. of Natural Products,* 59, pp. 139-144.

Terahara, N. et al., 1998. Eight New Anthocyanins, Ternatins C1-C5 and D3 and Preternatins A3 and C4 from Young Clitoria ternatea Flowers. *Journal of Natural Products,* 61, pp. 1361-1367.

Terahara, N., Saito, N., Honda, T. & Toki, K., 1989. Structure of ternatin D1, an acylated anthocyanin from Clitoria ternatea flowers. *Tetrahedron Letters,* 30(39), pp. 5305-5308.

Terahara, N. et al., 1990. Structure of ternatin A1, the largest ternatin in the major blue anthocyanins from Clitoria ternatea flowers.. *Tetrahedron Letters,* 31(20), pp. 2921-2924.

Terahara, N. et al., 1990. Structure of TERNATIN A2, one of Clitoria ternatea Flower Anthocyanins Having the Unsymetrical Side Chains. *Heterocycles,* 31(10), pp. 1773-1776.

Uma, B., Prabhakar, K. & Rajendran, S., 2009. Phytochemical Analysis and Antimicrobial Activity of Clitoria ternatea Linn Against Extended Spectrum Beta Lactamase Producing Enteric and Urinary Pathogens. *Asian Journal of Pharmaceutical and Clinical Research,* 2(4), pp. 94-96.

Vankar, P. S. & Srivastava, J., 2010. Evaluation of Anthocyanin Content in Red and Blue Flowers. *International Journal of Food Engineering,* 6(4), pp. Article 7.

Wang, L. & Ma, Q., 2018. Clinical benefits and pharmacology of Scutellarin: A comprehensive review. *Pharmacology & Theurapetics,* 190, pp. 105-127.

Xiao, J., Capanoglu, E., Jassbi, A. R. & Miron, A., 2016. Advance on the Flavonoid C-glycosides and Health Benefit. *Critical Reviews in Food Science and Nutrition,* 56, pp. S29–S45.

Yao, L. H. et al., 2004. Flavonoids in Food and Their Health Benefits. *Plant Foods for Human Nutrition* , 113–122, pp. 59.

Zakaria, N. et al., 2018. In vitro protective effects of an aqueous extract of Clitoria ternatea L. flower against hydrogen peroxide-induced cytotoxicity and UV-induced mtDNA damage in human keratinocytes. *Phytotherapy Research,* pp. 1-9.

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THE POTENCY OF CAJUPUTS CANDY IN MAINTAINING THE COMPETITIVE CAPACITY OF *STREPTOCOCCUS SANGUINIS* UPON *STREPTOCOCCUS MUTANS*

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ABSTRACT

Streptococcus mutans were competing *Streptococcus sanguinis* in biofilm formation. As pioneer colonizer, *S. sanguinis* were able to control *S. mutans* growth. This study was aimed to explore the ability of sucrose and non-sucrose cajuputs candies (SCC and NSCC) in maintaining the antagonistic relationship between the indigenous oral flora when they grew as dual-species biofilms (*S. sanguinis* and *S. mutans*). SCC and NSCC contained cajuput and peppermint oils as the flavor which the volatile compounds had been identified. Unflavored sucrose candy and unflavored non-sucrose candy were prepared similarly to the SCC and NSCC, respectively, except the flavor addition. SCC, NSCC, unflavored sucrose candy, unflavored non-sucrose candy, and the control were exposed in vitro to the biofilms. The biofilm was examined for biofilm inhibition capacity, DNA amount, and the expression level of spxB mRNA. The biofilm inhibition by SCC and NSCC were higher than the unflavored ones and were significantly different compared to the control. The SCC and NSCC managed to decrease the total DNA amount in the biofilm, but unflavored candies did not. The qPCR assays showed that the exposure of candies did not alter the proportion of *S. sanguinis* DNA to *S. mutans* DNA in the biofilms. Meanwhile, spxB mRNA expression indicated the ability of *S. sanguinis* to control *S. mutans* growth.

Keywords: *Biofilm; cajuputs candy; Melaleuca cajuputi; spxB gene; Streptococcus mutans; Streptococcus sanguinis.*

ABSTRAK

Streptococcus mutans bersaing dengan *Streptococcus sanguinis* dalam pembentukan biofilm. Sebagai pionir kolonisasi, *S. sanguinis* mampu mengendalikan pertumbuhan *S. mutans*. Penelitian ini bertujuan untuk mengeksplorasi kemampuan permen cajuputs sukrosa dan non-sukrosa (SCC dan NSCC) dalam menjaga hubungan antagonistik pada flora di mulut saat tumbuh sebagai *dual-species* (*S. sanguinis* dan *S. mutans*). Permen beraroma (SCC dan NSCC) mengandung minyak cajuput dan pepermint sebagai rasa yang telah teridentifikasi sebagai senyawa volatil. Permen tanpa rasa dibuat mirip dengan permen dengan rasa tetapi tanpa penggunaan rasa. Permen rasa, permen tanpa rasa, dan kontrol diekspos secara in vitro ke biofilm. Biofilm dianalisa untuk kapasitas penghambatan biofilm, jumlah DNA, dan tingkat ekspresi mRNA spxB. Penghambatan biofilm oleh permen dengan rasa lebih tinggi daripada yang tidak diberi rasa dan secara signifikan berbeda dibandingkan dengan kontrol. Permen rasa berhasil mengurangi jumlah DNA total dalam biofilm, tetapi sampel tanpa rasa tidak. Tes qPCR menunjukkan bahwa paparan permen tidak mengubah proporsi DNA *S. sanguinis* ke DNA *S. mutans* dalam biofilm. Sementara itu, ekspresi mRNA spxB menunjukkan kemampuan *S.sanguinis* untuk mengendalikan pertumbuhan *S. mutans*.

Kata kunci: *Biofilm; Melaleuca cajuputi; permen cajuputs; spxB gene; Streptococcus mutans; Streptococcus sanguinis.*

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INTRODUCTION

Dental caries is one of the most common diseases in oral cavity (Somaraj et al., 2017; Eslami et al., 2016;). Eco-systemic factors such as saliva, dietary habit, and microbial composition in biofilm contribute to its formation (Fejerskov, 2004; Becker et al., 2002). In addition, interaction among polymicrobial could lead to cases of dental caries (Becker et al., 2002; Kreth et al., 2005). *Streptococcus sanguinis* is a health-associated species (Percival et al., 2006; Kreth et al., 2005) and is considered as beneficial bacterium in regards to dental caries (Percival et al., 2006; Kreth et al., 2005; Magalhaes et al., 2016). On the contrary, *Streptococcus mutans* is the most commonly found species on dental caries (Oda et al., 2015) because it manages to grow in acidic environment and produces acidic compounds by fermenting carbohydrates (Percival et al., 2006; Becker et al., 2002; Kreth et al., 2005). Interestingly, *S. sanguinis* is able to antagonize *S. mutans* activity and protects the host from the negative effect of this opportunistic pathogen (Kreth et al., 2009).

S. sanguinis pioneers the development of oral biofilm, which have a role for the healthy dental plaque (Kreth et al., 2009). This bacterium is able to produce hydrogen peroxide (H_2O_2) that inhibits *S. mutans* growth under aerobic condition (Zheng et al., 2011a). *S. mutans* is not able to tolerate H_2O_2 (Zheng et al., 2011b). The H_2O_2 production in *S. sanguinis* was generated by pyruvate oxidase (SpxB). SpxB activity was controlled by SpxB gene (Zheng et al., 2011a). Expression of *spxB* mRNA represented the activity of *spxB* gene that corelated with the production of H_2O_2 by *S. sanguinis* (Magalhaes et al., 2016). Therefore, the physiological activity of *S. sanguinis* to inhibit *S. mutans* can be evaluated by the expression level of *spxB* mRNA (Zheng et al., 2011a).

Cajuputs candy is an Indonesian herbal-based candy that has been developed since 1997 as a functional food. Cajuputs candy was invented to increase the economic value of cajuput oil from *Melaleuca cajuputi* plant and to create a novel and distinctive Indonesian food product. Cajuputs candy has been patented (ID 0000385 S) (Wijaya et al., 2002) and developed into several variants including Sucrose Cajuputs Candy (SCC) (Wijaya et al., 2011) and Non-Sucrose Cajuputs Candy (NSCC) (Iftari et al., 2013). Both SCC and NSCC have been produced and marketed in certain area in Indonesia with good consumer acceptance.

Based on the previous studies, it is known that SCC and NSCC could suppress the growth of several pathogenic microbes in the oral system such as *S. mutans*, *S. sobrinus* (Wijaya et al., 2011) and *Candida albicans* (Wijaya et al., 2014). Iftari et al. (2013) reported that NSCC showed inhibition on biofilm formation and *gtfC* expression of *S. mutans* serotype c. Cajuputs candy activity could be caused by a synergistic effect between cajuput oil and peppermint oil, which were utilized as the main flavoring components. Cajuput oil contains α-terpineol and terpien-4-ol which had been reported having antimicrobial activity against *Streptococcus spp*. and *C. albicans* (Jedlickova et al., 1994). Peppermint oil, which contains menthol as the major component, had been also reported for its antimicrobial activity against cariogenic bacteria (Dwivedi et al., 2012; Galvao et al., 2012).

Antibiofilm potency of cajuputs candy formulas toward single type of pathogenic bacteria that related to dental caries has been proven (Wijaya et al., 2011; Iftari et al., 2013). However, its effect on polymicrobial biofilms, especially *S. sanguinis* and *S. mutans*, has not been reported. The aim of this study was to determine the ability of SCC and NSCC to maintain the competitive capacity of *S. sanguinis* toward *S. mutans*. The effect of cajuput oil and peppermint oil as flavor and the effect of sucrose and non-sucrose as the raw material were validated *in vitro* by biofilm assay, quantification of *spxB* mRNA expression level and total bacterial DNA using qPCR technique.

MATERIALS AND METHODS

Materials

Food grade cajuput oil distillated from *M. cajuputi* was obtained from Pulau Buru Maluku and sweeteners such as sucrose, liquid glucose, isomalt and acesulfame-K were obtained from local supplier while peppermint oil and honeydew flavor were from flavor houses. Analytical grade

chemicals were used: Brain Heart Infusion (BHI) agar and liquid (Acumedia), distilled water, milli-Q water, hexane (JT Baker), alkane standard (C_8-C_{20}), Nitrogen, carbon dioxide, violet crystal 1%, ethanol 95% (Merck), ethanol p.a. (Merck), phosphate buffer saline (PBS), trizol reagent (Invitrogen), isopropanol (Merck), chloroform, SYBR green (Kapa Biosystems), ultrapure distilled water (Invitrogen) and RT-PCR primer (1st BASE Custom Oligos, Singapore).

Preparation of candy formulas

Four candy formulas in this experiment are: (1) Unflavored sucrose candy, (2) Sucrose Cajuputs Candy (SCC), (3) Unflavored non-sucrose candy, and (4) Non-Sucrose Cajuputs Candy (NSCC). SCC and NSCC were prepared based on the procedures conducted by Wijaya et al. (2002) and Iftari et al. (2013), respectively. Similar procedures were conducted by removing flavor (cajuput and peppermint oil) to prepare unflavored sucrose and non-sucrose candy. For *in vitro* assay, all candy formulas were diluted 1:1(w/v) aseptically in sterile BHI broth. They will be used in biofilm inhibition assay and qPCR analysis. BHI broth without candy formula was used as a negative control.

Volatile compounds identification

SCC or NSCC (50 grams) were diluted with 20 mL distilled water and 5 mL hexane. They were put on a shaker (160 rpm) for 15 hours until all candies were dissolved. The supernatant was pipetted then added with sodium sulphate anhydrous. This water-free flavor extract was then pipetted into a new vial and added with 0.5 mL of 1,4-dichlorobenzene 1% (diluted with hexane) as an internal standard. The final extract solution was flashed with N_2.

Each extract (0.5 mL) was injected into the GC-MS (Agilent) on split mode (50:1 for SCC and 5:1 for NSCC) that was equipped with a DB-5 capillary column (60m length; 0.25mm i.d.; 0.25µm film thickness; helium carrier gas). The injector temperature was 250˚C, and detector was 280°C. The initial oven temperature of the column was 60°C (held for 5 min), increased to 250°C at 10°C/min and held constantly for 2 min. Alkane standard C_8-C_{20} (Fluka) was used as an external standard.

Qualitative identification of the constituents was performed by comparison of their linear retention indices (LRI) with the literature and their mass spectral data (NIST library) (Muchtaridi et al., 2010; Adams, 2009). The volatile profiles of SCC and NSCC were compared to volatile profile of cajuput and peppermint oil from Iftari et al. (2013).

Bacterial strains, media, and culture conditions

S. sanguinis ATCC 10556 and *S. mutans* XC from -70°C culture stocks were grown in BHI agar in an anaerobic jar under microaerophilic condition (CO_2 10%, N_2 80%, H_2 10%) and incubated for 24 hours at 37°C. For *in vitro* assay, the bacteria were harvested and adjusted to achieve 0.477 optical density in 490 nm (OD_{490}) for *S.sanguinis* and 0.061 in OD_{450} for *S. mutans*, which equaled to 1×10^6 colony forming units (CFU) mL^{-1}.

Preparation of dual-species biofilm

Preparation of dual-species biofilm for *in vitro* assay was conducted based on the method of Kreth et al. (2008) with modification. Two types of microplates were used, 96-well microtitre plate for biofilm inhibition assay and 6-well microtitre plate (Takara, Tokyo, Japan) for qPCR analysis (mRNA and DNA quantification). Briefly, 100 µL of *S. sanguinis* suspension (1×10^6 $CFUmL^{-1}$) was inoculated into 96-well microplate and 350 µL (1×10^6 $CFUmL^{-1}$) of the same culture was inoculated into 6-well microplate. They were incubated for two hours in anaerobic jars under a microaerophilic condition (CO_2 10%, N_2 80%, H_2 10%) at 37°C. In order to prepare dual-species biofilms, 100 µL (1×10^6 $CFUmL^{-1}$) and 350 µL (1×10^6 $CFUmL^{-1}$) of *S. mutans* suspensions were respectively added into the 96 and 6-well microplate containing *S. sanguinis* biofilm, and re-incubated for 18 hours in a similar condition.

In regards to the inhibition assay, dual-species biofilm on 96-well microplate was added with 200 µL of diluted candies in BHI broth (1:1 v/v). For qPCR analysis, 6-well microplate biofilm was added with 700 µL of diluted candies. Biofilm

added with only BHI broth was used as the negative control. The microplates were incubated for 18 hours, after which, the medium in 96 and 6-well plates were decanted and the remaining planktonic cells were removed by rinsing the wells with PBS pH 7.2) three times.

Biofilm inhibition assay

The inhibition effect of candy formulas toward dual-species biofilm was analyzed using method described by Yamanaka et al. (2004) After PBS rinsing, the plates were air dried and the adhered bacteria was stained with 200 µL of 0.5% crystal violet (CV, Sigma Aldrich) for 15 minutes at 37°C. After rinsing twice with 200 µL of PBS, bound dye was extracted from the stained cells using 200 µL of 95% ethanol. The OD_{490} of the extracted CV was measured with a microplate reader (Bio-Rad Laboratories, Hercules, CA). The absorbance value OD samples compared to OD control. Each experiment was performed in triplicate and repeated two times in separated occasions.

Bacterial DNA quantification

Microbial DNA was extracted using Trizol® reagent, following the instruction provided by the company. The DNA concentration was determined by spectrophotometer and standardized prior to qPCR analysis. The qPCR mixture for DNA quantification (10 µL) included 5 µL SYBR Green 1x Universal (KAPA Biosystem), 1 µL DNA (100 µg/mL), 3.2 µL DEPC water, and 0.3 µLof 5 mM forward and reverse real-time PCR primers that was Ss 16S rRNA, Sm 16S rRNA, and universal primers of 16S rRNA gene (Table 1). The qPCR protocol included one cycle of 95°C for 3 minutes, followed by 40 cycles of 95°C for 3 second and 60 minutes for 30 second. The bacterial load was determined based on the proportion of each species compared with total bacteria. It was determined by using the ΔC_t method (Yoshida et al., 2003).

Analysis of the expression of spxB mRNA

RNA extraction was performed in similar procedures with DNA extraction. It was reversed to cDNA using Superscript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Moreover, reactions mixture (10 µL) which contained 1×KAPA SYBR Green Master Mix (KAPA Biosystems), 1 µL cDNA, forward and reverse primers (0.5 µM) of spxBSs was centrifuged at 2000 rpm for 1 minutes. The house keeping gene (16S rRNA) was used as internal control. All primers used in this study are shown in Table 1. The real-time PCR cycle was carried out under the similar conditions as previous DNA quantification. The relative change in spxB mRNA expression was analyzed using 2-ΔΔCt (Suzuki et al., 2005).

Statistical analysis

Student's t test (SPSS Inc 17.0 software) was carried out to analyze the data significance on a p-value of <0.05 among the biofilms OD, *spxB* mRNA expression, and bacterial proportion of experimental samples to the control groups. One-way analysis of variance (ANOVA) by SPSS Inc 17.0 was used in biofilm inhibition analysis with significance value $p<0.05$. ANOVA analysis was carried out to support the assumption/discussion about significance biofilm inhibition between samples.

Table 1. Primers used for qPCR

Primer	Sequence (5′-3′)	Purpose	Ref.
Sm 16S rRNA forward	CCTACGGGAGGCAGC AGTAG	*S. mutans* quantification	(Shemesh et al., 2007)
Sm 16S rRNA reverse	CAACAGAGCTTTACG ATCCGAAA	*S. mutans* quantification	(Shemesh et al., 2007)
Ss 16S rRNA forward	CCGCCTAAGGTGGGA TAGATGATTG	*spxB* mRNA and *S. sanguinis* quantification	(Zheng et al., 2011a)
Ss 16S rRNA reverse	ACCTTCCGATACGGC TACCTTGTTAC	*spxB* mRNA and *S. sanguinis* quantification	(Zheng et al., 2011a)
spxB Ss forward	AATTCGGCGGCTCAA TCG	*spxB* mRNA quantification	(Zheng et al., 2011a)
spxB Ss reverse	AAGGATAGCAAGGAA TGGAGTG	*spxB* mRNA quantification	(Zheng et al., 2011a)
Universal forward	TCCTACGGGAGGCAG CAGT	Total bacteria quantification	(Suzuki et al., 2003)
Universal reverse	GGACTACCAGGGTAT CTAATCCTGTT	Total bacteria quantification	(Suzuki et al., 2003)

RESULTS

Volatile compounds contents

Volatile compounds of SCC and NSCC are presented in Table 2. The major compounds in SCC were 1,8-cineole and α-terpineol, while in NSCC were menthol and α-terpineol. Both SCC and NSCC contained 1,8-cineole, α-terpineol, β-caryophyllene, terpinen-4-ol, menthol, menthone, limonene, β-pinene, α-terpinene, γ-terpinene, and viridiflorol. Monoterpene groups, especially oxygenated monoterpenes, dominated these bioactive components.

Inhibition of dual species biofilm by Cajuputs candy

SCC, unflavored sucrose candy, NSCC, and unflavored non-sucrose candy were evaluated for their effect on the dual-species biofilm formation. Figure 1 shows that after incubation, both sucrose and non-sucrose candy showed inhibition activity.

The inhibition of all candy samples were significantly different from control ($p<0.05$). Moreover, the biofilm inhibition between samples were observed. OD values with different subset (a, b, ab, and c) mean they were significantly different with each other. As could be seen from Figure 1, the inhibition of SCC and NSCC were significantly higher compared with unflavored sucrose candy indicated the lower growth of the tested bacteria in biofilm mass of SCC and NSCC. The addition of cajuput and peppermint oils as flavoring ingredients in SCC and NSCC significantly reduced the biofilm formation by more than 50% compared to the control (growth medium cultured with bacteria without any formula addition). Meanwhile, the inhibition of SCC was not significantly different from unflavored non-sucrose candy ($p>0.05$). Biofilm inhibition was also observed in unflavored sucrose candy. Biofilm inhibition of unflavored sucrose candy was significantly lower compared to the control.

Table 2. Chemical composition of volatile compounds of Sucrose Cajuputs Candy (SCC) and Non-Sucrose Cajuputs Candy (NSCC)

No.	Compounds	LRI Ref[a]	SCC		NSCC		References for antimicrobial activities
			LRI exp[b]	%	LRI exp[b]	%	
1	α-pinene	939	940	0.98	-	-	
2	β-pinene	979	984	0.86	986	0.11	(Maggi et al., 2009)
3	p-cymene	1026	1031	1.63	1031	0.41	
4	D-limonene	1029	-	-	1039	1.92	(Inouye et al., 2001)
5	1,8-cineole	1031	1043	23.67	1044	8.48	(Maggi et al., 2009 ; Hamoud et al., 2012)
6	γ-terpinene	1059	1064	2.78	1065	0.51	(Inouye et al., 2001)
7	Terpinolene	1088	1094	1.9	1095	0.66	
8	Linalool	1096	1098	0.36	1100	0.34	(Dwivedi et al., 2012 ; Maggi et al., 2009)
9	Isopulegol	1145	1156	0.32	1158	0.63	
10	Menthone	1152	1165	3.3	1168	4.43	(Inouye et al., 2001)
11	Isomenthone	1162	1175	2.91	1178	4.34	
12	Menthol	1171	1185	7.29	1191	13.32	(Jedlickova et al., 1994 ; Inouye et al., 2001)
13	Terpinen-4-ol	1177	1189	1.86	1194	1.28	(Jedlickova et al., 1994 ; Maggi et al., 2009)
14	Neoisomenthol	1186	1195	0.26	1199	0.51	
15	α-terpineol	1188	1203	9.17	1209	9.97	(Dwivedi et al., 2012 ; Inouye et al., 2001)
16	Pulegone	1237	1252	0.47	1256	0.92	
17	Piperitone	1252	1267	0.29	1271	0.73	
18	Menthyl acetate	1295	1299	1.37	1302	2.56	
19	α-terpinyl acetate	1354	1359	3.66	1362	3.38	
20	α-copaene	1376	1398	0.34	1400	0.39	
21	β-elemene	1390	1409	0.32	1412	53	
22	β-caryophyllene	1419	1452	6.78	1456	7.64	(Heleno et al., 2011)
23	α-humulene	1454	1475	4.2	1489	4.67	
24	Viridiflorol	1592	1631	0.57	1635	0.77	(Iscan et al., 2002)

Note:
a: LRI reference (Adams, 2009) with DB-5 column; b: LRI experiment with DB5-MS column.

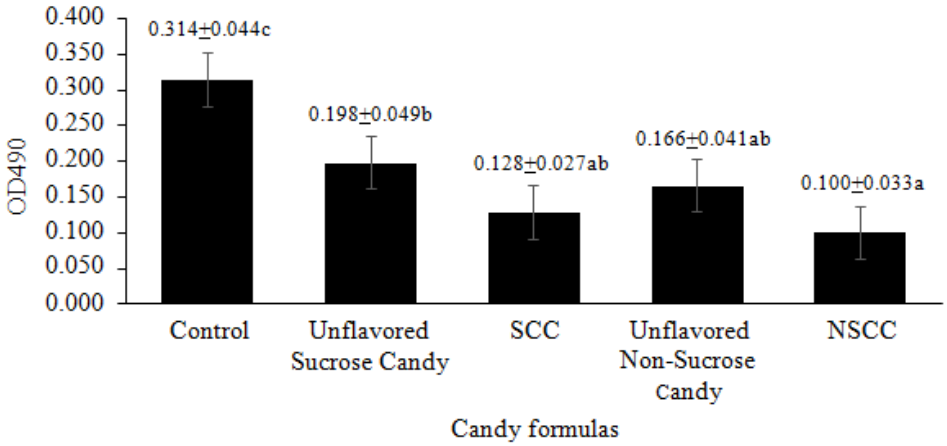


Figure 1. Inhibition of candies exposures on dual-species (*S. sanguinis* and *S. mutans*) biofilms

Bacterial DNA quantification: effect of candy exposure to the relative amount and the proportion of *S. sanguinis* and *S. mutans* in dual-species biofilm

Table 3 showed the quantification of relative amount of total DNA in the four formulas compared to the total DNA in the control group (defined as 100% growth). Our data showed that the exposures of SCC and NSCC decreased the amount of total bacterial DNA significantly, indicating the involvement of the flavor. In contrast, when the unflavored candies were exposed into the bacterial biofilm, the amount of bacterial DNA were significantly increased compared to those of control. Nevertheless, the DNA proportion of *S. sanguinis* and *S. mutans* in the biofilm did not show any significant difference between samples and control (Table 3). This study showed that the exposure of the candies did not alter the antagonistic interaction between *S. sanguinis* and *S. mutans, in vitro.*

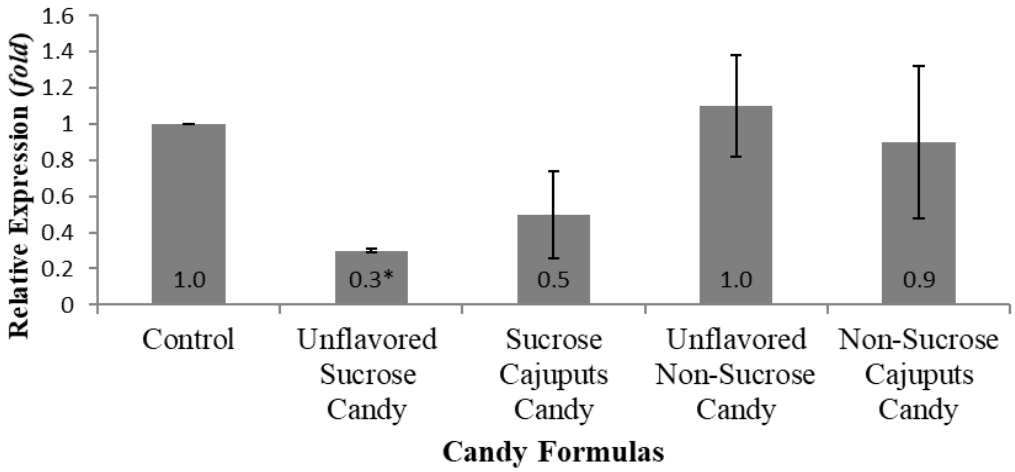
Tables 3. Effect of candies exposures to the relative amount of total DNA bacteria and DNA proportion of *S. sanguinis* and *S. mutans* in dual-species (*S. sanguinis* and *S. mutans*) biofilm using qPCR

Formula	Relative amount of total DNA bacteria (%)	Proportion	
		S. sanguinis (%)	*S. mutans* (%)
Control	100.00	41.47	58.53
Unflavored sucrose candy	184.77	35.58	64.42
SCC	1.20	35.39	64.61
Unflavored non-sucrose candy	221.29	36.35	63.65
NSCC	1.04	38.95	61.05

SCC: Sucrose Cajuputs Candy, NSCC: Non-Sucrose Cajuputs Candy
No significance difference in proportion was observed between sample and control in the analysis of student's t test with $p>0.05$

Effect of candy exposure on the expression level of *spxB* mRNA in the biofilms

Figure 2 showed that the exposure to all sample formulas did not eliminate the expression of *spxB* gene. No statistical difference was found between the expression of samples and the control group, which meant that the ability of *S. sanguinis* in producing H_2O_2 as its competing agent could be maintained. The *spxB* expression of NSCC and unflavored non-sucrose candy were higher than SCC.



*p<(0.05) indicated statistically significant difference between sample and the control group.

Figure 2. *spxB* mRNA expression level affected by candy formulas in dual-species (*S. sanguinis* and *S. mutans*) biofilm

DISCUSSION

Volatile profile

The volatile profiles of SCC and NSCC showed a similarity but vary in percentage (Table 2). Both candies were using cajuput and peppermint oil as the flavor. The flavor concentration in these candies were about 0.9%, that consisted of cajuput oil (0.7-0.8%) and peppermint oil (0.1-0.2%) based on their original formulations. Different heating temperature during production of SCC and NSCC may caused variation in their volatile percentage (Iftari et al., 2013). Table 2 showed that oxygenated monoterpenes (1,8-cineole, α-terpineol, menthol, menthone, and terpinen-4-ol) were dominated the bioactive volatiles in SCC and NSCC. Functional groups of bioactive compounds might contribute to their antimicrobial activities. As reported by Inouye et al. (2001), the antimicrobial activity of terpene alcohols (menthol, α-terpineol, and terpinen-4-ol) were higher than terpene ketone (menthone) as well as terpene ether (1,8-cineole), and terpene hydrocarbons (limonene, β-pinene, and γ-terpinene) were lowest in activity.

Biofilm inhibition

This study showed cajuputs candy exposures suppressed the formation of dual-species (*S. sanguinis* + *S. mutans*) biofilms. In comparison to the control, cajuputs candy (SCC and NSCC) were more effective in inhibiting biofilm formation than the unflavored candies (Figure 1). Meanwhile, biofilm inhibition of unflavored sucrose candy was significantly lower compared to the control, despite of the missing flavor as anti-bacterial agent. The potency of cajuput and peppermint oils as antibiofilm agent was due to their bioactive volatiles. Cajuput and peppermint oils contain highly hydrophobic substances, such as 1,8-cineole, menthol, and menthone (Table 2). These volatile components have been reported for their antimicrobial capacity (Inouye et al., 2001; Maggi et al., 2009; Hamoud et al., 2012; Iscan et al., 2002; Jedlickova et al., 1994; and Dwivedi et al., 2012). Direct contact of these oils to the biofilm at long period of incubation (over 18 hours) could inhibit biofilm growth. The inhibition mechanism can be vary by disturbing membrane function, inhibition of cells respiration, and alteration of ion transport processes which lead to the death of microbial cell (Hamoud et al., 2012), thus can reduce biofilm formation. The biofilm inhibition of NSCC was higher compared to SCC, which meant that the combination of flavor and non-sucrose carbohydrate in NSCC may play a role. However, further study is needed to elucidate the exact mechanism of SCC and NSCC in inhibiting biofilm formation.

Not only NSCC and SCC, the unflavoured non-sucrose candy in comparison with the control also exhibited a significant inhibition in biofilm formation. As shown in Figure 1, the inhibition of unflavored non-sucrose candy was not significantly different from SCC. Lower biofilm density produced when biofilms were exposed to non-sucrose candies than that were exposed to sucrose candies. It showed that the usage of isomalt to substitute sucrose in the candy formulation exhibited biofilm inhibition. In the present study, different kind of carbohydrate were used, and this may promote different biomass density. Therefore, the usage of isomalt in non-sucrose candy formulations will produce lower biomass density in comparison with sucrose or glucose containing candies (Mayo and Ritchie, 2009). Sucrose is naturally more available to be fermented by microorganism than isomalt (Childers et al., 2011).

The unflavored candies were also reported to have lower biofilm density than control (Figure 1). In this study, the unflavored sucrose candy contained high ammount of sucrose and glucose, while the unflavored non-sucrose candy contained high amount of isomalt. The sucrose and isomalt concentration were approximately 50% of the candy formulations (data not shown), meanwhile the growth medium (BHI broth) itself already contained sucrose and glucose. The high amount of such carbohydrates (e.g. sucrose and isomalt formulations) in the biofilm may cause environmental disturbance to bacterial growth. High content of external carbohydrates exposed to the biofilm will modify the growth environment, increases osmotic pressure, that will disturb bacterial growth (Touger-decker and Loveren, 2003). These disturbances were able to reduce the ability of bacteria to form biofilm.

In this study, the result of biofilm inhibition was presented by optical density (OD) value. OD value sometimes represents number of bacterial cell, but in term of biofilm, it does not merely indicating bacterial cell, but biofilm mass. Biofilm is a complex mixture of several materials consisting of bacterial cells, proteins, carbohydrates, water, lipids, including non-viable cell of bacteria that form a biofilm mass (Kreth et al., 2008). It means that OD value also represented biofilm density. Biofilm can have low OD value, eventhough its bacterial number (DNA) was high. As it can be shown, a contradictive result was found between OD value (Figure 1) and DNA amount (Table 3) of unflavored candies compared to the control.

DNA quantification

Both unflavored sucrose candy and unflavored non-sucrose candy were able to decrease biofilm formation compared with control (Figure 1). Meanwhile, higher bacterial DNA was detected in those unflavored candies (Table 3). It seemed that OD values from the biofilm inhibition measurement could not be used to distinguish the amount of bacterial load. Since the crystal violet assay did not only determine the number of bacterial cells but also measure the extracellular matrix within biofilm, low OD value did not mean low bacterial number. OD value is the determination of turbidity, which represents the biomass density of the biofilm (Bakke et al., 2001). Therefore, in contrast with crystal violet assay, qPCR is a sensitive method to quantify total and/or individual bacterial DNA in clumping cells, including the presence of viable and non-viable cells (Childers et al., 2011). Moreover, it can also be used to quantify specific bacterial species (Fortin et al., 2001).

In this study, unflavored non-sucrose candy had the higher number of bacterial DNA and even higher than that in unflavored sucrose candy (Table 3). This result indicated that isomalt was used in biofilm metabolism. However, isomalt can still be used in bacterial metabolism, thus can support bacterial growth. Mayo and Ritchie (2009) reported that the incubation time of 18-24 hours implied isomalt degradation by *Streptococci* isolates used. Frequent and prolonged contact of isomalt (an equimolar mixture of D-glucopyranosyl-1,6-sorbitol and α-D-glucopyranosyl-1,6-mannitol) used in “sugar-free” cough drop syrup could be degraded to produce glucose, sorbitol, and mannitol.

This study showed that NSCC had the greatest antibacterial activity and inhibition toward biofilm formation (Figure 1), which was significantly different from the control. NSCC inhibited biofilm formation by approximately 68.2% compared to

control and decreased bacterial DNA to 1% of the control (Table 3). These phenomena were due to antibacterial volatiles provided from the flavor, supported by the limitations of the substrate. Essential oils tend to inhibit more powerfully on Gram-positive bacteria than the growth of Gram-negative bacteria, which could be attributed to the different structure and cell membrane compositions (Guiterrez et al., 2008). *S. mutans* and *S. sanguinis*, which are Gram-positive bacteria, have single membrane structure with thick peptidoglycan layer that are sensitive to essential oil (Trombetta et al., 2005).

The DNA proportion was measured to determine the effect of cajuputs candy formulas (SCC and NSCC) on the ability of *S. sanguinis* to maintain its antagonistic competency upon *S. mutans* growth. Both SCC and NSCC were able to decreased bacterial load in the biofilm (Table 3) and inhibited biofilm growth (Figure 1), but the result of DNA proportion (Table 3) indicated that they did not show any capacity to interfere the natural interaction among *S. mutans* and *S. sanguinis*. The absence of cajuputs candy increased the cells number of both bacterial in biofilm. On the contrary, their presence simultaneously inhibited (Table 3). Kreth et al. (2005) reported that *S. sangiunis* and *S. mutans* have a competitive exclusion in the biofilm system caused by the production of diffusible substances that inhibited the other growth. *S. sanguinis* produces peroxidase, while *S. mutans* produces bacteriocin. This study showed that, the cajuputs candy exposures did not interfere such competitiveness effect (Table 3). Thus, the exposures may have a capability in maintaining the antagonistic competency of *S. sanguinis* upon *S. mutans* in the biofilm.

Gene Expression of *spxB* mRNA

We have demonstrated that the presence of cajuputs candy in biofilm development resulted in up-regulating of *spxB* mRNA expression, which implied the transcription activity of *spxB* gene of viable *S. sanguinis*. The *spxB* gene produces an enzyme responsible for production of H_2O_2 by *S. sanguinis* to inhibit the growth of *S. mutans* (Zheng et al., 2011a). The detectable expression of *spxB* mRNA in all of the tested formula (Figure 2) indicated the competitive capacity of *S. sanguinis* against *S. mutans* and showed that viable bacteria remained within the biofilms. No negative effect was found in the expression of *spxB* mRNA compared to control since there were no statistically difference between samples and control (Figure 2). This indicated that the bacterium still has capacity in controlling *S. mutans* as naturally occurred *in vivo* (Kreth et al., 2008).

The exposure of unflavored sucrose candy showed the lowest expression level of *spxB* mRNA compared to others formulas (Figure 2), whereas the amount of total DNA bacteria was very high (Table 3). Kreth et al. (2005) mentioned that in high sucrose concentration, *S. sanguinis* prefered to use the carbon source for growth rather than spend a lot of energy to produce H_2O_2 to suppress the growth of the *S. mutans*.

The *spxB* mRNA expression of that in NSCC exposure was higher than SCC exposure (Figure 2). The use of isomalt on NSCC might create a stress condition due to the lack of carbon source that could be metabolized (Lemos et al., 2005). Therefore, under these conditions, *S. sanguinis* would focused on producing H_2O_2 to maintain the bacteria coexistence (Kreth et al., 2005), and increase the bacterium competitive capacity in the biofilm (Zhu and Kreth, 2012).

It is known that the essential oil is a mixture of various components that are mostly hydrophobic compounds and have antibacterial properties. The mechanism essential oil biofilm inhibition were very diverse but mostly related to disruption of membrane function by penetration of hydrophobic compounds (Nazaro et al., 2013). Other proposed mechanisms include the alteration of the membrane fatty acids, degradation of cell wall, alteration of the proton motive force, increase of permeability of the membrane, and membrane protein damage (Nazaro et al., 2013). Even though SCC and NSCC exposure effectively inhibited the dual-species biofilm formation (Figure 1), the inhibition did not eliminate the ability of *S. sanguinis* to produce H_2O_2, as proven by the up-regulation of *spxB* mRNA expression because *S. sanguinis* is less susceptible to bioactive compounds in essential oil than *S. mutans* (Magalhaes et al., 2016). Therefore,

the survival *S. sanguinis* still has the ability to express the *spxB* gene. However, more studies are needed to fully understand the inhibition mechanism of cajuputs candy in the dual-species biofilm formation.

CONCLUSIONS

The data showed that the absence of flavor in the candy triggered greater biofilm growth than the control. Flavored candy exposures effectively inhibited the bacterial growth within the biofilms. Each tested candy showed the ability to maintain the antagonistic competency of *S. sanguinis* toward *S. mutans* in dual-species biofilms. The combination of active volatiles and non-sucrose material made NSCC exposures as the most effective formula to inhibit the biofilm growth without eliminating its competitive capacity of *S. sanguinis* toward *S. mutans* in dual-species biofilm. However, more studies are needed to fully understand the inhibition mechanism of cajuputs candy in polymicrobial biofilm formation. The data in this study can be used for further exploration of the SCC and NSCC potency in preventing dental caries *in vivo*.

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REFFERENCES

Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Carol Stream, IL: Allured Publ. Corp., 2009.

Bakke R, Kommedal R, Kalvenes S. Quantification of biofilm accumulation by an optical approach. J Microbiol Methods, 2001; 44: pp.13-26.

Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, Boches SK, Dewhirst FE, Griffen AL. Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol, 2002; 40: pp.1001–1009.

Childers NK, Osgood RC, Hsu KL, Manmontri C, Momeni SS, Mahtani HK, Cutter GR, Ruby JD. Real-time quantitative polymerase chain reaction for enumeration of *Streptococcus mutans* from oral samples. Eur J Oral Sci, 2011; pp.119:447-454.

Dwivedi D, Khandelwal G, Patidar RK, Singh V. Antimicrobial activity of *Menthaarvensis* against clinical isolates of human cariogenic pathogens- an *in vitro* study. Int J Pharm Sci Res, 2012;3: pp.1355-1360.

Eslami H, Zarandi A, Pouralibaba F, Fakhrzadeh V, Falsafi P, Sepas RR. Assesment of relationship between. *Streptococcus mutans*, dental caries, and TGF-β. Asian J Biomed Pharmaceutic Sci, 2016; 6 (56): pp.20-22.

Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res, 2004;38 pp.182–191.

Fortin NY, Mulchandani A, Chen W. Use of real-time polymerasechain reaction and molecular beacons for the detection of *Escherichia coli* O157:H7.Anal Biochem, 2001; pp.289:281–288.

Galvao LC, Furletti VF, Bersan SM, da Cunha MG, Ruiz AL, de Carvalho JE, Sartoratto A, Rheder VL, Fiqueira GM, Duarte MC, Ikegaki M, de Alencar SM, Rosalen PL. Antimicrobial activity of essential oils against *Streptococcus mutans* and their antiprolifrative effects. Evid based Complement Alternat Med, 2012;doi: http://dx.doi.org/10.1155/2012/751435.

Gutierrez J, Barry-Ryan C, Bourke P. The anti-microbial efficacy of plant essential oil combinations and interactions with food ingredients. Int J Food Microbiol, 2008; pp.124:91–97.

Hamoud R, Sporer F, Reichling J, Wink M. Antimicrobial activity of a traditionally used complex essential oil distillate (Olbas[R]

Tropfen) in comparison to its individual essential oil ingredients. Phytomedicine, 2012; 19: pp.969-975.

Heleno VC, Martins CG, Morais GO, da Silva EH, Wakabayashi K, Carvalho C, Critti AM. Antimicrobial activity of the essential oils and non-polar extracts from leaves and flowers of *Tithonia diversifolia* against cariogenic bacteria. Planta Med, 2011; 12: pp.77-PE47.

Iftari W, Fardiaz D, Bachtiar BM, Wijaya CH. The potency of non-sucrose Cajuputs candy in inhibiting the formation of biofilm by *Streptococcus mutans* serotype c. XIII Asean Food Conference, Singapore; 2013.

Inouye S, Takizawa T, Yamaguchi H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J AntimicrobChemother, 2001; 47: pp.565-573.

Işcan G, Kirimer N, Kürkcüoğlu M, Başer KHC, Demirci F. Antimicrobial screening of *Mentha piperita* essential oils. J Agric Food Chem, 2002;50: pp.3943-3946.

Jedlickova Z, Ery V, Mott O, Nguyen DC. Antibacterial properties of cajuput oil. Medicaments et Aliments: L'Approche Ethnopharmacologique, 1994; 2: pp.293.

Kreth J, Merritt J, Wenyuan S, Fengxia Q. Competition and coexistence between *Streptococcus mutans* and *Streptococcus sanguinis* in the dental biofilm. J Bacteriol, 2005;187: pp.7193-7203.

Kreth J, Zhang Y, Herzberg MC. Streptococcal antagonism in oral biofilms: *Streptococcus sanguinis* and *Streptococcus gordonii* interference with *Streptococcus mutans*.J Bacteriol, 2008, 190: pp.4632-4640.

Lemos JA, Abranches J, Burne RA. Responses of cariogenic *Streptococci* to environmental stresses.*Curr Issues Mol Biol*, 2005; pp.7:95-107.

Magalhães CF, de Siqueira EP, de Oliveira EA, Zani CL, Peres RL, dos SantosKV, Andrade AA. Antimicrobial activity of *Piper aduncumleaf* extracts against the dental plaque bacteria *Streptococcus mutans* and *Streptococcus sanguinis*. J Med Plants Res, 2016; 10: pp.331-337.

Maggi F, Bramucci M, Cecchini C, Coman MM, Cresi A, Cristalli G, Lupidi G, Papa F, Quassinti L, Sagratini G, Vittori S. Composition and biological activity of essential oil of *Achillea ligustica All. (Asteraceae)* naturalized in central Italy: ideal candidate for anti-cariogenic formulations. Fitoterapia, 2009; 80: pp.313-319.

Mayo JA, Ritchie JR. Acidogenic Potential of "Sugar-Free" Cough Drops. Open Dent. J, 2009; 3: pp.26-30.

Muchtaridi, Subarnas A, Apriyantono A, Mustarichie R. Identification of compounds in the essential oil of nutmeg seeds (*Myristicafragrans Houtt.*) that inhibit locomotor activity in mice. Int J Mol Sci, 2010;11: pp.4771–4781.

Nazzaro F, Fratianni F, de Martino L, Coppola R, de Feo V. Effect of Essential Oils on Pathogenic Bacteria. Pharmaceuticals, 2013;6: pp.1451-1474.

Oda Y, Hayashi F, Okada M. Longitudinal study of dental caries incidence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in patient with intellectual disabilities. BMC Oral Health, 2015; 15: pp.102.

Percival RS, Devine DA, Duggal MS, Chartronand S, Marsh PD. The effect of cocoa polyphenols on the growth, metabolism, and biofilm formation by *Streptococcus mutans* and *Streptococcus sanguinis*. Eur J Oral Sci,2006;114: pp.343-348.

Saharkhiz MJ, Motamedi M, Zomorodian K, Pakshir K, Miriand R, Hemyari K. Chemical composition, antifungal and antibiofilm activities of essential oil of *Mentha piperita L.*

ISRN Pharm, 2012; doi: http://dx.doi.org/10.5402/2012/718645.

Shemesh M, Ashyalom T, Steinberg D. Expression of biofilm-associated genes of *Streptococcus mutans* in response to glucose and sucrose. J Med Micobiol, 2007;56: pp.1528-1535.

Somaraj V, Shenoy RP, Panchmal GS, Kumar V, Jodalli PS, Sonde L. Effect of herbal and fluoride mouth rinse on *Streptococcus mutans* and dental caries among 12-15-year-old school children: a randomized controlled trial. Int J Dent, 2017; doi: http://doi.org/10.1155/2017/5654373.

Suzuki N, Yoshida A, Nakano Y. Quantitative analysis of multi-species oral biofilms by taqMan Real-Time PCR. Clin Med Res, 2005; 3: pp.176-185.

Touger-Decker R, Loveren C. Sugar and dental caries (suppl). Am J Clin Nutr, 2003; 78: pp.881S-92S.

Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G. Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother, 2005; 49: pp.2474–2478.

Wijaya CH, Halimah, Kindly, Taqi FM.(2002). The composition of Cajuput candy as throat lozenges (Komposisi permen cajuput untuk pelega tenggorokan). Indonesia Patent No. 0000385S. The Ministry of Justice and Human Rights, Republic of Indonesia.

Wijaya CH, Nurramdhan IF, Bachtiar BM. Ability of Cajuput candy in maintaining oral health: *in vitro* inhibition of cajuput candy flavor formula against *Streptococcus mutans* and *Streptococcus sobrinus*. International Food Conference. Surabaya, Indonesia; 2011.

Wijaya CH, Rachmatillah AR, Bachtiar BM.Inhibition of Cajuputs candy toward the viability of *Candida albicans* by using *in vitro* assay. *J Teknologi dan Industri Pangan*, 2014; 25: pp.158-167.

Yamanaka A, Kimizuka R, Kato T, Okuda K. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. Oral Microbiol Immunol, 2004; 19: pp.150-154.

Yoshida A, Suzuki N, Nakano Y, Oho T, Kawada M, Koga T. Development of a 5′ fluorogenic nuclease-based real-time PCR assay for quantitative detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis.* J Clin Microbiol, 2003; 41: pp.863-866.

Zheng L, Chen Z, Itzek A, Ashby M, Kreth J. Catabolite control protein A controls hydrogen peroxide production and cell death in *Streptococcus sanguinis*. *J Bacteriol*, 2011;193: pp.516-526.

Zheng L, Itzek A, Chen Z, Kreth J. Oxygen dependent pyruvate oxidase expression and production in *Streptococcus sanguinis*. Int J Oral Sci, 2011;3; pp.82-89.

Zhu L, Kreth J. The role of hydrogen peroxide in environmental adaptation of oral microbial communities. Oxic Med Cell, 2012; pp.1-10.

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IMPROVING METHODOLOGY OF SQUALENE EXTRACTION FROM PALM FATTY ACID DISTILLATE (PFAD) THROUGH ENHANCED PRE-TREATMENT PROCESS

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ABSTRACT

Indonesia has been accounted as one of the biggest palm oil producers in the world with annual production capacity reaching over 34 million tons. As a consequence, the amount of wastes resulting from this industry requires immense attention to be given. One of the wastes resulted is the Palm Fatty Acid Distillate (PFAD), which in previous researches has been proven to contain some beneficial bioactive compounds such as squalene. Squalene is known as one of the best natural emollients for pharmaceuticals and cosmetics, so that many researches have given the attempt to extract squalene from PFAD. Despite all attempts, large amount of impurities such as free fatty acids (FFA) were still to be found present in squalene extract. Therefore, in this research an effort to enhance the pre-treatment process of PFAD was done by combining saponification process and centrifugation, in order to remove FFA prior to extraction process. Three different pre-treatment scenarios in single stage liquid-liquid extraction (LLE) were compared in their effect on squalene content found in the extract using GC-MS analysis. The analysis showed that the squalene content increased from 5.370 to 9.320 % (w/w) when centrifugation was applied. Adding another round of saponification to this method has increased the content even further to 23.940 %. Furthermore, the application of multiple stage extraction could increase the squalene content to 37.450 %.

Keywords: *Liquid-liquid extraction; multiple stage extraction; Palm Fatty Acid Distillate; squalene.*

ABSTRAK

Indonesia merupakan salah satu negara penghasil minyak sawit terbesar di dunia, dengan kapasitas produksi melebihi 34 juta ton per tahun. Dengan besarnya volume produksi minyak sawit, jumlah limbah yang dihasilkan pun sangat besar dan memerlukan perhatian khusus. Salah satu limbah industri minyak sawit adalah Distilat Asam Lemak Minyak Sawit (PFAD), yang telah dibuktikan mengandung beberapa senyawa bioaktif, di antaranya adalah *squalene. Squalene* merupakan salah satu krim alami terbaik untuk pengobatan dan kosmetik, sehingga banyak upaya telah dilakukan untuk mengekstrak *squalene* dari PFAD melalui proses bertingkat tunggal dan jamak. Akan tetapi, beberapa pengotor seperti asam lemak bebas masih ditemukan dalam ekstrak *squalene* yang diperoleh. Oleh karena itu, dalam penelitian ini dilakukan upaya untuk mengoptimalkan proses perlakuan awal, dengan menggunakan teknik separasi untuk mengurangi jumlah asam lemak sebelum ekstraksi dilakukan. Dari percobaan ini, ekstraksi tingkat tunggal memperlihatkan hasil analisa GC-MS kandungan squalene yang meningkat dari 5,370 ke 9,320 % (b/b) jika dilakukan sentrifugasi. Penambahan proses penyabunan pada metode ini juga meningkatkan kandungan skualen lebih lanjut ke 23,940 %. Dengan penggunaan ekstraksi bertingkat jamak dapat meningkatkan kandungan skualen menjadi 37,450 %.

Kata kunci: *Distilat Asam Lemak Minyak Sawit (PFAD); ekstraksi bertingkat jamak; ekstraksi cair-cair; squalene.*

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INTRODUCTION

In recent years, palm oil has become one of the most potential resources of vegetable oils in the world. Indonesia has been one of the biggest countries that produces palm oil which yields over 30 million tons yearly where 80 % is exported. There are several waste products, which come from the production of palm oil such as empty fruit bunches, palm press fiber, palm oil mill effluent, palm kernel cake, palm kernel shell, and sludge cake (Prasertsan and Prasertsan, 1996). Due to the high production rate of palm oil in Indonesia, the high amount of palm oil waste offers high potency to be utilized. One of the wastes that is generated through the refinery process of crude palm oil is Palm Fatty Acid Distillate (PFAD), which can be used as feedstock for animal feed, soap industry, oleo chemical industry and combustion for local power generation (Zero & Rainforest Foundation Norway, 2015).

PFAD was also found to contain some beneficial bioactive compounds, among those is squalene (Yusuf, *et. al*., 2015), which can be used mainly for food supplements and pharmaceutical ingredients. In addition, it also can be considered as one of the best natural emollients in pharmaceuticals and cosmetics (Gapor, 2010). Moreover, squalene can be applied as a detoxification factor, as an eye and skin antioxidant. It has been widely known, that the demand for squalene is increasing from time to time, while there is limited availability from conventional sources such as shark liver oil. At the time being, the availability of shark liver oil is low due to many regulations prohibiting the killing of sharks. With this condition, the interest to get squalene from other sources has been arisen. Obtaining squalene from PFAD became very favorable to be explored, despite several obstacles faced related to its purity (Gapor, 2010).

Based on a previous study (Yusuf, *et. al*., 2015), squalene extract can be obtained by using organic solvents such as dichloromethane (DCM) through liquid-liquid extraction (LLE). Wandira, *et. al.* (2017) has proposed an optimum condition for the saponification and extraction process to extract squalene from PFAD, resulting in an extract with a squalene content of around 24 %-w. In a separate study, Sibuyo, *et. al*. (2017) has applied multiple stage LLE to extract squalene from PFAD using DCM, and has proven that compared to a single stage extraction, this method could increase squalene content up to 1.33 times, depending on the ratio used between PFAD and DCM. However, in these researches, the content of FFA in the extract was still considerably high. It could be assumed, that the saponification process conducted prior to LLE, was not able to remove the majority of FFA contained in the PFAD. Hence, this research was aimed at enhancing the pre-treatment process, and to study the effect of incorporating different sequences of PFAD pre-treatment methods on the squalene content in the extract.

MATERIALS AND METHOD

Materials

The materials used in this research are including PFAD sample, L – ascorbic acid (Merck, Germany), potassium hydroxide (Merck, Germany), ethanol 96 % (JT-Baker), dichloromethane (Mallincrodt, USA), iso-propanol (Mallincrodt, USA), toluene (Mallincrodt, USA), and distilled water. The sample of PFAD was taken from a palm oil refinery industry in Bekasi, Indonesia.

Research Methodology

In this experiment, several pre-treatment methods were applied (Figure 1). It comprised of three different research paths, which were then followed with acid base titration analysis. Research path 1, is principally the suggested squalene extraction method from previous researches (Sibuyo, *et. al*., 2017 & Wandira, *et. al*., 2017), whose results this research aimed to improve. In research path 1, PFAD would go under a saponification process prior to a liquid-liquid extraction, with the purpose to as much as possible reduce the amount of FFA, by converting it into glycerol and soap. After saponification, LLE process would proceed using dichloromethane (DCM) as a solvent, where afterwards, the extracted sample was analyzed by using gas chromatograph – mass spectroscopy (GC-MS) analysis for the determination of squalene content.

In the second and third research paths, a combination between saponification and centrifugation was applied, prior to LLE. Centrifugation is a process that can separate a mixture based on density differences by applying centrifugal force field. The product of saponification between PFAD and KOH are mainly glycerol and soap, with the rest of unreacted FFA and impurities contained in the mixture. Given that the density of glycerol was 1.26 g/cm^3 and the density of soap is 0.932 g/cm^3, centrifugation was considered to be a practical method to separate the two phases. Free fatty acid, with a density of 0.961 g/cm^3 is expected to be found in a bigger portion in the bottom layer together with glycerol, while squalene with a density of 0.858 g/cm^3 should be found in the upper layer together with the soap. This way, it is expected that adding a centrifugation process after saponification will give favor to the subsequent process, which is the extraction of squalene.

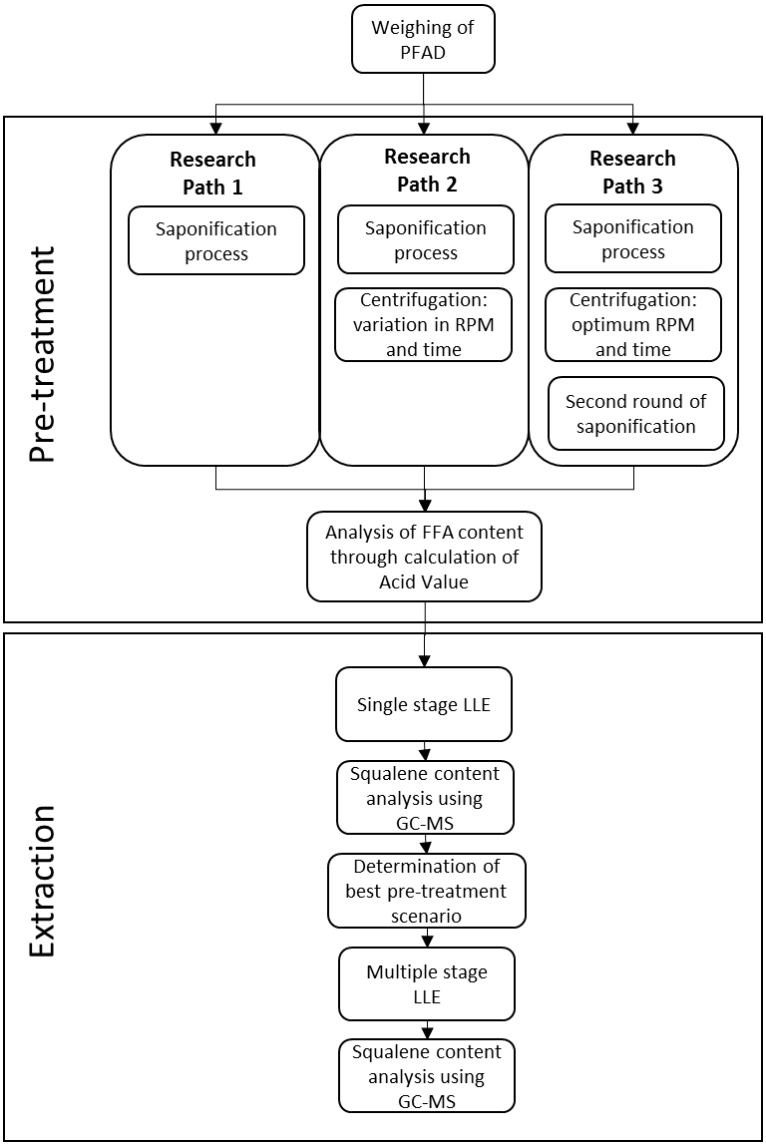

Figure 1. Experiment design

Multiple stage extraction process with 3 stages was applied in the latest part of the experiment in order to determine how much increase of squalene content can be achieved by using a combination of the selected pre-treatment scenario and the multiple stage extraction process.

Pre-Treatment Process

The pre-treatment process will comprise saponification and centrifugation processes. As much as 10 g of PFAD sample was added to 0.500 g of ascorbic acid in the three necks round bottom flask. Then, 88 ml of 96 % ethanol was added into the mixture. Heating was applied to maintain the temperature at 70˚C by using water bath while being continuously stirred using magnetic stirrer. The mixture was saponified with 10 ml of 50 %-w/v concentration of potassium hydroxide (KOH) using reflux condenser in water bath for 60 minutes. Then, centrifugation of the mixture occurred twice at 3,000 rpm and 5,000 rpm for 10 and 30 minutes. In the research path 3, the saponification procedure was repeated after the centrifugation process.

Extraction of Sample

Single Stage Extraction

The sample and 100 ml of distilled water was poured into 500 ml separator funnel at ± 25˚C and shook in vertical direction carefully. Following this step, 75 ml of dichloromethane was added into the mixture and left for approximately an hour until two separated layers were formed. The transparent layer of the mixture was removed and the remaining liquid was mixed with another 75 ml of dichloromethane and left for an hour for extraction. The transparent layer was removed once again to be collected with the previous obtained extract. This repetition was conducted three times, and the total volume of the transparent layer was measured and labelled as Extract 1.

Multiple Stage Extraction

The multiple stage extraction was conducted following the schematic diagram shown in Figure 2. Each circle in this figure represents a single extraction process, with the exact procedure to be performed as explained previously. The numbers written in the circles show different separator funnels used in each step. The procedure for multiple stage extraction as shown above is a means to batch-wisely approximate a continuous multiple stage process. The first three stages shown in the diagram is the pre-conditioning stage, where stages 4 until 6 are considered to be the approximated real condition. Hence, the analysis of squalene content was done on extracts E9, E12 and E15, and additionally also on extract E1, so that a comparison between squalene content prior and subsequent to multiple stage extraction can be made.

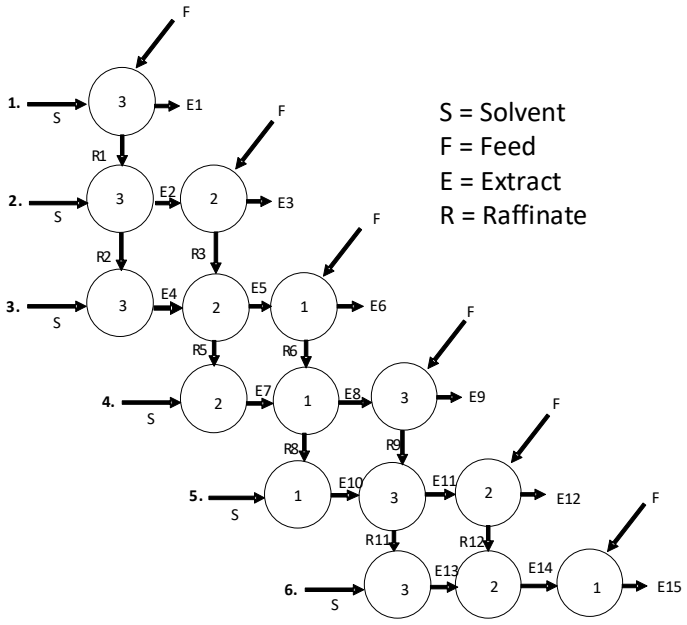

Figure 2. Multiple extraction process with 3 stages

Analysis Techniques

Titration Acid-Base Analysis

In order to determine the free fatty acid percentage in oil, titration acid-base analysis based on ASTM D 974 (American & Standard 2003) was conducted. This is the standard test method for acid and base number by color indicator titration. This method can be used to indicate the acidic or base constituents in petroleum products and lubricants that are soluble in mixtures of toluene and isopropanol. Titration was conducted in this study by the addition of 50 ml of iso-propanol and 50 ml of toluene into the sample in 250 ml conical flask. The addition of 20 drops of napthol benzene indicator into the solution then followed, and this mixture was titrated with standard alkali solution (potassium hydroxide 0.087M), while being vigorously rotated until dark green color was observed. The volume of standard KOH was used to determine the free fatty acid value.

Gas Chromatograph – Mass Spectrocopy (GC-MS) Analysis

Gas Chromatograph – Mass Spectroscopy (GC-MS) analysis was used to analyse the squalene content of the resulting extracts. The column that was used was HP Ultra 2 Capillary Column Length x Internal Diameter x Film Thickness = 30 m x 0.25 mm x 0.25 µm. Helium (He) gas was used as the carrier gas. The initial temperature of oven was set at 70°C and held for 0 minute, then rising at 3°C/min to 150°C. The instrument was then injected with 1 µl sample with the constant flow of 0.9 µl/min. Thus, it was being on hold for 1 minute and finally rising at 20°C/min to 280°C and was held for 26 minutes. The temperature was set for injection port at 250°C, ion source at 230°C, interface at 280°C and quadruple at 140°C. The detector used was coupled to mass spectrometry.

RESULTS AND DISCUSSION

Optimization of pre-treatment process

The extraction method by Sibuyo, *et. al*. (2017) & Wandira, *et. al*. (2017), which only suggested a single saponification method as a pre-treatment prior to LLE, was to be improved through addition of centrifugation and another round of saponification in this research. In order to analyze whether there is a decrease in FFA content in the PFAD sample in each path, titration acid-base analysis was conducted. The volume of KOH added to change the titrated sample color into dark green was used to calculate the FFA content (%-w).

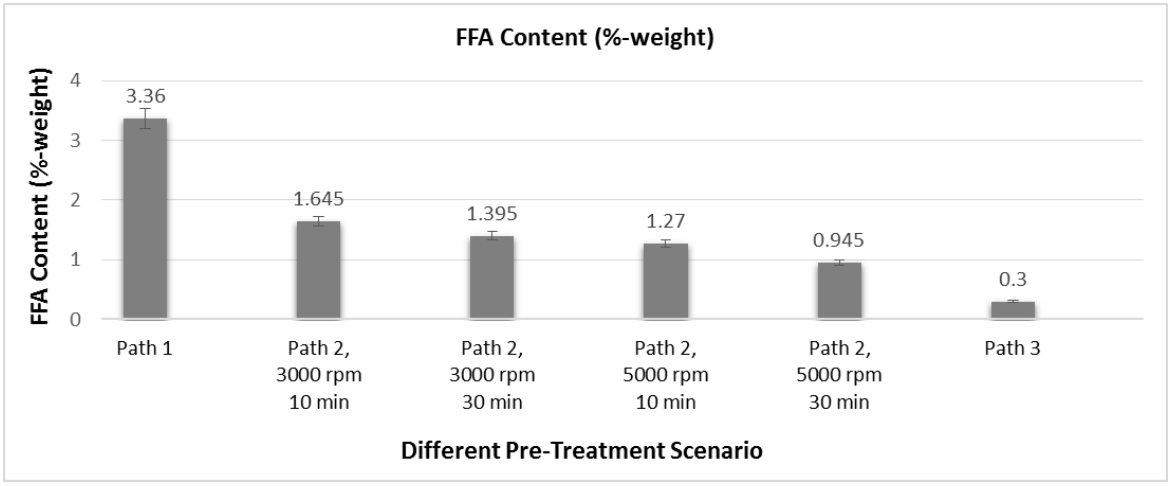

Figure 3. Free fatty acid content (%-weight) from different pre-treatment scenario

The result summarized in Figure 3 shows that by applying centrifugation after saponification process (research path 2), the FFA content can be reduced to less than half of its initial amount. The FFA content has decreased from 3.36 ± 0.03% to 0.945 ± 0.135% when a centrifugation at 5,000 rpm for 30 minutes took place subsequent to saponification process. Adding another round of saponification subsequent to the centrifugation process (research path 3) has shown further improvement in removing FFA. Research path 3, which applied a combination between centrifugation and double saponification, has shown results with lowest FFA content (0.3 ± 0.06%) and therefore is proven to be able to remove a large portion of FFA contained in PFAD.

In order to confirm that the application of research path 3 is not only going to remove FFA but will as well have an effect on the squalene content in the final extract, the observation was continued by performing single stage extraction subsequent to the pre-treatment. Squalene content analysis of the resulting extracts was then conducted by means of GC-MS analysis.

All extract samples resulting from the single LLE process and the squalene standard were injected into GC-MS to obtain the chromatograms and mass spectrums. The result of GC-MS was observed qualitatively by chromatogram, including the quantitative analysis by the area under each peak which was shown in the chromatogram. Based on the chromatogram of squalene standard as shown in Figure 4 below, the retention time of squalene was shown at 26.107 min. Therefore, there should be a peak with similar retention time in the chromatogram if the extract does contain squalene. An example of GC-MS analysis result on one extract is shown in Figure 5, where a similar peak to the squalene standard could be observed at an approximately same retention time. This indicates qualitatively that this extract indeed contains squalene. Afterwards, a quantification of the amount of squalene present in the extract was done by determining the area below the peak.

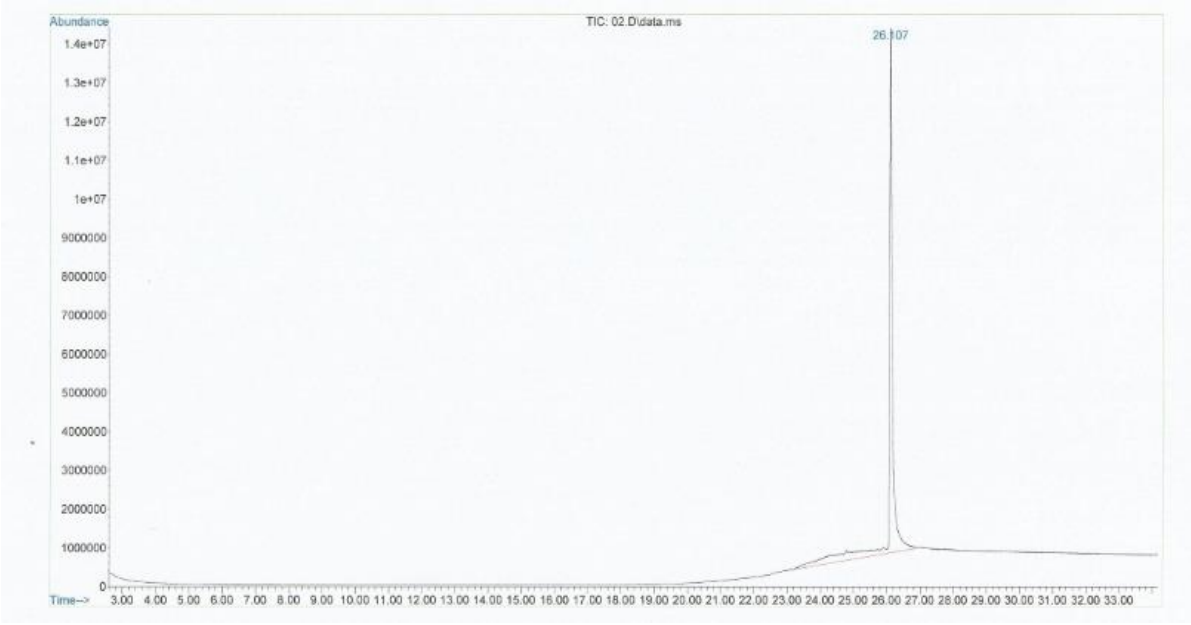

Figure 4. Chromatogram of squalene standard at retention time 26.107 min

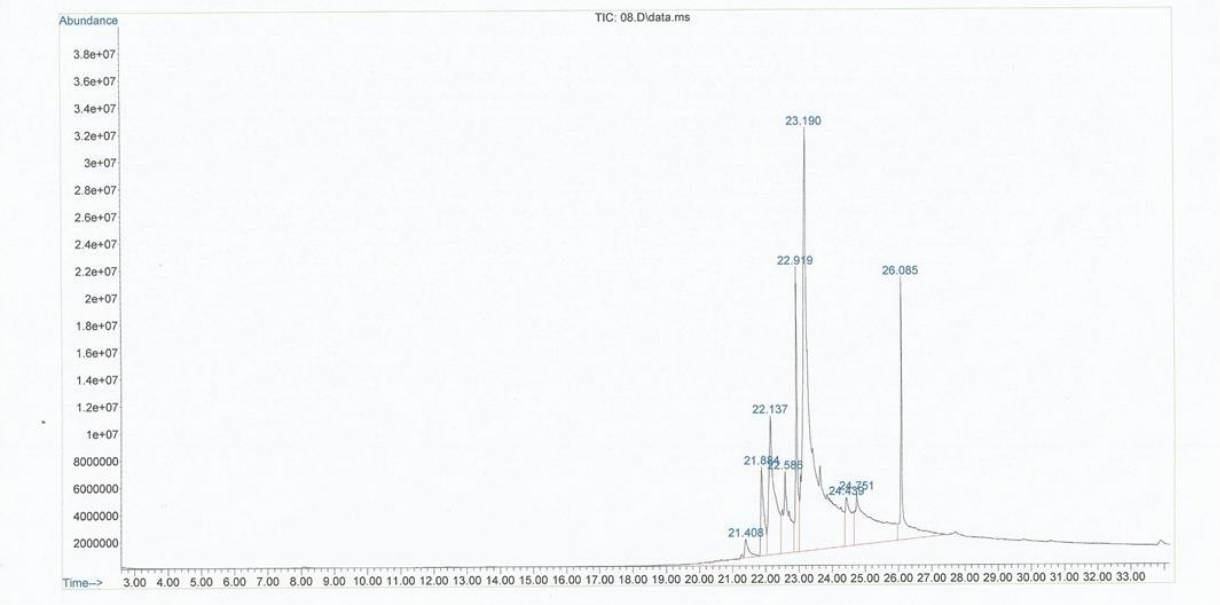

Figure 5. Chromatogram of an extract (research path 2 at 5,000 rpm and 30 minutes) at retention time 26.085 min indicating the existence of squalene

The three different pre-treatment scenarios performed previously were observed further to study their effects on the squalene content in the extract through GC-MS analysis and the results of these analyses are depicted in Figure 6.

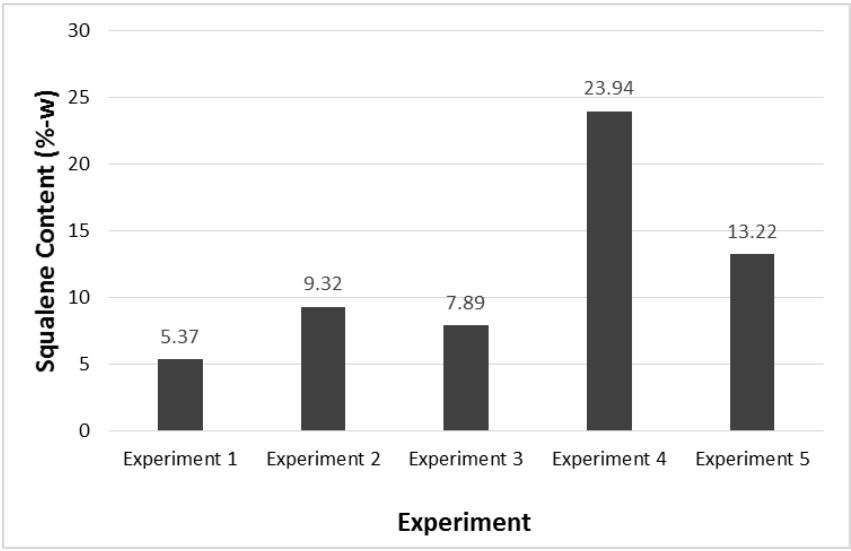

Figure 6. Squalene content in extracts resulting from 5 experiments based on GC-MS Result. Experiment 1: saponification process with 50 %-w/v KOH continued by LLE process (representing the research path 1). Experiment 2 and 3: saponification process with 50 %-w/v KOH, continued with centrifugation at 5,000 rpm for 30 minutes, and LLE (representing research path 2 in two replications). Experiment 4 and 5: saponification process with 50 %-w/v KOH, followed by centrifugation at 5,000 rpm for 30 minutes, second step of saponification with 50 %-w/v KOH, and LLE Process (representing research path 3 in two replications).

Figure 6 shows that the squalene content in the extract resulting from single stage extraction was increased from 5.370 % (research path 1) to 9.320 % (research path 2). However, the highest squalene content was found in experiment 4 (research path 3), where up to 23.940 % squalene content was obtained. Even though the replication of GC-MS analysis of the same research path (experiment 5) did not deliver as high squalene content as in experiment 4, it still showed an increase in squalene content compared to other experiments.

There are several reasons why research path 3 delivered highest squalene content. This research path combined a centrifugation and double saponification as pre-treatment of PFAD, and was proven to be able to remove a large portion of FFA in it. After the implementation of this combination of pre-treatment, the FFA content was reduced down to 0.3 ± 0.06 %. During extraction, squalene was expected to be more soluble in DCM, while soap phase will be distributed more in water phase. The extraction solvent DCM was chosen, because it had been proven by Yusuf (2015) to be the best solvent in extracting squalene from PFAD. However, FFA is also found to be highly soluble in non-polar organic solvent (Astuti, *et. al.*, 2010), so that it might also be found in DCM phase during extraction. Hence, reducing the amount of FFA as much as possible prior to the extraction will favor the selectivity of squalene.

Moreover, the centrifugation was also capable in removing a large portion of glycerol and soaps, which are not desired to enter the LLE process. Removing these impurities has been proven to have significant effect on the squalene content in the final extract. Hence, based on this result, an optimum pre-treatment process for squalene extraction from PFAD is decided to follow the research path 3, which combines a centrifugation and double saponification process.

Multiple Stage Extraction

Multiple stage process (Figure 2) was applied in this study in order to determine how far the squalene content can be increased by applying the previously chosen pre-treatment method. The first three stages were the preliminary stages, and for the analysis, stage 4 until 6 were observed. Each circle of the process represents a single stage extraction conducted in a separator funnel, where layers of extract and raffinate would be formed. The extract resulted from stage 4 to 6 were then analyzed using GC-MS to determine their squalene content. Additionally, extract coming from the first step was also analyzed in order to make a before-and-after comparison of the squalene content. The GC-MS results of these extracts are summarized in the Figure 7 below.

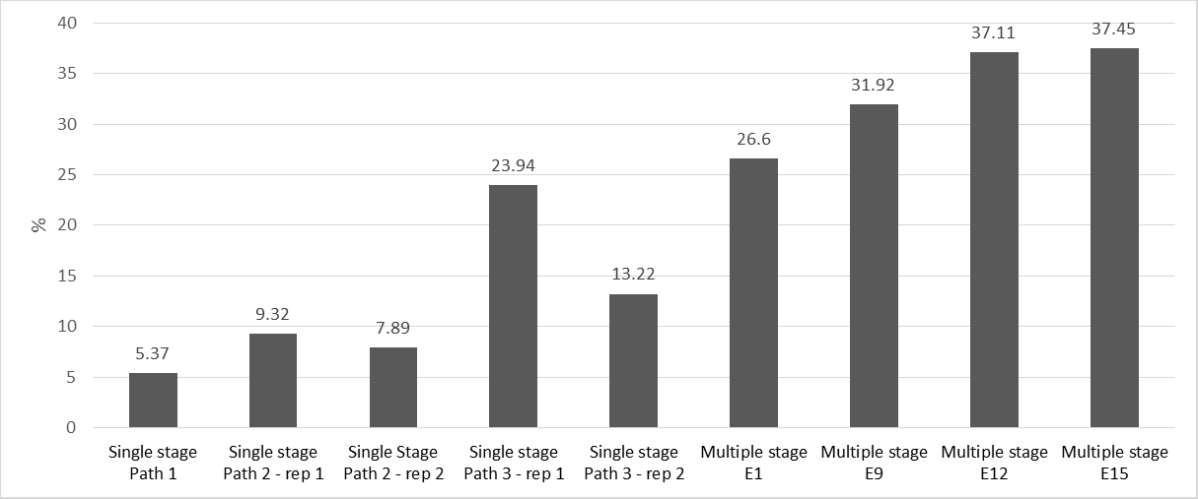

Figure 7. Comparison of squalene content in single and multiple stage extraction based on GC-MS result

As mentioned in previous section of this paper, extract E1 was analyzed to represent the single LLE using research path 3 as pre-treatment, whereas extracts E9, E12 and E15 were analyzed to represent the results of multiple stage LLE. The extract E15 is considered as the resulting final extract approximating real condition in a continuous multiple stage LLE process. Even though the experiment labelled with Single Stage Path 3 in Figure 7 was conducted with the exactly same procedure with E1, the GC-MS result showed slightly different value in squalene content. This could be caused since there was possibly a slight difference in the PFAD sample, as the waste specification taken from the palm oil industry might differ from day to day, depending on many factors during the production process.

The final squalene content resulting from the multiple stage process (extract E15) was 37.450 %, which was significantly higher than the squalene content in the extract obtained from single-stage extraction, which was 26.600 %. It has been shown, that the application of pre-treatment processes combined with multiple stage extraction was proven to be able to significantly increase the squalene content in the extract. However, the removal of FFA prior to the LLE process did not necessarily reduce its content in the extract to the lowest possible amount. A relatively large amount of FFA was still detected in the GC-MS analysis results, which were mostly the oleic and palmitic acid. In order to reduce the FFA content in the extract even further, hence, increasing the squalene content, the application of other separation techniques need to be explored. An extract purification must also be taken into consideration, in order to remove impurities from the squalene extract.

CONCLUSION

This research has proven that by applying centrifugation process in a combination with double saponification, the free fatty acid (FFA) content in PFAD can be reduced down to 0.3 ± 0.06 %-w. Further combination between this pre-treatment and a multiple stage extraction process was found to be able to increase the squalene content in the extract up to 37.450 %-w. Compared to a single stage extraction with saponification alone as pre-treatment, this number shows an increase of squalene content of around six folds. It was also concluded that despite this positive result, there exists a big room for improvement to be explored in future works. The impurities were still found as majority in the extract, and mostly was identified as free fatty acids. This indicates that the removal of FFA in the pre-treatment must be supported also with extract purification at the end.

REFERENCES

ASTM D974-02, 2002. Standard Test Method for Acid and Base Number by Color-Indicator Titration, ASTM International, West Conshohocken, PA, http://www.astm.org/cgi-bin/resolver.cgi?D974

Astuti, E., Haryadi, W. and Matsjeh, S., 2010. Extraction of palm oil's free fatty acids by triethylammine (TEA) in polar-nonpolar mix-solvent. *Indonesian Journal of Chemistry*. 2(1), pp.48-54.

Chongkhong, S., Tongurai, C., Chetpattananondh, P. and Bunyakan, C., 2007. Biodiesel production by esterification of palm fatty acid distillate. *Biomass and Bioenergy*. 31(8), pp.563-568.

Gapor Md Top, A., 2010. Production and utilization of palm fatty acid distillate (PFAD). *Lipid Technology*. 22(1), pp.11–13.

Prasertsan, S. and Prasertsan, P., 1996. Biomass residues from palm oil mills in Thailand: an overview on quantity and potential usage. *Biomass and Bioenergy*. 11(5), pp.387-395.

Sibuyo, L., Widiputri, D. and Legowo, E., 2017, January. Optimization of squalene extraction from Palm Fatty Acid Distillate (PFAD) in multistage process. In *AIP Conference Proceedings* (Vol. 1803, No. 1, p. 020060). AIP Publishing. doi: 10.1063/1.4973187.

Wandira, I., Legowo, E.H. and Widiputri, D.I., 2017, January. Optimization of squalene produced from crude palm oil waste. In *AIP Conference Proceedings* (Vol. 1803, No. 1, p.

020061). AIP Publishing. doi: 10.1063/1.4973188.

Yusuf, N.R.K., Legowo, E.H. and Sutanto, H., 2015. Utilization of Crude Palm Oil Waste to Produce Squalene. In *Proceedings of the International Conference on Innovation, Entrepreneurship and Technology*.

Zero & Rainforest Foundation Norway, 2015. Palm Fatty Acid Distillate (PFAD) in biofuels. pp.1–5.

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EXTRACTION OF HYALURONIC ACID FROM *ALOE BARBADENSIS* (ALOE VERA)

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ABSTRACT

Hyaluronic acid have a high moisture preservation and biocompatibility characteristic, thus allowing various usage of this substance in pharmaceutical, medicinal, and skin care products. Present manufacturing process of hyaluronic acid requires extraction of animal cells or through other methods utilizing bacteria. The aim of this research is to investigate an alternative source of hyaluronic acid extraction using plant based which is *Aloe barbadensis* (aloe vera). Three main parts of aloe vera (rind, mesophyll and gel) underwent several steps of extraction process and the result was compared to the sample of hyaluronic acid from goat brain. The evaluation including comparison of total carbohydrates, reducing sugars and degradation using heat treatment. The results of extraction were analyzed using UV-Spectrophotometer at 230 nm and compare to the extraction result of goat brain to ensure the presence of hyaluronic acid. It was found out that the rind part of aloe vera have the highest potential of high content of hyaluronic acid.

Keywords: *Aloe vera; extraction; hyaluronic acid.*

ABSTRAK

Asam hialuronat memiliki kemampuan untuk mempertahankan kelembapan serta biokompatibilitas yang tinggi, hal ini menjadi alasan asam hialuronat banyak digunakan dalam produk farmasi baik yang berhubungan dengan obat maupun perawatan kulit. Pada proses pembuatan asam hialuronat, ektraksi dari sel hewan masih merupakan sumber utama disamping penggunaan metode alternatif menggunakan beberapa jenis bakteria. Tujuan dari penelitian ini adalah untuk menginvestigasi sumber alternatif pengekstrasian asam hialuronat menggunakan bahan dasar tumbuhan yaitu *Aloe barbadensis* (liday buaya). Terdapat tiga bagian dari lidah buaya yang melewati beberapa tahap ekstraksi (kulit, mesofil, dan jel), hasil dari ekstraksi kemudian dibandingkan dengan sampel asam hialuronat dari otak kambing. Evaluasi mencakup perbandingan karbohidrat total, penurunan kadar gula, dan degradasi molekul menggunakan panas. Analisis terakhir menggunakan UV-Spektrofotometer di panjang gelombang 230 nm dan dibandingkan dengan hasil ekstraksi dari otak kambing untuk memastikan keberadaan asam hyaluronat. Hasil analisis menunjukan bahwa kulit lidah buaya memiliki potensi mengandung asam hialuronat yang cukup tinggi.

Kata kunci: *Asam hialuronat; ekstraksi; lidah buaya.*

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INTRODUCTION

Hyaluronic acid (HA) is a biological occurring polymer which has substantial biological functions in almost every organism (Necas, et.al., 2008). In humans, HA can be found in skin, vitreous of the eye, umbilical cord, and synovial fluid, but it is also present in body's tissues such as skeletal tissues, heart valves, lungs, brain, and many others (Meyer K., Palmer, J.W., 1934). Hyaluronic acid was located predominantly within extracellular and peri cellular matrix, although correspondingly existed on the intracellular cell (Balazs, et al., 1986).

Resources to gain hyaluronic acid were commonly taken from various animal tissues such as human umbilical cords, rooster combs, bovine vitreous humor, and bovine synovial fluid (Liu, et. Al., 2011). At present day, even though production through animal-based tissues still remain unshaken to be the major pathway for large HA production, another possibility of production systems have been demanded because of some disadvantages of the existing process. Due to the grinding procedure and several repetition of using acid and organic solvents, both practical and mechanical issues will always happened in animal extraction in terms of cost and safety (Widner, et. al., 2005).

Another issue is that HA from animal tissues may remain connected to a HA-specific binding cellular proteins of hyaluronidase (Fraser, et al., 1997). Hyaluronidase is undesirable since it may trigger the risk to prohibit an immune response. Furthermore, transmitter of infectious diseases in form of nucleic acids, prions, and viruses may well increases within extraction procedure (Shiedlin, et al., 2004). Lastly, the procedure are expensive and require a long period of time, labor, and advanced facilities to accommodate processes involved from animal extraction until purification of HA (Shlini, et al., 2017). Hence, it is preferred to generate hyaluronic acid via an animal cell-free system that could reduce contagion of undesirable contaminant and expense of manufacturing (Widner, et al., 2005) and (Yu & Stephanopoulos, 2008). Therefore, this research was arranged to find another pathway of extracting hyaluronic acid from a plant source, which according to (Shlini, et al., 2017) has proved to be successfully done from sweet potato and tapioca (Sana, et al., 2017). Moreover, aloe vera *(A. barbadensis)* was chosen due to its popularity to the public and considerably easy to be harvested in Indonesia.

In this research *A. barbadensis* is chosen as the potential source of HA due to similarities with HA in compositions and biological activities. Both aloe vera and hyaluronic acid proven to promotes wound healing (including dermatology applications), anti-inflammatory and therapeutic benefits. Moreover, *A. barbadensis* and hyaluronic acid have been used for dermatology purposes due to their abilities to retain water. There are three major parts of *A. barbadensis* used in this research, those are: rind, mesophyll, and gel. Rind is the external surface waxy cuticle which performs as a wall in a contradiction to moisture loss. Rind covers several levels of structures, with slight beneath from the waxy cuticle remains an area where the aloe related bacteria live (Sushruta, et al., 2013). Mesophyll is a liquid yellow-brownish part of aloe vera which holds the xylem and phloem vascular bundles. Mesophyll has the biggest concentration of anthraquinones and chromones of the whole aloe vera. Last part of aloe vera is the gel which located inside the inner parenchyma part of aloe vera. It consist of two components: juice of the gel and fibrous pulp enriched with cellulose.

Commercial manufacturing of hyaluronic acid is built on either animal-based extraction or genetically modified strains of bacterial fermentation. Both of these pathways are commonly applied and proved to manufacture hyaluronic acid products with molecular weights above 10 kDa that was suitable for medicine and dermatology usage (Liu, et al., 2011). Biological properties of hyaluronic acid are connected with its molecular weight, hence there is a great interest in HA degradation and evaluation of the biological behavior of HA fragments. Mechanisms of the HA cleavage into its smaller fragments involve enzymatic, free radical, thermal, ultrasonic, and chemical methods such as acid and alkaline hydrolysis (Soltes, et al., 2007).

MATERIALS AND METHOD

Materials

All aloe vera (*A. barbadensis)* and fresh goat brain were purchased from a market in Tangerang, Indonesia. The chemicals used for this research were acetone (Amresco), chloroform (Merck), methanol (FULLTIME), sodium acetate (CV. Bina Sejahtera), L-cysteine (Merck), acetic acid (Merck), 37% hydrochloric acid (Sigma Aldrich), ethylenediaminetetraacetic acid/EDTA (Disolvin), distilled water, sodium chloride (HiMedia Laboratories), absolute ethanol (FULLTIME), sulfuric acid (J.T Baker), ice cubes, sea salt, sodium carbonate (Merck), anthrone (Merck), sodium hydroxide (Merck), potassium sodium tartrate tetrahydrate (PUDAK Scientific), dinitrosalicylic acid/DNS (Sigma Aldrich).

Equipment

M254A BEL Engineering Weighing balance, water filtration system (Hydro Water Solution PT. Hydro Water Technology), hotplate stirrer (WiseStir MSH-20D), MColorpHast pH-indicator strips, centrifuge (Type 80-2 China), refrigerator (Electrolux), autoclave HG 50 Hirayama, Phillips food processor/grinder, PG Instruments T60 UV-Visible Spectrophotometer, and VWR V-1200 Visible Spectrophotometer.

Extraction Process

The extraction methodology is based on the studies being performed by (Shlini, et al., 2017) with sweet potato (*Ipomoea batatas)* and (Sana, et al., 2017) with tapioca (*Manihot esculenta)*. In this research, aloe vera (*A. barbadensis)* will be taken as the plant source and goat brain as sample of pure hyaluronic acid. The samples were washed thoroughly, parts of aloe vera were separated by knife and each of the four samples were homogenously crushed. 50 g of each sample was submerged in 50 mL of acetone and stirred for an hour. Chloroform and methanol with ratio 2:1 was used to incubate 100 mL sample for 24 hours at 25°C. Followed by digestion buffer (100mM sodium acetate pH 5.0, 5.0mM cysteine and 5.0mM disodium EDTA) that arranged in a ratio 2 mL of buffer to 100mg of tissue. The sample was hydrated inside the digestion buffer for 44 hours at 5°C before centrifuge at 3200rpm for 30 minutes. The solvents was removed and the solid filtrate was splashed by 3 mL of 2.0M sodium chloride and followed by absolute ethanol. Absolute ethanol was inserted in ratio of 2:1 and kept for 24 hours at -16°C. The next procedure was centrifugation at 3200 rpm for 30 minutes. Sequentially, the supernatant was taken away and the solid filtrate was washed with 80% ethanol. Second centrifugation was done as previous one before supernatant was discarded and the solid filtrate dried for 24 hours at 25°C. The final solid was re-suspended in 5 mL of distilled water and stored inside a test tube.

Total Carbohydrate Analysis using Anthrone's Method (Hodge, et al., 1962)

0.1 g of sample was boiled for 3 hours with 5 mL of 2.5N-HCl, then cooled to room temperature with ice and salt. The sample was neutralized by adding solid sodium carbonate until the effervescence ends. The sample was made up to the volume of 100 mL and centrifuged at 3200 rpm for 15 minutes. The supernatant was collected to prepare 1mL aliquots for analysis. The sample was added by 4mL of fresh anthrone reagent (dissolve 0.2 g of anthrone in 100 mL of ice cold H_2SO_4) and heated for 8 minutes in a boiling water. The sample was rapidly cooled with ice and sea salt and observed at absorbance of 630 nm in a visible spectrophotometer.

Reducing Sugar Analysis using DNS Method (Garriga, et al., 2017)

DNS reagent was prepared by making two mixtures; Solution A (1 g of DNS was dissolved in 20 mL of NaOH 2M) and Solution B (30 g of potassium sodium tartrate tetrahydrate was dissolved in 50 mL of distilled water). Solution A was added into Solution B, heated, and mixed on a hot plate at 300°C and 370 rpm. This new solution was completed to the volume of 100 mL with distilled water and stored in amber bottle at refrigerator (4°C). This solution was named as DNS reagent. 1 mL of each sample was placed into a test tube and added by 1 mL of DNS reagent. The test tube was heated in a boiling water for 5 minutes and cooled

by ice and sea salt to room temperature. The sample was added by 8 mL of distilled water and read at 540 nm in a visible spectrophotometer.

Fragmentation of Hyaluronic Acid (Lowry and Beavers, 1994) and (Botner, et al., 1988)

Degradation of the pre-assumed HA sample and goat brain sample were done through thermal degradation. 10 mL of each sample was taken into small bottle and inserted into the autoclave for 4 hours at 128°C. Sequentially, the sample was observed using UV-spectrophotometer in 230 nm wavelength.

RESULTS AND DISCUSSION

Total Carbohydrate Measurement

Hyaluronic acid is a carbohydrate compound, more specifically a repeated glycosaminoglycan (GAG) which formed of ß4-glucuronic acid and ß3-N-acetylglucosamine (Meyer K, 1934). Hyaluronic acid occurred in a high molecular weight due to the repetition of glucuronic acid and *N*-acetylglucosamine that able to goes up to a thousand repetition even further as can be seen from figure 1.

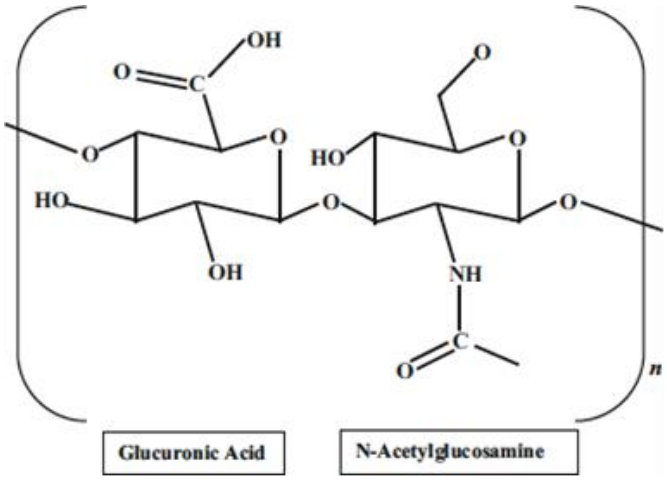

Figure 1. Structure of hyaluronic acid monomer (Cowman & Matsuoka, 2005)

Anthrone's method was used to measure total carbohydrate content from three different part of aloe vera samples (rind, mesophyll and gel) to be compared to total carbohydrate content of hyaluronic acid from natural source, in this case goat brain. This method used as the initial stage to identify hyaluronic acid.

As can be seen from Figure 2, all of aloe vera's parts (rind, mesophyll, and gel) were proved to show some value of absorbance at 630 nm, which showed that aloe vera does contains carbohydrate.

Goat brain as the hyaluronic acid source showed highest peak with the value of absorbance of 0.034 followed by rind with absorbance of 0.023. From three parts of aloe vera (rind, mesophyll and gel), rind part showed highest and closest absorbance to hyaluronic source from goat brain, but to be certain further analysis through reducing sugar needs to be done.

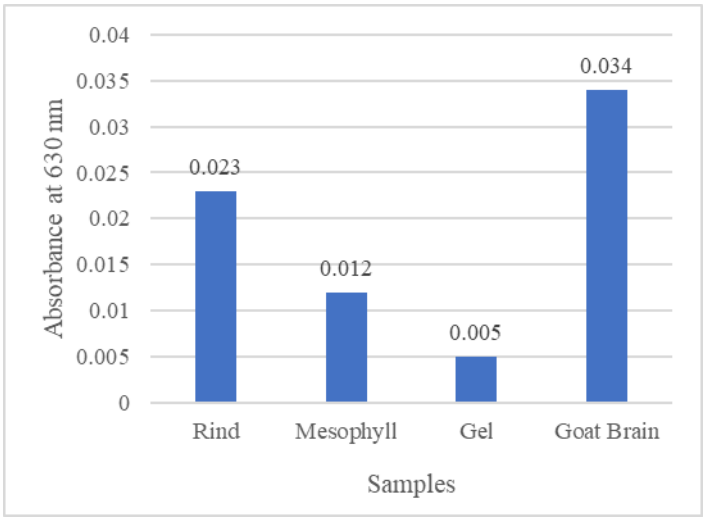

Figure 2. Graph showing comparison of total carbohydrate of aloe parts with goat brain

Reducing Sugar Measurement.

Anthrone method only cover the general picture of finding carbohydrate, hence another method is used to observe more specific compositions of carbohydrate which downgrade the structure from polysaccharides into smaller fragments of carbohydrates; reducing sugar. Moreover, hyaluronic acid chemical structure is particularly included a form of reducing sugar: ß-D-glucose (Gunawardena, 2015), which made the essential on doing DNS is highly proposed.

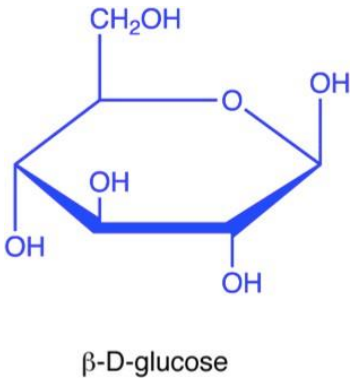

Figure 3. ß-D-glucose (Gunawardena, 2015)

DNS method was done as a complement procedure from anthrone's result to specifically qualify any reducing sugars inside the sample. Total carbohydrate analysis through anthrone's methods already showed that rind and goat brain has highest and closest absorbance compared to other part of aloe vera. Figure 4 below showed that all parts of *A. barbadensis* have shown value of absorbance which suggested contains reducing sugar. It should be highlighted that both in anthrone and DNS method, rind part of aloe vera showed the highest absorbance 0.333 in comparison to mesophyll and gel. In addition, rind have the closest absorbance to goat brain (0.288) that contain high concentration of hyaluronic acid in both anthrone and DNS method thus conforming that rind have a very high chance to contain hyaluronic acid. Based on these findings, rind was chosen to undergo further analysis step which is thermal degradation.

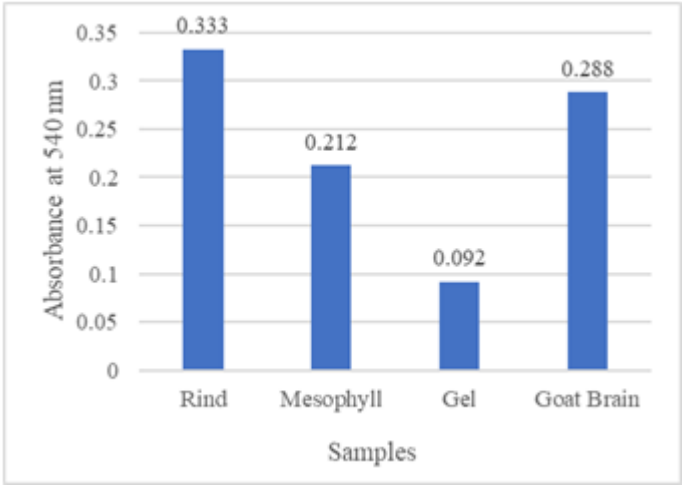

Figure 4. Graph showing comparison of reducing sugar of aloe parts with goat brain

Thermal Degradation

Hyaluronic acid is naturally occurred in a high molecular weight, but since there are various applications which came from different sizes of molecular weight of hyaluronan, it prompted a HA cleavage method to be performed. There were numerous ways to decrease the molecular weight of hyaluronic acid into smaller fragments which engage with enzyme, free radical, heat, ultrasound, and chemicals. Unfortunately, most of those methods will produce unwanted toxic impurities and demand a high cost. Thermal degradation of hyaluronic acid proved to be successfully done by (Botner, et al., 1988) at 128°C in an autoclave.

Based on total carbohydrate and reducing sugar measurement, rind has the highest chance of containing hyaluronic acid, hence thermal degradation analysis was done to conforming the presence of hyaluronic acid in rind compared to goat brain. Hyaluronic acid was proved to be existed on the wavelength of 230 nm based on several studies being done by (Shlini, et al., 2017) and (Sana, et al., 2017). Therefore, the rind sample and goat brain were gone through UV-spectrophotometer before and after thermal degradation to showed the existence of hyaluronic acid.

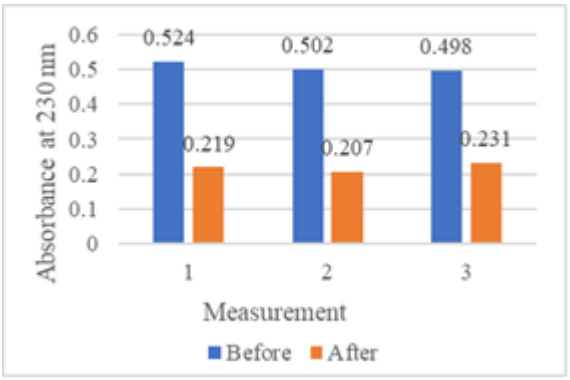

Figure 5. Absorbance of rind before and after thermal degradation

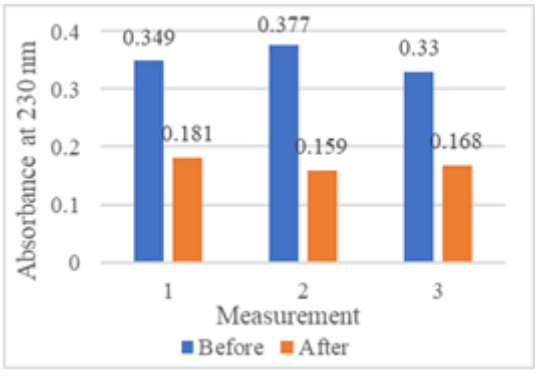

Figure 6. Absorbance of goat brain before and after thermal degradation

Hyaluronic acid is naturally occurred in a high molecular weight, but since there are various applications which came from different sizes of molecular weight of hyaluronan, it prompted a HA cleavage method to be performed. As can be seen from Figure 5 and Figure 6, three repetitions of both samples showed a decrease of absorbance with very similar value, hence showed degradation process using heat treatment to be successful and hyaluronic acid component from both samples was successfully fragmented as the end product. One law that affirm molecular weight of the end product after degradation will decreased is the law of conservation of mass. The law stated that mass is neither created nor destroyed in chemical reactions (Sterner, R and Hood, J., 2011). Since thermal degradation was not a chemical reaction, it only shrinks the structure molecules which produced a less bulky compound with smaller weight of mass.

Another supportive evidence to show the declining of its molecular weight is the smaller value of the concentration after degradation procedure. If the chemical structure of HA were cut during thermal degradation, it ends with less bulky chemical compounds which leads to smaller value of concentration. The concentration of the sample was declined after degradation as can be seen in the decreased of absorbance value. This can be explained through the Lambert Beer's Law, expressed through:

$$A = \varepsilon c l \qquad \text{(Equation. 1)}$$

Whereas A is absorbance, ε is molar absorption coefficient, c is molar concentration and l is optical path length passed by the UV light. Since the value of absorbance after thermal degradation was lower compared from before degradation process, it concluded that concentration after degradation was also dropped due to proportionally equivalent value of absorbance and concentration according to the Equation. 1. It can be clearly seen that the drop of concentrations was constant through three repetitions of sampling using UV-spectrophotometer which referring back to Figure. 5 and Figure. 6.

This result also supported by the fact that rind is highly composed by one of the hyaluronic acid structures; carboxyl group which are richly present in form of oxalic acid inside rind. Moreover, rind has anti-inflammatory property due to chromones which someway equaled with hyaluronic acid's anti-inflammation property. Chromones also have skin protection effects which matched with one of hyaluronic acid's benefits for skin; protection of water loss to the skin. Lastly, on just below the waxy cuticle of rind, there is an area where aloe correlated bacteria live. Gram-positive microbes (including Group A and group C *Streptococci)* which able to produce hyaluronic acid through bacterial pathway, were only found on the surface of aloe vera (*A. barbadensis)*, whereas *coccobacilli (streptococcus morbillorium, enterococcus faecium,* and other Gram-negative rods) are observed only in gel part.

CONCLUSION

Anthrone method showed that all parts of aloe vera containing carbohydrate with rind has the highest absorbance, just below the absorbance of goat brain. This result was confirmed by DNS method which showed that again rind has the highest absorbance just like the goat brain. Furthermore, thermal degradation process was done to degrade high molecular weight HA into small molecular weight HA. The result of thermal degradation can be seen through UV-Spectrophotometer which showed constant and very similar decrease of absorbance on both rind and goat brain sample, thus showed that rind is containing hyaluronic acid. For further studies, isolation and purification of hyaluronic acid and quantification of its concentration, ion exchange chromatography is preferred due to anionic nature of hyaluronic acid. The elution obtained by ion exchange chromatography can be further purified using gel permeation chromatography and for determination of precise structure of HA, NMR (Nuclear Magnetic Resonance) followed by FT-IR can be used in future research.

REFERENCES

Balazs, E., Laurent, T., Jeanloz, R. 1986. Nomenclature of hyaluronic acid, *Biochemical Journal*, 235, pp.903.

Botner, H., Waaler, T., Wik, O. 1988. Limiting viscosity number and weight average molecular weight of hyaluronate samples produced by heat degradation, *International Journal Biology Macromolecules*, 10, pp.27-291.

Cowman, M. & Matsuoka, S., 2005. Experimental approaches to hyaluronan structure. *Carbohydrate Res*, Volume 340, pp. 791-809.

Fraser, J., Laurent, T., Laurent, U. 1997. Hyaluronan: its nature, distribution, function and turnover, *Journal International Medicine*, 242, pp.27-33.

Garriga, M., Almaraz, M., Marchiaro, A. 2017. Determination of reducing sugars in extracts of Undaria pinnatifida (harvey) algae by UV-Visible spectophotometry (DNS Method), *Actas de Ingenieria*, 3, pp.173-179.

Gunawardena, G., 2015. The Elements of Organic Chemistry: A Compendium of Terminlogy, Definitions and Concepts for the Beginner. In: California: Linus Publications, pp. 540.

Hodge, J., Hofreiter, B., Whistler, R. 1962. Determination of reducing sugars and carbohydrates, methods in carbohydrate chemistry, *Carbohydrate Chemistry, pp.*17-22.

Liu, L., Liu, Y., Li, J., Du, G., Chen, J. 2011. Microbial production of hyaluronic acid: curent state, challanges, and perspective, *Microbial Cell Fact*, 10, pp.99.

Lowry, K., Beavers, E. 1994. Thermal stability of sodium hyaluronate in aqueous solution, *Journal Biomed Mater Res*, 28, pp.1239-1244.

Meyer, K., Palmer, J.W. 1934. The polysaccharide of the vitrous humor, *Journal of Biological Chemistry*, 107, pp.629-634.

Necas, J., Bartosikova, L., Brauner, P., Kolar, J. 2008. Hyaluronic acid (hyaluronan): a review, *Veterinarni Medicina*, 53, pp.397-411.

Sana, Fathima, M., Shambhavi, U., Shlini, P. 2017. Isolation and purification of hyaluronic acid like components from Manihot esculeta (tapioca), *International Journal of Pharmaceutical Science Invention*, 6(10), pp.35-41.

Shiedlin, A., Bigelow, R., Arbabi, S., Yang, L., Maier, R.V., Wainwright, N., Childs, A., Miller, R.J. 2004. Evaluation of hyaluronan from different sources: Streptococcus zooepidemicus, rosster comb, bovine vitrous, and human umbilical cord, *Biomacromolecules*, 5, pp.2122-2127.

Shlini, P., Sana, F.M., Shambhavi, U. 2017. Isolation and purification of hyaluronic acid like components from Ipomoea Batatas (sweet potato), *European Journal of Pharmaceutical and Medical Research*, 4, pp.282-286.

Soltes, L., Kogan, G., Stankovska, M., Mendichi, R. 2007. Degradation of high-molecular hyaluronan and characterization of fragments, *Biomacromolecules*, 8(9), pp.2697-2705.

Sterner, R., Small, G., Hood, J. 2011. The conservation of mass, *Nature Education Knowledge*, 3(10), pp.20.

Sushruta, M., Amol, B., Achana, B., Anubha, K. 2013. Aloe vera – a review, *International Journal of Pharmaceutical and Phytopharmacological Research*, 3(3), pp.203-211.

Widner, B., Behr, R., Dollen, S.V., Tang, M., Heu, T., Sloma, A., Sternberg, D., DeAngelis, P.L., Weigel, P.H., Brown, S. 2005. Hyaluronic acid production in Bacillus subtilis, *Applied and Environmental Microbiology*, 71, pp.3747-3752.

Yu, H., Stephanopoulos, G. 2008. Metabolic engineering of Escherichia colli for biosynthesis of hyaluronic acid, *Metabolic Engineering*, 10, pp.24-32.

Journal of Functional Food and Nutraceutical

CHOCOLATE BAR WITH MORINGA AND DATES AS CALCIUM-RICH FOOD WITH LOW GLYCEMIC INDEX FOR ENDURANCE ATHLETES

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ABSTRACT

This research aims to provide chocolate for endurance athletes by utilizing the potential of highly nutritious local foods. The ingredients used are chocolate, moringa leaves (*Moringa oleifera*), and dates (*Phoenix dactylifera*). This research method uses a Completely Randomized Design (CRD) with One Way Anova statistical analysis. Samples were analyzed by testing in a food laboratory. In this study, moringa powder was used for its calcium content and dates were used for its carbohydrates content with a low glycemic index. In 100 grams of developed chocolate contains 3.27 g of water, 1.83 g of ash, 30.4 g of fat, 5.32 g of protein, 59.1 g of carbohydrate, 0.38 g of crude fiber, 427.07 mg of calcium.

Keywords: *Chocolate; dates; endurance athletes; low GI; moringa.*

ABSTRAK

Penelitian ini bertujuan untuk menyediakan cokelat bagi atlet *endurance* dengan memanfaatkan potensi pangan lokal yang bernilai gizi tinggi. Bahan-bahan yang digunakan adalah cokelat, daun kelor (*Moringa oleifera*), dan kurma (*Phoenix dactylifera*). Metode penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan analisis statistik One Way Anova. Sampel dianalisis dengan pengujian di laboratorium pangan. Dalam penelitian ini, penggunaan moringa powder didasari oleh kandungan kalsiumnya dan kurma oleh kandungan karbohidratnya yang memiliki indeks glikemik rendah dalam 100 g cokelat yang dikembangkan adalah sebagai berikut 3,27 g air, 1,83 g abu, 30,4 g lemak, 5,32 g protein, 59,1 g karbohidrat, 0,38 g serat kasar, 427,07 mg kalsium.

Kata kunci: *Atlet endurance; cokelat; GI rendah; kelor; kurma.*

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INTRODUCTION

In the competition period, the supply of food must meet the quantity and quality of nutrition, namely the amount of energy and balanced nutritional composition. It is recommended that the consumption of carbohydrate-source foods as a reserve of muscle and liver glycogen needed during the match. This aims to prevent the occurrence of hypoglycemia, prevent fatigue and maintain muscle working power. Feeding needs to be arranged so that before the competition begins the process of food digestion is complete. This is important because, during the competition, blood flow is concentrated into the muscles to deliver nutrients and oxygen needed when the muscles contract. Usually, 2-3 hours before the match has given snacks such as bread/crackers (Welis and Syafrizal, 2009). Refill carbohydrate deposits with a pre-event meal or snack for 1-4 hours before the competition around 1-4 g / kg BB (Louise Burke and Greg Cox, 2010).

In this case, the concept of the glycemic index (GI) was developed, which is a level of food according to its effect on blood sugar levels. Consumption of foods with low GI (± 2 hours before a competition) can guarantee the release of glucose into the bloodstream constantly during the game. This is because foods with a low GI are digested slowly so that their storage is also slow. Extra glucose will be available until the end of the game because muscle glycogen is stored slowly (Welis and Syafrizal, 2009). Low GI has characteristics that can cause the digestive process in the stomach to run slowly, so the rate of emptying the stomach (gastric emptying rate) also takes place slowly. This results in a suspension of food that has undergone digestion in the stomach (chyme) more slowly reaching the small intestine so that further digestion of carbohydrates and absorption of glucose in the small intestine occurs slowly. Likewise, in low-GI foods, most glucose uptake occurs in the upper small intestine (duodenum) and the middle part (jejunum). In the end, fluctuations in blood glucose levels are relatively small. With these metabolic characteristics, low-GI foods can reduce the glycemic and insulin responses (Hoerudin, 2012). Dates contain high carbohydrates with a low glycemic index so that it is potentially a food source of energy for endurance athletes. Dates are useful as a substitute for the energy lost when competing because of the natural sugar content. So, it does not make blood sugar pressure soaring sharply. Dates also contain potassium which can strengthen muscle function so that it is not easily injured. 100 g of dates can provide 314 kcal of energy with the main components being monosaccharides (Dayang *et al*., 2014).

High-intensity exercise causes a decrease in bone mass. Calcium plays an important role in bone health, especially for athletes who prioritize physical contact (Nguyen, 2010). Adequate calcium consumption helps maintain healthy bones and reduces the risk of injury to bones when competing. Athletes are encouraged to consume calcium-fortified foods as an alternative source of calcium to meet calcium needs per day. Calcium helps optimize bone density and prevents osteoporosis in athletes (Amiruddin and Yusni, 2015). One source of calcium is found in Moringa leaves. Moringa leaves are referred to as magic plants based on their use, especially related to medicine and nutrition. In 100 g fresh Moringa leaves contain at least 1077 mg of calcium (Fahey, 2005). The calcium content is increased when the Moringa leaves are dried.

Fortification can also be done on processed cocoa bean products, namely chocolate. Some types of chocolate products such as dark chocolate made from cocoa paste with the addition of a little sugar, milk chocolate made from cocoa paste, cocoa butter, sugar and milk powder, and white chocolate made from cocoa butter, sugar and milk powder. Also, there are couverture chocolates which are premium or high-quality chocolates that are often used by professionals in the industry to make pastries or cakes (Agus, 2012).

Nowadays athletes tend to want food they like and are practical, also consider food in terms of nutrition. The availability of local food-based snacks from chocolate, Moringa, and dates for athletes is still rarely found, especially in Indonesia. Therefore, it needs to be developed as an effort to utilize local food potential.

METHODOLOGY

Materials and Method

This study used an experimental method with a completely randomized design. Data analysis used One Way ANOVA and continued with the Duncan test. Samples were analyzed in a food laboratory, carbohydrate using the By Difference method, protein using the Kjeldahl method, fat using the Sokhlet method, crude fiber using Gravimetric method, ash using Gravimetric method, moisture using Gravimetric method, and Calcium using AAS method.

The ingredients consist of white chocolate, moringa powder and dates with a comparison of formulations in Table 1. The equipment consists of heat-resistant glass bowls, scales, chocolate molds, pans, spoons, aluminum foil, and refrigerators.

Steps to make chocolate with the addition of moringa flour and dates is chopped chocolate or cut it into small pieces to make it easy to melt, then put it in a heat-resistant glass bowl. Then melt the chocolate using the double boiler technique. By preparing a pan whose top surface fits with a chocolate bowl, then fill about half of the water, cook until it boils. Then place the chocolate bowl on the pan. This aims to keep the water vapor from mixing with chocolate because it will make the chocolate clot quickly and the texture is not good. Stir chocolate until melted. Turn off the heat, stir continuously then add the Moringa flour and dates that have been cut into small pieces. Then pour the chocolate into a mold and put it in the refrigerator until hard. The last, pack with aluminum foil.

Table 1. Comparison Formulations of Chocolate, Moringa, and Dates (g)

Raw Material	F1	F2	F3
Chocolate	40.00	40.00	40.00
Moringa Powder	5.00	10.00	15.00
Dates	15.00	10.00	5.00

Results and discussion

The results of ANOVA mean analysis presented in a pie chart. The pie chart explains the total average of nutritional content of chocolate bars in 100 grams.

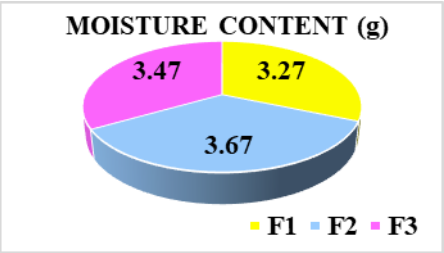

Figure 1. Nutrition-related claims in chocolate bar products (Moisture)

Based on the analysis of nutrients found the highest moisture content is F2 3.67 g. The moisture content contained in the product is affected by the constituent ingredients.

According to Rahmadi (2010), dates are a fairly high contributor to water content. A study by Sinaga *et al*., (2019) that the addition of Moringa leaf powder can increase ash content.

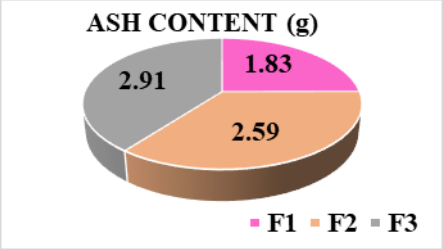

Figure 2. Nutrition-related claims in chocolate bar products (Ash)

Based on the analysis of nutrients found the highest ash content is F3 2.91 g. Because the content of Moringa powder is more than other ingredients. Moringa contains calcium that are high enough so that the more moringa powder,

ash content will increase. About 96% of food consists of organic matter and water. The rest consists of mineral elements known as organic substances or ash content. Ash content indicates the mineral content contained in an ingredient (Fajri *et al.*, 2013).

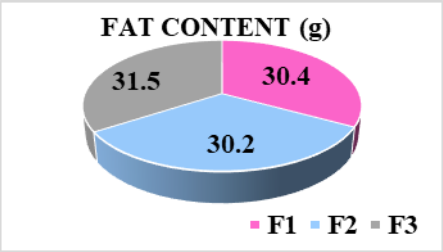

Figure 3. Nutrition-related claims in chocolate bar products (Fat)

Based on the analysis of nutrients found the highest fat content is F3 31.5 g. The fat content of this product is affected by the addition of dates and chocolate. White chocolate contains a high fat compared to other constituent ingredients. Fat in the body acts as an energy source, especially in sports with moderate intensity in a long time, for example endurance sports (Rismayanthi, 2015).

According to Burke *et al.*, (2004) fat is an important source of energy for muscle contraction during endurance sports. High-fat consumption (> 30% of total calories) can reduce carbohydrate intake, so muscle glycogen cannot be maintained. According to Fink and Mikesky (2015), the recommended consumption of fat for athletes per day is 20–35% of total energy, which includes 7–10% SFA, 10% MUFA, 10% PUFA.

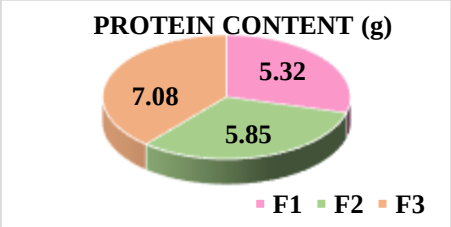

Figure 4. Nutrition-related claims in chocolate bar products (Protein)

Based on the analysis of nutrients found the highest protein content is F3 7.08 g. Because the concentration of Moringa powder is higher than other formulations.

Athlete's protein requirements are in the range of 1.2–1.6 g/ kg body weight per day. Increased protein requirements because athletes are more at risk of damage to muscle tissue, especially when training or strenuous exercise (Irawan, 2007).

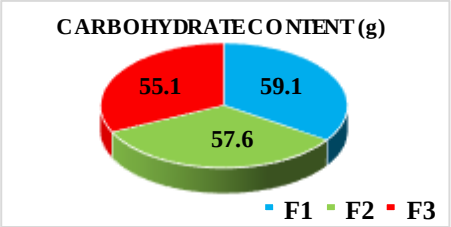

Figure 5. Nutrition-related claims in chocolate bar products (Carbohydrate)

Based on the analysis of nutrients found the highest carbohydrate content is F1 59.1 g. Because of the concentration of dates more than other ingredients. Dates contain high carbohydrates with a low glycemic index so that it is potentially a food source of energy for endurance athletes (Hoerudin, 2012). Research by Hafidha (2018) that the addition of dates can increase carbohydrate levels. Research by Al-Shahib and Marshall (2003) that dates contain a high percentage of carbohydrates (total sugar, 44-88%).

According to Louise Burke and Greg Cox (2010), the carbohydrate requirement of endurance athletes is 1–4 g per kg of body weight. Carbohydrates play a role in maintaining blood glucose levels and the speed of carbohydrate metabolism in the body to reduce the occurrence of fatigue in athletes who have endurance, carbohydrates contained in chocolate will be absorbed by the body slowly so that it can become a continuous source of glucose. Adding dates to this chocolate product can provide energy quickly for endurance athletes (Burke *et al.*, 2004).

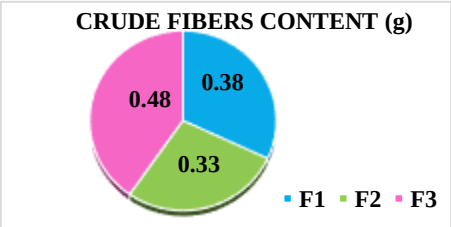

Figure 6. Nutrition-related claims in chocolate bar products (Crude Fibers)

Based on the analysis of nutrients found the highest crude fibers content is F3 0.48 g. Because the concentration of Moringa powder is higher than other formulations. Besides, the addition of dates also contributed to the levels of the fiber of chocolate dates and moringa.

According to Rock (2009) dates contain 2.49–12.31% food fiber. The comparison between the addition of dates and moringa leaf powder affects the levels of crude fibers of chocolate moringa and dates.

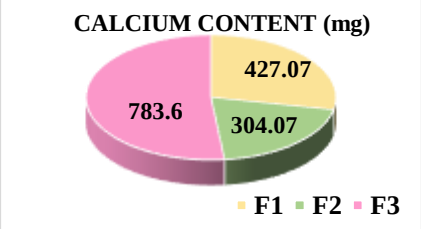

Figure 7. Nutrition-related claims in chocolate bar products (Calcium)

Based on the analysis of nutrients found the highest calcium content is F3 783.6 mg. Because the concentration of Moringa powder is higher than other formulations. According to Mahmood *et al.*, (2011) that Moringa contains good nutrients one of which is calcium. In 100 g of Moringa leaves contain at least 2003 mg of calcium (Fahey, 2005). Athletes who exercise with high intensity require calcium intake to maintain bone health to reduce the risk of injury to the bones during exercise or competition. According to Nguyen (2010) in the NSCA's Performance Training Journal that the athlete's calcium adequacy rate is Upper Level (UL) around 2500 mg/day.

CONCLUSION

Based on this research, F1 is the best formula. In 100 grams of developed chocolate contains 406.16 kcal, 3.27 g of water, 1.83 g of ash, 30.4 g of fat, 5.32 g of protein, 59.1 g of carbohydrate, 0.38 g of crude fiber, 427.07 mg of calcium. The final product is served as much as 30 grams. So, it contains ± 120 kcal, ± 18 g carbohydrates, ± 9 g fat, ± 1.6 g protein, ± 0.1 g crude fiber, ± 130 mg calcium. Based on the daily value of 2475 calorie diets, 30 grams of this product can meet the daily needs of calcium 10.6%, carbohydrates 5.2%, protein 2.2%, fat 10.9%. It can be concluded that this product can be a snack for endurance athletes with good nutritional content. Health claims may also contribute to the improvement of industrial competitiveness (Fadlillah *et al.*, 2019). Chocolate with calcium content and low glycemic index is a new finding in the food industry, so it needs to be developed.

Nutrition Facts		
1 Serving Per Container		
Serving Size		30 g
Amount per serving		
Calories		**120 kcal**
		% Daily Value*
Total Fat	**9 g**	10.9%
Protein	**1.6 g**	2.2%
Carbohydrate	**18 g**	5.2%
Calcium	130 mg	10.6%
Crude Fiber	0.1 g	0.31%

***Percent Daily Value are based on 2475 calories diet. Your daily value may be higher or lower depends on your calories needs.**

Figure 8. Chocolate Bar with Moringa and Dates (30 g) and Nutrition Fact

ACKNOWLEDGEMENT

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REFERENCES

Al-Shahib and Marshall. 2003. The fruit of the date palm: its possible use as the best food for the future. *International Journal of Food Sciences and Nutrition*. 54(4), pp.247-59.

Amiruddin and Yusni. 2015. Pemenuhan Kebutuhan Kalsium Dan Besi Atlet Sepak Bola Junior Banda Aceh. *Jurnal Sport Pedagogy*. 5(2).

Burke, Louise and Cox, Greg. 2010. *Complete Guide to Food for Sport Performance*. Australia: Griffin Press.

Burke, L., Kiens, B., and Ivy, J. L. 2004. Carbohydrates and Fat for Training and Recovery. *Journal of Sports Sciences*. 22, pp.15-30.

Dayang, J.F., Reuben, C.R., and Raji, F. 2014. Nutritional, socioeconomic and health benefits if dates. *International Journal of Food and Nutritional Sicence*. 3(6), pp.63–73.

Fadlillah, H.N., Nurkhoeriyati, T., Felanesa, L., and Utomo, A.W. 2019. Study on Functional Ingredients and Claims of Ready to Drink (RTD) Fruit Juice in Modern Retail. *Journal Functional Food and Nutraceutical*. 1(1), pp.13-22.

Fahey, J. W. 2005. Moringa oleifera: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties Part 1. *Trees for Life Journal*. 1, pp.5-30.

Fajri, R., Basito., and Muhammad, D. R. A. 2013. Karakteristik Fisikokimia Dan Organoleptik Food Bars Labu Kuning (Cucurbita Máxima) Dengan Penambahan Tepung Kedelai Dan Tepung Kacang Hijau Sebagai Alternatif Produk Pangan Darurat. *Jurnal Teknologi Hasil Pertanian*. 6(2).

Fink, H. H., Mikesky, A. E. 2015. *Practical Applications in Sport Nutrition*. 4 th. Dearborn R, Fabery S, editors. United States: Jones & Bartlett Learning. pp.60-125.

Hafidha, K., dan Ismawati, R. 2018. Pengaruh Penambahan Tepung Sukun (Artocarpus Communis), Pisang Hijau (Musa Paradisiaca L.), Cokelat (Theobroma Cacao L.) dan Kurma (Phoenix Dactylifera) Terhadap Daya Terima dan Nilai Karbohidrat Egg Rol. *Jurnal Media Gizi Indonesia*. 13(1), pp.81-88.

Hoerudin. 2012. Indeks Glikemik Buah Dan Implikasinya Dalam Pengendalian Kadar Glukosa Darah. *Jurnal Buletin Teknologi Pascapanen Pertanian*. 8(2), pp.81-89.

Irawan, M.A., 2007. Glukosa dan Metabolisme Energi. *Sport Science Brief*. 1(4), pp.1-12.

Mahmood, K. T., Tahira, Mugal., and Ikram Ul Haq. 2011. Moringa oleifera: a natural gift-A review. *Journal of Pharmaceutical Sciences and Research*. 2(11), pp.775-781.

Nguyen, V.H. 2010. Calcium for Athletes to Improve Bone Strength and Health. *NSCA's Performance Training Journal*. 9, pp.11-13.

Rahmadi, A. 2010. Phoenix Dactylifera Food Tecnologist. *Jurnal Neurobiologist, Pharmacologist*. 4(1), pp.19-23.

Rismayanthi, C. 2015. Sistem Energi dan Kebutuhan Zat Gizi yang Diperlukan untuk Peningkatan Prestasi Atlet. *Jurnal Ilmu Keolahragaan*. 11(1), pp.109-121.

Rock, W. 2009. Effects of Date (Phoenix dactylifera L., Medjool or Hallawi Variety) Consumption by Healthy Subjects on Serum Glucose and Lipid Levels and on Serum

Oxidative Status: A Pilot Study. *Journal of Agricultural and Food Chemistry*. 57(17), pp.8010- 8017.

Sinaga, H., Purba, R. A., and Nurminah, M. 2019. Pengaruh Penambahan Tepung Daun Kelor (Moringa Oleifera) Dalam Pembuatan Kue Onde-Onde Ketawa Menggunakan Tepung Mocaf. *Jurnal Ilmu dan Teknologi Pangan Fakultas Pertanian USU Medan*. 3(1) 29 – 37.

Sudibyo, Agus. 2012. Peran Cokelat Sebagai Produk Pangan Derivat Kakao Yang Menyehatkan. *Jurnal Riset Industri*. 6(1), pp.23-40.

Welis dan Syafrizal. 2009. *Gizi Olahraga*. Padang: Wineka Media.

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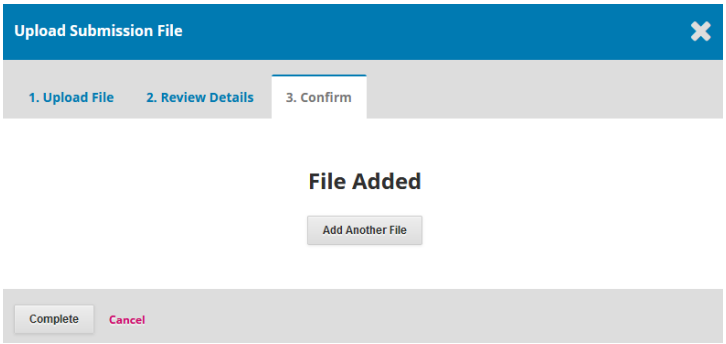

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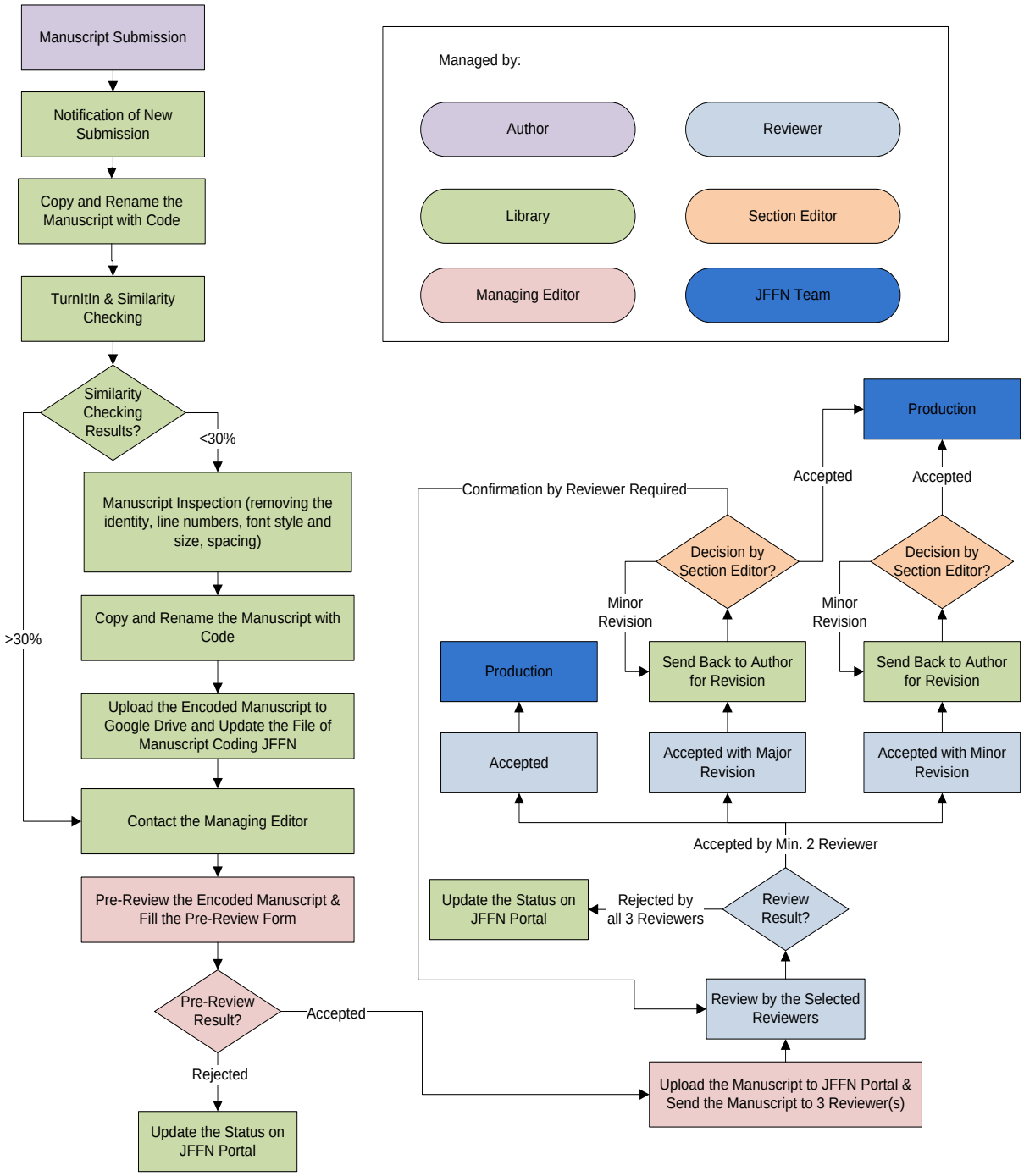

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Perhimpunan Penggiat Pangan Fungsional dan Nutrasetikal Indonesia (P3FNI) juga mengembangkan kontribusinya di kancah internasional bersama dengan International Society for Nutraceutical and Functional Food (ISNFF). Secara internasional terdapat klaster ISNFF seperti di Korea dan China. Untuk kepentingan percaturan internasional P3FNI menggunakan nama Indonesian Society for Functional Food and Nutraceutical (ISFFN).

Keterlibatan P3FNI atau ISFFN ini memberi manfaat anggotanya untuk memberikan kontribusi ilmu pengetahuan dan teknologi serta mengikuti pemutakhiran pangan fungsional dan nutrasetikal. Bersosialisasi dan berkesempatan dalam pertemuan ilmiah bersama penggiat pangan fungsional dan nutrasetikal dari berbagai negara untuk terus-menerus mengarah pada optimalisasi penggunaan pangan untuk kesejahteraan manusia secara bijaksana dan menjaga kelestariannya.

Siapa Yang Perlu Menjadi Anggota?

1. Akademisi dan peneliti yang terus-menerus mengembangkan dan memajukan ide alternatif dan kreatif untuk menuju kemajuan dan kesejahteraan manusia dengan menggunakan data basis ilmiah.
2. Praktisi kesehatan maupun industri yang menerapkan pangan fungsional dan nutrasetikal.
3. Mahasiswa sebagai penerus masa depan untuk melestarikan praktik-praktik pengadaan dan penggunaan pangan fungsional dan nutrasetikal secara bijaksana dan berkelanjutan.
4. Memberi advokasi dalam kasus-kasus pangan dan kesehatan.
5. Individu yang memiliki perhatian dalam pangan fungsional dan nutrasetikal.

Fasilitas Anggota P3FNI

1. Link Internasional untuk kegiatan atau program riset dan pembelajaran pangan fungsional dan nutrasetikal meliputi direktori laboratorium dan professor, lembaga kesehatan, LSM/NGO, skim hibah, dan internship/magang di industri.
2. Biaya partisipasi dalam pertemuan ilmiah yang diselenggarakan P3FNI.
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