ISOLATION AND CYTOTOXIC ACTIVITY OF THE B-CAROTENE COMBINATION OF TRIGONA HONEY AND NAMNAM LEAVES EXTRACT (*Cynometra cauliflora*)

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Abstract: Isolation and cytotoxic activity of the β-carotene combination of Trigona honey and Namnam leaves extract (*Cynometra cauliflora*) were conducted. The urgency of isolating β-carotene compounds because of their known anticancer activity. Namnam leaves are macerated using methanol, then combined with Trigona honey. Fractionation and isolation of β-carotene in combination samples were conducted out by chromatography method. The β-carotene was analyzed using UV-Vis and FTIR spectrophotometer. The cytotoxic activity test was conducted using the Brine Shrimp Lethality Test (BSLT) method with mortality analysis using Probit Analysis with SPSS v20 and Lethal Concentration 50 (LC_{50}) as parameters. The result showed that the cytotoxic activity of Trigona honey after combination reached 36.6% with an LC_{50} value of 168.2 ppm, an increase from the Trigona honey sample (LC_{50} = 265.2 ppm) and Namnam leaves extract (LC_{50} = 196.12 ppm). The highest cytotoxic activity belongs to the n-hexane fraction (LC_{50} = 77.6 ppm). The best eluent for β-carotene isolation is petroleum ether. There was increased cytotoxic activity in isolates (LC_{50} of 22.85 ppm). Isolates were analyzed by UV-Vis and FTIR and compared with standards compounds. FTIR analysis results showed that the isolates had functional groups of -CH_3 (υ 2850-2960 cm^{-1}), -CH_3 aliphatic (υ 1350-1470 cm^{-1}), C=C (υ 2100-2350 cm^{-1} and 675-870 cm^{-1}), and C-O (υ 1000-1300 cm^{-1}). Thus, the combination of Trigona Honey and Namnam leaf extract can be developed as an alternative chemopreventive agent.

Keywords: β-carotene, BSLT, cytotoxic, Namnam leaves extract (*Cynometra cauliflora*), Trigona honey

Abstrak: Isolasi dan aktivitas sitotoksik β-karoten kombinasi madu trigona dan ekstrak daun namnam (*Cynometra cauliflora*) telah dilakukan. Urgensi mengisolasi senyawa β-karoten karena telah diketahui adanya aktifitas antikanker. Daun namnam dimaserasi menggunakan metanol, untuk kemudian dikombinasikan dengan madu trigona. Fraksinasi dan isolasi β-karoten pada sampel kombinasi dilakukan dengan metode kromatografi. Kandungan senyawa β-karoten dianalisis menggunakan spektrofotometer UV-Vis dan FTIR. Uji aktifitas sitotoksik dilakukan dengan metode *Brine Shrimp Lethality Test* (BSLT) dengan analisis mortalitas menggunakan *Probit Analysis* dengan software SPSS v20 dan *Lethal Concentration* 50 (LC_{50}) sebagai parameter. Hasil analisis menunjukkan bahwa aktivitas sitotoksik madu trigona setelah dikombinasikan mencapai 36,6 % dengan nilai LC_{50} sebesar 168,2 ppm, meningkat dari sampel madu trigona dengan (LC_{50} = 265,2 ppm), dan ekstrak daun namnam dengan (LC_{50} = 196,12

ppm). Aktifitas sitotoksik tertinggi dimiliki oleh fraksi n-heksana (LC_{50} = 77,6 ppm). Eluen terbaik untuk isolasi β-karoten adalah petroleum eter. Terjadi peningkatan aktifitas sitotoksik pada isolat hasil pemisahan (LC_{50} sebesar 22,85 ppm). Isolat dianalisis dengan UV-Vis dan FTIR dan dibandingkan dengan senyawa standar. Hasil analisa FTIR menunjukkan isolat memiliki gugus fungsi -CH_3 (υ 2850-2960 cm^{-1}), -CH_3 alifatik (υ 1350-1470 cm^{-1}), C=C (υ 2100-2350 cm^{-1} dan 675-870 cm^{-1}), dan C-O (υ 1000-1300 cm^{-1}). Dengan demikian, kombinasi Madu Trigona dan ekstrak daun namnam berpotensi untuk dikembangkan sebagai agen kemoprefentif alternatif.

Kata kunci: β-karoten, BSLT, sitotoksik, ekstrak daun Namnam (*Cynometra cauliflora*), madu Trigona

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Introduction

Cancer is a non-contagious disease responsible for most global deaths (Bray et al., 2018). Typical cancer treatments are surgery, radiation therapy, and chemotherapy (Mount Elizabeth Hospital, 2021). Unfortunately, the Available treatment methods have not completely overcome cancer and have a toxic effect on normal tissue (Dos Santos et al., 2019). Based on these reasons, it is necessary to research safe and selective treatments. One of them is by exploring chemopreventive agents from natural or herbal ingredients that can slow or prevent the development of cancer cells.

In the Qur'an, Q.S An Nahl 16:69, there are instructions about the benefits of honey for humans.

ثُمَّ كُلِيْ مِنْ كُلِّ الثَّمَرٰتِ فَاسْلُكِيْ سُبُلَ رَبِّكِ ذُلُلًاۗ يَخْرُجُ مِنْ بُطُوْنِهَا شَرَابٌ مُّخْتَلِفٌ اَلْوَانُهٗ فِيْهِ شِفَاۤءٌ لِّلنَّاسِۗ اِنَّ فِيْ ذٰلِكَ لَاٰيَةً لِّقَوْمٍ يَّتَفَكَّرُوْنَ

"Then eat of all fruits (flowers)! Fly (wander around) in the ways of your Lord that He commanded. There comes forth out of their bellies a fluid (honey) of varying hues, in which there is a healing for mankind. Most surely there is a Verse (proof) in this for a people who reflect" (LPMQ, 2021)

Honey is one of the ingredients often used as herbal medicine, which is believed to cure several diseases. Scientifically, one of the pigment contents is the carotenoid group. Honey is known to contain chlorophyll, β-carotene, lycopene, carotenoids, and flavonoids (Mungai et al., 2017; Strapáč et al., 2016). Honey contains 9.49 mg/kg of β-carotene and 6.12 mg/kg of lycopene (Ferreira et al., 2009). Besides being a natural dye source, carotenoid compounds also have anticancer properties (Mezzomo & Ferreira, 2016). β-carotene is known to inhibit neuroblastoma tumors (Kim et al., 2019) and inhibit Prostate Cancer Cell Line (PC-3) (Jayappriyan et al., 2013). Indonesian honey is known to have potential as

an anticancer (Sumarlin et al., 2014) and laryngeal cancer Hep-2 cell inhibitor (Sumarlin et al., 2019). Other studies have shown that honey has antioxidant, antibacterial, antiseptic, and anti-inflammatory activities. There is a possibility that compounds have activity as a chemopreventive agent (Afrin et al., 2020).

The use of honey as medicine is not only in the form of pure honey but also in combination with other ingredients has been done since ancient times. The results of the research by Sumarlin et al. (2015) showed that the combination of methanol extract of Namnam leaves (EMDN) and Trigona Honey (MT) had strong antioxidant activity. The content of β-carotene in the combination of MT and EMDN is higher than in the single form. According to Tajudin et al. (2012) that Namnam fruit extract can inhibit HL-60 leukemia cells. Thus, trigona honey, methanol extract of Namnam leaf (Cynometra cauliflora L) in single or in combination form can be developed as a source of antioxidant and chemopreventive agents in the form of functional food.

However, studies on the cytotoxic ability of the combination of Trigona Honey (MT) and methanol extract of Namnam leaves (EMDN) related to the role of β-carotene are still rare. Therefore, information regarding the role of active β-carotene compounds in the combination of the two ingredients is important to know. In the long term, it can be used to study the use of combination ingredients as anticancer

Materials and Methods

Trigona honey was obtained from beekeepers in the Luwuk area, Central Sulawesi. Namnam leaves (Cynometra cauliflora L.) were obtained from Cintaratu Village, Parigi District, Pangandaran Regency, West Java and identified at the Herbarium Bogoriense Botanical Sector, Biology Research Center LIPI, Bogor. The chemicals used are methanol p.a. (Merk Millipore & JT Baker), n-hexane p.a. (JT Baker), petroleum ether p.a. (JT Baker), β-carotene standard p.a. (Sigma Aldrich), dimethyl sulfoxide (DMSO) p.a. (Merk Millipore), acetic acid p.a. (Merk Millipore), filter paper Whatman No. 4 (Merk Millipore), TLC plate of silica gel 60 F254 and column silica (Merk Millipore), 10% H_2SO_4, NaCl, capillary tube, aluminium foil, and Arthemia eggs (IPB). The tools used include glassware, analytical balance, micropipette, blender (Arte, Indonesia), rotary evaporator (Laborata 4000 Heidolph, USA), oven, stopwatch, magnetic stirrer, laminar airflow, UV-Vis spectrophotometer, and FTIR.

Samples were prepared by the maceration method. Namnam leaves with moisture content 9-10% finely grinded. 100 grams of grinded sample are macerated in 100 mL methanol for 24 hours. Maceration is carried out five times in stages. The filtrate was evaporated with a rotary evaporator at 40 ° C to obtain a condensed extract. The extracts obtained were tested for carotenoid and cytotoxic content. The combination of honey and methanol extract of Namnam leaf was prepared using a ratio of 1: 1 (w/w). Combination samples were partitioned

sequentially using n-hexane, ethyl acetate, butanol, and water as solvents. Trigona honey, methanol extract, combination sample, and fractions were tested for their cytotoxic activity.

Isolation of β-carotene was conducted on n-hexane extract. Selection of n-hexane extract based on preliminary research (Sumarlin et al., 2015). β-carotene separation using chromatographic methods Analytical Thin Layer Chromatography, Gravity Column Chromatography, and Preparative Thin Layer Chromatography. The mobile phases for Analytical Thin Layer Chromatography were petroleum ether; toluene: n-hexane (1: 9); petroleum ether: acetone (9: 1); and n-hexane: petroleum ether (1: 1). Solvents with the best separation were used in gravity column chromatography and Preparative Thin Layer Chromatography. The isolates obtained from Preparative Thin Layer Chromatography were analyzed using a UV-Vis spectrophotometer and FTIR.

Meyer method was used for cytotoxic test using larvae of *Artemia salina* Leach. Shrimp eggs are hatched in a hatchery container and 3.5% salt solution medium. The salt solution is filled in half of the total volume. The part of the box containing the shrimp eggs is covered with aluminium foil, while the part of the box that is not closed is placed under 18 watts of fluorescent light. After 48 hours, the larvae are ready for use for cytotoxic testing.

The samples for the cytotoxic test were prepared in a series of concentrations of 25; 125; 250; 500; 1000; and 2000 ppm. A concentration of 0 ppm was made as a control. The samples were prepared by dissolving 100 mg of the extract with 10 ml of the solvent to obtain 10,000 ppm. Sample solution of 1 mL, 0.5 mL, 0.25 mL, 0.125 mL, 0.0625 mL, 0.0125 mL is put in a vial and the solvent is evaporated. Once dry, 100 µL (0.1 mL) DMSO and 1 mL of 3.5% salt solution were added to each vial. In each vial, 10 larvae were added, and the volume of salt solution was added up to 5 mL so that the target concentration was obtained. The solution was incubated with 18 watts of fluorescent light. After 24 hours, the dead shrimp larvae from each vial were counted, and LC_{50} values were analyzed. Mortality was calculated and processed using Probit Analysis using SPSS v20 software. A substance that is said to be active or toxic if the LC_{50} value is <1000 ppm for extracts and <30 ppm for a pure compound.

Results

Preliminary research

Some data were obtained in preliminary research (Sumarlin et al., 2015). λmax scans of MT and EMDN samples show the maximum absorption at a given wavelength. This result is confirmed by (Britton et al., 2004), which states that the maximum absorption of β-carotene is at wavelengths of 425, 450, and 477 nm. This illustrates that the MT and EMDN samples contain β-carotene with the difference between adjacent wavelengths. In the EMDN sample, there is a lot of maximum absorption due to the presence of other compounds.

Measurement of β-carotene content was conducted using a UV-Vis spectrophotometer and pure β-carotene standard (Biswas et al., 2011; Riyanti et al., 2018). The β-carotene content in namnam leaf extract was 1.013 mg βCE/g sample, greater than the trigona honey (MT) (0,014 (mg βCE/g sample). Therefore, the β-carotene content will increase when MT and EMDN are combined. This shows that using the combination of the two materials is very potential to obtain β-carotene function.

There was an increase in β-carotene concentration when the combination sample was partitioned with n-hexane (about six times). The concentration of β-carotene before partition was 3.15 ± 0.500 mg βCE/g to 18.83 ± 0.030 after partition extraction. The increase in β-carotene levels in terms of the structure and properties of β-carotene. β-carotene is a carotenoid group that is non-polar, so it is easily dissolved in non-polar solvents according to the like dissolves like principle. It is this n-hexane fraction that continues for the cytotoxic activity test.

Cytotoxic activity test of the extract

The cytotoxic test used shrimp larvae (*Artemia salina Leach*) as bioindicators. The development of this method is based on the characteristics of the larvae, which can accept all kinds of substances and materials without prior selection. The Brine Shrimp Lethality Test (BSLT) is used as an initial screening method to find anticancer components in higher plants (Meyer et al., 1982).

The test was conducted in vitro with shrimp larvae aged 48 hours in certain concentrations. The solvent from the samples was evaporated so as not to be toxic to shrimp larvae. The test medium used was the salt solution with a concentration of 3.5%, which resulted in not all extracts being soluble. So that DMSO is used, which functions as a surfactant so that all extracts can dissolve. This test is carried out for 24 hours in a vial bottle. LC_{50} was analyzed based on the mortality in shrimp larvae after 24 hours. The results of the cytotoxic test can be seen in table 1.

Table 1. The results of the cytotoxic test

Sample	LC_{50} (ppm)
Trigona Honey (MT)	265.2
Methanol extract of Namnam leaves (EMDN)	196.12
Combination of MT + EMDN	168.2
n-Hexane fraction	77.6
Ethyl acetate fraction	124.94
Butanol fraction	140.35
Water fraction	264.68

Single form, combination, and partition results indicate potential as anticancer, and this is because LC_{50} <1000 ppm. Trigona honey has an LC_{50} of 265.2 ppm, and this is not much different from Malaysian honey, which has a

cytotoxic activity of LC_{50} 235.4 ppm (Kassim et al., 2010). The combination sample MT + EMDN (1: 1) showed an increase in cytotoxic activity with an LC_{50} of 168.2 ppm.

LC_{50} values were processed using the probit analysis method with SPSS 20 software. Increased cytotoxic activity of trigona honey after being combined with namnam leaf extract reached 36.6%. This shows a synergistic relationship with increased activity. This synergistic relationship is the same as the research of Patel et al. (2011), which combined honey and ginseng extract, which showed increased antibacterial ability. The same thing also happened to the combination of honey and olive oil in increasing antibacterial activity (Al-Waili, 2005; McLoone et al., 2016). In addition, the combination of honey with other ingredients in medicine has been shown to increase activity since ancient times (Samarghandian et al., 2017).

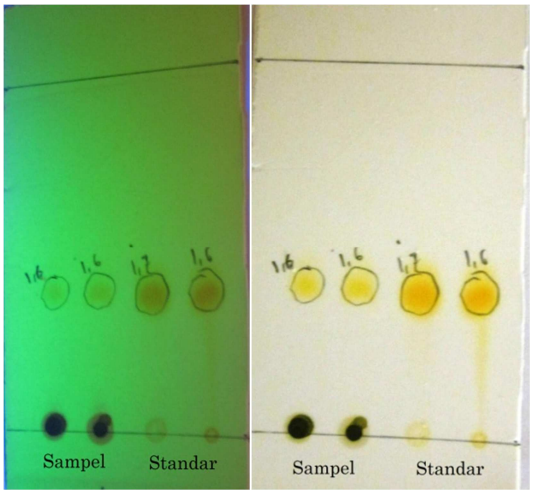
The cytotoxic activity showed an increase in the fractions of n-hexane, ethyl acetate, and butanol. But different results in the water fraction (residue) that decreased activity. This is because there is a lot of sugar in the water fraction, characterized by a caramel-shaped extract.

In the butanol fraction, it is predicted that the flavonoids that play a role in cytotoxic activity are flavonoids. Flavonoids act as anticancer agents both in vitro and in vivo (Kopustinskiene et al., 2020). The cytotoxic activity of the n-hexane fraction increased by 53.8% and had the highest ability compared to other fractions. In the n-hexane fraction, it can be predicted that those that play a role in cytotoxic activity are the terpenoids, steroids, carotenes, and other non-polar compound groups. The suspected role of carotenes in cytotoxic activity correlates with carotenoid levels (Singh et al., 2016). The highest carotenoid levels are found in n-hexane extract compared to other extracts Sumarlin et al., (2015). In their research, Tanaka et al. (2012) stated that the carotenoid group has potential as an antitumor due to its cytotoxicity both in vitro and in vivo. Bolhassani et al. (2014) stated that biochemically, the immunological activity and chemopreventive properties of natural saffron and carotenoids could be used as cancer drugs. Carotenoids can inhibit proliferation and regulate the expression of peroxisome proliferation by activating gamma receptors (PPARγ). When PPARγ is active, it can inhibit the growth of K562 leukaemia cells. Carotenoids that can inhibit K562 leukaemia cells are β-carotene, astaxanthin, capsanthin, and biksin (Zhang et al., 2011). The high carotene content and cytotoxic activity in n-hexane extracts are considered for selecting samples for the separation process. The separation process was conducted by the chromatography method.

β-carotene isolation

Isolation of β-carotene was conducted on n-hexane extract, selecting n-hexane extract based on preliminary research (Sumarlin et al., 2015) and confirmed by the highest cytotoxic activity. The separation methods used were

column chromatography and thin-layer chromatography (TLC). According to Britton et al. (1995a), the eluent to be tried is petroleum; acetone: petroleum ether (1: 9); and n-hexane: petroleum ether (1: 1). The TLC chromatogram showed that petroleum ether as eluent produced the optimum separation. The spot in the TLC plate was confirmed as a carotenoid when compared to the standard β-carotene with an Rf value of 0.67 (Figure 1.). Based on TLC, petroleum ether is used as an eluent in gravity column chromatography.



(a) (b)

Figure 1. Sample and Standard TLC chromatogram (a) under UV254 lamp (b) without the UV lamp

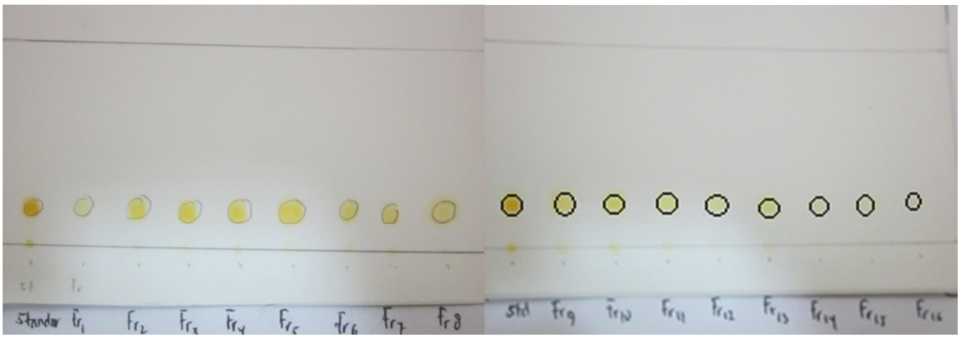


Figure 2. TLC chromatogram of the Gravity Column Chromatography eluate under UV254 lamp

Gravity column chromatography produces 16 fractions. TLC analysis of each fraction and β-carotene standard delivered spots resembling each other (Figure. 2). Due to the similarity of the spots, all fractions were combined for subsequent analysis. To ensure the content in the gravity column chromatography fraction, UV-Vis and FTIR analyses were conducted.

The λ_{max} of carotenoids belong to the conjugated diene system (C = C-C = C). The π orbitals of separate alkene groups combine to form new orbitals, namely two bonding orbitals called π_1 and π_2; and two anti-bonding orbitals called π^*_3 and π^*_4. The relative energies of the orbitals $\pi \rightarrow \pi^*$ require very low energy due to

conjugation. This low energy results in absorption at longer wavelengths. Long conjugated diene systems of beta carotene will show λmax in the range 425, 450, and 477 nm (Britton et al., 2004).

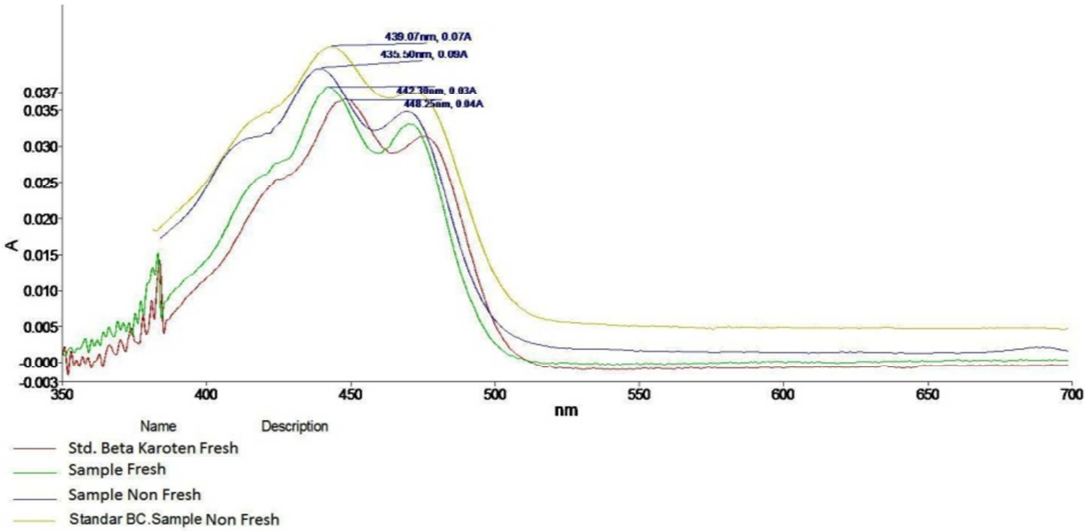


Figure 3. UV-Vis Spectrum of Sample and Standard β-carotene

The UV-Vis spectrum (Figure 3) shows the small difference in λ_{max} between the sample and the standard β-carotene. Britton et al. (2004) stated that λ_{max} β-carotene with petroleum ether solvent is 450 nm, not much different from sample and standard. There was a shift of λ_{max} in the standard and sample due to impurities, and it was suspected that β-carotene oxidation occurred by air, temperature, and light. There is a difference between the fresh and non-fresh samples (stored several hours at room temperature). This difference is due to the storage process that causes oxidation so that β-carotene changes to other carotenoid derivatives. According to Britton et al. (2004), λmax 439 nm is γ, γ-carotene. The structures of γ, γ-carotene, and β-carotene differ at the double bond location in the cyclic (Figure 4). The measured λ_{max} indicate isolates as β-carotene. To confirm, a functional group characterization test was conducted using FTIR.

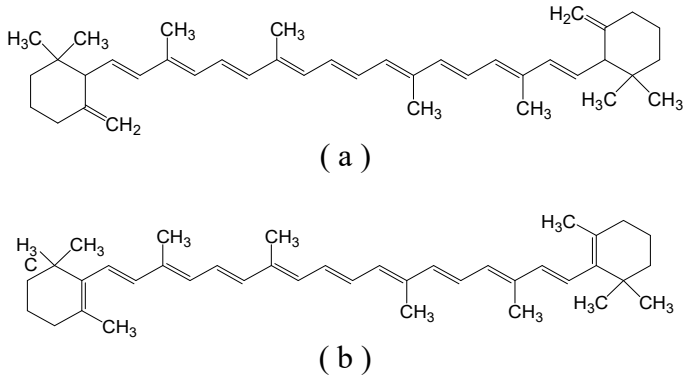


Figure 4. Structure of γ, γ-carotene (a) and β-carotene (b)

FTIR analysis was performed on column chromatography fraction, isolate, and standard β-carotene (Table 2). The FTIR spectrum of the column chromatography fraction (fractions 1-16) shows the presence of absorption at wave number (υ) 2677.31; 3358.21, and 3190.4 cm^{-1}, this shows the –OH with hydrogen bonds. The supporting peak of the OH or C-O group is found in the fingerprint area at υ 1242.21 and 1168.91 cm^{-1}. Absorption in the fingerprint region confirms the presence of the OH group. FTIR spectrum shows that the column chromatography fraction is still not pure β-carotene, this is because OH and aldehyde are detected.

Table 2. Prediction of FTIR Spectrum of column chromatography fraction, isolate, and standard β-carotene

Sample	Wave number (cm^{-1})	Reference (cm^{-1})	Functional group
Column chromatography fraction (Fr 1-16)	3358.21 ; 3190.40	3200-3450	O-H Stretching (bonded)
	2921.32	2850-2960	$–CH_3$ Stretching
	2677.31	2400-3400	O-H Stretching (bonded)
	1742.76	1690-1740	C=O Stretching (aldehyde)
	1648.24	1642-1657	C=C Stretching
	1457.28 ; 1372.41	1350-1470	C-H Bending (Aliphatic)
	1242.21 ; 1168.91	1000-1300	C-O Stretching
	720.44	675-870	C = C Bending (Aromatic)
Isolate	2947.23 ; 2873.94	2850-2960	$-CH_3$ Stretching
	2349.30	2100-2350	C=C Stretching
	1460.11 ; 1377.17	1350-1470	C-H Bending (Aliphatic)
	1151.50	1000-1300	C-O Stretching
	734.88	675-870	C = C Bending (Aromatic)
Standard β-carotene	2951.09 ; 2924.09	2850-2960	$–CH_3$ Stretching
	1741.72	1690-1740	C=O Stretching (aldehyde)
	1467.83	1350-1470	C-H Bending (Aliphatic)
	1230.58 ; 1168.86	1000-1300	C-O Stretching
	964.41	960-975	trans-RCH-CHR Bending (alkene)

The FTIR spectrum of the isolates was similar to the standard β-carotene (Figure 6). The spectrum of the isolates showed the presence of C-H (υ 2947.23 and 2873.94 cm^{-1}) and alkenes (C = C) (υ 2349.30 cm^{-1}). The fingerprint area showed the presence of aromatic alkenes (υ 734.88 cm^{-1}), C-H aliphatic (υ 1460.11 and 1377.17 cm^{-1}), and C-O groups (υ 1151.50 cm^{-1}). An aldehyde group is thought to have originated from the oxidation process of the isolate and the standard β-carotene. β-carotene is very sensitive to air and light so that it can be easily oxidized.

According to Britton et al. (1995b), polyene carotenoids' structure generally provides a weak absorption between υ 1650-1550 cm^{-1}. The fingerprint

area shows a strong absorption between υ 990-960 cm^{-1} (stretch C = C) and υ 675-870 cm^{-1} (aromatic alkenes). This has similarities to the spectrum of isolates and standard β-carotene.

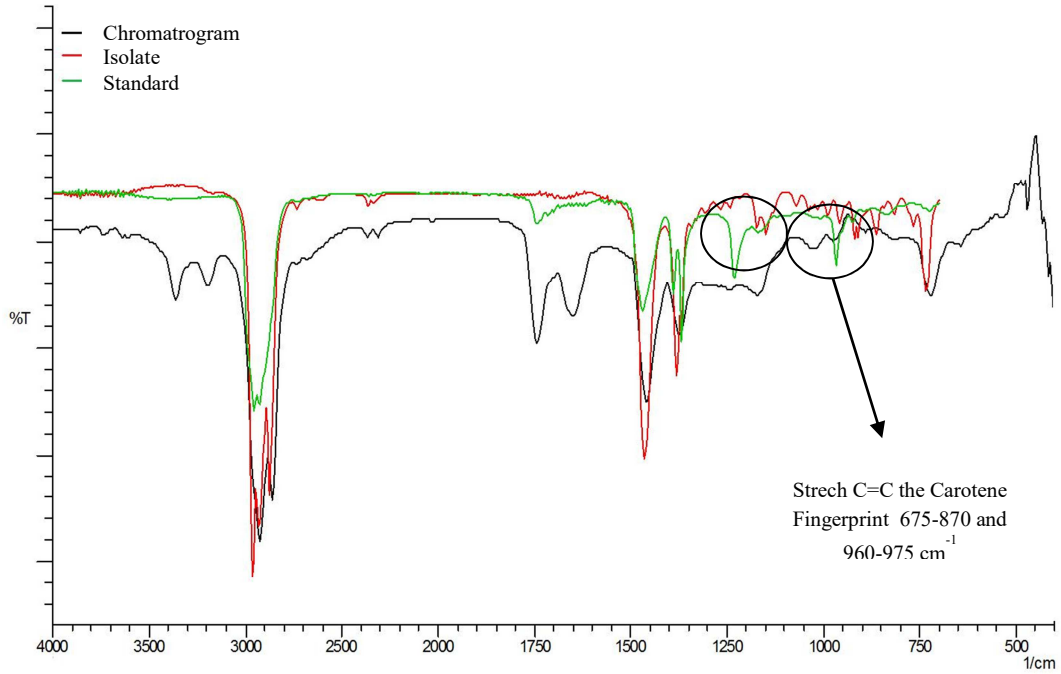


Figure 6. FTIR Spectrum of column chromatography fraction, TLC isolates from column chromatography, and standard β-carotene

Cytotoxic activity test of the isolate

Cytotoxic activity test of isolates was compared with standard β-carotene. The results of cytotoxic testing showed that the activity of the isolates (LC_{50} 22.85 ppm) was higher than the β-carotene standard (LC_{50} 33.62 ppm) (Figure 7). This is consistent with Jayappriyan et al., (2013) research that the isolated β-carotene is 5% more effective than the standard β-carotene (synthesis). This is because several β-carotene isomers, such as α-carotene, γ-carotene, and others, affect their activity in isolates. According to Tanaka et al. (2012), α-carotene was found to be more active than β-carotene.

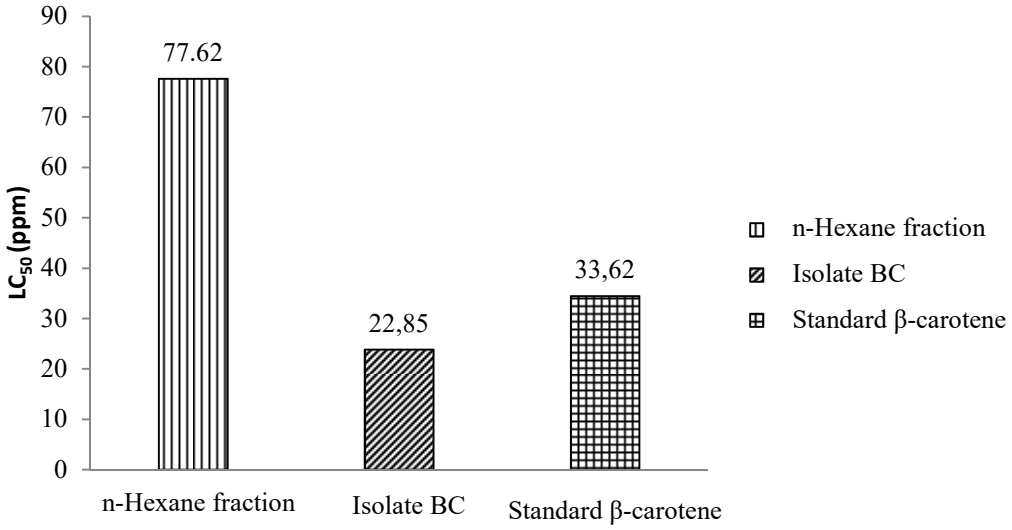


Figure 7. LC_{50} comparison of n-hexane fraction, isolate, and standard β-carotene

The artemia used is in the nauplius phase. In this phase, Artemia is most actively splitting by mitosis, which is identical to cancer cells. This causes the BSLT test to be often used as a preliminary study of anticancer activity. Cytotoxic activity is an activity that can cause cell death (Hassan et al., 2018). One of the mechanisms of action of anticancer drugs is based on cytotoxicity. The mechanism of cytotoxic activity in Artemia salina is not certain.

The mechanisms that have been identified for carotenoid compounds in inhibiting cancer are antioxidant, anti-inflammatory, anti-proliferative, anti-angiogenesis, immune system regulation, enzyme regulation, growth factor regulation, inducing apoptosis, and inducing cell differentiation (Bolhassani et al., 2014). Each type of carotenoid has a different mechanism. β-carotene undergoes different metabolic pathways into vitamins A, 8 ', 10', and 12'-apo-carotenal. All of which have the same function as anticancer. Metabolism of vitamin A is required as an antioxidant to protect against various forms of cancer, including epithelial cancer of the skin, cancer cells of the stomach, lung, bladder, breast, and other organs. β-carotene pathways to 8 ', 10', and 12'-apo-carotenal modulate transcription engines to regulate proteins so that apoptosis occurs (Mukherjee et al., 2011).

β-carotene is not efficiently metabolized to vitamin A (retinol) and is largely absorbed intact. In the intestinal mucosa, the retinals are reduced to vitamin A (retinol) by retinaldehyde reductase, and enterocytes take up free retinol. In erythrocytes, retinol forms complex bonds with type-II cellular proteins. This complex is believed to assist the re-esterification of retinol by the enzyme retinol acyltransferase. Free retinol is also esterified by acyl-CoA and acyltransferase. This ester then enters the chylomicron and is finally secreted into the lymph to transport the target tissue (Harrison, 2012; Mukherjee et al., 2011).

In metabolism, 8', 10', and 12'-apo-carotene are oxidized into two retinoic acid isomers. The two main active metabolites of all-trans-retinoic-acid (ATRA) and 9-cis-retinoic acid have a high affinity for ligands to protein receptors. All-trans-retinoic-acid (ATRA) has a high affinity for the RAR protein receptor-ligand, whereas 9-cis-retinoic acid has an affinity for the RXR protein receptor-ligand. These two protein receptors form homodimers (RXR / RXR) and heterodimers (RXR / RAR). Retinoic acid functions to induce protein transcription regulators and bind to DNA sequences in the promoter region of genes to control gene function. The portions of this sequence are commonly known as retinoic acid response element (RARE) and retinoid response element X (RXRE). Thus they play an important role in controlling the transcription of various genes. This causes the DNA to become inaccessible to transcription machines. This resulted in inhibited cancer at the cellular level (Mukherjee et al., 2011).

Conclusion

The cytotoxic activity of the combination of trigona honey and namnam leaf extract increased to 36.6%. β-carotene levels in the combination form increased to 3.15 (mg βCE / g sample). The n-hexane fraction had the highest cytotoxic activity, and β-carotene levels, the increase in cytotoxic activity reached 53.8% with an LC_{50} value of 77.6 ppm. The cytotoxic activity of the isolates increased to 70.55%, with an LC_{50} value of 22.85 ppm. The FTIR spectrum of the isolates was similar to β-carotene. The difference is in the trans-RCH-CHR (alkene) functional group (υ 960-975 cm^{-1}). The combination of Trigona Honey and Namnam leaf extract can be developed as an alternative chemopreventive agent.

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