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Research paper on “ On Kenaf Seed ”use in medicinal potential

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Abstract

Kenaf belongs to the family Malvaceae noted for their economic and horticultural importance. Kenaf seed is a valuable component of kenaf plant. For several years, it has been primarily used as a cordage crop and secondarily as a livestock feed. The potential for using kenaf seeds as a source of food-based products has not been fully exploited. Consumers are becoming more interested in naturally healthy plant-based food products. Kenaf seed, the future crop with a rich source of essential nutrients and an excellent source of phytocompounds, might serve suitable roles in the production of value-added plant-based foods. In this study, kenaf seed oils obtained by Sonication.Soxhlet and supercritical carbon dioxide fluid extraction (SFE) .Kenaf seed is a valuable component of kenaf plant. At present kenaf seed and its value-added components have not been effectively utilized for both their nutritional and functional properties as either ingredient or major constituent of food products. This research focuses on the possible food applications of kenaf seed and its value-added components based on their nutritional composition and functional properties available in literature, with the purpose of providing an overview on the possible food applications of this underutilized seed. The research focuses on a brief introduction on kenaf plant, nutritional function, lipids and proteins composition and food applications of the seed. The review elaborately discusses the seed in terms of; bioactive components,anti-hypercholesterolemic, antioxidants, anticancer,anti-inflammatory,anti-thrombotic, antimutagenic property as edible flour, as edible oil and a source of protein in food system. The research closes with discussion on other possible food applications of kenaf seed. The need for food scientists and technologists to exploit this natural agricultural product as a value-added food ingredient is of great significance and is emphasized.

Keywords: kenaf seed,food application,medicinal property,or potential.

Introduction

Kenaf (Hibiscus cannabinus L.) is a seasonal herbaceous plant, which belongs to the family Malvaceae. Cultivated broadly in Asia and Africa, that mostly cultivates in tropical and temperate regions . Ezzadin, Neven Azad et.,al.(2022) [1] who reported that in the United States agriculture department determined kenaf as a high potential crop for use as a source of fiber in pulp and paper industry. Ayadi et al. (2017) [2] defined kenaf as a substitute crop which might be a sustainable cellulose supply, and environmentally friendly and economically practical. Therefore, kenaf plant is sowed for its fiber, but its leaves and seeds that have been used in traditional medicine in Africa and India for a variety of diseases. As well as, kenaf is a major ligno-cellulosic feedstock for production of bioenergy. who declared that derivatives of kenaf such as leaves and seeds were institute to exhibit high bioactive chemicals and prospective effect of skin whitening, indicating its potential for cosmetic submissions. Kenaf seeds and leaves might be situated as promising source of bio-active chemicals and phytonutrient. Since, a study conducted by Sim and Nyam (2019) [3] review about the phrase "return to nature" have been widely employed in cosmetic industry, utilization of botanical extracts resulted in consumer acceptability. KLE (kenaf leaves extract) showed promising anti-oxidant and anti-tyrosinase capabilities, and it has the potential to be exploited as an added value to component in creation products for cosmetic. It's critical to produce safe and stable formulation incorporating KLE, since it includes a high concentration of polyphenol chemicals that have been shown to have skin whitening and anti-aging characteristics . In addition, (WHO) World health organization reports according to one study approximately 80 % of the people in the world populations utilizes herbal plants to treat human illnesses. indicated that kenaf is an active plant biologically, were its seeds and leaves had extensive pharmacological submissions for example anti-oxidant and anti-infammatory activities. Earlier, extracted cellulose from kenaf was used to produce AgNPs, the sucrose were utilized as a dropping agent and NPs showed strong anti-bacterial properties against E. coli . Further, seeds of kenaf contain a putative major bioactive component known as kaempferol' which has been used to treat cancer (Wang et al., 2019; Imran et al., 2019) [4] .

Taxonomy of Kenaf Seeds

Alexopoulou et al. (2013) [5] Exposed that post pollination capsules of seeds are made, which are 1.3 – 1.9 cm wide and 1.9 – 2.5 cm long. The seed grows in five – lobed capsules. Every capsule has five segments, and a total of 20 – 26bseeds capsules-1 . Small hairy structures surround the seed capsules, which causes irritating for human skin. The grown type's capsules are typically indehiscent and persist intact for numerous weeks once accomplish its maturity. Under normal conditions of storage, the seed is viable for around eight months. Generally, kenaf plant seeds are similar. Nevertheless, there are some color and size differences. Kenaf seeds are around 4 mm wide and 6 mm long, which are wedge-shaped and have black color as showed in (Fig 1) (Salih, 2016) [6] . who told that kenaf seeds are triangular, sharp angles, ash gray with pointed pale yellowish warty patches. Brown hilum color and are relatively small.

Fig 1: Seeds of kenaf plant .

2. Methods and materials

2.1. Extraction of kenaf seeds

Kenaf seeds were purchased from the National Tobacco Board, Pasir Putih, Kelantan, Malaysia. The variety of kenaf seeds was Quiping . Kenaf seeds were cleaned, soaked in water at ambient temperature for 24 h and dried at constant temperature (50 ◦C) overnight in an oven (FD 115, Fisher Scientific, Germany). Yazan, Latifah Saiful et al; (2011) [7] The detailed procedures for kenaf seed oil extraction have been previously reported in the literature (Chan and Ismail, 2009)[8]. Briefly, for Sonication or conventional ultrasonic assisted solvent extraction (SONIC), 25 g of kenaf seeds were ground in a stainless steel Waring blender for 1 min and homogenized with 300 ml of n-hexane at 13,500 rpm for 3 min (Ultra-turax T25). Subsequently, the mixture was sonicated for 90 min in an ultrasonic bath (Power sonic 505, Microprocess Controlled Benchtop Ultrasonic Cleaner, Germany). After Sonication, the mixture was filtered through a filter paper (Whatman No. 1) and the filtrates were evaporated using a rotary vacuum evaporator (Buchi, UK). For Soxhlet extraction, 50 g of kenaf seeds were ground by a stainless steel Waring blender for 1 min and equally divided into two extraction thimbles. Each thimble was then transferred into a Soxhlet extractor. Prior to extraction, 300 ml of n-hexane was added into the round bottom flask. After extraction was initiated, the solvent flow rate was manually adjusted to 7 min/cycle and the extraction was terminated after 20 cycles (rapid Soxhlet extraction, SOX/S) and 100 cycles (classic Soxhlet extraction, SOX/L), respectively. For SFE, kenaf seeds were extracted by using the supercritical carbon dioxide fluid extractor (Thar 1000 F, USA) at 9 different combinations of pressure (bars) and temperature (◦C). The combinations were 200/40, 200/60, 200/80, 400/40, 400/60, 400/80, 600/40, 600/60 and 600/80. Briefly, kenaf seeds were ground in a stainless steel Waring blender for 1 min and 100 g of kenaf seeds were placed into a 1 l extraction vessel. After the extraction vessel was tightly sealed, the desired temperature and pressure were set. The flow rate of carbon dioxide was set at 25 g/min and regulated by an automated back pressure regulator. The SFE extraction was initiated after the desired temperature and pressure were achieved. The whole extraction lasted for 150 min and the yield was measured.

2.3. Treatment

Cells were treated with different concentrations of kenaf seed oil (100–5000g/ml) for 72 h.

2.4 Determination of cytotoxicity

2.4.1. MTT assay

Briefly, 10l medium with 0.5 mg/ml MTT in PBS was added into each well. The plate was incubated at 37 ◦C for 4 h. Next, the medium was totally removed and 200l Tris-DMSO solutions were added to each well. (Abd Ghafar, K.W., Tahir)et.al (.2011) [9] Effect of kenaf seed oil from different ways of extraction towards ovarian cancer cells. *Food and Bioproducts Processing*. The absorbance, which was proportional to cell viability, was measured at 570 nm and a reference wavelength of 630 nm by using an ELISA plate reader (Bio-Rad, Hercules, CA, United States). A graph of percentage of cell viability versus concentration of extracts was plotted and the concentration that gave 50% inhibition of the cell viability (IC50) was determined.

2.4.2. Trypan blue dye exclusion method

Briefly, 15l of cell suspension were mixed with 15l of trypan blue. Subsequently, the mixture was transferred to a haemocytometer with an overlay of cover slip and viewed under an inverted light microscope. The cell viability was then determined. A graph of percentage of cell viability versus concentration of extracts was plotted and the concentration that gave 50% inhibition of the cell viability (IC50) was determined.

2.5. Morphological changes

The cells were treated for 72 h and viewed under an inverted light microscope.

2.6. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 16.0. Results were analyzed by one-way analysis of variance (ANOVA). Data were expressed as mean ± standard deviation (mean ± SD). A difference was considered to be significant at $p < 0.05$

Applications of kenaf seed oil

As mentioned previously, kenaf seed has relatively high oil content with unique fatty acid composition and similar to cottonseed oil. This made kenaf seed oil a suitable substitute for cottonseed oil, which is rather expensive. Kenaf seed oil can be used in many applications. Firstly, it can be used as salad dressing. In comparison to cottonseed oil, kenaf seed oil has milder odour and thus will be more acceptable by consumers. The high amount of monounsaturated and polyunsaturated fatty acid in kenaf seed oil also provides benefits for cardiovascular health. Besides, kenaf seed oil can be used as cooking oil. However, this type of oil is not suitable for cooking at high temperature and long duration. This is because oil high in unsaturated fatty acid particularly polyunsaturated is unstable and oxidation occurs at a rapid rate and form free radicals and carcinogenic compounds (Whitaker,et.,al 2003) [10]. In addition, kenaf seed oil can be used in soap manufacturing especially

those hard types. The process of making soap is called saponification and the main ingredient is oil . The oil used can be of animal or plant source. During saponification, sodium hydroxide will be added into oil and the mixture is heated and stirred. Sodium chloride is added at the end of saponification to precipitate the soap . Furthermore, kenaf seed oil can also be used in cosmetics products such as lipsticks and milky lotion. (Chang Wing-Yan, and Kar-Lin Nyam.at.l.(2016) [11] "Kenaf seed oil: A potential new source of edible oil." *Trends in Food Science & Technology* . The oil functions to enhance penetration, control moisture evaporation and hydrate skin. Before addition, the oil will usually undergo processing in order to remove odours and colour or become hardened oil . Apart from that, kenaf seed oil has found applications in industry as lubricants and biofuel . Seed oil is increasingly used in industry as an alternative of mineral oils for several reasons. Firstly, seed oil can have good lubricating properties and the effect is comparable to the conventional lubricants. Using seed oil is also more environmentally-friendly as they are more biodegradable than mineral oil . More importantly, kenaf seed oil is cheaper, renewable and with constan supply. This ensures the continuous industrial operation and production.

Nutritional Functions of Kenaf Seed

Kenaf seeds have many nutritional functions and could be used as a valuable natural source of ingredient for production of functional foods. Kenaf seeds are an important source of dietary fibers, oil and proteins. (Giwa Ibrahim et al(.2019) [12] "Kenaf (Hibiscus cannabinus L.) seed and its potential food applications: a review." *Journal of food science* . The oil has been suggested to be used as a new source of functional edible oil with high antioxidant activity and anticancer properties . Several researchers have reported the value of kenaf seed-derived products like kenaf seed flour (KSF), defatted kenaf seed meal (DKSM) and kenaf seed protein concentrates (KSPC); they have health supporting activities . The potential of kenaf seed as a source of functional edible oil seems to be excellent as it contains alpha-linolenic acid, an essential omega-3 fatty acid with anti-inflammatory and antithrombotic activities and also chemo-preventive activity.(Roselina Karim, Nazamid Saari, .et.al.2019) [13]"Kenaf (Hibiscus cannabinus L.) seed and its potential applications: a review." *Journal of food science* . The relatively high oil composition like that of cottonseed oil, and appreciable quantities of phospholipids and phytosterols suggest that kenaf oil can be used for culinary purposes and the seeds could serve feed and food purpose. Researchers have shown interest in isolating the bio-active components (phytosterol) of kenaf seed oil for the production of healthy and nutritious foods (Holser et al., **2004**) [14]. The phenolic and flavonoid compounds present in kenaf seed have been reported as potential inhibitors of angiotensin I-converting enzyme and in the peroxidation of lipids **.**

The significance of DKSM as an alternative source of dietary protein. Protein concentrates prepared from DKSM exhibit satisfactory functional properties and are suitable to be used as functional ingredients in food products. DKSM contains reasonably high amounts of total phenolic compounds which could be used in functional food industry as phenolic compounds such as flavonoids and phenolic acids are strong antioxidants against food lipid peroxidation. Vital phenolic compounds of DKSM have been reported as playing the roles of powerful antioxidants in different foods and when tested in experimental animal. Thus, the addition of DKSM

to food products could offer beneficial effect against lipid deterioration during food processing and storage; as it prevents free radicals chain reactions of lipid peroxidation in food products. Report that DKSM is an effective secondary antioxidant as it can prevent the decomposition of hydroperoxides. The study also suggests the use of DKSM as a functional food ingredient, as food preservatives and in nutraceutical formulations due to its potent antioxidative properties.

Lipids Composition of Kenaf Seed

Fatty acids content of kenaf seed is a well-studied area and several researchers have reported that high values of crude lipid of kenaf seed ranging from 20.4% to 24.8% (Alexopoulou et al.[15] as an attractive alternative source of edible oil, rich source of unsaturated fatty acids such as oleic, linoleic, linolenic, and also containing important steroids and tocopherols. The polyunsaturated fatty acids content of kenaf seed is similar to that of Roselle seed oil . Palmitic acid is the major saturated fatty acids, whereas oleic, and linoleic acids are predominant unsaturated fatty acids in kenaf seed oil . Similar to the fatty acid composition of Roselle seed oil . Several phytosterol have also been reported to be present in kenaf seed oil with β-sitosterol being the predominant sterol followed by campesterol and stigmasterol . However, the phytosterol content of kenaf seed oil is much lower than that of Roselle seed oil (Nyam et al., **2009**) [16]. Kenaf seed oil has also been characterized to contain wide varieties of phospholipids such as phosphatidic acid, phosphatidylcholine, phosphatidylserine, lysophosphatidylcholine, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl glycerol, and sphingomyelin .

Chemical Composition of Kenaf leaves

The leaves of kenaf are high in bio-active compounds including catechin hydrate, caffeic acid, kaempferol and chlorogenic acid (Kho et al. 2019; Haw et al. 2020) [17]. Leaves of kenaf contains alkaloid 0.28%, saponin 0.15%, tannin 0.003%, glycoside 0.10%, steroid 0.002%, flavonoid 20 mg/100 g and carotenoid 569.55 mg/100 g . There more, indicated that seeds have the greatest phenolic and flavonoid levels as compared to the leaves. Water extract has greatest total phenol concentration for both kenaf seeds (754.6 3.14 mg/100 g dry extract) and leaves (418.7 3.47 mg/100 g dry extract). The highest flavonoid concentration likewise found in water extract (425.33 4.39 and 299.17 3.43 mg/100 g dry extracts) extracts of kenaf seeds and leaves. Other liquid seed extracts of ethyl acetate, followed by ethanol extract and n-Hexane extract, as well as leave extracts of ethanol extract, followed by ethyl acetate and n-Hexane extract, was shown to be significant for total phenolic content and total flavonoid content (Fig 2).

The extract of kenaf leaves made it has been claimed that it might be used as potential natural antioxidant source, antibacterial, and anti-tyrosinase constituents in the food, cosmetic, and pharmaceutical industries. Personal care products, such as skin and hair care products are pharmaceutical preparations with therapeutic characteristics that protect against degenerative disorders. They are becoming increasingly popular in current formulations as a result of customer worries about the safety of synthetic constituents .

Demanded that natural produces for cosmetic has quantifiable therapeutic efficiency on skin, as the medications and formulations has evolved from skin, body to hair and are used to treat a variety of ailments such as damage of hair, photo aging, wrinkles, dryness of skin, un-even tone of skin, pigmentation, etc.

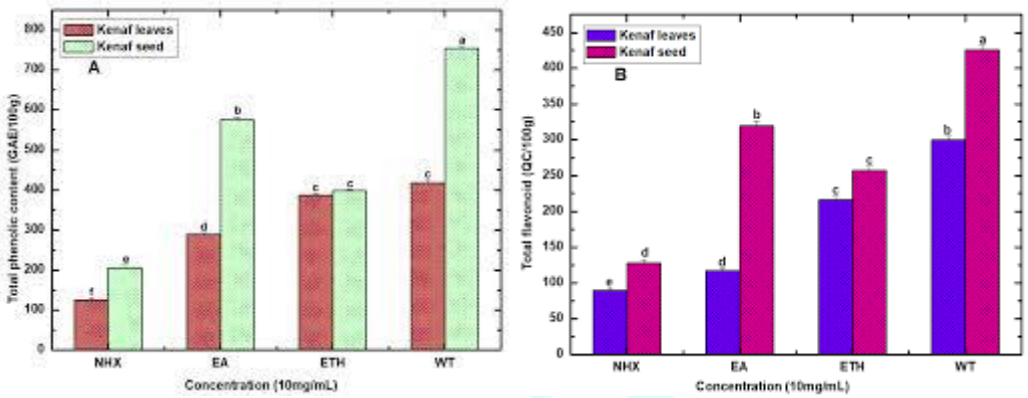


Fig 2. Total phenolic (A) and flavonoid (B) content of various solvent extracts (NHX, EA, ETH, and WT) of Kenaf leaves and seed. Values are expressed as mean ± SD (n = 3). Values marked by different letters in each column are significantly different by t-test ($p < 0.05$). GAE = Gallic acid equivalent, QC = Quercetin equivalent, NHX = n-Hexane extract, EA = Ethyl acetate extract, ETH = Ethanol extract, and WT = Water extract 5

Kenaf Seed in Food Applications

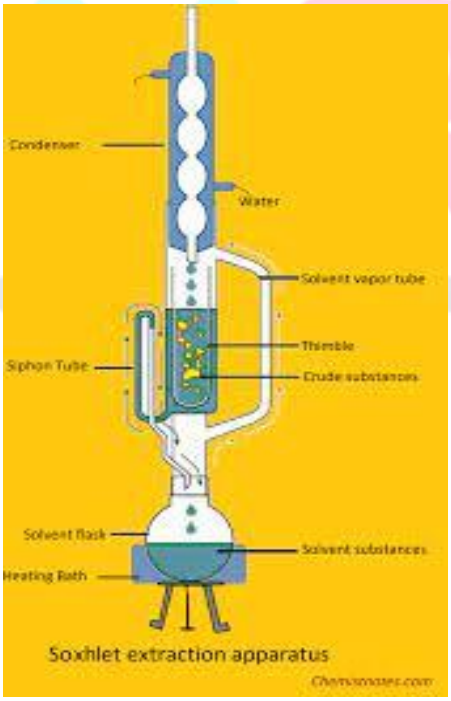
Owing to the excellent nutritional qualities of kenaf seed, it could be used in food application as whole seed or be processed into different forms. The kenaf seed could be made into flour by grinding into different particle sizes, depending on the purpose which is intended to be used for. The seed could also be subjected to defatting process, which will increase the protein content of the flakes. Researchers had used different methods to determine the quantity and quality of the oil obtained. Mariod et al. (2010) [18] have extracted oil from ground kenaf seed using Soxhlet extraction method. Sonication method had been used to extract oil from ground kenaf seed. This method has been reported to have advantages such as reduction in extraction time, lesser destruction of antioxidant activity and it also allows high solvent diffusion into sample and increases the contact surface area between the solute and the solvent in contrast to Soxhlet extraction in which the solvent used will contaminate the final product and further processing will be required to remove it. 19) Wong,lau,et.,al (2014) [19] Supercritical fluid extraction (SFE) method had also been investigated. Temperature and pressure are important factors that affect the yield of kenaf seed oi. Previous studies reported that pressure plays a more important role than temperature in determining the quantity of kenaf seed oil. SFE method has been recommended as the most preferred method of oil extraction as it is friendly, time-saving and completely removes solvent from oil . Although reported that Soxhlet extraction method can give a higher yield of oil than supercritical fluid extraction method. Another form of kenaf seed flour is protein concentrates. Kenaf protein concentrates are more refined than the flour and grits and normally have 70% or more protein content, higher than that in flour and grits . The concentrates are obtained from defatted kenaf flour or flakes by separating the saccharides, ash, and other minor components in any of the Sabapathy. The first method requires washing defatted flakes or flour with about 80% alcohol. By this process, the oligosaccharides, ash and other minor

components are dissolved and separated from the protein. The second method uses acid at pH 4.5 to separate sugars from the proteins. The third method uses moist heat to modify the proteins in the flour and subsequently using water separates the sugars and other minor components from the protein. Protein concentrates obtained by either of the methods may contain 70% more of proteins, but the physical properties may vary according to the processing method . Protein concentrates have decreased flavor in contrast to flour due to processes involved during its production which remove some of the flavor components. Kenaf seed proteins isolates are more refined, obtained by removing the oligosaccharides, insoluble polysaccharides, and minor components. Shows the protein content of Kenaf seed increases because of further processing during the production of kenaf protein isolates. Protein isolates may contain 90% or more of protein with other minor constituents . Defatted kenaf seed flour and protein concentrates are rich protein sources, with high essential amino acid levels than the whole seed . Protein concentrate has reduced flavor, low flatus and saccharides features which may promote product quality and acceptability .

Extraction of kenaf seed oil

As aforementioned, kenaf seeds have high oil content and they can be extracted through several methods. In recent studies, the commonly used methods to extract kenaf seed oil are solvent extraction and supercritical fluid extraction (SFE). Generally, the latter is more preferable as it is cost-effective, environmental friendly and time-saving and does not create safety issue due to incomplete removal oil solvent . Solvent extraction uses organic solvents such as hexane and petroleum ether to extract oil from kenaf seeds. This method is further divided into classic (SOX/L), rapid (SOX/S) and ultra-sonic assisted (SONIC). The difference between classic and rapid extraction is the number of cycles to extract oil where classic method stops the extraction after 100 cycles whereas rapid extraction will be terminated after 20 cycles. On other hand, the conventional ultra-sonic assisted solvent extraction involves homogenization and sonication and pulsed mode is usually chosen to carry out the processes it requires lower electrical energy consumption, high extraction time reduction and the effect on antioxidant activity is lesser. Mentioned that ultrasound-assisted extraction provides advantages over conventional Soxhlet extraction because it enables greater solvent penetration into sample matrix, increase the contact surface area between solid and liquid phase resulting in higher diffusion rate of solute into solvent. Soxhlet extraction is an established method to extract oil but it presents some problems. The organic solvent used during Soxhlet extraction will pollute the final products and further processing is necessary to remove the residual solvent. Besides, Table 1 World production of kenaf and allied fibre from . Trends in Food Science & Technology other lipid soluble compounds are extracted along with fatty acids as the organic solvents used do not provide desired selectivity. Therefore, another method known as supercritical fluid extraction is suggested to be an alternative to the conventional method. Supercritical fluid extraction (SFE) in turn utilizes supercritical fluid solvents, such as carbon dioxide, ethane and nitrous oxide. Supercritical fluids are dense gases that completely fill a container and possess solvating power . They are also low in surface tensions, viscosities and have moderately high diffusion coefficient thereby enhancing the mass transfer . This method has been used over a decade in food analysis especially for fatty food. SFE has also expanded application to extracting oil from many plant sources, seeds and kernels. The most popular SFE solvent is carbon dioxide because

triglycerides, fatty acids and cholesterol are quite soluble in it . Furthermore, carbon dioxide is inexpensive, non-toxic, nonflammable and available at high purity (Goodship & Ogar, 2004) [20]. In comparison to Soxhlet extraction, this method avoids the problem of contamination of oil by residual solvent and enables the complete removal of solvent at final stage of extraction. This saves the cost of purification of kenaf seed oil that is required when using conventional solvent extraction. Besides, thermal degradation is less likely to occur since temperature and pressure can be manipulated. This method is also cost-effective, time esaving, nonexplosive, safe and environmental friendly. However, in terms of yield, conventional soxhlet extraction (SE) can give higher yield than supercritical fluid extraction (SFE). Compared the yields of three extraction methods; supercritical fluid extraction with carbon dioxide at different temperature and pressure, Soxhlet extraction and conventional ultra-sonic assisted solvent extraction. The yields for SOX/L, SOX/S and SONIC were 24.81%, 22.40% and 21.08% respectively. While for SFE-extracted oil, the yield was ranging from 2.12% to 20.18%. It can be seen that classic solvent extraction gave higher yield than ultrasonic assisted and SFE. This implies that heat treatment is an efficient treatment in extracting oil from seeds. In spite of the higher yield, classic Soxhlet extraction took more than 12 h to complete the entire process which is not convenient from the perspective of mass production. For SFE, temperature and pressure are two important factors affecting yield of kenaf seed oil and biological activities , when pressure increases at constant temperature, the density of carbon dioxide will also increase and this results in enhanced solute (oil) solubility and subsequently the yield. While at constant pressure, increasing temperature will decrease the density of carbon dioxide and increase the vapour pressure of analytes. This increases the tendency of components to pass through critical fluid and to be extracted thereby increasing the oil yield . Extracted kenaf seed oil using SFE at nine different parameters with varying temperature and pressure and both studies reported that highest yield was obtained at a pressure of 600 bars and temperature of 80 C.) Ng et al.(2015)[21] From the result, the yields of kenaf seed oil are generally higher at higher pressure. Hence, it is suggested that pressure play a more dominant role than temperature in affecting the solubility of oil triglycerides.



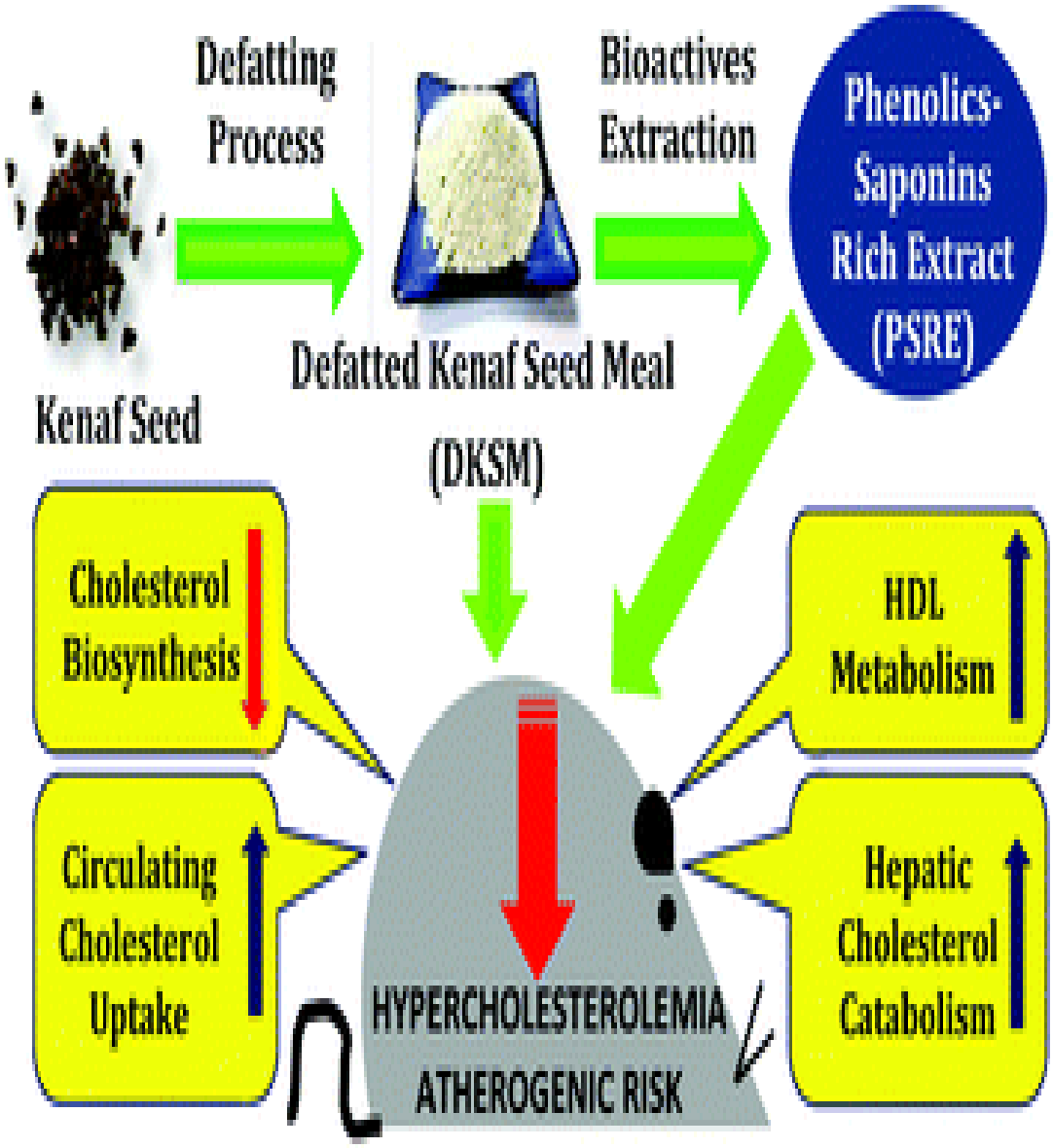
Medicinal activities of kenaf seed oil

Extensive research revealed that kenaf seed oil has several functional properties which especially beneficial to human health contributed to the phytochemicals and compounds present. This review focuses on the beneficial biological activities of kenaf seed oil such as anti-hypercholestrolemic, antioxidant, anti-cancer, antiinflammatory, anti-thrombotic, anti-mutagenic properties and also applications of kenaf seed oil in various fields.

Anti-hypercholesterolemic effect

Hyperlipidemia is characterized by elevated blood cholesterol (hypercholesterolemia), low density lipoproteins cholesterol and/ or triglyceride level along with decreased high density lipoprotein cholesterol. Hypercholesterolemia, as one of the causes of hyperlipidemia could contribute to the occurrence of atherosclerosis and abnormal lipid metabolism under the influence of oxidative stress. (Beery and workman et,al.2011) [22] Carried out a study on anti-hypercholestrolemic effect of kenaf samples (kenaf seed oil, microencapsulated kenaf seed oil, defatted kenaf seed meal (DKSM) and kenaf seed extract (KSE)) on rats. Elevated malodialdehyde (MDA) in serum is a biological indicator

of lipid peroxidation as a result of reaction between reactive oxygen species (ROS) with unsaturated fatty acids. It was reported that MDA level of rats treated with kenaf samples were lower than those untreated and the results had no significant difference with commercial hypocholesterolemic drug, simvastatin. In addition, the effects of kenaf samples on total cholesterol and total triglyceride level were also comparable to simvastatin where no significant difference was reported. From this study, it was shown that the highest anti-hypercholestrolemic effect was found in KSE followed by KSO, MKSO and DKSM. Although kenaf seed extract had higher anti-hypercholesterolemia effect than microencapsulated kenaf seed oil and kenaf seed oil, the cholesterollowering effects of kenaf seed oil and encapsulated kenaf seed oil were comparable to the commercial drug, simvastatin. Therefore, this study suggested that kenaf seed oil could be used as an alternative to hypocholesterolemia drug and this property enables it to find more application in food industry. The anti-hypercholestrolemic effect of kenaf seed oil is due to the cholesterol lowering ability of polyunsaturated fatty acids (PUFAs), phytosterols and tocopherols. The phytosterols content in KSO and MKSO were 6510.3 ± 54.2 mg/100 g and 4680.4 ± 171.9 mg/100 g, respectively while total tocopherols of KSO and MKSO were 186.83 ± 2.70 mg/100 g and 154.89 ± 3.15 mg/ 100 g, respectively . The mechanism of unsaturated fatty acid to lower serum cholesterol remains unclear but it is proposed that polyunsaturated fatty acid decreases the production of low density lipoprotein (LDL), increase catabolism of LDL and conversion of polyunsaturated fatty acid into ketone bodies instead of being incorporated into very low density lipoprotein. On the other hand, phytosterols such as beta-sitosterol, campesterol and stigmasterol reduce cholesterol level by replacing the cholesterol in micelles (the compound formed to facilitate fat absorption) thereby decreasing its absorption in intestine. The excess cholesterol will be excreted with feces. Besides, phytosterols increase the level of high density lipoprotein (HDL) cholesterol and also prevent esterification of cholesterol to further reduce absorption.



Antioxidant property

Oxidation and presence of free radicals are the culprits of deterioration in sensory properties, nutritional quality, safety and acceptability. As oxidation is a process that poses many challenges, synthetic antioxidant, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are added during food processing to prolong shelf life. However, several physiological disorders related to the use of synthetic antioxidants have been reported. Therefore, natural antioxidants are still preferred due to safety issue. Different tests have been used to evaluate the antioxidant activity of kenaf seed oil and 2,20 -azino-bis(3- ethylbenzothiazoline-6-sulphonic acid)(ABTS), beta-carotene bleaching (BCB), 2,2- diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays show that kenaf seed oil does possess antioxidant property. This is also supported That compared antiradical activity of several commercial edible oils and reported that kenaf seed oil is high antioxidative oil. The method and condition of extraction will affect the antioxidant activity of kenaf seed oil to some extent. For ultrasonic assisted extraction, pulsed mode is selected because it will have less effect on antioxidant activity on kenaf seed extract (Wong, Lau, et al., 2014)[23]. If kenaf seed oil is extracted at higher temperature, the antioxidant activity will be lowered as some of the antioxidant compounds are destroyed by heat. This is in line with the study carried by where the peroxide value (an indicator of primary oxidation) of MKSO produced at 200 C was higher than those produced at 160 C and 180 C. The higher peroxide value is attributed to the lower total phenolic content as a result of destructive effect of high temperature. In terms of concentration of antioxidants, according to ethanol is a better solvent than hexane in extracting phenolic compounds from seeds. This is because ethanol is polar solvent and its polarity enables it to extract hydrophilic phenolic compounds more effectively. Free radicals are generated from reactive oxygen species (ROS) and reactive nitrogen species (RNS) from chemical reactions in the body, exposure to physicochemical conditions or pathophysiological states. Free radicals can cause alterations of proteins, lipids, DNA and other biomolecules which subsequently result in abnormal metabolism and mutagenesis. A condition known as oxidative stress results when the levels of free radicals and prooxidants (ROS and RNS) outgrow the ability of natural antioxidants in body to remove them. Kenaf seed oil is suitable to be used as natural antioxidant because it contains polyphenols, flavonoids and tocopherols that can act as free radical scavengers, reducing agent or quenchers of singlet oxygen. Antioxidant trends in Food Science & Technology neutralizing free radicals at different stages, namely prevention, interception and repair. At preventive stage, antioxidants inhibit the formation of reactive oxygen species whereas free radicals and peroxyl radicals will be scavenged or chain breaking is inhibited during propagating oxidation at interception stage . On other hand, enzymes are usually involved in the repair stage.

Anti-cancer property

Several studies have been carried out to investigate the anticancer property of kenaf seed oil against different human cancer cell lines such as human cervical cancer, ovarian cancer, breast cancer, colon cancer, lung cancer and leukemic cancer cell lines. Positive results were obtained and kenaf seed oil showed cytotoxic effect towards all studied cancer cell lines aforementioned (Ghafar et al., 2013) [24] Studies reported that kenaf seed oil extracted from variety V36 under pressure of 600 bars and 40 C showed strongest cytotoxic activity towards breast cancer

(MDA-MB-231), leukemic cancer cell lines (MOLT-4), human promyelocytic HL-60, murine myelomonocyticWEHI-3B and human chronic myelogenous K562 . On the other hand, among the cancer cell lines investigated. It was discovered that kenaf seed oil showed strongest cytotoxic activity toward colon cancer cells was observed followed by breast cancer cells, lung cancer cells and cervical cancer cells. These findings show that kenaf seed oil could be a promising anti-cancer agent. Besides, it is also mentioned in study that polyphenols and conjugates must remain in the gut lumen and gastrointestinal tract in order to inhibit abnormal cell proliferation and protect against cancer. Similar to antioxidant property of kenaf seed oil, IC50 is also used to determine the cytotoxic activity of kenaf seed oil. Oil with IC50 value between 125 and 5000 mg/ml is a potential cancer therapeutic agent (Wong, Tan, et al., 2014). Table 6 indicates the effectiveness of kenaf seed oil from variety V36 as anti-cancer agent towards different cancer cell lines. In addition, an inverse relationship was found between cell viability and concentration of kenaf seed oil (Yazan, Foo, et al., 2011) [25]. This reflects that cytotoxic activity is dose-dependent where higher concentration of oil results in lower cell viability. The mode of cancer cell death was also studied and it was revealed that apoptosis and necrosis were two main mechanisms by which cancer cells are killed. In comparison to necrosis, apoptosis is more favorable because it does not trigger inflammatory responses. This confirmed the results obtained by where apoptotic cells were significantly higher than necrotic cells. The features of apoptosis include membrane blebbing, chromatin condensation, nuclear margination and fragmentation . That linoleic acid, alpha-linolenic acid, phytosterols and vitamin Eare the components in kenaf seed oil responsible for the cytotoxic effects of kenaf seed oil. Linoleic acid is found to be able to inhibit proliferation of human skin, breast, colon, stomach and leukemia in vitro and in vivo . On the other hand, phytosterols inhibit growth of cancer cells through cell cycle arrest and induction of apoptosis as well as reducing metastatic ability of cancerous cells. This is further supported that phytosterols can act as anticancer dietary components. It is also proposed that the phytosterols exert effects on membrane structure, integrity and fluidity, membrane-bound enzymes, signal transduction pathway, apoptosis, immune function of host tissues. Similar to phytosterols, vitamin E was found to induce programmed cell death (apoptosis) in human colon cancer cell while inhibiting growth of prostate cancer cell. Apart from that, the anti-cancer property of kenaf seed extract and oil is contributed to certain extent by polyphenols and flavonoids. These two compounds are believed to reduce the risk of cancer. It is also interesting to note that extraction method can affect the anti-cancer property of kenaf seed oil. Kenaf seed oil extracted from supercritical carbon dioxide fluid extraction has greater cytotoxic effect than from solvent extraction. This may be due to destruction of some heat sensitive bioactive compounds by high temperature used in solvent extraction method.

Anti-thrombotic property

Thrombosis is a condition characterized by formation of blood clot, known as thrombus in blood circulatory system. This is a fatal disease and it can lead to vascular blockage and acute coronary disorders. Thrombosis occurs when the balance between two systems responsible for creating and breaking down blood clots (thrombus) is upset. Thrombus can obstruct blood flow when it grows bigger. The antithrombotic drugs available in the market can be classified into two, antiplatelet and anticoagulant. The drugs are working based on the principles of preventing formation of clot and inhibiting the production of chemicals in platelet which

cause platelets to clump. Therefore, the compounds in kenaf seed oil might as well prevent thrombosis according to the principles mentioned previously. Phytochemicals present such as tannins, phenolic compounds and polyphenols contributed to the anti-thrombotic effect of kenaf seed oil. Low, Mnonopi, Davids, Naude, and Frost studied the antithrombotic or anticoagulation of selected medicinal plants and found out that the activities were attributed to the presence of tannins in plant. Antithrombotic/anticoagulation effect was not significant when tannins are removed. Tannins were found to be able to reduce ADP-induced platelet aggregation . Platelet aggregation, one of the factors causing thrombosis, is modulated by dietary compounds such as polyunsaturated fatty acid (PUFA), vitamins, alcohols and phenolic compounds. Kenaf seed oil is rich in PUFA and phenolic compounds and this explain its anti-thrombotic effect (Natella et,al.2005) [26]. In addition, flavonoids can act as anti-thrombotic agent apart from a strong antioxidant. Flavonoids can reduce the concentration of thromboxane A2, a platelet aggregator in blood. They also function to scavenge free radicals and maintain concentrations of endothelial prostacyclin and nitric oxide (a platelet inhibitor and vasodilator) at appropriate level. One study showed that flavonoids are powerful antithrombotic agents in vitro and in vivo because of their inhibition of the activity of cyclooxygenase and lipoxygenase pathways. which platelet aggregation occurs. These include inhibition of the transduction pathway mediated by phospholipase C (PLC), phospholipase A2 (PLA 2) and thromboxane A2 (TXA2), inhibition of cytoplasmic calcium increase, inhibition of protein kinase cascades and effect on calcium channels. With regards to that, polyphenols exert anti-thrombotic effect by inhibiting platelet aggregation and interaction between platelet and leukocyte. The risk of thrombosis can be reduced through nutritional supplements that have cholesterol-lowering, anti-inflammatory, antioxidants and blood thinning effects. From here, we can see that kenaf seed oil is a potential dietary component to prevent thrombosis and effective as a treatment of cardiovascular diseases since it has the mentioned functions.

12. Anti-mutagenic property

Mutation is regarded as a permanent change in DNA sequence and this process can be spontaneous or induced (Hawley & Richards, 2011) [27]. Spontaneous mutations are due to normal metabolism of DNA whereas induced-mutations are caused by mutagens such as chemicals, ultraviolet light and ionizing radiation . explored the antimutagenic, antioxidant and total phenolics of clove (Syzgiumaromaticum L.) seed extracts and reported that there was a strong correlation between total phenolics and the biological activities Table 6 IC50 values of the kenaf seed oil from variety V36 towards various cell lines. Cell line IC50 (mg/ml) 5000 MDA-MB-231 (breast cancer) 483.35 ± 31.97 >5000 >5000 4T1 (breast cancer) >5000 >5000 >5000 HeLa (cervical cancer) >5000 >5000 >5000 A549 (lung cancer) >5000 >5000 >5000 MOLT-4 (leukemic) 153.26 ± 25.43 1657.42 ± 72.83 >5000 HL-60 (leukemia) 178.78 ± 10.52 320.48 ± 11.35 >800 WEHI-3B (leukemia) 189.43 ± 11.63 380.32 ± 15.21 >800 K562 (leukemia) 213.33 ± 15.45 472.34 ± 13.12 >800 CaOV3 211.67 ± 3.79 187.00 ± 5.20 188.33 ± 10.12 Adapted . Mentioned. It is observed that antimutagenic activity of cloves seed is directly related to the availability and concentration of phenolic antioxidants. This implies the additional role of phenolic compounds in kenaf seed oil as the antimutagenic agent. Pharmacological investigations have shown that genus Hibiscus possesses many biological activities. Research showed that Hibiscus rosasinensis possesses antitumor, antidiabetic, anticonvulsant and antidiarrheic activity whereas Hibiscus syriacus has antipyretic activity and

used as anthelmintic. On the other hand, methanolic extract of Hibiscus tiliaceus flowers displayed antioxidant and antimutagenic activities. It is reported that tannic acid is the major compound in kenaf seed extract and this compound possesses antioxidant, antimutagenic and anticarcinogenic properties. Since tannins are also present in the oil, this supports that kenaf seed oil has the ability to prevent cell mutation. Tannic acid is found to suppress mutagenesis in Escherichia coli and enhance excision-repair of DNA system in E. coli. Another compound in kenaf seed oil, saponins (steroid or triterpene glycosides) have also been shown that they have significant antimutagenic . Furthermore, antioxidants appear to be antimutagenic and anticarcinogenic too. For instance, vitamin E, a well-studied molecule that acts as lipophilic antioxidant can reduce free-radical-induced DNA damage. As cancer is always with mutation, compounds with antimutagenic capacity could well be linked to anticarcinogenic activity .

Conclusion

kenaf seed oil has emerged as a new source of oil with functional properties to be used in various areas such as food, industrial and medicinal fields. The utilization of kenaf seed, an agricultural waste product to produce kenaf seed oil no doubt leads to better waste management and ensure sustainable production. It is proven that kenaf seed oil, which is rich in unsaturated fatty acid and phytochemicals, provides myriad essential health benefits. More research has yet to be carried out on kenaf seed oil to discover the method for mass production at lower cost and increase the oil yield. Stability of kenaf seed oil is a main concern that needs to be addressed since it is high in unsaturated fats and shorten the shelf life. Comprehensive study can be carried out on kenaf seed oil to explore the industrial potential and discover more applications.

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