Bioactive compounds in foxtail millet (Setaria italica) - extraction, biochemical activity, and health functional: A Review

Donald John Calvien Hutabarat¹* & Valerie Aditya Bowie¹

¹Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia, 11480

*Email: donald.calvien@binus.ac.id

Abstract. Foxtail millet (Setaria italica) is one of the important millets in south-east Asia yet it is less exploited. Foxtail millet contains bioactive compounds as phenolics, bioactive peptides, carotenoids, and tocols that has health physiological function. This review presents information on the extraction technique, biochemical activities, health-functional properties of foxtail millet. The extraction efficiency of bioactive compounds is influenced by the method and solvent. Fermentation of foxtail millet which produces bioactive peptides can also increase the antioxidant content. Phenolics in foxtail millet are most bioavailable in gastrointestinal digestion and protein hydrolysate had the highest antioxidant activity in hydrophobic form. Bioactive compounds were also displayed many health benefits and biological activities, including anti-proliferative, hyperglycaemia, and hypertensive prevention.

1. Introduction

Foxtail millet (Setaria italica), which belongs to the Poaceae family along with corn and sorghum, is a round-shaped cereal available in variety of colors and sizes. It can easily grow in any soil condition, even in tropical or subtropical regions of the world such as Africa or Asia [1]. Foxtail millet also grown in Europe, China, India and Indonesia, and the Korean peninsula. The production of all types of millet in 2012 until 2018 ranged from 26.6 to 31-million-tonnes worldwide, where India being the top producer of foxtail millet [2]. Foxtail millet is considered one of the major millets, yet it receives less attention compared to other cereals [1].

In Indonesia, foxtail millet is known by various different local names such as ba’tan (Toraja), jawa (Palembang), jaba ikur (Batak), jaba ure (Toba), jelui (Riau), sekui (Melayu), sakuih (Minangkabau), randau (Lampung), tarreang or bailo (Majene and Polewali Mandar), and jawae (Dayak). The processing of foxtail millet in West Sulawesi is still limited such as porridge, dodol, cookies, wajik, noodle, or rice substitute [3].

Nutrients that are present in foxtail millet can give a physiological function that can prevent the occurrence of non-communicable diseases. The type of grain like foxtail millet contains a small amount of gluten so it can be consumed by coeliac disease [4]. Maintaining these nutritional qualities is important for the development and processing of healthy foxtail millet-based products.

Foxtail millet are excellent sources of biologically active compounds such as dietary fibers, bioactive peptides, proteins, minerals, amino acids, phenolic compounds, sterols, tocols, phytic acids, carotenoids, unsaturated fatty acids, and several anti-nutritive compounds [5,6]. Bioactive compounds
of foxtail millet are becoming important functional food ingredients. The phenolics in proso millet exhibit antidiabetic, antioxidant, anti-inflammatory, and antimicrobial potential [7]. Phenolic acid in foxtail millet also has an antiproliferative effect against MDA human breast cancer and HepG2 human liver cancer cells [8].

Even though the number of bioactive compounds is actually small, their biological activities give a positive impact on human health. Some studies found that bioactive compounds available in foxtail millet have fairly high antioxidant capacity extracted from different parts of its grain. Various techniques have been implicated to obtain and identify the phytochemicals in foxtail millet, such as extraction using solvents (with few common solvents is as follows: hexane< chloroform< ethyl acetate< acetone< methanol< water), isolation and purification by spectroscopy (i.e mass spectroscopy, UV-visible, infrared, nuclear magnetic resonance) and chromatography (i.e gas chromatography, high-performance liquid chromatography) [9].

The bioactive compounds must be released from the matrix and modified in the gastrointestinal (GI) tract. Therefore, it is also important to analyze whether digestion affects the stability of the bioactive compounds, then their bioavailability and bioaccessibility will affect the possible health benefits [10]. The increase of interest in using foxtail millet is very small compared to other cereals. Understanding the potential utilization of foxtail millet in Indonesia contributes to the development of millet into a functional food alternative. This review aims to give information on the extraction process of the bioactive compounds in foxtail millet and the impact on health diseases.

2. Bioactive Compounds
Extraction process is the crucial first step in analyzing potential functional food. The extraction method will affect the biological activity of plant extracts, so it is important to choose the optimal extraction method. Foxtail millet might also associate with other components such as sugar, organic acids, and fat, so these undesirable substances must be pre-treated (e.g. hexanes to remove crude fat) and may proceed by air-drying or lyophilizing (freeze-dried). Generally, samples that are treated with freeze-drying have a high level of phytochemicals than air-drying [11]. Several methods are used for the extraction of bioactive compounds (i.e atmospheric liquid extraction with maceration, soxhlet extraction, supercritical and subcritical fluid extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), pulsed electric field (PEF) assisted extraction, enzyme-assisted extraction (EAE) and green extraction) [12].

The concentration of bioactive compounds usually still contains a large amount of carbohydrate or lipid material. Before analysis of the bioactive compounds, the purification and fractionation are firstly strategized to obtain concentrated and bioactive compound-rich fractions, such as sequential extraction, liquid-liquid partitioning or solid-phase extraction (SPE) [11]. Hexane, dichloromethane or chloroform are used to eliminate lipid, based on polarity. A compound such as sugar and organic acids are able to be removed by polar solvents.

2.1. Phenolic extraction
Phenolics have an aromatic ring with a hydroxyl group that makes them polar. The most common procedure to extract phenolic is solvent extraction. Samples may contain phenolics in different amounts from simple to highly polymerized substances. Additional steps are also required if the phenolic compounds are associated with other components such as carbohydrates and proteins. Selecting the particular solvent may affect the quantity and level of polyphenols extracted. Methanol, ethanol, acetone, ethyl acetate, and mixtures thereof were used for phenol extraction with water in varying proportions. Because methanol is relatively polar compared to other solvents (petroleum ether, benzene, chloroform), it was the optimal solvent for extraction of phenolic compounds and flavonoids from whole wheat and bran-rich millet fractions [13].

Acidified methanol (methanol and 1% HCl in methanol) also has the ability to enhance the extraction of phenolic. In antioxidant studies, acidified solvents have been shown to be able to extract
phenolic components (methanol and 1% HCl), and alkaline treatment (CaO) in a shaker for 1h 28°C would reduce the amount of phenolic damaged by leaching out during cooking [14].

Extraction of dissolved phenolic from foxtail using acidified methanol (1% HCl) extraction (1:50, w/v) combined with heat treatment (boiling for 30 minutes) of defatted raw, germinated, steamed, and microwaved foxtail millet flours, showing phenolic content which was high in germinated foxtail millet (Table 1) [15]. Biosynthesis of phenolic compound increased due to germination. The acidified phenolic extraction (80% methanol, 1% HCl v/v) were combined with heat treatment (50ºC, 1 h) of the bran, dehulled, hull, pearled, and whole of foxtail millet, showing the highest total phenolic contents were in the hull of foxtail millet [16].

| Foxtail millet | Extraction method | Total phenolic content (mg of FAE/100g) | Reference |
|----------------|-------------------|-----------------------------------------|-----------|
| Raw            | Methanol (1% HCl) 1:50, boiling for 30 minutes | 72.70 ± 2.29 | [15]       |
| Germinated     |                   | 170.14 ± 2.10                           |           |
| Steamed        |                   | 76.22 ± 1.53                            |           |
| Microwave      |                   | 104.46 ± 0.96                           |           |
| Whole          | 80% methanol, 1% HCl v/v, 50ºC, 1 h | 6.02 ± 0.37 | [16]       |
| Dehulled       |                   | 1.89 ± 0.04                             |           |
| Pearled        |                   | 0.79 ± 0.01                             |           |
| Hull           |                   | 17.99 ± 0.42                            |           |
| Bran           |                   | 10.44 ± 0.27                            |           |

The result was also aligning with the study on finger millets using acidified methanol, which indicates extraction using pure solvents at room temperature was not as effective as another method of solvent extractors, but solvent acidification improves extraction, furthermore, refluxing the sample with 1% HCl-methanol seems to be very effective for extraction. Increasing the temperature of the extract softens the tissue and weakens the phenol-protein and phenol-polysaccharide bonds, allowing polyphenols to migrate into the solvent [17]. Ethanol is also often used in the extraction of free phenolic compounds to initiate the extraction of bound phenolic compounds by the alkaline hydrolysis method. Extraction of phenolic and flavonoid (free and bound) of un-germinated and germinated foxtail millet using 80% chilled ethanol showed high phenolics and flavonoids total in germinated foxtail millet [18].

Several techniques had been evolved which can be implemented within the extraction of phenolic in plants, one in every of that is ultrasound-assisted extraction (UAE). The UAE does not require complicated instruments and relatively low costs. The mechanism for UAE involves shearing forces with cavitation bubble impulses at mechanical wave propagation (kHz) which can disrupt the membrane to release extractable bioactive compounds and increase solvent penetration into cells and enhance mass transfer. According to Table 2, UAE was able to offer an effective alternative for phenolic extractions. This method indicated that ultrasound enhanced the efficiency of the extraction rapidly and lowering the volumes of organic solvents needed. However, this method did not increase the yield comparing with the conventional solvent extraction. Also in this method, acetone-water is more commonly used than ethanol in extracting polymeric phenols. It was proven that acetone was able to extract phenolic compounds due to its semi-polarity.

Phenolic profiles that were dominantly present in foxtail millet samples were the free form and insoluble bound [19]. Extractions of soluble and insoluble phenolic compounds generally use the alkaline hydrolysis method. The phenolics identified in foxtail millet are hydroxybenzoic acid and hydroxycinnamic acid. The phenolic content of raw, processed (soluble and bound) foxtail millet is present in Table 3, the extraction used acidified methanol. The results also showed that benzoic acid derivatives and cinnamic acid derivatives were found in raw and processed grains of foxtail millet.
Treated foxtail millet (germinated and steamed) can increase benzoic and cinnamic content, foxtail millet (hull) has high total cinnamic both bound and free [15]. Ferulic acid (hydroxycinnamic) and vanillic acid (hydroxybenzoic) were found to be the major phenolic acids [15], whereas caffeic, gallic, and p-hydroxybenzoic acid were present in the lowest amount [16]. Caffeic, ferulic, and sinapic acids were the major phenolic acids in the soluble form, while p-coumaric acids and ferulic were present in higher levels in bound form [16].

Table 2 Phenolic content indexes from foxtail millet samples by ultrasound-assisted extraction

| Ultrasound-assisted extraction | Phenolic content | Reference |
|-------------------------------|------------------|-----------|
| 70% (v/v) acetone, ultrasonic bath (25 min, refluxed 60°C) | 10.8 ± 0.82 (whole grain), 3.80 ± 0.22 (dehulled), 3.67 ± 0.04 (cooked), 22.8 ± 0.88 (hull) µmol FAE/g defatted grain | [20] |
| 70% (v/v) acetone, ultrasonic bath (25 min, refluxed 60°C) | Approximately 200 µmol FAE/g defatted crude extract | [21] |
| 70% ethanol, sonicated at 55°C for 2 h | TPC ranged from 18.0 ± 2.3 to 26.5 ± 0.8 GAE mg/g extract of 8 cultivars of foxtail millet | [22] |
| 70% (v/v) acetone, ultrasonic bath (25 min, in refluxed condition) | TPC: 4.49 ± 0.2 µmol FAE/g defatted grain | [19] |
| 70% (v/v) acetone, ultrasonic bath (25 min, in refluxed condition) | TPC: 215.9 ± 16.7 µmol FAE/g, TFC: 25.15 ± 0.68 µmol cathecin equivalent/g | [23] |
| 65% ethanol, sonicated for 50 min at 70°C | TPC (mg GAE/100g): 33.17 ± 0.15 (native) and 57.17 ± 0.78 (germinated) TFC (mg Rutin Equivalent/100g): 28.10 ± 0.19 (native) and 57.72 ± 2.22 (germinated) | [24] |

Table 3 Soluble and bound phenolic acids in foxtail millet phenolic extract

| Sample | Total Benzoics µg/g defatted meal | Total Cinnamics µg/g defatted meal | References |
|--------|-----------------------------------|------------------------------------|------------|
| Raw    | 59.25 ± 6.65                      | 326.37 ± 4.36                      | [15]       |
| Germinated | 114.77 ± 2.40                  | 425.72 ± 4.81                      |            |
| Steamed | 61.00 ± 5.70                      | 185.85 ± 3.45                      |            |
| Microwave | 55.53±3.88                     | 174.71 ± 3.42                      |            |
| Soluble |                                   |                                    |            |
| Whole | 10.35 ± 0.86                      | 287.55 ± 6.24                      | [16]       |
| Dehulled | 4.47 ± 0.09                       | 118.83 ± 2.71                      |            |
| Pearled | 2.98 ± 0.09                       | 40.91 ± 1.17                       |            |
| Hull    | 33.39 ± 1.28                      | 653.48 ± 12.63                     |            |
| Bran    | 44.38 ± 1.19                      | 574.78 ± 8.46                      |            |
| Bound   |                                   |                                    |            |
| Whole | 33.84 ± 1.53                      | 289.71 ± 7.71                      | [16]       |
| Dehulled | 2.96 ± 0.05                      | 23.87 ± 0.68                       |            |
| Pearled | 3.16 ± 0.07                       | 13.12 ± 0.31                       |            |
| Hull    | 59.51 ± 1.17                      | 8667.49 ± 148.00                   |            |
| Bran    | 65.43 ± 1.36                      | 1885.25 ± 45.45                    |            |
2.2. Bioactive peptide extraction
Bioactive peptides have a lower molecular weight than protein. Grains such as wheat, barley, rice, rye, oat, millet, sorghum, and corn are sources of bioactive peptides [25]. Millet contains four main types of protein, such as albumin, globulin, prolamin, and glutelin. Prolamins are one of the primary fractions of protein in millet with over of 50% constituents of total protein [26].

Bioactive peptides can be produced by enzymatic hydrolysis or microbial fermentation [27,28,29]. Proteins can be hydrolyzed using proteolytic enzymes to produce hydrolysates containing peptide sequences. Foxtail millet extract fermentation by Lactobacillus paracasei Fa032 produced hydrolyzed protein with hydrophobic amino acids (41.47-51.39%), hydrophobic index (8.10-8.47 Kj/mol amino acid residues), and molecular weight (180-5000 Da) [28]. RP-HPLC was used to amino acid analysis and SE-HPLC was used to molecular weight distribution.

Analysis of fractionated defatted foxtail millet hydrolysate, prepared with alcalase from Bacillus subtilis (pH 8.0 and temperature 50°C for 3 h) resulted in 4 protein hydrolysate fractions using gel filtration [29]. The fourth fraction (FIV) has the highest hydrophobic amino acid (51.93%). All fractions were found to be rich in alanine, arginine, aspartic acid, glutamic acid, leucine, phenylalanine, proline, and serine. HPLC system using gel permeation chromatography was used to determine the molecular size of the fractions, the results revealed that the fourth fraction (FIV) had the lowest molecular weight with peaks located at 99-420 Da (73.82%), 420-1040 Da (18.25%), and 1040-1040 Da (6.94%) associated with higher antioxidant activity [29].

Recently, there are many technological developments for protein modifications such as thermal treatment, microwave-assisted method, pulsed electric field and ultrasonic waves. Combining ultrasonic and heat treatment with enzyme hydrolysis was effective to obtain two antioxidant novel peptides from foxtail millet prolamins [26]. The extraction started with a dispersion of foxtail millet flour in 80% ethanol-water (w/v). The foxtail millet prolamin was precipitated gradually at 0-4°C for 24 h. NaCl was removed by dialysis, and the extracted protein was lyophilized and stored at -20°C. After ethanol extraction, the foxtail millet prolamins were barely soluble in water. Ultrasound (frequency: 20 kHz, 400 W, 15-30 min) and heat treatment (15-30 min at 90°C) were used to reconstitute the protein to promote protease hydrolysis in water. Pre-treated millet prolamin was then subjected to enzymatic hydrolysis by alcalase (pH 9, 55°C). Alcalase was usually preferred over another enzyme because it can hydrolyze almost any peptide bond within a protein molecule. After enzymatic hydrolysis, the hydrolysate was fractionated using ultrafiltration membranes. Purifications of the alcalase-hydrolyzed fractions were using RP-HPLC and gel filtration chromatography were successfully isolated and identified new bioactive peptides, PFLF (MW 522.3 Da, Proline-Phenilalanin-Leusin-Phenilalanin) and IALLPF (MW 785.5 Da, Isoleusin-Alanine-Leusin-Leusin-Isoleusin-Proline-Phenilalanin) [26].

2.3. Carotenoid extraction
Two classes of carotenoid found in nature: β-carotene that consist of linear hydrocarbons that can be cyclized at one end or both ends of the molecules, and xanthophylls, the oxygenated derivatives of carotenones such as lutein, violaxanthin, neoxanthin and zeaxanthin. The most efficient carotene extraction is determined by the right selection of solvent. HPLC and LC-MS were used to identify carotenoids and the effect of the cooking method on foxtail millet, by extracting the carotenoids with several different solvents (80% methanol, 80% ethanol, methyl tert-butyl ether, and water-saturated 1-butanol) [30]. As a result, it was shown that saturated 1-butanol was the most efficient solvent for millet carotenoid extraction and was used in the following analysis (water-saturated 1-butanol> methyl tert-butyl ether> 80% ethanol> 80% methanol). n-butanol can maximize the extraction of carotenoid derivatives of foxtail millet, identified the major carotenoids are zeaxanthin and lutein [31].

Foxtail millet was mixed with MgCO3 and extracted with methanol/tetrahydrofuran (1:1 v/v) at 75°C 5 min, showed that xanthophyll and zeaxanthin (primary carotenoid) were detected in HPLC [8]. Tetrahydrofuran (THF) is frequently used with methanol as the first extraction because of its high solubility of carotenoid. However, the use of tetrahydrofuran must be considered because of its ability
to form peroxide which can degrade carotenoid and form the second product. Peroxide formation can be prevented by treating THF with sodium metal before extraction. Most methods that are used to remove peroxides are highly reactive agents, time-consuming, and call for extreme caution [32].

2.4. Tocols extraction
Tocols describing the bioactive compound both tocopherol and tocotrienol, found in the unesterified form. Tocols concentrate isolate depends on the sources and extraction techniques applied. The extraction process includes esterification, saponification, liquid-liquid extraction, crystallization, distillation, ion exchange, and adsorption chromatography. However, solvent extraction combined with chromatography (HPLC) is the method often used in foxtail millet products [33].

Common vitamin E extraction in foxtail millet (de-husk and de-bran) was carried out by methanol mixing for one hour. The extracts are then centrifuged to obtain the supernatant and stored until subjected to RP-HPLC for vitamin quantification. Vitamin E in foxtail millet is substantially better than in rice and wheat [34].

Bran oil in foxtail millet was the source of vitamin E using several solvents extraction (SE). Extraction of semi-non-polar compounds in foxtail millet can use supercritical carbon dioxide (SCE); subcritical propane extraction (SPE) can be as effective as other solvent extractors including SCE. These three extraction methods were compared on the oil yield (%), tocopherol, and fatty acid from foxtail millet bran oil [35]. SPE is the most effective extraction method among other methods and is a better solvent for triacylglycerol than SCE. Fatty acids in foxtail millet were detected, such as oleic, linoleic, and linolenic acids (major unsaturated fatty acids), while stearic and palmitic acids (major saturated fatty acids), with composition linoleic > oleic > palmitic > stearic > linolenic. The fatty acids in foxtail millet can be utilized as a functional food [36].

3. Antioxidant potential in foxtail millet
The bioactive compounds in foxtail millet, such as phenolic acids, peptides, carotenoids, and tocols known to have antioxidant properties. Fermentation and germination processes can increase the antioxidant content [15,18,29].

3.1. Phenolic acids
Flavonoids and phenolic acids are found in foxtail millet, the quantities of flavonoids quite small [37]. Foxtail millet mostly contains phenolic acids in bound form, while others are in free form. Ferulic, chlorogenic acids, and p-coumaric were more abundant in bound form [8,10]. The cinnamic acid derivative ferulic acid is a secondary plant metabolite [38]. The antioxidant action mechanism of ferulic acid is inhibition of ROS (reactive oxygen species) or nitrogen and free radical scavenging [39].

By in vivo shown that phenolics from various extracts are bioavailable Before bioavailability, bioactive compounds must be discharged from matrix and altered in the gastrointestinal tract (in vitro). In vitro study assay of foxtail millet (cooked dehulled) used to release phenolic compound and allows to evaluate the stability of the phenolic compound in digestion system and microbial colonic fermentation [40].

Foxtail millet phenolics were mostly available (30.08±0.98 μmol FAE/g), had high antioxidant (155±19.4 μmol TE/g; 11.8±0.88 μmol AAE/g; 591±2.28 μmol EDTA/g), and had high radical scavenging (1.94±0.46 μmol TE/g; 33.2±4.42 μmol FAE/g; 1873±23 μmol FAE/g) at GI digested phase, after gastric digestion [40]. This indicates that gastrointestinal pH can increase antioxidant activity and total phenolic of foxtail millet extract. During the gastric phase, protein-bound phenolics were released. The released phenolics may be absorbed, have some antioxidant effects, and increase their solubility in the digestive system [40]. Fermentation of bran in the colon also released phenolic, intestinal extract analysis by HPLC, phenolic bound to insoluble fiber were released [41]. Mainly product colonic fermentation of foxtail millet (cooked) was acetic acid followed by propionic acid and butyric acid [40].
3.2. Protein hydrolysate

The previous discussion [29] on defatted foxtail millet protein can be one of the formulations of functional food and nutraceuticals to prevent oxidative stress-related damage in human diseases. Natural antioxidants are alluring since they can be utilized at any concentration without any side harmfull impact [42]. Four fractions of the foxtail millet protein hydrolysate were conducted to several antioxidant assessments, such as inhibition of linoleic acid autoxidation, ABTS (2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) radical scavenging activity, DPPH radical scavenging activity, metal-chelating activity, and reducing power.

Fraction (FIV) exhibits the highest ABTS radical scavenging activity (at 6.67 μg/mL), metal chelating activity (5 mg/mL), and DPPH radical scavenging activity (at 1.0 mg/mL). The highest free radical scavenging and antioxidant activity can be caused by the fraction's high total amino acid content. The fourth fraction (FIV) shows a significant content of hydrophobic amino acids (amino acid residue 6.62 kJ/mol), so the hydrophobicity of the fraction increases fat solubility and improves antioxidant activity [43]. The high proportion of hydrophobic amino acids could freely penetrate into target organs due to their hydrophobic interactions with the lipid bilayer of the membrane due to their hydrophobicity, which may indicate a significant ability to trap radicals [44].

Peroxidation of fatty acids in food can cause a harmful effect if consumed including biotoxicity [44]. All fractions were compared with α-tocopherol and BHT to characterize their capacity against oxidation of linoleic acid. Fraction (FIV) had the highest antioxidant activity (85.71%) close to α-tocopherol (86.27%) but lower than BHT (92.44%). The high activity about the inhibition of linoleic acid lipid peroxidation is because of the small size of the peptide (< 1 kDa). The heavier weight of the peptide sequence will have the effect of diluting the radical scavenging peptide. [46].

3.3. Carotenoids and tocols

Bioaccessibility and antioxidant activity of carotenoid and tocols in foxtail millet are needed to be studied for further research to provide more comprehensive information on bioactive compounds. Carotenoids are valuable as antioxidants, possess beneficial functions in human health like prevention of atherosclerosis, support of immune system, and cells in the eye. Carotenoids act as antioxidants, absorbing the energy of singlet oxygen, converting it into triplet oxygen without changing its chemical structure, and preventing oil oxidation by light [47]. The levels of carotenoids in foxtail millet are generally zeaxanthin and lutein, but most contents found were lutein [31].

Vitamin E or tocols functions as a natural antioxidant, protect fat in membranes around cells from mutagens, and prevent from carcinogenesis, cardiovascular disease, and aging. Tocols act as an antioxidant by inhibiting lipid peroxidation in biological membranes. The highest yield of tocols were found in the bran of foxtail millet, which mostly contains α- and β-tocopherol [48].

4. Health functional

4.1. Anti-inflammatory

The ethanolic extract of foxtail millet has an anti-inflammatory effect in the Swiss albino mice experiment, given carrageenan to induce local inflammation that causes edema of the hind legs [49]. The extract of foxtail millet at 400mg/kg body weight (C4SI) indicated a significant anti-inflammatory effect with 20.16, 65.44, 65.5, and 71.15% inhibition of paw edema after 1st, 2nd, 3rd, 4th hours of carrageenan injection. Every increase of time, the effect in anti-inflammatory also increased with the peak at 4th hours this also compared to anti-inflammatory drug (Diclofenac).

Foxtail millet prolamins peptides (MPP), MPH-A-I (MW<1kDa, hydrolysate by alcalase), PPLF (Pro-Phe-Leu-Phe), and IALLIPF (Ile-Ala-Leu-Leu-Ile-Pro-Phe), have a significant anti-inflammatory effect on RAW267.4 murine macrophage (stimulation of inflammation using lipopolysaccharide), by suppressing the production of nitric oxide (NO) and pro-inflammatory cytokines, with IL-1β, IL-6, and TNF-α [50]. Inhibitory effect of IALLIPF had a better on NO as its concentration decrease from
15.1μM to 9μM when the corresponding peptide ranging from 100 to 250 μg/mL as well as its effect on other pro-inflammatory mediators.

The mechanism is comparable to the activity of foxtail millet bran-derived bound polyphenol (BPIS) in LPS (lipopolysaccharide)-induced HT-29 cells and mice. BPIS suppress levels of IL-1β, IL-6, IL-8 (pro-inflammatory cytokines), and increase IL-10 (anti-inflammatory cytokine) by blocking NF-κB p65 translocation [51].

Macrophages RAW264.7 have a unique way of responding to LPS in the cell wall of Escherichia coli, and inflammatory cell patterns can be recognized by LPS stimulation. Stimulated by LPS, RAW 267.4 produces NO and other pro-inflammatory cytokines that act as signaling molecules that allow tissues and cells to engage the immune system to initiate inflammation and robustly respond to invading viruses and bacteria [50].

4.2. Anti-proliferation
Carcinogens (cancer) such as aristolochic acids (AA), heterocyclic aromatic amines (HAAs), ionizing radiations (IR), nitrosamines, mycotoxins, polycyclic aromatic hydrocarbons (PAH), and ultraviolet (UV), can also cause changes in DNA sequence [52]. Foxtail millet showed remarkable ability to scavenge peroxide radicals and antioxidant activity, possibly due to its high content of carotenoids and phenolic acids. Furthermore, foxtail millet extract inhibits the growth of HepG2 liver cancer and MDA breast [8].

Foxtail millet phenolic compounds were tested on mutant epithelial cells (HT-29 cells tested from range 10% to 39% activity) of colorectal adenocarcinoma, they have an antiproliferative effect in terms of DNA stratification inhibitory activity, which is important for the control of early and advanced stage carcinogenesis [23]. BPIS has anti-proliferative activity in HT-29 cells and its nude mice model. The mechanism relies on the reversal of glycolysis (aerobic), and this effect is achieved by increasing miR149 expression [53]. miR149 plays an important role in cell metastasis, apoptosis, proliferation, chemical resistance and tumorigenesis in human cancers [54]. A novel 35 kDa foxtail millet bran protein (FMBP) significantly inhibited colon cancer grown in a time and dose-dependent manner. FMBP exhibited cytostatic and cytotoxic effects in colon cancer cells without disrupting the normal colon epithelial cells. FMBP can inhibit colon cancer cell by inducing arrest of the G1 phase (causing mitochondrial transmembrane potential loss) that induces caspase-dependent apoptosis [55].

4.3. Hyperglycaemia prevention
The major enzymes involved in the breakdown of starch and uptake of glucose in the intestine are intestinal α-glucosidase and pancreatic α-amylase [56]. They hydrolyze polysaccharides into glucose, then transport them into the bloodstream, which spikes the postprandial glucose in the blood. Phenolic is known to be effective in preventing post-prandial hyperglycemia by blocking access to the active site of the enzyme [57].

Dietary fiber from foxtail millet is expected to reduce the level of glucose absorption. First, fiber increases the viscosity of the small intestine and prevents the diffusion of glucose. Second, fiber binds the glucose and decreases its concentration in the small intestines, and retard the starch digestive enzyme [58]. Raw and germinated foxtail millet with high phenolic content can inhibit activity of α-amylase and intestinal α-glucosidase [15].

4.4. Hypertensive prevention
Protein hydrolysates of foxtail millet can prevent the activities of angiotensin-converting enzyme (ACE) which leads to reducing angiotensin II and has a direct effect in lowering blood pressure amongst hypertensive rats (SRHs) [59]. Hypertensive prevention treatment with captopril and hydrolysate had no significant difference. It is also confirmed that hydrolysates of foxtail millet protein can reduce cardiac damage and left ventricular hypertrophy. Consumption of foxtail millet does not show any adverse effect on the liver as well as kidney functions as indicated by no change in percentage weight of liver and kidney [59]. The anti-hypertensive effect of the foxtail millet diet in
healthy men and women (40–65 years) with mild hypertension (130 mmHg ≤ SBP ≤ 139 mmHg, 80 mmHg ≤ DBP ≤ 89 mmHg), showed a significant reduction SBP (4.13 mmHg) and DBP (3.49 mmHg) after 12 weeks. Fat mass, body fat percentage, and body mass index decreased and increased fasting blood glucose of the subjects [60].

5. Conclusion
Extraction techniques of the bioactive compounds are a crucial process in analyzing the potential health functional of foxtail millet. The efficient extraction techniques for each of bioactive compounds is ultrasound-assisted extraction for phenolic compounds, enzymatic hydrolysis combines with ultrasonic heat treatment for bioactive peptide and protein hydrolysate, methanol solvent extraction for tocots, and subcritical propane extraction for carotenoids, and subcritical propane extraction for tocots.

Each of the bioactive compounds has several physiological effects on health. It was proven in vitro that phenolics were mostly available and has the highest antioxidant activity in gastrointestinal digestion after consumption. While hydrophobic protein hydrolysates were proven to have the highest antioxidant activity against free radicals. Biological activity in foxtail millet occurs as: anti-inflammation effect against inflammatory and liver cancer inflammation effect against inflammatory antioxidant activity against free radicals. Biological activity in foxtail millet occurs as: anti-inflammation effect against inflammatory-related illness; anti-proliferative effect against colon, breast, and liver cancer; inhibition of α-glucosidase and α-amylose in increasing blood sugar, and; inhibition of angiotensin-converting enzyme to lower blood pressure.

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