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REVIEW

The bioactivities of legumes: a review

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Abstract

Legumes are dehiscent plants which belong to the family of Fabaceae and are rich in nutrients. Although legumes are known for their beneficial health effects, the consumption of legumes is still low due to gastrointestinal problems after consumption as well as long cooking time. Hence, this review aimed to discuss the bioactivities of legumes exerted in regard to the different extraction methods namely solvent (SE), microwave-assisted (MAE), ultrasound-assisted (UAE), supercritical fluid (SFE) and pressurized liquid extraction (PLE). Generally, the MAE legume extract could potentially exert the highest antioxidant activity than UAE and SE. Meanwhile, UAE legume extract displayed higher strength in anti-diabetic activity as compared to MAE while SFE legume extract showed higher anti-diabetic activity than PLE. The effectiveness of antimicrobial and anti-inflammatory activity of legumes by different extraction techniques can be predicted by the amount of total phenolic compound. The higher total phenolic compound of legumes contributed to the strength of the bioactivity of legumes. The legume extract can also be applied to additives, nutraceutical products and skin care products. This review aimed to provide a better understanding of the suitable extraction techniques that potentially exert the highest bioactivity in legumes and hence increase the consumption of legumes as well as reduce prejudice towards eating legumes.

1. Introduction

Legumes belong to the family of Fabaceae or Leguminosae and consist of three subfamilies namely Faboideae, Mimosoideae and Caesalphinioidae. It is a dehiscent plant that is ranked as the third largest family of angiosperms composed of more than 20000 species and 750 genera (Cakir *et al*., 2019). It is rich in in protein with essential complex carbohydrates, amino acids, unsaturated fats, dietary fibre, vitamins and essential minerals, but the consumption of legumes worldwide is still considerably low which is below 3.5 kg per capita per year (Gilham *et al*., 2018). The main reason for this barrier could be due to gastrointestinal problems such as excessive intestinal gas or flatulence faced by some people when consuming legumes and long cooking time due to its impermeable hard shell and scelerma properties.

Several findings have reported that legumes are excellent sources of antinutrient compounds known as bioactive compounds which are considered undesirable forms as they can lead to undesirable physiological effects. usually described as toxins or food toxicants that are harmful to health (Muzquiz *et al*., 2012). In contrast, it can exhibit different bioactivities including anti-carcinogenic, anti-allergenic, anti-artherogenic, antioxidant, anti-inflammatory, antimicrobial and analgesic activities that are beneficial to our health (Singh *et al*., 2017). To achieve these bioactivities, extraction of the bioactive compounds is very crucial as it may affect the yield of the compounds desired (Che Sulaiman *et al*., 2017). Years ago, scientists used conventional methods or traditional methods which used solvents to extract these compounds, but in recent years, newer non-conventional methods have been derived. The non-conventional extraction methods such as MAE, UAE and SFE offered various advantages over conventional extraction methods like SE such as requiring a lesser volume of solvents, higher extraction yield, shorter extraction duration and lesser impact on the environment. However, research documented on the bioactivity of legumes using non-conventional extraction methods is still scarce. Hence, the main objective of the review is to discuss and compare the bioactivities of legumes extracted by SE, MAE, UAE, SFE and PLE. Consequently, choosing the best extraction method in

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extracting the bioactive compounds. Meanwhile, this review can provide a better understanding better extraction method that exhibit the highest bioactivity and hence reduce the prejudice of society toward eating legumes as well as promoting the intake of legumes in daily life.

2. Legumes

2.1 Classification and species of legumes

According to McCrory *et al*. (2010), legumes are divided into four major groups which include vegetable crops, sow crops, pulses and oil seeds. Vegetable crops are legumes that are eaten as vegetables while sow crops are legumes that are purposely for sowing. The pulses are legumes that are harvested for dry grain and oil seeds are used for oil extraction. The Food and Agriculture Organization of the United Nations have been classifying legumes into eleven classes, including, dry bean, dry board bean, dry pea, chickpea, dry cow pea, lentil, Bambara bean, vetches, lupin and minor pulses (Maphosa and Jideani, 2017).

2.2 Structure and morphology of legumes

The legume fruit normally will develop into a pod that encloses the legume seeds. The legume pod is a one-celled seed container made up of two sealed parts known as valves. Legume pods are connected by two valves and will break along the seam. This feature is called dehiscent which means to gape or burst open in Latin word. Nevertheless, each species of legume has the shape of a pod. Not all legume pods have a smooth surface and dehiscent characteristic, some pods may wing or indehiscent. The length, thickness, curve and fleshy nature of the pods can differ between the species as summarized in Figure 1 (Allaire and Brady, 2008). Generally, legume seeds are composed of seed coats (testa), cotyledons and embryos. All legume seeds have similar structures, but different in shape, colour, size and thickness of the seed coat. The colour of the seed coat can be brown, black, purple, reddish, green, yellow and mottled (Amit *et al*., 2020; Vohra *et al*., 2019).

Additionally, the legume leaf contains the petiole or so-called stalk attached to the node and paired, leaflike appendages called stipules fused at the base of the petiole. The shapes of stipules vary such as pointed tips and long slender points as well and margins vary from smooth to irregular indentations (Champagne *et al*., 2007). Furthermore, the flower of a legume normally has five petals and an ovary with one carpel, cavity and style. The legume flowers usually follow the pattern of the papilionaceous design as in Figure 2, which denotes butterfly-like or pea-like were formed by one large petal, namely standard, two narrower petals called wings and two other petals named keel due to their shape (Jennings and Foster, 2020).

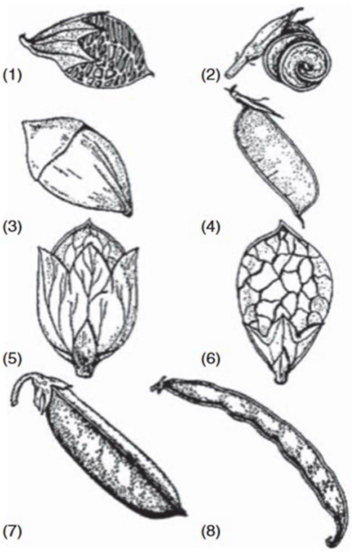

Figure 1. Different types of legumes pod. Legume pods are from (1) sweet clover, (2) alfalfa, (3) red clover, (4) hairy vetch, (5) common lespedeza, (6) korean lespedeza, (7) fieldpea and (8) cowpea. Source: Allaire and Brady (2008).

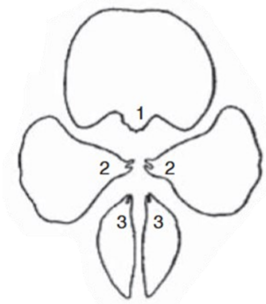

Figure 2. Structure of legume flower. (1) standard, (2) wings, (3) keel. Source: Jennings and Foster (2020)

2.3 World production and consumption of legumes

Global legume production has increased by around 34% from 88.33 million metric tons (1971) to 450.18 million metric tons (2017) with soybeans, beans, peas and chickpeas being the most abundant crops in 2017 in terms of total production (Magrini *et al*., 2019). Strong market demand for nutrition has led to an increase in the production of legumes because legumes serve as a cost-effective protein source or meat substitutes consisting of about 15% of protein consumption in developing countries (Getachew, 2019). Apart from that, India is the world's largest producer and consumer of legumes, accounting for about 29% of the world region and 19% of the world population respectively (Singh *et al*., 2015). This is because India is still the largest vegetarian country in the world and relies heavily on vegetative sources to fulfil its daily protein requirements.

Globally, the average level of consumption of legumes has been stagnant for the last three decades at about 21 g per capita per day (FAO, 2019). Regions such

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as Caucasus and Central Asia, East Asia and Europe have a very low consumption of legumes. A study by Winham and Hutchins (2011) stated that the difficulty of consuming legumes in certain countries might be due to barriers such as flatulence, long cooking time and unfamiliarity.

2.4 Nutritional composition of legumes

Most of the legumes follow the pattern where they consist of high-value carbohydrates followed by protein and moisture content. According to Hedley (2000), most legumes have the largest portion of starch, and it accounts for approximately 35–45% of the legumes' weight depending on the species. The majority of legumes have protein content ranging from 196 mg/g to 360 mg/g except for black beans with significantly higher values of protein (882 mg/g) as compared to others (El Tinay *et al*., 1989; Kamboj and Nanda, 2018; Kudre *et al*., 2013). Black beans are an excellent source of protein and contain even higher protein content than meat, egg and milk which can act as meat substitutes. Most legumes are low in fat content and have no cholesterol, but there is an exception for Marama bean and soybean since they are in the oil seed category (Mosele *et al*., 2011). Table 1 shows the proximate composition of the legumes.

In addition, according to Dias (2012), legumes are rich in source of vitamin B-complex which includes niacin, riboflavin, folic acid and thiamine but lack vitamin C and fat-soluble vitamins. Furthermore, magnesium and potassium are found to be present in most legumes and range from 4.2-4.8 mg/100 g and 874-1280 mg/100 g respectively (Iqbal *et al*., 2006). Besides, a previous study also stated that minerals such as phosphorus, copper, iron, calcium and magnesium are present in legumes in significant amounts (Rebello *et al*., 2014).

2.5 Roles of legumes in human nutrition

Even though many findings proved the health benefits of consuming legumes such as the decreased risk of high blood pressure, heart disease, stroke and type 2 diabetes, ironically, only about 8% of United States adults are reported to consume legumes (Mitchell *et al*., 2009; Polak *et al*., 2015). According to the 2007 National Children's Nutrition and Physical Activity Survey, Australian children aged two to sixteen were eating only 4 g to 12 g of legumes per day (Kouris-Blazos and Belski, 2016).

Firstly, legumes possess anti-diabetic activity which can help to prevent the risk of suffering from type 2 diabetes known as insulin-independent diabetes mellitus. Mojica et al. (2017) found that anthocyanin compounds in black beans such as delphinidin-O-glucoside, petunidin-O-glucoside and malvidin-O-glucoside had the ability to inhibit the activity of α-amylase by 35.6%. This is because anthocyanin compounds act as an inhibitor and compete with the substrates to bind to the active site of α-amylase. This can help to reduce the risk of suffering type 2 diabetes. In another study, cowpea was proven to contain a significant amount of resistant starch which can resist digestion of amylase in the small

Table 1. Proximate composition of legumes (All values are in unit mg/g).

Legumes	Moisture	Ash	Protein	Fat	Crude Fiber	Carbohydrates	References
Black bean	35	40	882	18	-	25	Kudre *et al*. (2013)
Broad bean	64	26	294	16	64	547	El Tinay *et al*. (1989)
Chickpea	60	34	229	44	45	598	El Tinay *et al*. (1989)
Cowpea	94	4.2	247	48	-	-	Iqbal *et al*. (2006)
Field bean	96	-	249	8	14	601	Kamboj and Nanda (2018)
Green pea	78	36	249	15	-	-	Iqbal *et al*. (2006)
Jack bean	64	65	262	20	11	578	Olalekan and Bosede (2010)
Kidney bean	92	39	201	25	68	-	Qayyum *et al*. (2012)
Lablab bean	83	40	254	26	-	598	Kilonzi *et al*. (2017)
Lima bean	140	43	250	31	20	516	Fasoyiro *et al*. (2006)
Lentil	66	29	255	12	27	600	El Tinay *et al*. (1989)
Marama bean	53	30	323	400	-	194	Mosele *et al*. (2011)
Moth bean	108	-	236	11	45	565	Kamboj and Nanda (2018)
Mung bean	94	39	237	19	68	549	Abbas and Shah (2007)
Pigeon pea	-	33	178	118	112	-	Oboh (2006)
Red gram	134	-	223	17	15	576	Kamboj and Nanda (2018)
Rice bean	105	36	193	25	34	-	Bajaj (2014)
Soybean	47	46	360	198	73	274	El Tinay *et al*. (1989)
Velvet bean	67	36	202	63	87	-	Vadivel and Janardhanan (2000)
White bean	65	46	196	13	41	639	El Tinay *et al*. (1989)

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intestine. Besides, it also can inhibit glucagon-like peptide 1 and delay the absorption of glucose into blood (Carneiro da Silva *et al*., 2019).

In addition, legumes reduce the risk of developing cancer such as oral, breast and prostate cancer. A study by Kumar *et al*. (2014) showed that chickpeas can inhibit p38 α MAP kinase at a concentration of 37.5 g/mL. Since lectin C-25 that exists in chickpeas would inhibit p38 MAP kinase and bind to p38 α to inhibit the production of inflammatory cytokines. Thus, it can inhibit the oral cancer cell line growth by targeting p38 α MAP kinase. Furthermore, legumes like lentils were proved can decrease low-density lipoprotein (LDL) cholesterol and increase high-density lipoprotein (HDL) cholesterol. Soluble non-starch polysaccharides that are present in lentils can increase biliary cholesterol and decrease biliary phospholipids could lead to a hypolipidemic effect. Hence, it can reduce the risk of cardiovascular diseases (CVD) (Faris *et al*., 2013).

Legumes are high in magnesium, potassium and fibre which have a beneficial effect on reducing blood pressure. According to Limón *et al*. (2015), kidney beans can lower blood pressure by inhibiting angiotensin converting enzyme (ACE) activity and thereby enhance cardiovascular health. Meanwhile, sweet lupin can also prevent hypertension by exhibiting hypotensive activity. Bioactive compounds that exist in sweet lupin, and apigenin compete with angiotensin I for the active site of ACE and thus exhibited hypotensive activity (Arnoldi *et al*., 2015). The summary of various beneficial effects of legumes is summarised in Table 2.

2.6 Phytochemical compound of legumes

Legumes are abundant in phytochemical or bioactive compounds that play a crucial role in many metabolic and physiological processes of humans. Legumes have antinutrient compounds that can interfere with the assimilation of some nutrients. For example, tannins, phytic acid, total free phenolics and L-Dopa. Normally, these compounds are considered undesirable, but the truth is these bioactive compounds may be beneficial for health (Champ, 2002). Apart from being able to scavenge the free radical activity, these compounds also possess anti-thrombotic, anti-carcinogenic, anti-ulcer, anti-allergenic, anti-atherogenic, antioxidant, anti-inflammatory, immunomodulating, antimicrobial, vasodilatory, cardioprotective and analgesic activities (Singh *et al*., 2017). Table 3 shows some of the phytochemical compounds that are present in legumes.

3. Bioactivities of legume extract

In this review, the antioxidant, antimicrobial, anti-inflammatory and anti-diabetic activity of legumes extracted by SE, MAE, UAE, SFE and PLE are compared. SE is a distribution of a solute between two immiscible liquid phases in contact with each other. The process of solvent extraction begins when solutes from one solvent transfer to another solvent and mainly depends on the solubility and polarity of the two types of immiscible solvents used (Todd, 2014).

Meanwhile, MAE is a technique that makes use of microwave energy to draw analytes from the plant matrix. The mechanism involved in the MAE is divided

Table 2. Beneficial effects of legumes.

Legumes	Beneficial Effect	Mechanism	Compound of Interest	References
Black bean	Reduced the risk of type 2 diabetes by inhibiting α-amylase with 35.6%.	Compete with the substrate to bind with the active site of the α-amylase.	Anthocyanins	Mojica *et al*. (2017)
Chickpea	Lowered the risk of oral cancer cell lines by inhibiting p38 α MAP kinase at a concentration of 37.5 g/mL.	Lectin C-25 would inhibit p38 MAP kinase and bind to p38 α to inhibit the production of inflammatory cytokines.	Lectin	Kumar *et al*. (2014)
Cowpea	Reduced the risk of type 2 diabetes by resistant starch present in cowpeas.	Inhibit glucagon-like peptide 1 and delay the absorption of glucose into blood.	Resistant starch	Carneiro da Silva *et al*. (2019)
Kidney bean	Lowered blood pressure by inhibition of angiotensin-converting enzyme (ACE) activity and thereby enhanced cardiovascular health.	Inhibiting ACE activity.	NA	Limón *et al*. (2015)
Lentils	Reduced the risk of cardiovascular diseases (CVD) by decreased low-density lipoprotein (LDL) cholesterol and increased high-density lipoprotein (HDL) cholesterol.	Increased biliary cholesterol and decreased biliary phospholipids could lead to a hypolipidemic effect.	Soluble non-starch polysaccharide	Faris *et al*. (2013)
Sweet Lupin	Prevention of hypertension by exhibited hypotensive activity.	Compete with angiotensin I for the active site of ACE.	Apigenin	Arnoldi *et al*. (2015)

Table 3. Phytochemical compounds from various legumes.

Legumes	Main Identified Compounds	References
Black bean	Delphinidin-3-O-glucoside, petunidin-3-O-glucoside and malvidin-3-O-glucoside	Mojica *et al*. (2017)
Kidney bean	Feruloyl hexoside acid, feruloyl aldaric acid, hesperetin glucuronide-hexoside, eridictyol hexoside and quercetin-O-hexoside	Limón *et al*. (2015)
Lentil	Dimers procyanidins, hydroxybenzoic acids, gallocatechin, phytic acid, tannins and saponins	Faris *et al*. (2013)
Sweet lupin	Apigenin, 7-O-β-apiofuranosyl-6,8-di-C-β-glucopyranoside, p-hydroxybenzoic acid, gallic acid, protocatechuic acid and caffeic acid	Arnoldi *et al*. (2015)
Azuki bean	Chlorogenic acid, caffeic acid, p-coumaric acid, sinapic acid and ferulic acid	Yao *et al*. (2011)
Yellow soybean	Trans-cinnamic, gallic, chlorogenic, p-coumaric, caffeic and ferulic	Đurović *et al*. (2018)
Black soybean seed coat	Pitunidin-3-O-glucoside and cyanidin-3-O-glucoside	Kumar *et al*. (2019)
Velvet bean	L-dopa, tannins, free phenolics and phytic acids	Dhanani *et al*. (2015)
Mung bean hull	Ferulic acid, sinapic acid, resveratrol, catechin, quercetin, luteolin and p-coumaric acid	Singh *et al*. (2017)
Round purple bean	Proanthocyanidins, catechin derivatives and catechin glucoside.	García-Lafuente *et al*. (2014)
Black bean coat	Syringic acid, chlorogenic acid, rutin hydrate, quercetin, myricetin, ferulic acid and kaempferol	Hsieh-Lo *et al*. (2020)
Pigeon pea leaves	A-humulene, α-bisabolene, β-caryophyllene, longifolene and ylangene	Qi *et al*. (2014)

into three steps. First, isolation of the solutes under high temperature and pressure from the active sample matrix sites. Then, the solvent is dispersed throughout the sample matrix and finally, the bioactive compound is gradually released from the sample matrix to the solvent (Khan *et al*., 2019). For UAE, it is used ultrasonic waves in the extraction process. The UAE is highly effective because the dissolution and diffusion of the solution are accelerated by ultrasound which acted as heat transfer that caused cavitation. According to McDonnell and Tiwari (2017), cavitation is the result of the high compression and low refraction pressure exerted into microscopic gas bubbles in the medium. The size of the gas bubbles will keep on growing until unstable size and finally collapse known as acoustic cavitation.

In addition, SFE is considered environmentally friendly because it uses solvent fluids in their supercritical states. The supercritical solvents that can be applied in this extraction method are carbon dioxide, methanol and ethane. Supercritical fluids which are relatively high in diffusivity and low in viscosity, may transport faster and diffuse easily through solid materials that can enhance extraction rates. However, carbon dioxide is most often used in the industry as supercritical fluid due to its attractive properties such as low cost, low toxicity, compatibility with foods, easy separation from extracted solutes and flexibility to be used when low temperatures are needed (Dai and Mumper, 2010; Khan *et al*., 2019).

Moreover, PLE is a technique used at high pressure and high temperature to isolate bioactive compounds beyond their normal boiling point. Although the bioactive compounds are heated at a high temperature beyond their normal point, the solvents remain in the liquid state at a high pressure. As a result, the high solubility and diffusion rate of lipid solutes in the solvent have been achieved and the penetration rate of the solvent in the matrix also has been improved. Consequently, the extraction time consumption is significantly reduced and the solvent can be used more repeatable compared to other methods (Khan *et al*., 2019; Kumar *et al*., 2019).

3.1 Antioxidant activity

Antioxidant activity is the extent of the ability to scavenge oxygen reactive species. The antioxidant activity can be measured by using 2,2-Diphenyi-l-picrylhydrazyl (DPPH) assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay, ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC), total antioxidant activity (TAA) and β-carotene bleaching assay.

According to Yao *et al*. (2011), the antioxidant activity of 70% ethanolic mung bean extracted by SE via DPPH assay contained the highest (45.36 µmol TE/g) followed by jack bean extract (37.81 µmol TE/g) and cow bean extract (37.27 µmol TE/g). This can be correlated to the total phenolic content of mung bean extract (8.14 mg GAE/g) was higher compared to jack bean extract (3.77 mg GAE/g) and cow bean extract (3.94 mg GAE/g). This is an agreement with Peng *et al*.

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(2008) when they noted that the mung bean contains the highest total phenolic content as compared to black bean, soybean and cow bean. Besides, mung bean contains a high amount of p-coumaric which gives high antioxidant activity due to electron delocalization between the aromatic ring and propenoic group. Thus, total phenolic content was found to be strongly positively correlated with antioxidant activity.

The antioxidant activity of pea (SE) extracted by methanol-water (13.67 g/g DPPH, IC_{50}) was noted to be higher than the acetone-water extract (29.40 g/g DPPH, IC_{50}) (Nithiyanantham *et al*., 2012). This is because methanol-water which is considered more polar could give a high affinity toward antioxidant compounds in peas as compared to acetone-water that is less polar. However, the total phenolic content of the acetone-water extract of the pea (12.91 mg/g) was slightly higher than the methanol-water extract (12.88 mg/g). In general, total phenolic content gives a positive correlation with the antioxidant activity but it is opposed in this case. This may indicate that the phenolic compounds in the pea mostly exist in the non-polar form in nature (Yao *et al*., 2010).

Moreover, based on the study of Ballard *et al*. (2010), peanut skin extracted by MAE (2789 µmol TE/g) possesses a higher value of ORAC as compared to SE extract (2149 µmol TE/g). Besides, the total phenolic content of peanut skin extract (MAE) was 144 mg GAE/g, considerably higher as compared to SE (118 mg GAE/g). This proved that MAE had the ability to extract a larger number of phenolic antioxidants that exerted higher antioxidant capability than SE. An explanation for this might be due to the sudden increase of temperature and internal pressure by microwave treatment which affect the structure of the cell by dipole rotation and ionic conduction. Consequently, the constituents inside the cell would diffuse into the solvent (Nayak *et al*., 2015).

Furthermore, the antioxidant activity of methanolic yellow soybean extracted by MAE (345.21 µmol TE/g) via DPPH assay was higher than UAE extract (289.12 µmol TE/g) (Đurović *et al*., 2018) and this was also seen by Dhanani *et al*. (2015), in their study about velvet bean. MAE possess better antioxidant activity for yellow soybean extract as microwave directly interacts with molecules in the solvent system causing damage to plant tissues and resulting in the diffusion of constituents into the solvent. However, cavitation of UAE could generate a shearing force that can increase diffusion and solubility of solid into solvent and consequences reduced extraction time. However, ultrasound was still unable to break some of the ether or ester bonds to release phenolic acid. This statement was also in agreement with the result obtained in a study where the p-coumaric and ferulic acids were absent in the UAE yellow soybean extract (Albuquerque *et al*., 2017).

In the study by Singh *et al*. (2017), both SE (76.75%) and UAE (86.31%) extract for mung bean hull exhibited higher antioxidant activity followed by whole mung bean and mung bean cotyledon. Similarly, Kanatt *et al*. (2011) proved that mung bean hull exerted higher antioxidant activity than its cotyledon. Apart from that, UAE extract of whole mung bean (65.27%), hull (86.31%) and cotyledon (32.61%) showed a higher value of DPPH compared to SE. UAE released high energy due to the collusion of acoustic bubbles which resulted in microstructure breaking of the conjugate bond in phenolic esters, glycosides and bound complexes. However, SE consumed a longer time of extraction than UAE and might cause degradation of the thermolabile compound and thus reduced antioxidant activity (Wang and Zuo, 2011).

On the other hand, 50% methanolic of round purple bean extract indicated a higher value of antioxidant activity from DPPH and ORAC assay compared to white kidney bean extract in UAE at 45 kHz (García-Lafuente *et al*., 2014). The results obtained were supported by the study by Madhujith *et al*. (2004), where coloured bean extracts such as red bean and brown bean extract showed a stronger antioxidant activity than white bean extract. Owing to the fact that the coloured bean had higher total phenolic content compared to white or pale bean. This fact was supported by the total phenolic content that was obtained from the round purple bean was 13.41 mg GAE/g while the white kidney bean was 4.63 mg GAE/g facilitated by the Folin-Cicauteau assay.

In the study of Hsieh-Lo *et al*. (2020), the DPPH value of black bean coat by PLE and SFE at IC_{50} were 0.158 mg C3GE/g and 0.078 mg C3GE/g respectively. This indicated that SFE extract exhibited higher antioxidant capacity than PLE extract. Pereira *et al*. (2019) suggested that the antioxidant activity could depend on the structure of the phenolic compounds. For instance, the presence of a catechol group or the addition of an alkyl group in a flavonoid compound can lead to the potential of antioxidants. Apart from the anthocyanin compounds, SFE could also extract the insoluble-bound phenolics compound which results in an increase in antioxidant activity (Vukoja *et al*., 2019). Besides, PLE showed lower antioxidant activity than SFE as it used high temperatures beyond the boiling point which could lead to the decomposition of thermolabile bioactive compounds in black bean coats (Bursać Kovačević *et al*. 2018).

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In short, the total phenolic content of the legumes commonly demonstrated a positive correlation to the antioxidant activity. However, in some cases, the total phenolic content showed no correlation with the antioxidant activity since it depends more on the individual or structure of polyphenolic compounds. For example, the presence of a catechol group or the addition of an alkyl group in a flavonoid compound can lead to the potential of antioxidants. Furthermore, the antioxidant activity of the legumes also relies on the nature of the legumes itself. For instance, legumes with darker colours would exhibit higher antioxidant potential than pale colour legumes. This is due to darker colour legumes such as red round bean and black bean consisting of a higher amount of phenolic content than pale colour beans. Besides that, part of the legumes used are also one of the factors that affect their antioxidant activity. In general, legume hulls would exhibit the highest antioxidant capacity than whole legume beans and legume cotyledons.

Based on the reviewed that has been done, MAE extract exhibits higher antioxidant activity as compared to UAE and SE extracts since microwaves could create internal superheating of water molecules in legumes which enhances the disruption of cellular and weak hydrogen bounds by molecular movement and rotation of liquids with a permanent dipole. This caused dissolved ion migration to increase the penetration of solvent into the matrix and thus promote analyte solving. Meanwhile, SFE extract showed higher antioxidant activity than PLE extract because the PLE method used high temperature beyond the boiling point which could lead to the decomposition of thermolabile bioactive compounds in legumes. Table 4 summarizes the antioxidant activity of legumes extracted by different extraction techniques.

3.2 Antimicrobial activity

The methods commonly used in determining the antimicrobial activity are disc diffusion and broth dilution methods. The method of dilution can be used to determine the minimum inhibitory concentration (MIC), defined as the lowest antimicrobial concentration required to inhibit the growth of a microorganism after overnight incubation.

According to Roy *et al*. (2020), kidney beans extracted by SE had the strongest microbial inhibition on *Escherichia coli* which is similar to the result obtained by Ma *et al*. (2013). This is probably due to the presence of chitosan in the kidney bean, a positively charged amino group that can interact with the microbial cell membrane that is negatively charged. Consequently, the leakage of microorganisms' intracellular constituents could also occur (Ma *et al*., 2013).

Besides, MAE pigeon pea leaves extract at 660 W for 44 min showed effective antimicrobial activity toward *Propionibacterium acnes* and *Bacillus subtilis* with MIC value of 0.13 mg/mL and 1.06 mg/mL by serial 2-fold dilution (Qi *et al*., 2014). MAE extract was observed under a scanning electron microscope and images showed that the MAE samples were destroyed. This may be due to the heat transfer in MAE occurring from the samples center to the outer and generating pressure within the gland and hence causing rupture (Hosni *et al*., 2013). However, pigeon pea leaf extract only showed significant inhibition against *Bacillus subtilis*, but small inhibition against *Cutibacterium acnes*. It was proved that sesquiterpenes such as humulene, bisabolene, caryophyllene, longifolene and ylangene in pigeon pea leaves exhibited antimicrobial activity (Qi *et al*., 2014).

By using direct bioautography assay, red bambara groundnut hull extracted by 70% methanol, 70% ethanol and milli-Q water via UAE at 42 kHz possessed antimicrobial action on *Klebsiella pneumoniae* subsp. *pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* subsp. *aureus*, where it appeared creamy or white zone on the media plate. This indicated that the tested microorganism was cidal and possessed antimicrobial activity. In contrast, both extracts showed no inhibition on *Acinetobacter baumannii* and *Candida albicans* where the colour of the plate became purple which indicated the presence of living microorganisms (Province, 2017). Brantner *et al*. (1996) agreed with this outcome, noting that gram-positive bacteria were susceptible to plant extract relative to gram-negative bacteria. The gram-negative bacteria are more resistant to antimicrobial agents since they have lipopolysaccharides on their outer membrane which protect them from the action of antimicrobial agents (Gao *et al*., 1999).

According to Anthika *et al*. (2015), a butterfly pea petal extracted with water by UAE at a condition of 3% power and 30°C for 30 mins showed inhibition on *Staphylococcus aureus* (14.75 mm). The water is polar and suitable for extracting phytochemical compounds such as anthocyanins. Long extraction time can aid in obtaining higher yields of total monomeric anthocyanin because of the formation of large bubbles that lead to violent disruption. This allowed more penetration of water into the cell constituent of butterfly pea petals, hence resulting in a higher yield of total monomeric anthocyanin. The study by Ma (2014) suggested that the mechanism of the antimicrobial agent butterfly pea petal extract worked by binding to the cell wall of the

Table 4. Antioxidant activity of legumes extracted by different extraction techniques.

Extraction Technique	Optimum Parameter	Assay	Legumes	Biological Effect	References
SE	70% ethanol, 2 hrs, room temperature	DPPH	Mung bean	45.36 µmol TE/g	Yao *et al*. (2011)
			Jack bean	37.81 µmol TE/g	
			Cow bean	37.27 µmol TE/g	
SE	Methanol-water (80:20), 25°C, 48 hrs	DPPH	Pea Pea	13.67 g/g DPPH (IC_{50})	Nithiyanantham *et al*. (2012)
	Acetone-water (70:30), 25°C, 48 hrs			29.40 g/g DPPH (IC_{50})	
SE	30% methanol, 52.9 °C, 12.2 mins	ORAC	Peanut skin	2149 µmol TE/g	Ballard *et al*. (2010)
MAE	30% ethanol, 90% power, 150 s, 1.5 g			2789 µmol TE/g	
MAE	-	DPPH	Yellow soybean	345.21 µmol TE/g	Đurović *et al*. (2018)
UAE	Methanol, 20 kHz, 2-15 min			289.12 µmol TE/g	
MAE	Water acidified with hydrochloric acid, 15 min	DPPH	Velvet bean	5.0 µg/mL (IC_{50})	Dhanani *et al*. (2015)
UAE	480 W, 35 kHz, 25 °C, 15 min			6.8 µg/mL (IC_{50})	
SE	-	DPPH	Mung bean hull	76.75%	Singh *et al*. (2017)
			Mung bean cotyledon	20.12%	
			Whole mung bean	28.13%	
UAE	Acetone, 45 kHz	DPPH	Mung bean hull	86.31%	
			Mung bean cotyledon	32.61%	
			Whole mung bean	65.27%	
UAE	50% methanol, 45 kHz	DPPH	White kidney bean	109.14 ($1/EC_{50}$)	García-Lafuente *et al*. (2014)
			Round purple bean	171.06 ($1/EC_{50}$)	
		ORAC	White kidney bean	217.97 µmol TE/g	
			Round purple bean	304.24 µmol TE/g	
SFE	Carbon dioxide, ethanol-water 50% (v/v), 60°C, 300 bar	DPPH	Black bean coat	0.078 mg C3GE/g (IC_{50})	Hsieh-Lo *et al*. (2020)
PLE	Ethanol-water 50% (v/v), 250 bar, 60°C			0.158 mg C3GE/g (IC_{50})	

microorganism led to the formation of pores. Therefore, microorganisms would suffer from leakage of intracellular constituents and slowly die due to the inability to grow.

Furthermore, pigeon pea hull which was extracted by SFE that used carbon dioxide as a solvent showed inhibition on the gram-positive bacteria. This is because pigeon pea hull extract exhibited the strongest microbial inhibition on *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Proteus vulgaris* with a MIC value of 0.0039 mg/mL. The supercritical fluid-carbon dioxide extract exerted higher antimicrobial activity on gram-positive bacteria than gram-negative bacteria. The situation may be due to the structural difference between both gram-positive and negative bacteria. The gram-negative bacteria's cell wall was made of the lipopolysaccharide which prevents the diffusion of the hydrophobic compounds. Meanwhile, the cell wall of the gram-positive bacteria is much simpler, without the present of the lipopolysaccharide and hence is more permeable and easier to penetrate (Zu *et al*., 2010).

Moreover, the ethyl acetate extract of sophora with PLE possesses inhibition against *Streptococcus pyogenes* and *Listeria monocytogenes* at MIC values of 15.62 µg/mL and 125 µg/mL (Dorla *et al*., 2019). The sophora extract showed strong inhibition against *S. pyogenes* and moderate inhibition against *L. monocytogenes*. According to Holetz *et al*. (2002), strong antimicrobial activity is defined as those extracts with a MIC value less than 100 µg/mL, while moderate antimicrobial activity has a MIC value of 100-500 µg/mL and weak antimicrobial activity has much higher MIC value of above 1000 µg/mL. Based on the result of this study, the sophora extract only exhibited antimicrobial activity on the gram-positive bacteria which is supported by findings by Ríos and Recio (2005). Similar reasons were noted in the susceptible pattern of the gram-positive bacteria to plant extract as compared to the gram-negative bacteria which is mainly due to the difference in their cell wall's composition.

In conclusion, the effectiveness of the antimicrobial activity relies on the types of microorganisms tested either the gram-positive or gram-negative bacteria. Commonly, gram-positive bacteria are more susceptible to the legume extract than gram-negative bacteria due to the difference in cell wall structure. The gram-negative bacteria are surrounded by a layer of lipopolysaccharides which obstruct the invasion of other molecules. Unfortunately, the comparison between the extraction techniques on the antimicrobial activity of legumes cannot be clearly concluded. However, the effectiveness of the antimicrobial activity of legumes might be estimated by the total phenolic content obtained from each type of extraction technique as summarized in Table 5.

3.3 Anti-inflammatory activity

Inflammation can be summarized as the sequential release of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukins-1β (IL-1β), interleukins-6 (IL-6) and inflammatory mediators including prostaglandin E2 (PGE2) and nitric oxide (NO) which are synthesised by inducible nitric oxide synthase (Inos) and cyclooxygenase (COX) respectively. The production of reactive nitrogen species is also considered as one of the factors that exacerbate the inflammatory damage (García-Lafuente *et al*., 2014). The anti-inflammatory activity worked by inhibition of COX enzyme that involved biosynthesis of prostaglandins (PGS) and thromboxane A synthase (TXS). Methods that are commonly used to determine anti-inflammatory are colourimetric COX (ovine) inhibitor screening kit, 15-lipoxygenase (15-LOX) inhibitor screening assay kit, inhibition of IL-6, IL-1β and iNOS assay.

Šibul *et al*. (2016) investigated the anti-inflammatory potential among soybean, common bean, pea, broad bean, chickpea, white lupin and grass pea extracted by SE via COX-1/12-LOX pathway inhibition assay. The results showed that most legumes extract inhibited biosynthesis of icosanoids with IC_{50} values ranging from 2.09-5.50 mg/mL for 12-hydroxyeicosatraenoic acid (12-HETE), 3.08-5.89 mg/mL for 12-heptadecatrienoic (12-HHT) and 2.94-5.84 mg/mL for thromboxane B_2 (TXB_2). However, common bean showed the most potent inhibition of TXS and 12-HETE. This indicates that legume extracts possess excellent inhibitory activity towards 12-LOX and COX which vary depending on the types of legumes.

Besides, both ethanolic MAE darkening and non-darkening cranberry bean extract increased gene expression of barrier function promoting genes including mucin 1 (Muc1), mucin 2 (Muc2), mucin 3 (Muc3), resistin-like molecule β (Relmβ) and regenerating islet-derived protein (Reg3γ). At the same time, they also reduced proinflammatory cytokines such as IL-1β, IL-6, interferon-gamma (IFNγ) and TNF-α. The promoting genes like Relmβ can secrete hormones which help to improve colonic barrier function and attenuate inflammation effect (Monk *et al*., 2016). According to Chen *et al*. (2017), both darkening and non-darkening cranberry bean extracts consisted of catechin glucoside, catechin, epicatechin, ferulic acid and p-coumaric acid that exerted anti-inflammatory activity. However, a bioactive compounds like flavonoids are only presence

Table 5. Antimicrobial activity of legumes extracted by different extraction techniques.

Extraction Technique	Optimum Parameter	Assay	Legumes	Biological Effect		References
SE	-	Disc diffusion	Kidney bean		**Inhibition zone**	Roy *et al.* (2020)
				Escherichia coli (−)	20.26 mm	
				Pseudomonas aeruginosa (−)	NI	
				Bacillus subtilis (+)	13.26 mm	
				Staphylococcus aureus (+)	12.06 mm	
				Klebsiella pneumonia (−)	NI	
MAE	660 W, 44 min	Serial two-fold dilution	Pigeon pea leaves		**MIC**	Qi *et al.* (2014)
				Cutibacterium acnes	0.13 mg/mL	
				Bacillus subtilis	1.06 mg/mL	
UAE	70% methanol, 70% ethanol and milli-Q water, 42 kHz, 30 mins, 25°C	Direct bioautography	Red bambara groundnut		**Colour**	Province (2017)
				Klebsiella pneumoniae subsp. *pneumoniae* (-)	White	
				Pseudomonas aeruginosa (-)	White	
				Acinetobacter baumannii (-)	Purple	
				Staphylococcus aureus subsp. *aureus* (+)	White	
				Candida albicans	Purple	
UAE	Water, 30°C, 3% power, 30 min	Agar well diffusion	Butterfly pea petal		**Inhibition zone**	Anthika *et al.* (2015)
				Staphylococcus aureus (+)	14.75 mm	
SFE	Carbon dioxide	Serial two-fold dilution	Pigeon pea hull		**MIC**	Zu *et al.* (2010)
				Staphylococcus epidermidis (+)	0.039 mg/mL	
				Staphylococcus aureus (+)	0.039 mg/mL	
				Bacillus subtilis (+)	0.020 mg/mL	
				Escherichia coli (-)	>2.50 mg/mL	
				Pseudomonas aeruginosa (-)	>2.50 mg/mL	
PLE	Ethyl acetate, 40 °C, 100 bar	Disc diffusion	Sophora		**MIC**	Dorla *et al.* (2019)
				Pseudomonas aeruginosa (-)	NI	
				Escherichia coli (-)	NI	
				Streptococcus pyogenes (+)	15.62 µg/mL	
				Listeria monocytogenes (+)	125 µg/mL	

in darkening cranberry bean extract, thereby, it showed higher attenuation on IL-8 secretion which exhibited anti-inflammatory activity.

According to the previous study, production of the NO for macrophages that is stimulated by lipopolysaccharides at 1 μg/mL which was added with round purple bean extract (UAE) (28%) was lower than white kidney bean extract (UAE) (52%). This showed that round purple bean extract had high inhibition of NO compared to white kidney bean by iNOS (García-Lafuente *et al*., 2014). Oomah *et al*. (2010) suggested that the anti-inflammatory strength was totally related to the extract's antioxidant activity and phenolic content. However, in this study, anti-inflammatory activity was affected by the types of phenolic compounds. The coloured bean (round purple bean) gave a high concentration of phenolic compounds, and most were catechin derivatives, while the white bean (white kidney bean) is rich in phenolic acids but is absence in catechin derivatives. The catechin derivatives might be the reason of round purple bean extract exhibited higher anti-inflammatory activity than white kidney bean extract (Aguilera *et al*., 2010).

An experiment was conducted by Paun *et al*. (2020), evaluating the extraction methods that are suitable for extracting the anti-inflammatory agent of yellow sweet clover. Based on the result, the PLE extract showed higher total phenolic content and total flavonoid content in yellow sweet clover than the UAE extract. The explanation for this could be bioactive compounds in yellow sweet clover were extracted more efficiently by the PLE method. This result is supported by the study of Nayak *et al*. (2015). Though PLE extract contains a high value in total phenolic content and total flavonoid content, UAE chose to extract an anti-inflammatory agent of yellow sweet clover. The reason for this is due to PLE which consumed a very long time to extract the needed amount in further process. UAE can obtain the same amount of extract in a shorter time than taken by PLE. Through UAE, yellow sweet clover extract contains LOX and hyaluronic acid (HYA) inhibition at IC_{50} values, 109.4 μg/mL and 16.5 μg/mL respectively. The polyphenolic content such as rosmarinic acid, chlorogenic acid, luteolin, isoquercitin and rutin were responsible for anti-inflammatory activity in yellow sweet clover.

According to Contreras *et al*. (2020), water-ethanol extract of black bean coat in SFE showed inhibition on COX-2 and iNOs with 30.63% and 32.33% respectively. Apart from showing anti-inflammatory effect, the black bean coat also exhibited antioxidant activity measured by DPPH and ABTS radical scavenging activity at IC_{50}, 143.8 mg GAE/g and 1.197 mg GAE/g respectively. This has shown a good link between antioxidant and anti-inflammatory activity (Oomah *et al*., 2010). Having said the above, there is still very little research done on the anti-inflammatory activity of legumes by SFE due to its high capital setup.

According to the study by Patel *et al*. (2014), an experiment to determine the anti-inflammatory activity between Indian medical plants extracted with PLE measured the inhibition of production of TNF-α, IL-1β and NO in lipopolysaccharide. Based on the result obtained, the ethyl acetate extract of *Mimosa pudica* showed significant inhibition of TNF-α (IC_{50}, 31.7 μg/mL), IL-1β (IC_{50}, 47.2 μg/mL) and NO (IC_{50}, 34.4 μg/mL) as compared to the dichloromethane and methanol extracts. Similarly, Mukarram Shah (2015) also found that the ethyl acetate extract showed the strongest effect on the anti-inflammatory activity.

To sum up, the anti-inflammatory activity of legumes is positively correlated with the total phenolic content and antioxidant activity. In some cases, anti-inflammatory activity depends on the types of bioactive compounds such as catechin derivatives. Besides, choosing the effective extraction techniques to extract the anti-inflammatory agent of legumes, the extraction time must also be considered. Due to the long-time of extraction by PLE, the UAE might become an alternative to PLE since both techniques were proven to efficiently extract the anti-inflammatory agents in a short time. The anti-inflammatory activity of legumes as implicated by different extraction techniques is summarized in Table 6.

3.4 Anti-diabetic activity

The International Diabetes Federation study reports that the number of deaths caused by diabetes increased to 5 million in 2015, which is equal to one death every six seconds. Diabetes mellitus is divided into type 1 and type 2 where type 2 diabetes is the most prevalent and accounts for 95% of the entire diabetes population. The bioactive compounds existing in legumes have been shown to have a higher potential to reduce the risk of type 2 diabetes than type 1 diabetes. There are many assays used to determine anti-diabetic activity for type 2 diabetes including α-glucosidase inhibition, α-amylase inhibition, dipeptidyl peptidase IV (DPP-IV) inhibition, glucose uptake in vitro and computational docking (Mojica *et al*., 2017) as summarized in Table 7.

A study reported that 24% (v/v) ethanol extract of black bean coat extracted using SE possesses inhibition on α-glucosidase, α-amylase and DPP-IV were 38.7%, 35.6% and 34.4% respectively (Mojica *et al*., 2017). This indicates that the bioactive compounds found in the

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Table 6. Anti-inflammatory activity of legumes extracted by different extraction techniques.

Extraction Technique	Optimum Parameter	Assay	Legumes	Biological Effect	References
SE	80% methanol, 90 min, room temperature	COX-1/12-LOX pathway inhibition assay	Soybean	3.08-5.89 mg/mL for 12-HHT 2.09-5.50 mg/mL for 12-HETE 2.94-5.84 mg/mL for TXB_2	Šibul *et al*. (2016)
			Common bean		
			Pea		
			Broad bean		
			Chickpea		
			White lupin		
			Grass pea		
MAE	60% ethanol, 80°C, 600 W	Colon mRNA expression assay	Darkening cranberry bean	Muc1 9.97	Monk *et al*. (2016)
				Muc2 0.76	
				Muc3 9.41	
				Relmβ 3.95	
				Reg3γ 80.9	
			Non-darkening cranberry bean	Muc1 4.29	
				Muc2 0.91	
				Muc3 13.15	
				Relmβ 4.89	
				Reg3γ 97.4	
UAE	Methanol, 30 mins, 60°C, 45 kHz	Nitric Oxide assay	Round purple bean	28%	García-Lafuente *et al*. (2014)
			White kidney bean	52%	
UAE	Ethanol 50% (v/v), 10-15 mins	LOX inhibitory assay	Yellow sweet clover	109.4 μg/mL (IC_{50})	Paun *et al*. (2020)
		HYA inhibitory assay	Yellow sweet clover	16.5 μg/mL (IC_{50})	
SFE	Water-ethanol 50% (v/v)	COX-2 inhibitory assay	Black bean	30.63%	Contreras *et al*. (2020)
		NO inhibitory assay	Black bean	32.33%	
PLE	Ethyl acetate, 100 bar, 60°C	TNF-α inhibitory assay	*Mimosa pudica*	31.7 μg/mL (IC_{50})	Patel *et al*. (2014)
		IL-1β inhibitory assay		47.2 μg/mL (IC_{50})	
		NO inhibitory assay		34.4 μg/mL (IC_{50})	

Table 7. Anti-diabetic activity of legumes extracted by different extraction techniques.

Extraction Technique	Optimum Parameter	Assay	Legumes	Biological Effect	References
SE	24% ethanol, 29	α-amylase inhibition assay	Back bean coat	35.6%	Mojica *et al*. (2017)
		α-glucosidase inhibition assay	Back bean coat	38.7%	
		DPP-IV inhibition assay	Back bean coat	34.4%	
SE	HCl acidified methanol, 40 mins	α-amylase inhibition assay	ox-eyebean	82.17%	Vadivel and Biesalski (2011)
		α-glucosidase inhibition assay	ox-eyebean	91.26%	
MAE	Purified water, 10 mins, 40°C	α-amylase inhibition assay	White kidney bean	4100 U/mg	Ye *et al*. (2010)
UAE	80% ethanol, 5 mins	α-amylase inhibition assay	Fenugreek	370.56 µg/mL (IC_{50})	Dsouza *et al*. (2018)
UAE	Methanol	α-amylase inhibition assay	*Sesbania sesban* Merrill	81.43%	Vadivel *et al*. (2012)
		α-glucosidase inhibition assay		67.05%	
SFE	Carbon dioxide, ethanol-water 50% (v/v), 300 bar, 60°C	α-glucosidase inhibition assay	Black bean coat	31.30 µg C3GE/g (IC_{50})	Hsieh-Lo *et al*. (2020)
		DPP-IV inhibition assay	Black bean coat	0.195 mg C3GE/g (IC_{50})	
PLE	Ethanol-water 50% (v/v), 250 bar, 60°C	α-glucosidase inhibition assay	Black bean coat	61 µg C3GE/g (IC_{50})	
		DPP-IV inhibition assay	Black bean coat	0.25 mg C3GE/g (IC_{50})	

black bean coat extract have the ability to lower the gastrointestinal system's glucose uptake and inhibit DPP-IV, an enzyme associated with the secretion of insulin. The anthocyanin compounds present in the black bean coat extract were believed to play important roles in anti-diabetic activity. These findings were similar to previous findings in Mexican black bean cultivars in relation to their anthocyanin composition (Aguilera *et al*., 2016). These anthocyanin compounds may react with α-glucosidase, α-amylase and DPP-IV through hydrogen bonds, polar interaction and hydrophobic interaction. In addition, a similar structure with the substrate maltose and glucosyl group makes anthocyanins function as a competitive α-glucosidase inhibitor.

Other than that, acidified methanolic ox-eye bean extract (SE) had α-amylase and α-glucosidase inhibition which represented 82.17% and 91.26% respectively. This is due to the total free phenolic content of acidified methanolic ox-eye bean extract was 14.80g CAE/100 g, considerably quite high as compared to other types of legumes (Vadivel and Biesalski, 2011). According to Zhang *et al*. (2015), phenolic compounds in legumes can inactivate α-amylase and α-glucosidase by non-specific binding to enzymes and thus lower the potential of having type 2 diabetes. The phenolic compounds can act as inhibitor to α-amylase which bind to α-amylase's active site and alter the catalytic activity resulting in lower blood sugar level (Ranilla *et al*., 2008). Besides, phenolic compounds also may serve as α-glucosidase inhibitors which can delay the rate of glucose absorption into the intestine by competitive and reversible inhibition of α-glucosidase.

White kidney bean extract possesses inhibition of α-amylase at 4100 U/mg by MAE at 40°C for 10 mins. The white kidney bean is composed of one of the isoforms of α-amylase inhibitor, which is phaseolamin (α-AI 1). The phaseolamin can bind to the active site of the pancreatic α-amylase. Through this, the α-amylase inhibitor can block and obstruct the substrate such as starch binds with the active site of pancreatic α-amylase, thereby, reducing the risk of diabetes. There are another two isoforms of the α-amylase inhibitor which are α-AI 2 and α-AI 3, however, only α-AI 1 is widely distributed in the wide range of legumes. Although each type of legume has a different primary structure of α-AI 1, still it showed the same inhibitory activity on human amylase (Micheli, 2019).

Moreover, the UAE extract of fenugreek gave higher yields of total phenolic content than that of MAE. This is caused by the ultrasound waves that are capable of generating agitation in the solid-solvent mixture whereas MAE is only facilitated by the heating process during extraction (Wani *et al*., 2016). Other than that, the inability to control the increase in temperature in the microwave might lead to the degradation of some bioactive compounds that are sensitive to heat (Kaufmann and Christen, 2002). On top of that, the UAE extract showed α-amylase inhibition by 370.56 μg/ml (IC_{50}). Fenugreek is rich in steroidal saponin, and diosgenin which have a significant level to inhibit α-amylase which can aid in reducing the high blood glucose level. In another research, diosgenin was proved to lower the blood glucose level of Wistar rats significantly and also inhibited α-glucosidase activity and thus exerted anti-diabetic activity (Dsouza *et al*., 2018).

Additionally, methanol extract of *Sesbania sesban* Merrill, a type of wild legume grain that is underutilized showed α-amylase (81.43%) and α-glucosidase (67.05%) inhibition in UAE. The α-amylase inhibitor is able to bind to α-amylase's active site which aided in altering the catalytic activity hence able to lower the blood sugar level. For the α-glucosidase inhibitor, it can slow the rate of glucose absorption into the small intestines since it is competitive and reversible. Thus, α-amylase and α-glucosidase inhibitors a good alternative source and anti-diabetic agent to reduce the risk of type 2 diabetes (Vadivel *et al*., 2012; Alrugaibah, 2016).

Based on the previous research, the SFE extract of the black bean coat showed stronger inhibition on α-glucosidase and DPP-IV than the PLE extract (Hsieh-Lo *et al*., 2020). This phenomenon is due to the ability of SFE to increase the stability of anthocyanins by means of co-pigmentation and isolated insoluble-bound phenolic. The glycosyl group of anthocyanins had similar structural with the substrate that competed with α-amylase and α-glucosidase to bind with the active site. Besides, phenolic compounds and the hydroxyl groups of anthocyanins were capable of changing the molecular configuration of the active sites by interacting with their polar group (Aguilera *et al*., 2016).

Overall, anti-diabetic activity is positively correlated with total phenolic content. This is because it can act as an inhibitor for α-amylase and α-glucosidase as they have a similar structure with the substrate. Consequently, phenolic compounds can bind to the active site of the α-amylase and α-glucosidase and thus exert anti-diabetic activity. Based on the review that has been done, UAE extract exhibits higher strength on the anti-diabetic activity of legumes as compared to MAE as ultrasound waves are able to generate agitation in the solid-solvent mixture whereas MAE is only facilitated by the heating process during extraction. Meanwhile, SFE extract showed higher anti-diabetic activity of legumes than PLE because SFE increases the stability of anthocyanins.

4. Potential application of legume extract

4.1 Food industry

According to a study by Marín-Manzano *et al.* (2020), naturally occurring oligosaccharides from peas (*Pisum sativum* L.) can act as supplemented products for infants. This is because non-fructosylated α-galactooligosaccharides from peas were able to mimic the function of human-milk oligosaccharides which is an important dietary ingredient to modulate the infant microbiome. The non-fructosylated α-galactooligosaccharides can reach the large intestine and be fermented by gut microbiota there as it is not hydrolysed in the upper gastrointestinal tract. As a result, non-fructosylated α-galactooligosaccharides from peas can exhibit prebiotic properties. Besides, non-fucosylated α-galactooligosaccharides from pea was proof safe to consume by an infant in a concentration of up to 8 mg/mL (Kruger *et al*., 2017).

Furthermore, lentil protein isolates can also act as stabilizers for the emulsion system. The lentil protein isolates will migrate to the oil-water interface during the formation of the emulsion. Then, they will rearrange themselves to orient hydrophilic groups toward the water phase and hydrophobic groups toward the oil phase. As a consequence, this can help to lower the interfacial tension of emulsion. Aggregation of protein can create a viscoelastic interfacial film that can stabilize oil droplets from coalescence and gravitational separation (Khazaei *et al*., 2019). In addition, lentil protein isolates can also serve as antioxidants that can inhibit lipid oxidation in food products. Previously, synthetic antioxidants like butylated hydroxyltoluene (BHT), butylated hydroxyanisole (BHA), ethylenediaminetetraaceticacid (EDTA) and propyl gallate (PG) have been commonly used in the food industry, but recently their use is declined because of consumer interest in more clean-label products. The lentil protein can inhibit lipid oxidation by binding with metal ions and preventing them from reaching the lipid droplet surfaces. It also possesses antioxidant side groups that can scavenge free radicals (Gumus *et al*., 2017).

Moreover, legume extract can also be used as a natural food colourant in the food and beverages industry. For instance, anthocyanin compounds from black beans will become red and purple colour at pH 1 whereas blue colour between pH 2 and 4. However, anthocyanin compounds are easily degraded when exposed to light, oxygen, the presence of enzymes and unsuitable temperatures. Therefore, the stability of anthocyanin can be optimised by increasing anthocyanin concentration, inactivating enzymes and removing oxygen (Mojica *et al*., 2017).

4.2 Nutraceutical industry

Parkia speciosa or common name known as stinky bean rich in antioxidant and anti-hypertensive bioactive peptides which can act as nutraceutical products. A total of 29 bioactive peptides were successfully derived from the seeds using the enzyme alcalase. These bioactive peptides that are derived from stinky beans consist of pharmacological properties to the human body (Chhikara *et al*., 2018). Besides, legumes have the potential to be used as an anti-inflammatory agent. This is due to the Bowman-Birk inhibitor, a type of protease from soybean that can exhibit anti-inflammatory activity in denatured form. Thus, Bowman-Birk inhibitor protease achieved investigational new drug status by the Food and Drug Administration (Barman *et al*., 2019).

Legumes such as *Mucuna pruriens* and *Vicia faba* contain a significant amount of L-Dopa, an amino acid precursor of the neurotransmitter's dopamine, norepinephrine and adrenaline. The L-Dopa can use to treat Parkinson's disease which is caused by neuronal degeneration in the substantia nigra and lead to a decrease in dopamine values and the ability to control movement and feeling. This is because L-Dopa can restore neurotransmission which helps to reduce Parkinson's disease symptoms (Priego-Poyato *et al*., 2021).

4.3 Cosmetic industry

Melissa officinalis extracts have been proven good for skin care especially in the treatment of postoperative circulatory and problematic wounds because of significant amounts of flavonoids and coumarins. The *Melissa officinalis* extracts also contain other bioactive compounds such as umbelliferone, melilotin, quercetin and triterpene sapogenin which can be applied for skin care by exerting anti-inflammatory, lentive and soothing effects (Pastorino *et al*., 2017). Apart from this, Semelil derived from *Melissa officinalis* can be used in the treatment of diabetic foot ulcers. Semelil is composed of bioactive compounds such as coumarin and flavonoid and it is present in gel form. Chorepsima *et al.* (2013) found that Semelil with a dose of up to 10 mL had significant improvement in the foot ulcer area.

In the past three decades, the prevalence of atopic dermatitis has increased two to three-fold in industrialized countries where children account for 10-20% while adults account for 1-3%. Generally, glucocorticoids are used for the treatment of atopic dermatitis, but they only can be used in the short term and for specific skin regions. Prolonged usage of glucocorticoids will bring side effects to human health and thereby researchers are searching for natural extracts

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as medicine for atopic dermatitis. According to research by Collantes *et al*. (2012), azuki bean is potent enough to become a natural medicine for atopic dermatitis. In the study, NC/Nga mice that were treated with 250 mg/kg of azuki bean extracts were able to relief the symptoms of atopic dermatitis by preventing transepidermal water loss, reducing irritancy threshold and preventing dryness. Other than that, NC/Nga mice that were treated with 250 mg/kg azuki bean extracts also showed less histopathological skin lesions, with minimal dermal and epidermal thickness as well as decreased manifestation of acanthosis and spongiosis.

5. Conclusion

In conclusion, the total phenolic content in legumes was positively correlated with its antioxidant, antimicrobial, anti-inflammatory and anti-diabetic activity. However, it is also important to note that in some findings, the antioxidant and anti-inflammatory activity must also consider the presence of other individual polyphenolic compounds. Regarding to antioxidant activity of legumes, MAE extract exhibits higher antioxidant activity as compared to UAE and SE extracts whereas SFE extract showed higher antioxidant activity than PLE extract. Based on the data reviewed, UAE extract exhibits higher strength in the anti-diabetic activity of legumes as compared to MAE while SFE extract showed higher anti-diabetic activity of legumes than PLE extract. However, the effectiveness of antimicrobial and anti-inflammatory activity of legumes between different extraction techniques cannot be clearly concluded as studies on these were still lacking. Finally, it can be concluded that legumes are rich in various types of bioactive compounds that can be further employed in the food industry as antioxidant, antimicrobial, anti-inflammatory and anti-diabetic agents. It is also hoped that with this review, the related industry gains more information on suitable extraction techniques that exhibit the highest bioactivity of legumes and thereby consumption of legumes can be improved as well as reduce prejudice toward eating legumes worldwide.

Conflict of interest

The authors declare no conflict of interest.

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