

Review Article

A Review on the Phytochemicals of *Parkia Speciosa*, Stinky Beans as Potential Phytomedicine

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Received: 28 June 2019; **Accepted:** 15 July 2019; **Published:** 22 July 2019

Abstract

Plants phytochemicals are extensively known for their advantageous in health to promote biochemical benefits in the area of reactions, cofactors and inhibitors of enzyme, absorbents/sequestrants that bind to and eliminate undesirable elements in the human body. Some research findings have supported the beneficial role of phytochemicals against cancers, coronary heart disease, diabetes, high blood pressure, inflammation and many other diseases. This review discussed phytochemical compounds properties of *Perkia specios* (*PS*) in Malaysia. The plants of focus are smelly legumes/stink beans; commonly grown and cultivated in Southeast Asian countries, including Malaysia, Indonesia and in some parts of Northeastern India. The young leaves, flowers and fruits that are collected from the wild are consumed as vegetables and herbal medicines. The seeds are either eaten raw or cooked and half-ripe seeds can also be traditionally prepared as a pickle in brine. The review will be useful for future studies through current knowledge on the phytochemical elements and medicinal functions to a possible magnitude with relevant data as these plants have potential to be developed as phyto-/herbal-/botanical medicine.

Keywords: Phytochemicals; Phenolic constituents; Terpenoid; Alkaloids; Saponins; Cyclic polysulfides; Tannins; *Parkia speciosa*

1. Introduction

Parkia speciosa Hassk is abundantly found in tropical regions, especially in South East Asian countries: Malaysia, Indonesia, Thailand and Philippines [1-4]. This rainforest tree belongs to the genus '*Parkia*', family '*Fabaceae*' and is also found in India, Africa, Madagascar and Fiji [4, 5]. *Parkia* genus is divided into three sections namely *Parkia*, *Platyparkia* and *Sphaeroparkia*. The *Parkia speciosa* is group under *Parkia* section with three other species:

P. cachimboensis, *P. decussate* and *P. discolor* [5]. This species thrives well in rainforest, on sandy, loamy and podzolic soils, waterlogged locations, freshwater swamp forest and areas nearby the riverbanks [3, 5].

The matured plant can grow up to 40 m height and it bears bulb shape flowers that hang oblong and twisted pods which contain 15-18 seeds. The light green seeds are encapsulated with seed coats in these pods. The seeds have foul, peculiar, but unique and distinctive smell with elliptical shape that can be eaten raw, roasted, cooked or blanched [3-5]. In Malaysia, PS seeds were mostly eaten as 'ulam' namely raw or boiled seeds consumed with chilies mixed with prawn paste as the main ingredient. Only a small portion of PS seeds were consumed after blanching or boiling [6, 7]. It is also cooked in chili paste mixed with seafood, boiled in coconut milk with varieties of vegetables or added as an ingredient in many other dishes, including fried rice or stir-fried food [5]. Matured seeds are usually pickled in brine and are among popular pickle products in the country [8]. Although PS seeds are widely used as a cooking ingredient [9], in traditional medicine, the seeds are consumed as remedies to clean and detoxify the kidneys and urinary tract, to treat diabetes and headache [10-12]. It is also a traditional remedy for itchiness [13], inflammation, oedema, liver failure and to eliminate intestinal worms [3].

PS seeds contain many nutritional values such as protein, fatty acids, carbohydrate, minerals, vitamins etc. [4, 5]. The seeds also rich in bioactive compounds either essential or non-essential from the secondary metabolites that have abundant therapeutic benefits. Phytochemicals are natural plant elements occurred in the leaves or roots which are useful as defense instruments to protect them from various diseases that over time have been discovered and used by diverse groups of people for treatment of various diseases [14]. Beneficial phytochemicals in plants may enhance the needs of the human body as they are rich in antioxidants and other health benefits as well.

2. Phytochemicals of PS

Primary and secondary metabolites are bioactive phytochemicals, which produced in plant tissues and they are well known for their advantages in medicinal benefits to human beings [14, 15]. Chlorophyll, amino acids, proteins, common sugars or simple carbohydrate, membrane lipids, purines and pyrimidines of nucleic acids [16-18] are an example of the primary constituents that play recognized roles in biochemical reactions in plants for example the major activities such as photosynthesis and respiration [17]. The secondary compounds are the terpenoid, alkaloids, phenolic compounds, lignins, plants steroid, curcumines, saponins, flavonoids, glucosides [16, 18] etc. and these compounds are synthesized as part of the defense system of the plants [15, 17]. These constituents protected plants from diseases and damages subsequently enhanced the plant's color, aroma and flavor. Phytochemicals also serve as a defense mechanism to protect plant cells from unfavorable conditions such as environmental pollution, climate change, UV and pathogens exposure [16]. These compounds accumulated in the whole parts of the plant, especially the three main parts which are the roots, stems and leaves. The pigment molecules are abundance in the chloroplasts of the plant. Their levels vary from plant to plant depending upon the variety, growing conditions, processing and cooking methods [16].

Literature review indicated that phenolic compounds (45%) are the most numerous and structurally diverse plant phytoconstituents, followed by the terpenoids and steroids (27%), alkaloids (18%) and others (10%) [16]. Harborne identified and delineated the major classes of plant chemicals of recently recognized as health benefits specific phytochemicals [19]. These compounds were cited by Dillard et al. and listed in Table 1 [20]. These divisions of categories were constructed from their biosynthetic origin [15]. Alkaloids, tannins, flavonoids and phenolic compounds are among the most important bioactive constituents that are still being studied under phytochemistry or natural product chemistry [21]. Among phytochemicals, flavonoids, polyphenols, stilbenes, carotenoids and anthocyanins are several compounds that are vital for health promoting effects [22]. Several of these elements have been extracted from PS and researched for pharmacological activity. Terpenoids [23-25], polyphenols and flavonoids [23-29], alkaloids [23-25, 27-28], saponins [23, 24, 27, 28], steroids [23, 25, 28], tannins [23, 27] and phytosterol [30, 31].

No	Classes	Compounds
1	Terpenoids	monoterpenoids, iridoids, sesquiterpenoids, sesquiterpene lactones, diterpenoids, triterpenoids, saponins, steroid saponins, cardenolides and bufadienolides, phytosterols, cucurbitacins, nortriterpenoids, other triterpenoids and carotenoids.
2	Phenolic metabolites	anthocyanins, anthochlors, benzofurans, chromones, coumarins, minor flavonoids, flavonones and flavonols, isoflavonoids, lignans, phenols and phenolic acids, phenolic ketones, phenylpropanoids, quinonoids, stilbenoids, tannins and xanthones.
3	Alkaloids	amaryllidaceae, betalain, diterpenoid, indole, isoquinoline, lycopodium, monoterpene, sesquiterpene, peptide, pyrrolidine and piperidine, pyrrolizidine, quinoline, quinolizidine, steroidal, and tropane.
4	Nitrogen-containing plant constituents	non-protein amino acids, amines, cyanogenic glycosides, glucosinolates, and purines and pyrimidines.

Table 1: Harborne [19] identified and delineated the major classes of plant phytochemicals as cited by Dillard and German [20].

2.1 Phenolic constituents

Phenolics consist of the aromatic ring (C₆) bonded directly to at least one (phenol) or more (polyphenol) hydroxyl group (-OH) and other substituents such as methoxyl (CH₃O-) or carboxyl (COOH) groups. These substituents either give the hydrophilic or hydrophobic character to the compounds [32]. These compounds occurred in conjugated forms with one or more sugar residues linked to hydroxyl group inclusive direct linkages of sugar to an aromatic carbon. The association forms with other compounds as well, for example carboxylic and organic acids, amines, lipids and other phenol [33]. Such structural diversity resulted of these compounds often referred to as polyphenols. These compounds are classified based on the number of phenol rings where the structural elements

bind these rings to one another [34]. In the wide range, phenolic compounds can be categorized into several classes as shown in Table 2 and were divided into four classes (Figure 1) [34]. Of these, phenolic acids, flavonoids and tannins are the main dietary phenolic compounds [35]. Plant phenolic compounds arise from phenylalanine or shikimic acid [33] where the aromatic ring of phenolic compounds is synthesized in the shikimic acid pathway from amino acid phenylalanine. The initial step is simple detachment of the amino group (-NH₂) from phenylalanine which convert to cinnamic acid. This reaction is catalyzed by enzyme phenylalanine ammonia-lyase [33].

Class	Structure
Simple phenolic, benzoquinones	C ₆
Hydroxybenzoic acids	C ₆ -C ₁
Acetophenones, phenylacetic acids	C ₆ -C ₂
Hydroxycinnamic acids, phenylpropanoids (coumarins, isocoumarins, chromones, chromenes)	C ₆ -C ₃
Naphthoquinones	C ₆ -C ₄
Xanthones	C ₆ -C ₁ -C ₆
Stilbenes, anthraquinones	C ₆ -C ₂ -C ₆
Flavonoids, isoflavonoids	C ₆ -C ₃ -C ₆
Lignins, neolignans	(C ₆ -C ₃) ₂
Biflavonoids	(C ₆ -C ₃ -C ₆) ₂
Lignins	(C ₆ -C ₃) _n
Condensed tannins (proanthocyanidins or flavolans)	(C ₆ -C ₃ -C ₆) _n

Table 2: Classes of phenolic compounds in plants [35].

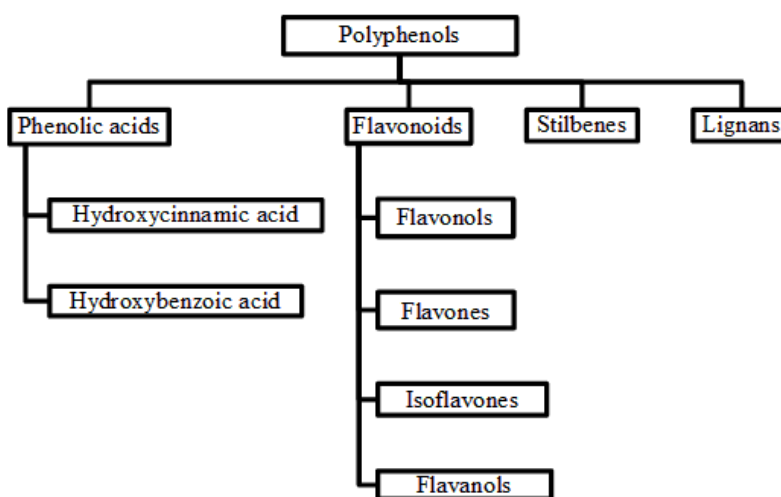


Figure 1: Classification of major plant polyphenols [34].

Phytochemicals screening of PS revealed the presence of pharmacologically active compound in many parts of the plants especially the empty pods. A study conducted by Ko et al. [29] indicated that the antioxidant activities of empty pods of PS extracts vary with the type of assays due to different responses of bioactive compounds towards these assays. Results showed that ethanol extracts (EE) of PS possessed good DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity than an aqueous extracts (AE) due to higher total phenolic and flavonoid contents. Specific analysis tracked total flavonoids was almost five times higher in EE compared to AE [29]. The most abundant phenolic constituents in EE were gallic acid while this compound with the other three compounds, the catechin, ellagic acid and quercetin were found in a moderate amount in the AE. Other phenolics detected with significant amounts were chlorogenic acid, vanillic acid, caffeic acid, epicatechin and kaempferol [29]. In earlier studies, phenolics (gallic acid) and flavonoids have also found in AE and EE of PS seeds [36, 37].

Hasim et al. [23] conducted a qualitative phytochemical screening of PS Hassk peels extract and reported that the n-hexane extract contained saponins, flavonoids, tannin and steroids. The ethyl acetate and ethanol extracts contained similar compounds of alkaloids, saponins, flavonoids, tannins and triterpin except for flavonoids, these compounds were not detected in the ethanol extract [23]. Ghasemzadeh et al. [24] reported that PS seeds collected from different locations in Malaysia contain alkaloids, terpenoids, flavonoids and phenolic compounds. Six flavonoid compounds (quercetin, rutin, kaempferol, catechin, luteolin and myricetin) were identified from PS seed extracts. Five phenolics (gallic acid, caffeic acid, ferulic acid, trans-cinnamic acid and *p*-coumaric acid) were also identified. The study indicated that the phytochemical composition and biological activity of PS seeds vary significantly and it depends on where the PS tree is grown. Their results showed highest antioxidant and antibacterial activities in PS seeds grown in Perak followed by Negeri Sembilan and Johor states of Malaysia [24].

Mustafa, et al. [38] identified quercetin in the hexane and ethyl acetate extract at 5.84 mg/100g dried empty pods of PS but none of flavonoid compounds identified in the methanol extracted samples in study conducted by Mian, et al. [39]. Notwithstanding, a preliminary phytochemical screening revealed the methanolic extract of PS empty pods contained alkaloid, flavonoids, tannins, phenolic compounds and saponin with flavonoids and tannins been the most prominent compounds [27]. Santha Maria, et al. [40] reported on the presence of flavonoids in ethanol extract of PS pods. Recent study by Sonia et al. [26] identified alkaloid, saponin, steroid, flavonoid, tannin, coumarins, quinines, phenols, terpenoid and other compounds as well in the extract of methanol, hydromethanolic and aqueous of PS seeds. The chromatographic profile of polyphenol compounds which comprising of gallic acid, ellagic acid, catechin and quercetin were detected by Kamisah and co-workers at 254 nm [4]. These compounds were identified in the methanolic extract of PS pods and the extract contained 65.39 mg quercetin per 100 g dry PS pods extract. They also reported on 17 peaks detected from the LC-MS chromatogram where the major compounds were epigallocatechin gallate, quercetin, epicatechin/cathecin, coumaric acid, tangeritin and apigenin. However, two other major peaks could not be identified. Recent study by Mustafa, et al. [38] reported on identification of quercetin alone at concentration of 5.84 mg/100 g dry weight of ethyl acetate extract of PS pods.

Phenolic acids account for about a third of the polyphenolic compounds in our diet and in all other plant materials

inclusive in pods and seeds of PS, but are particularly abundant in acidic-tasting fruits [33]. Phenolic acid consists of two subgroups; the hydroxybenzoic acids, which in common to have C₆-C₁ structure (gallic, p-hydroxybenzoic, protocatechuic, vanillic and syringic acids) while hydroxyl cinnamic acids, the aromatic compounds with a three-carbon side chain (C₆-C₃) (cafeic, ferulic, *p*-coumaric and sinapic acids) [35, 41]. Eight phenolic acids were identified in PS (gallic acid (GA), ellagic acid (EA), ferulic acid (FA), chlorogenic acid (CGA), vanillic acid (VA), Caffeic acid (CA), trans-cinnamic acid and *p*-coumaric acid) are some common phenolic acids [33] and structures of these compounds are shown in Figure 2.

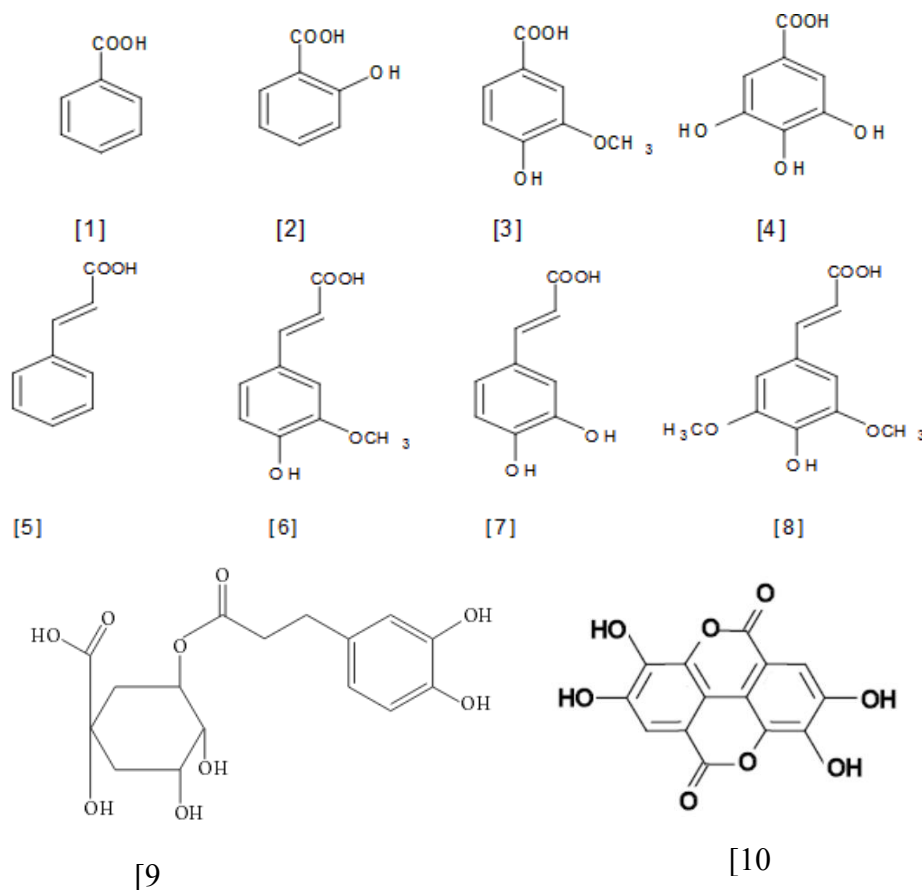


Figure 2: Structures of the important naturally occurring phenolic acids in PS. (Hydroxybenzoic acid are 1-Benzoic acid, 2-Salicylic acid, 3-Vainillic acid, 4-Gallic acid; Hydroxycinnamic acid are 5-Cinnamic acid, 6-Ferulic acid, 7-Sinapic acid, 8-Caffeic cid) [16], 9-chlorogenic acid [45], 10-ellagic acid [91]).

GA, (3,4,5-trihydroxybenzoic acid), a naturally occurring low molecular weight tri-phenolic compound, has been suggested to possess strong antioxidant activities. This compound provides effective protection against oxidative damage caused by reactive species often encountered in biological systems. These species include hydroxyl (HO•), superoxide (O₂^{•-}), and peroxy (ROO•) and the nonradicals, hydrogen peroxide (H₂O₂) and hypochlorous acid (HOCl). In addition, GA derivatives (GADs) can exhibit both antioxidant and prooxidant characteristics, displaying a dual edge sword behavior [42]. Another compound, EA is an increasingly popular dietary supplement and exhibits

powerful anticarcinogenic and antioxidant properties [43]. The constituent is an active agent to induce vasorelaxation, oxygen scavenging, hypolipidemic, antiinflammatory and anticarcinogenic activities either *in-vitro* or *in-vivo* in various animal preparation [43]. FA, 4-hydroxy-3-methoxycinnamic acid, is widely distributed in beverages, fruits, vegetables, cereals, flowers and nuts. It is a caffeic acid derivative formed by the action of the enzyme caffeate O-methyltransferase [41]. FA is recognized for its ability to remove free radicals. As a result, its' potential therapeutic effects cover in the medication in either infectious or noninfectious diseases such as cancer, diabetes and cardiovascular diseases, hepatic and antimicrobial and anti-inflammatory activities [41, 44] and great attention is now directed toward its incorporation into cosmetic emulsions for topical application [41].

CGA is another important phenolic acid identified in PS and it is produced from the esterification of caffeic acid and L-quinic acid (Figure 2). It is also extensively scattered in coffee and fruits (apples, pears, etc.) and is among the important hydroxycinnamic acid derivatives in plants [41]. This compound exhibits many biological properties and reported in numerous publications that support its potential as antibacterial, antioxidant, and anticarcinogenic activities, particularly hypoglycemic and hypolipidemic effects, anti-inflammatory, antiviral, and anti-tyrosinase agent metabolic disordered conditions [41, 45]. While VA is a widely used as flavoring and perfumery that corresponds to the oxidized form of the aldehyde vanillin [46]. CA is one of the most commonly determined in plants, especially in fruits, vegetables, mushrooms and herbs. It is biosynthesized by hydroxylation of p-coumaric acid. This compound covers a wide range of medicinal properties inclusive antioxidant, antitumor, anti-inflammatory, antimicrobial and antidiabetic activity [41]. This compound is also actively involved in plant physiology and mechanisms of stress tolerance primarily utilized by plants for the synthesis of lignin. The process ultimately caused cell walls thickening and increase the resistance to ion toxicity sodium and heavy metal stress.

It also resolves the absorption of high energy radiations in mesophyll cells under drought stress. The mechanism involves the production of FA through the ethylation of CA catalyzed by O-methyltransferase [47].

Cinnamic acid and its derivatives were shown in Figure 3 and the most common are cinnamic acid, CA, FA, isoferulic acid, and *p*-hydroxycinnamic acid [48]. Among various biological activities, cinnamic acid and its derivatives are associated with the diabetes and its complications. These compounds act on various actions, including to promote insulin secretion, to improve pancreatic β -cell function, to inhibit hepatic gluconeogenesis, to enhance glucose uptake, to increase insulin signaling pathway, to delay carbohydrate digestion and glucose absorption, and to inhibit protein glycation and insulin fibrillation ([48]. While, hydroxycinnamic acids and their derivatives display antioxidant, anti-collagenase, anti-inflammatory, antimicrobial and anti-tyrosinase activities and act as a UV protective tool. These suggested that they can be utilized as anti-aging and anti-inflammatory agents, preservatives and hyperpigmentation-correcting ingredients [41]. *p*-Coumaric acid is a phenolic acid, which is synthesized mainly from tyrosine and phenylalanine. It is the main pathway in the synthesis of other phenolic acids, CA, CGA, rosmarinic and FA. *p*-coumaric acid and its conjugated forms showed properties, such as antioxidant, antimicrobial, antitumor, anti-inflammatory, antiplatelet aggregation and many other interesting health benefits. Their major properties are the potential for depigmentation, antioxidant, anti-collagenase, antimicrobial and anti-inflammatory activities. These were among the most important properties in the cosmeceutical industries [41].

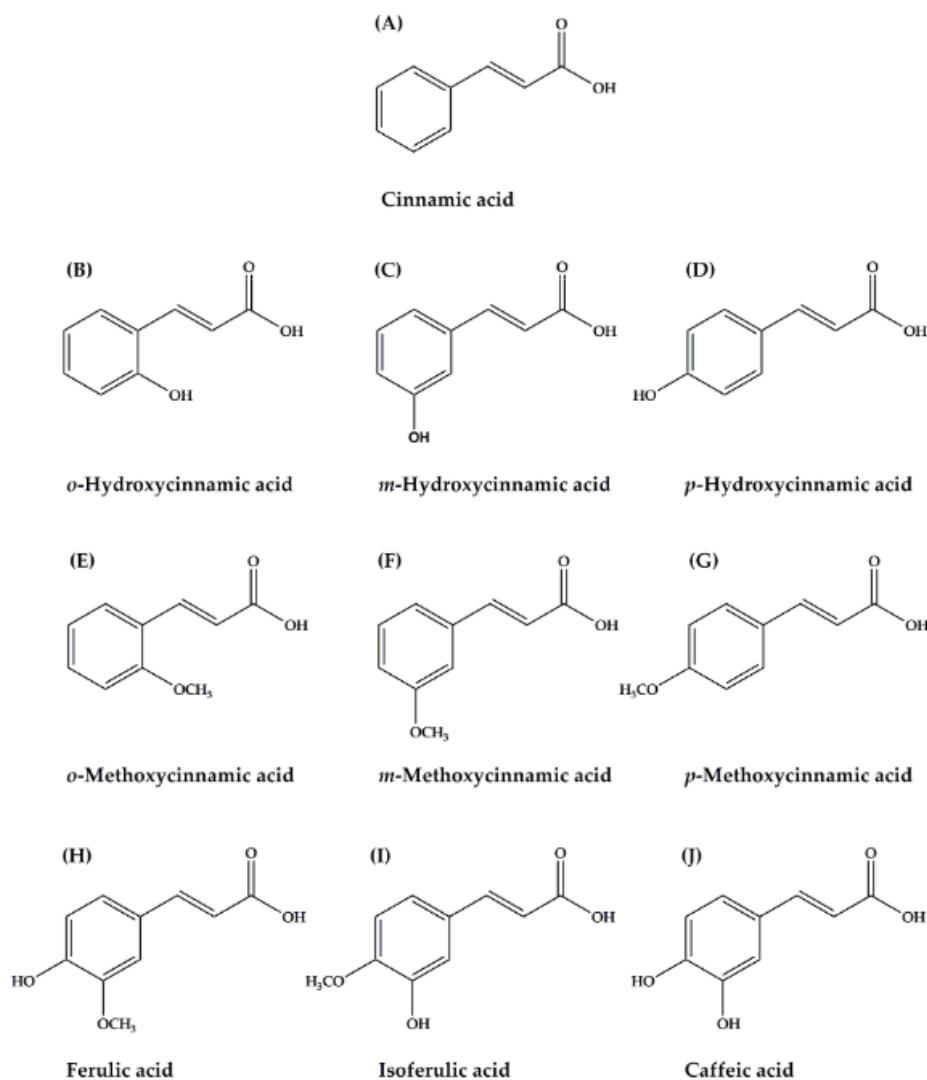


Figure 3: The chemical structure of cinnamic acid and its derivatives. (A) Cinnamic acid; (B) *o*-Hydroxycinnamic acid; (C) *m*-Hydroxycinnamic acid; (D) *p*-Hydroxycinnamic acid; (E) *o*-Methoxycinnamic acid; (F) *m*-Methoxycinnamic acid; (G) *p*-Methoxycinnamic acid; (H) Ferulic acid (I) Isoferulic acid; (J) Caffeic acid [48].

Other than phenolic acids, flavonoids are another group of phenolic constituents identified among major phytochemicals in PS. Eight flavonoids were identified in its pod and seed extracts; catechin, epicatechin, quercetin, kaempferol, rutin, luteolin and myricetin [24, 29, 38] (Figure 4). Flavonoids are low molecular weight compounds arranged in a C₆-C₃-C₆ or C₁₅, configuration consist of two aromatic rings join by a three carbon bridges usually in the form of three heterocyclic rings [35] or heterocyclic skeleton of flavan (2-phenylbenzopyrane) [22, 49]. Variation in these heterocyclic rings resulted in variety of flavonoid classes. The heterocyclic rings condensed with the benzene ring, is either flavones and flavonols or flavanones and flavan-3-ols. These position divides the flavonoids into two classes, which are flavone and isoflavone. Most natural flavonoids associated with sugar in conjugated form and may be characterized as monoglycosidic, diglycosidic, etc. The glycosidic linkage is normally

located at position 3 or 7. While the carbohydrate unit can exist in L-rhamnose, D-glucose, glucorhamnose, galactose or arabinose [16].

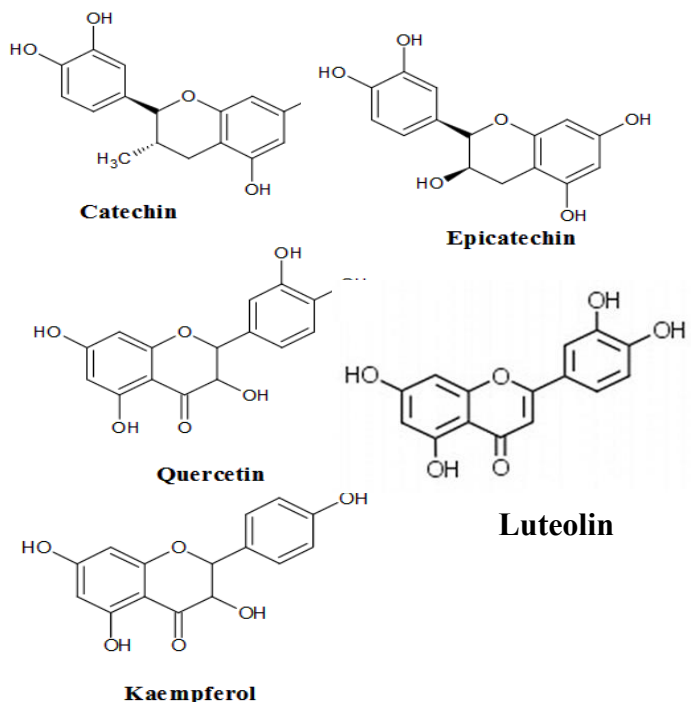


Figure 4: Some flavonoid compounds identified in PS seeds and pods.

Major classification of flavonoids were chalcones, flavones, flavonols, flavandiols, proanthocyanidins and their derivatives anthocyanidins, condensed tannins and aurones [16, 22, 32, 50] (Figure 5). Flavones and flavonols are major compounds occurred in plants and were structurally diverse [32, 35]. These compounds are widely distributed in nearly all plant species. Recently, more than 9,000 flavonoid compounds have been identified. Majority of them have been reported to possess many useful properties, including anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, antiallergic, vascular and cytotoxic antitumor activity [22, 32, 49, 51, 52]. For later properties, flavonoids could prevent through blocking of several points in the progression of carcinogenesis. Those points include cell transformation, invasion, metastasis, and angiogenesis. This carcinogenesis is progressing through inhibiting kinases, reducing transcription factors, regulating the cell cycle, and inducing apoptotic cell death [53].

Epicatechin is also reported as major flavonoid compounds in green tea (*Camellia sinensis*) and comprises of about 30-40 % of extractable solid from dried green tea leaves [54, 55]. Major catechins identified are epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate [54]. Bitter flavor of tea is attributed from the presence of compounds such as oligomeric quinines which are products of catechin derivatives during tea fermentation and also from the quercetin, kaempferol and myricetin, the non-water-soluble flavonoids [54].

Epicatechins reduces the risks of diabetes mellitus and cardiovascular diseases. It also reduced the pharmacological effect such as anti-hyperlipidemic, anti-inflammatory, antioxidative effects, anticarcinogenic, and cytoprotective [54, 55]. These flavonoids can be used individually or in combination with other synthetic drugs and antibiotics as therapeutic agents. These will produce cost effective, highly biocompatible and low toxicity of new generation phytopharmaceuticals [55]. On the other hand, luteolin is a glycosylated in plants, and the glycoside is hydrolysed to form free luteolin during adsorption. Plants rich in luteolin have been used as Chinese traditional medicine for hypertension, inflammatory diseases and cancer [53]. While, rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside), is a glycoside comprising of flavonolic aglycone quercetin together with disaccharide rutinose. It has been reported in many pharmacological activities, for example, antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities [56, 57]. Rutin is also used in the treatment of chylothorax in dogs [57].

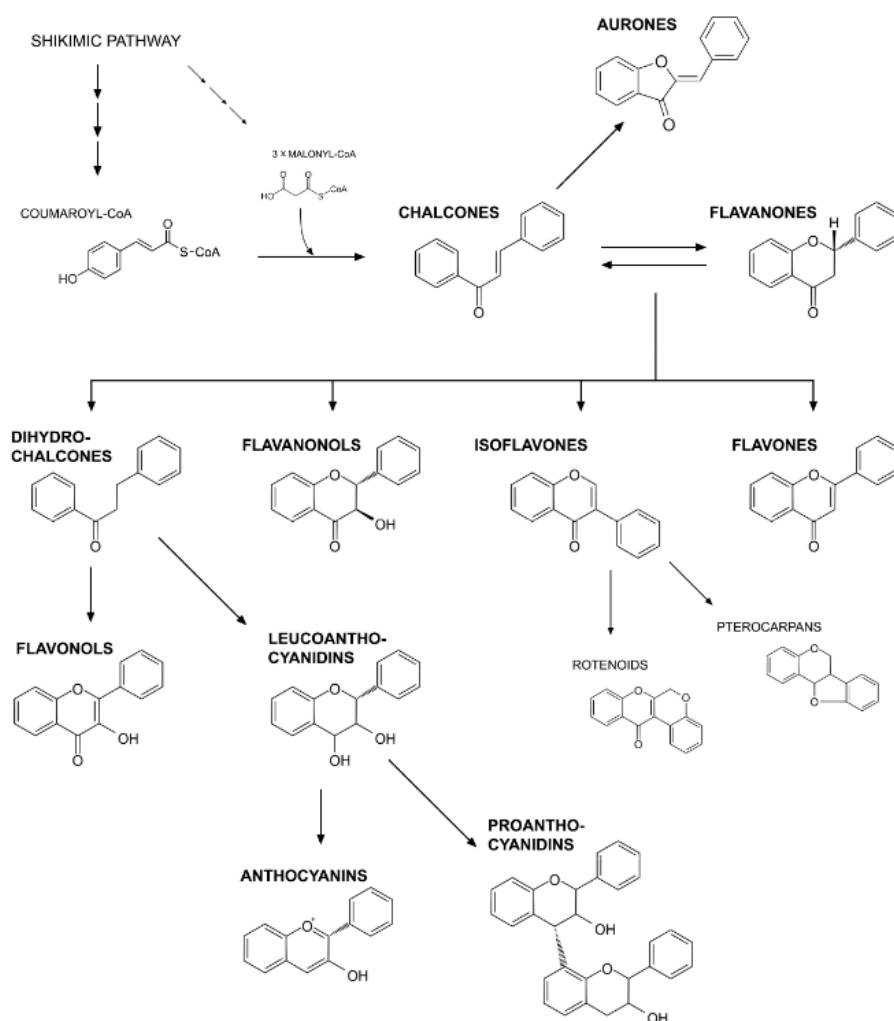


Figure 5: Their biosynthesis pathway is initiated with the condensation of one p-coumaroyl-CoA molecule with three molecules of malonyl-CoA. This is to produce chalcone (4', 2', 4', 6'-tetrahydroxychalcone) and catalyzed by chalcone synthase (CHS). The next step is isomerization of chalcone to flavanone through enzymatic reaction (chalcone isomerase-CHI). Onwards, the pathway branches to form several different flavonoid classes. These classes

include auronones, dihydrochalcones, flavanonols, isoflavones, flavones, flavonols, leucoanthocyanidins, anthocyanins and proanthocyanidins [51].

Quercetin give colors to plants, most often the brilliant citron yellow and this compound is insoluble in water but quite soluble in alcohol [32, 58]. Quercetin is 3,3',4',5,7-pentahydroxyflavanone or its synonym 3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one. It has hydroxyl group (OH) attached at positions 3,5,7,3', and 4'. Kaempferol is different from quercetin as it lacks of the OH group at position 3'. While, myricetin has an extra OH group at position 5' [58]. Furthermore, quercetin is an aglycone which is lacking of attached sugar [58]. In addition, quercetin is an aglycone which is lacking of attached sugar [58]. In food, quercetin is bounded with sugars, phenolic acids, alcohols etc. [58]. This compound with its derivatives has been studied for their pharmacological properties including, antiviral, antioxidant, anticancer, antimicrobial, anti-inflammatory, neurological effects, cardiovascular, and hepatoprotective [58]. While, kaempferol supplementing body's antioxidant defense against free radicals, which promote the development of cancer. At the molecular level, this compound could modulate a number of key elements in cellular signal transduction pathways. These are linked to apoptosis, angiogenesis, inflammation, and metastasis [59]. Kaempferol also significantly inhibits cancer cell growth and angiogenesis and induces cancer cell apoptosis. On the other hand, kaempferol appear stop reserve normal cell viability and in some cases exerting a protective effect [59].

2.2 Terpenoids

Santha Maria and co-workers [40] reported on the presence of terpenoid active compounds when conducting a screening on the ethanol extract of PS pods. In earlier specific study, terpenoid compounds were identified in PS seeds at different temperature and pressure using gas chromatography/time of flight mass spectrometry from the extraction by supercritical carbon dioxide: β -sitosterol and squalene were identified in all conditions studied, while campesterol, stigmasterol, stigmasterolmethylether and lupeol were identified in certain condition of temperature and pressure of supercritical fluid extraction (SFE) system [60]. In addition, the highest extracted yield was achieved at the smallest percentage of oil yield extracted at low pressure and high temperature conditions [60, 61]. Later, squalene, campesterol, stigmasterolmethylether, stigmasterol, stigmastan-6, 22-dien, 3, 6, 3dehidro-(3-sitosterol) were also identified in PS seeds by SFE in hexane extract [31]. In earlier study, stigmast-4-en-3-one was identified in PS empty pod and this compound is assumed as a necessary intermediate in the metabolism of β -sitosterol [30]. Similar study also showed that the hypoglycaemic effect of PS seed is due to the synergistic action of β -sitosterol and stigmasterol [62]. Some of terpenoids identified in PS were shown in Figure 6.

Terpenes/terpenoids constitute one of the largest classes of natural products that can arise from the biochemical transformation of the prenyldiphosphate, a polymer chain of prenyl units. [63]. Various lengths of this starter units are the backbones of all terpenes/terpenoids. The ionization of the diphosphate group, caused the remaining allylic carbocation intermediates to be coaxed into complex chemical cascades. Thus, will lead to diverse linear and cyclized hydrocarbon backbones [64]. These can then be further modified with a wide range of functional groups, for example, alcohol and ketones; and other substituent additions as well; sugars and fatty acids). Terpenoids can be

classified as hemiterpenes, monoterpenes (C₁₀), sesquiterpenes (C₁₅), diterpenes (C₂₀), sesterpenes (C₂₅), triterpenes (C₃₀), tetraterpenes (C₄₀) and polyterpenes (>C₄₀) based on the basis of C₅ units [63, 65, 66]. As these chemicals are diverse, they are widely used in the industry for flavors, fragrances, high grade lubricants, biofuels, agricultural chemicals and medicines [64].

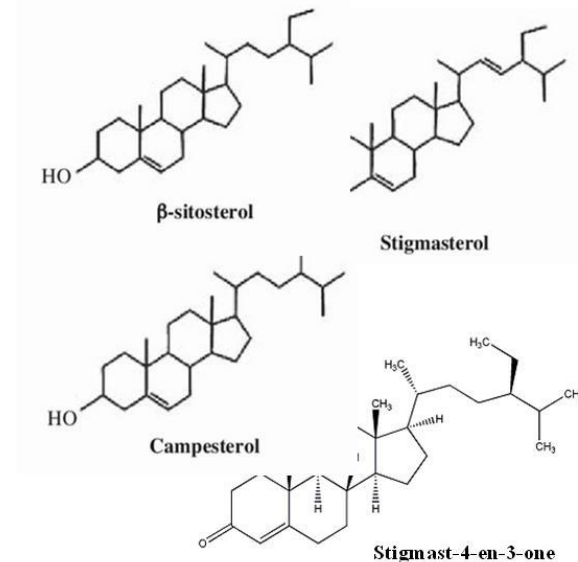


Figure 6: Sterols identified in PS seed and pods.

Terpenoids has numerous therapeutic properties including anticancer, antimicrobial, antifungal, antiparasitic, anti-allergenic, antihyperglycemic, anti-inflammatory and immune-modulatory properties [57]. β -sitosterol which identified in different extraction conditions in PS is among several terpenes under studies of clinical trials, to prevent and relieve prostate symptoms, to lower cholesterol and also suggested to help strengthening the immune system [66]. It is also usually used for treatment of rheumatoid arthritis, tuberculosis, cervical cancer and hair loss [68]. Another compound, the squalene is considered a potent chemo preventive and chemotherapeutic agent. This compound could inhibit the tumor growth in the colon, skin, lung and breast. It can also stimulate the immune system for the application of drugs in the treatment of diseases such as HIV, H1N1, leukemia, papilloma and herpes [69]. These compounds are dispensable for plant growth and development, but are indispensable for plant survival [63]. Those terpenoids have huge structural diversity, and intraspecific variability within plant taxa, even among genotypes. This enables the plants to secure individual habitats from natural enemies, competitors and friends [65].

2.3 Alkaloids

Alkaloids are other compounds identified when researchers screened phytochemicals in either the seeds, seed coat or pods/bark of PS [23-28]. Liliwirianis and co-workers [25] reported on the presence of alkaloid in the acidic aqueous phase of the PS seed, bark (pods) and its seed coat when conducted a screening on phytochemical in ulam and fruits from Malaysia. While Sihombing and co-workers [28] following similar methodology and reported the presence of alkaloids in PS peel samples. Current study by Ghasemzadeh and co-workers [24] reported the presence of alkaloids

in the ethanol extract of PS seeds collected from three states of Malaysia (Perak, Negeri Sembilan and Johor). Alkaloids were also determined in the methanolic extract [23, 27], ethanol and ethyl acetate extracts [23] of PS pods. While, Sonia and co-workers [26] analyzed seeds of PS in water, methanol and hydromethanolic extracts and denotes an average of alkaloids presence in the later extract compared to the other two extracts.

All analysis of alkaloids in PS identified currently was at screening or qualitative stage, none was conducted quantitatively. This is due to various chromatography methods need to be involved to isolate and purified the extract. In addition, identification of the isolated alkaloids was based on their spectral data and various spectroscopic methods [70]. Unless the study aimed to characterize these compounds in specific/selected plants is conducted as alkaloids vary in polarity and thermal stability. Their separation is still remaining a big challenge for the process of identification and characterization of the compounds. Their efficient isolation, determination and characterization process may need a combination of conventional chromatography methods (thin layer chromatography, column chromatography, etc.) and instrumentation technologies (CTLC, HLPC, LCMS etc.) [71]. Major methods for characterization of alkaloids involved spectroscopic with MS and NMR or in combination with chromatographic methods [70]. Besides that, non-chromatography techniques alone, such as immunoassay, screening assay and fourier-transform infrared spectroscopy (FTIR) is been used to obtain and facilitate the identification process [72].

Nitrogen atom in the heterocyclic ring of alkaloids are derived from amino acids. The main role of alkaloids in plants toxicity is to protect against predators and pathogens [73]. These compounds have been classified into different categories based on their biosynthetic precursor and heterocyclic ring system. Different types of alkaloids are inclusive indole, tropane, piperidine, purine, imidazole, pyrrolizidine, pyrrolidine, quinolizidine and isoquinoline alkaloids [70, 73, 74]. Different alkaloids have their own specific properties and act useful for the medicinal purposes. For example, indole alkaloids associated with about 2,000 compounds inclusive vinblastine and vincristine, which are often used as anticancer drugs. Scopolamine, hyoscyamine, cocaine and atropine are among tropane alkaloids are known to have anticholinergic activities. Whereas, quinoline possessed important biological activities viz. antimalarial, antibacterial, antifungal anthelmintic, cardiotonic, anticonvulsant, anti-inflammatory and analgesic activity. Isoquinoline exhibit biological activities like antihyperglycemic, antitumor and antibacterial activity and etc. [70]. Pure extracted plant alkaloids and their synthetic derivatives are utilized as common medicinal ingredients for their analgesic, antispasmodic, bactericidal effects and some alkaloids are used as an antiseptic due to its antibiotic activity [74]. Recent review focused on the potential efficacy of plant-derived alkaloids which possesses therapeutic effects against several neurodegeneratives disorder (NDDs) by attenuating the development of NDDs through their vast mode of action. These actions may inhibit the activity of acetyl-cholinesterase (AChE) enzyme or increasing the level of gamma-aminobutyric acid (GABA) or acting as antagonist of NMDA and many more [75].

2.4 Saponins

Determination of saponins in PS also involved only screening or qualification phases in all studies identified. Phytochemical screening of PS revealed saponins present in peels/pods of PS [23, 27, 28], seed extract [26, 67] and

its seed coats [25]. In most studies, saponin was present in methanolic extracts, only few studies reported on positive identification of these compounds in ethanolic, n-hexane and ethyl acetate extracts [23, 26, 27, 28, 66].

Foam test is a qualitative standard protocol for detection of saponins [26]. This natural detergent is made from a combination of hydrophobic aglycone backbone and hydrophilic sugar molecules that allows foaming and emulsifying properties of the compounds [75, 76]. Saponins with one sugar chain have the best foaming characteristics compared to with two or three chains, though saponins without foaming character have also been observed [76]. Saponins consist of a glycan moiety linked to aglycon or genin or sapogenin (triterpenoid saponins with 30 carbon atoms) and steroidal saponins (27 carbon atoms with a 6-ring spirostane or a 5-ring furostane skeleton) (Figure 7) [75]. Based on the carbon skeleton of the aglycon, saponins are sometimes further classified into 12 main classes (dammaranes, tirucallanes, lupanes, hopanes, oleananes, 23-nor oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes, and steroids) [75]. Plant-derived triterpenoid and steroidal saponins have been used in the production of steroid hormones in the pharmaceutical, food additives, fire extinguishers and other industries. Other interesting biological applications include their use in anti-inflammatory, hypocholesterolemic, immune-stimulating remedies, cytotoxicity, antitumor, antimutagenic, antiviral, cardiac activities, hyperglycemic/hypolipidaemic activities, antifungal/antimicrobial, etc. [76-78].

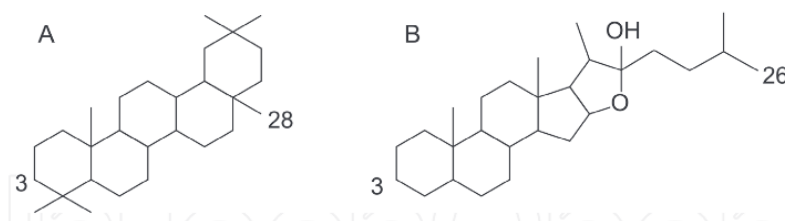
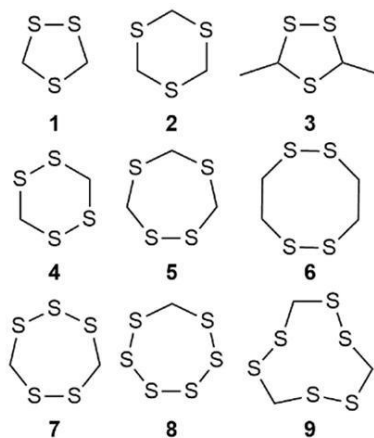


Figure 7: Structures of (A) triterpenoid and (B) steroidal saponins [76].

2.5 Cyclic polysulfides/organosulfur compounds

Cyclic polysulfides/organosulfur compounds which were found in plant seeds are accountable for a significant pungent smell and taste. Earlier studies indicated that 1,2,4-trithiolane, 1,2,4,6-tetrathiane, lenthionene, 1,2,4,5,7,8-hexathionine and 1,2,4,6,7- or 1,2,4,5,7-pentathiocanewere the major constituents from a chloroform extract of PS crushed beans that incubated in water [9] (Figure 8). Later, Suvachittanont and co-workers [79] reported on the formation of thiazolidine-4-carboxylic acid, a thioproline was significantly increased after boiling of PS seeds. In year 2001, Miyazawa and Osman [80] reported on the abundance of hydrogen sulfide in the headspace constituent of PS. Salman and co-workers [60] also reported on the present of 1,2,4,5-tetrathiane and 1,2,3,5,6-pentathiepane (lenthionene) in PS seeds.



(1) 1,2,4-trithiolane; (2) 1,3,5-trithiane; (3) 3,5-dimethyl-1,2,4-trithiolane; (4) 1,2,4,5-tetrathiane; (5) 1,2,4,6-tetrathiepane; (6) 1,2,4,5-tetrathiocane; (7) 1,2,3,5,6-pentathiepane (lenthionine); (8) 1,2,3,4,5,6-hexathiepane; (9) 1,2,4,5,7,8-hexathionane.

Figure 8: Cyclic organosulfide from PS [92].

Frèrot and co-workers [81] reported that sulfur compounds make up the vast majority of the identified compounds and was classified into three categories: cyclic polysulphides, linear polysulphides and hetero sulphur compounds. 1,2,4-trithiolane was the major component for cyclic polysulphides followed by 1,2,4,6-tetrathiepane, 1,2,3,5-tetrathiane and lenthionine. They also reported for the first identification of 2,4-dithiapentane, 2,3,5-trithisixane and 2,4,6-trithiaheptane in cooked beans of PS (Figure 8). Other cyclic polysulfides that have been identified in PS were 1,2,3,4,5-pentathiane, 1,3,5,7-tetrathiocane, 1,2,3,4,6-pentathiepane, 1,2,3,4,5,6-hexathiepane, 1,2,3,4,6,7-hexathiocane or 1,2,3,5,6,7-hexathiocane, isomerix hexathionanes, 1,2,4,6-tetrathiepane, methyl-1,2,4,5-tetrathiane and 3-methyl-1,2,4-trithiolane [81]. Linear polysulphides identified in PS seeds were 1,3-dithiabutane, 1,3,5-trithiahexane, 2,4-dithiapentane, 2,5,7-trithiaheptane 2,4,5-trithiahexane. 1,3-dithiabutane is a strong odorant that possessing an incredible strong alliaceous note formed via unstable methanedithioles [81, 82]. Sulphur compounds containing other heteroatoms identified in this study were 4,5-dihydrothiazole, 2,5-dihydrothiazole and 2,3-dihydrothiazole [81].

In a current study, thirteen sulphurous compounds were identified in unripe, mid-ripe and ripe PS, where during ripening these compounds increased from 7.1 to 20.13%. Major components are 1,2,4-trithiolane, methanethiol and hydrogen sulfide [83]. Djenkolic acid identified in PS is formed from the dithiocetal (S-C-S), might be formed from formaldehyde and two molecules of cysteine [81]. Blockage of urinary tubules is connected to low solubility of djenkolic acid in acid condition subsequently precipitate as crystals which can cause pain, haematuria and sometimes death to human [84]. This is especially so when ingestion of djenkol beans that contain large amount of the djenkolic acid, in the range of 0.3-1.3 g/100g wet weight or 93% of acid exist in a free state [85]. In traditional medicine, PS has been used for antibacterial and antifungal effects on kidney, ureter and urinary bladder and later was found to be the effects from cyclic polysulfides [84]. PS is also suggested as potential functional food with H₂S donation property where 1,2,4-trithiolane slowly release H₂S donor through reaction with glutathione. During this

reaction, the thiolate attacks the carbon which lead to an opening of the trithiolane ring. This resulting in conjugation of H₂S and several glutathione-methylene-sulphides [86].

2.6 Tannin

Tannins are undesirable compounds when plant-parts are consumed as food but they are useful as a protection from biotic-stress and are also important in reinforcing plant tissues [87]. Tannins are polyphenolic secondary metabolites of higher plants. They were divided into four groups based on their structural characteristic: Gallotannins, ellagitannins, complex tannins and condensed tannins. Gallotannins is comprises of polyphenolic and a polyol residue, the simplest hydrolysable tannins. Most of these compounds contain a polyol residue derived from D-glucose. Ellagitannins are derived from gallotannins by the oxidative coupling of at least two galloyl units. Complex tannins are a combination of gallotannin or an ellagitannin unit and a catechin unit. While, condensed tannins are oligomeric and polymeric proanthocyanidins that consist of coupled flavan-3-ol (catechin) units [88] (Figure 9).

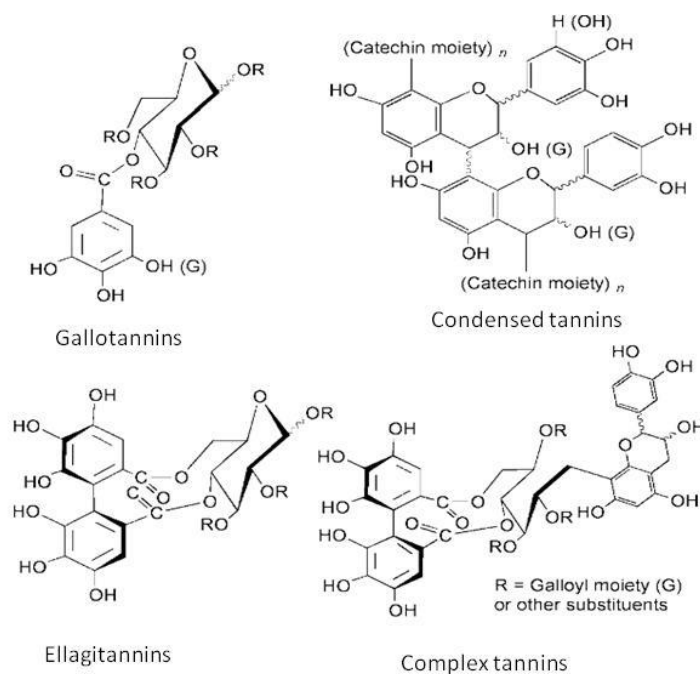


Figure 9: Classification of tannins [88].

In PS, phytochemicals inclusive tannins were screened from the plant extracts using standard procedure, the ferric chloride test [26]. This is a simple method for tannin quantifications as these compounds are differ in their sensitivity and specificity. Due to this fact, condensed tannins are the easiest and safest tannin group to be quantified, since the spectrophotometric HCl–butanol assay is highly specific to this group. The test depolymerised plant condensed tannins into colourful monomeric units that are then detected and quantified at approximately 550 nm [89]. Balaji and co-workers [27] reported on positive identification of tannins in methanolic extracts of PS, which is contradicted to findings from Chanu and co-workers [67] where identification of tannins were in aqueous extracts only. Hasim and co-workers [23] reported on positive identification of tannins in all three extracts tested (n-hexane, ethyl acetate and ethanol) with abundance identification in 70% of ethanol extracts. While Sonia and co-

workers [26] denoted an average identification of tannins in hydromethanolic extract compared to the aqueous and methanol extract.

Higher amount of tannins was identified in antimicrobial herbal which selected from the vegetation in Northern Brazil compared to antidiarrheal and hypoglycemia and/or antidiabetic activities [90]. Tannins have been a target compound in many studies on antioxidant and anti carcinogens activities and there is ample proof of their anti-inflammatory, cicatrizant and anti-HIV functions [87].

3. Conclusion

Parkia speciosa, or stink bean, is a Malaysian favourite culinary ingredient. The seed of *PS* contains important chemical and medicinal properties including its pods that always been discarded as a waste. In this present paper, we have reviewed the relevant literature to congregate the phytochemicals secondary metabolites and pharmacological information on *PS*. *PS* seeds and its pods are rich in biologically active compounds such as phenolic acids, flavonoids, alkaloids, saponins, terpenoids, cyclic polysulfides and tannins. Phenolic constituents specifically, the gallic acid is the most abundant phytochemicals identified in *PS* pods. This is followed by the catechin, ellagic acid and quercetin. The terpenoids, β -sterol, squalene, campesterol and stigmasterol were identified in *PS* seeds. Cyclic polysulfides, compounds that responsible for a strong pungent smell and taste in *PS* were also identified in *PS* seeds, with 1,2,4-trithiolane being the most abundant compound identified.

Other cyclic polysulfides which were also among most identified in *PS* seeds were 1, 2, 4, 6-tetrathiepane, 1, 2, 3, 5-tetrathiane and lenthionine. Alkaloids, saponins and tannins were only reported in qualitative phytochemical screening of *PS* in all articles reviewed. The alkaloids and saponins were reported to be abundant in the methanolic extracts but tannins were in ethanol extracts. These phytochemicals are beneficial in terms of antioxidant activity, hypoglycemic activity, antitumor/antimutagenicity, antimicrobial activity and current research showed some evidence of their effects on the cardiovascular system. Many scientific studies have been performed to prove on antioxidant, hypoglycemic activity as well as antitumor/antimutagenicity by different model either *in vitro* or *in vivo*. The studies also revealed that plants with high antioxidant activity contained high total phenolic and flavonoid compounds while for hypoglycemic effect is from the synergistic effect of terpenoid compounds; the β -sterol and stigmasterol. The antimicrobial properties is possesses from both the seed and pod of *PS*, however, the spectrum of the activity depends on the type of the extract.

Acknowledgement

We would like to thank the Director General of Health Malaysia for his permission to publish this Chapter. We are also grateful to the Director of the Institute for Medical Research (IMR), Kuala Lumpur, for the continuous support and encouragement. We thank Ms Sumarni Mohd Ghazali from Epidemiology and Bio-Statistic Unit, IMR for editing and proof reading the text.

Author Contributions

Nurul Izzah Ahmad and Salina Abdul Rahman drafted the manuscript; Leong Yin Hui and Nur Hayati Azizul edited and commented on the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interests.

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Citation: Nurul Izzah Ahmad, Salina Abdul Rahman, Yin-Hui Leong, Nur Hayati Azizul. A Review on the Phytochemicals of *Parkia Speciosa*, Stinky Beans as Potential Phytomedicine. Journal of Food Science and Nutrition Research 2 (2019): 151-173.



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