

Stereospermum fimbriatum as a Potential Source of Phytochemicals: A Review of *Stereospermum* Genus

Anis F.I. Awang¹, Sahena Ferdosh², Md. Zaidul I. Sarker^{1*}, Hassan I. Sheikh³, Kashif Ghafoor⁴ and Kamaruzzaman Yunus³

¹Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia, Kuantan Campus, 25200 Kuantan, Pahang, Malaysia; ²Department of Plant Science, Faculty of Science, International Islamic University Malaysia, Kuantan Campus, 25200 Kuantan, Pahang, Malaysia; ³Department of Biotechnology, Faculty of Science, International Islamic University Malaysia, Kuantan Campus, 25200 Kuantan, Pahang, Malaysia; ⁴Department of Food Science and Nutrition, King Saud University, Riyadh 11451, Saudi Arabia

Abstract: *Stereospermum fimbriatum* is one of the medicinal plants that has been claimed to be used traditionally to treat several illnesses such as stomachache, earache, skin irritation and postpartum illness. The genus of this plant is known to possess medicinal properties in every part of the plant. Therapeutic potential of *S. fimbriatum* is anticipated based on numerous previous studies that documented variety of phytochemical contents and bioactivity of the genus. The most reported bioactivities of its genus are antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, anti-diarrheal and analgesic activities. *S. fimbriatum* is a rare species that has not been discovered yet. Thus, this review aims at highlighting the potentials of *S. fimbriatum* by collecting available data on the bioactivities of its genus and set the directions for future research on this plant.



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1. INTRODUCTION

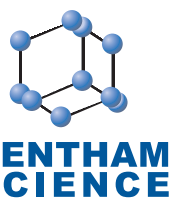
Stereospermum fimbriatum (Fig. 1a) belongs to the Bignoniaceae family which is a well-known ornamental trees, shrubs and climbers with different colours of funnel-shaped flowers ranging from a showy orange, yellow or purple to pale pink or white. The medium-sized plant could reach up to 27 m high and 150 cm in diameter [1]. It had a light grey, cranny and scaly bark (Fig. 1b) with pinnate leaves of 4 to 6 pairs of elliptic leaflets covered by yellowish sticky hairs (Fig. 1c). The arrangement of flowers on its shoot or the inflorescence was a type of terminal panicle [2]. The flowers were hairy outside and glabrous inside (Fig. 1d). In a dry weather, the tree shedding its leaves and the flowers started to bloom from the month of February until June.

The “stereos” prefix is a Latin word that means firm or solid, while the “sperm” means seed. The species name “fimbriae” describes the deeply fringed white to pale-pink

corolla lobes. *S. fimbriatum* is locally called as “Chicha” or “lempoyan” and also known as a snake tree due to its long coiled fruits (Fig. 1e). The habitat of this species is often lowland and hill forest. It is widely distributed in Peninsular Malaysia specifically in Kelantan, Terengganu, Kedah, Malacca, Langkawi, and Tioman Island. This plant is also found in Myanmar, Laos, and Sumatra [3]. Traditionally, different parts of *S. fimbriatum* were used as a remedy for several illnesses. The shoot and root of this plant were decocted to cure stomachache and postpartum illness, respectively [3]. Meanwhile, the leaves were crushed until become juicy to cure earache and also pounded with lime to apply on itchy skin [2]. Based on local people in Malaysia, the dried flower was used in their dessert as a flavor.

Recently, the use of medicinal plants for drug discovery was remarkably increased in order to meet the pharmaceutical need of new effective drugs. However, the discovery of plant-based drugs faces the problem of having limited resources due to cutting down of plants, mainly for its wood. Therefore, scientists have come up with solutions to chemically synthesize the isolated compounds derived from natural resources for large scale [4]. Furthermore, the structures of these isolated compounds are improved to increase their

*Address correspondence to this author at the Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia, Kuantan Campus, 25200 Kuantan, Pahang, Malaysia; Tel/Fax: +609-5704000 (Ext: 4841); E-mail: zaidul@iiu.edu.my



bioavailability and reduce their toxicity. The medicinal plants are also cultivated as one of the agro-technology approach to generate and conserve their valuable therapeutic properties [4]. As for *S. fimbriatum*, even though it is a rare plant species, its promising traditional values should not be neglected and must be promoted to unleash its undiscovered therapeutic potential so that new chemical entities can be identified for the development of plant-based product. Therefore, this review aims at highlighting the potential of *S. fimbriatum* as a new source of medicinal compounds based on the great and already proven potential of its related species and traditional uses. The reported studies on *Stereospermum* genus were reviewed particularly in terms of their phytochemicals content and the bioactivities.



Fig. (1a). *S. fimbriatum* tree.



Fig. (1b). Stem bark of *S. fimbriatum*.



Fig. (1c). Leaves with coiled fruit of *S. fimbriatum*.



Fig. (1d). Flower of *S. fimbriatum*.

2. SCREENING AND EXTRACTION OF PHYTOCHEMICALS COMPOUNDS FROM *STEREOSPERMUM* GENUS

Plant's pharmaceutical properties are derived from specific parts of a plant species. The phytochemicals found in leaves, stem barks, and roots of *Stereospermum* genus such as alkaloids, tannins, saponin, flavonoids, coumarins, anthroquinones, phenols, terpenoids, terpenes, and sterols are summarized in the Table 1 [5-17]. Phytochemical contents of closely related species or from the same genera and family mostly produce similar chemical constituents or secondary metabolites. Different plant parts may provide several phytochemicals in different amount owing to their distinctive gene expression in synthesizing the compounds [18]. There were various phytochemical contents accumulated in different plant parts of genus *Stereospermum*. However, flavonoid, saponin and tannin seemed to be the most abundant phytochemicals reported in all species of *Stereospermum*. The

Table 1. Phytochemicals screening of genus *Stereospermum*.

Species	Leaf		Stembark/bark		Root		References
	Extract	Phytochemicals	Extract	Phytochemicals	Extract	Phytochemicals	
<i>S. kunthianum</i>	PE	Sterols/triterpenes, coumarins.	-	-	-	-	[5]
	Aqueous	Antraquinone, sterols, flavonoids, terpenes, phenolic nucleus, tannins.	-	-	-	-	[6]
	Methanol	Alkaloids, saponin, flavonoids, tannins.	-	-	-	-	[7]
	-	-	Aqueous	Alkaloids, tannins, saponins, phlobatannins, cardiac glycosides, anthracene derivatives.	-	-	[8]
	-	-	Ethanol	Tannins, saponins, flavonoids, phlobotannins, terpenes, sterols and terpenes.	-	-	[9]
			^a PE ^b EtOAc fraction of ethanol	(Tannins, flavonoids, saponins) ^{a, b} , terpenoids ^a			
<i>S. colais</i>	-	-	-	-	Methanol	Phytosterols, saponins, and flavonoids.	[10]
	^b EtOAc ^c CF ^d Methanol ^f Hexane	(Flavonoids, alkaloids, quinones, cardiac glycosides, terpenoids, steroid, triterpenoids) ^f , tannins ^{b, c, d} , coumarin ^{c, d}	-	-	-	-	[11]
	^b EtOAc ^c CF ^e Ethanol ^f hexane ^g Aqueous	(Tannins, phenols, iridoid & flavonoids) ^{b, e, g} , terpenoids ^{b, c, f} , saponin ^{e, g} , cardiac glycosides ^{b, e} , steroids ^{f, c}	-	-	-	-	[12]
	-	-	-	-	^b EtOAc ^e Ethanol	(Flavonoids, tannins, phenols, glycosides) ^{b, e} , saponins ^e	[13]
	-	-	-	-	^a PE ^b EtOAc ^c CF ^e Ethanol	Terpenoids ^a , (steroids, anthroquinones, quinones) ^{d, e} , saponin ^e , glycosides ^{b, c, e} , (flavonoids, tannins, phenols) ^{c, e}	[13]
<i>S. suaveolens</i>	-	-	-	-	-	-	[14]
	-	-	EtOAc fraction of ethanol	Flavonoids, tannins, alkaloids, saponins, glycosides.	-	-	[15]
	-	-	Methanol	Alkaloids, phenols, saponins, flavonoids, tannins	-	-	[16]
<i>S. chelonoides</i>	Methanol	Alkaloid, tannin, glycoside, flavonoid, steroid.	Methanol	Alkaloid, tannin, saponins, flavonoid, steroid.	-	-	[16]
	-	-	-	-	Methanol	Saponins, flavonoids, glycosides.	[17]
<i>S. tetragonum</i>	-	-	-	-	Methanol	Saponins, flavonoids.	[17]
<i>S. acuminatis-simum</i>	Aqueous	Antraquinone, sterols, flavonoids, terpenes, tannins, resins, phenolic nucleus.	-	-	-	-	[6]

^a: PE=Petroleum ether; ^b: EtOAc=Ethyl acetate; ^c: CF=Chloroform; ^d: Methanol; ^e: Ethanol; ^f: Hexane; ^g: Aqueous.

phytochemical contents in leaf, bark and root of genus *Stereospermum* were extracted in higher amounts using polar solvents especially methanol. Similar observation on methanol efficiency in extracting bioactive compounds was also reported in a study by Iloki-Assanga *et al.* [19] whereby methanol extract yielded higher amount of flavonoid, phenolic and tannin compounds in Toji Oak as compared to other solvent. Thus, the extraction of phytochemicals must consider a suitable solvent with respect to their polarity to optimize the extraction of targeted compounds.

Bioactive compound can be defined as a substance that is originated either from nature or synthetic source and has a broad range of possible biological activity [20]. The bioactive compounds from genus *Stereospermum* had been extracted with several methods such as maceration, soxhlet, percolation and decoction. The fractionation and isolation of the compounds were done previously using Vacuum Liquid Chromatography and Gravity Column Chromatography, Preparative Thin Layer Chromatography (TLC) and Preparative High Performance Liquid Chromatography (HPLC). A number of studies that isolated the bioactive compounds from genus *Stereospermum* have been reviewed and summarized in Table 2 [21-31]. From these documented studies, a great numbers of compounds had been isolated from different parts of various *Stereospermum* species especially from the stembark with maceration as the most employed method of extraction. This finding indicates that the stembark of *Stereospermum* genus contained the highest sources of bioactive compound as compared to the other plant parts.

3. BIOACTIVITY OF GENUS STEREOSPERMUM

The species under *Stereospermum* genus are known for various bioactivities led by their traditional uses such as to cure toothache, bronchitis, ulcers, cough, gastritis, leprosy, diarrhea [32-34], fever, indigestion [35-36], and wound healing [37]. The most studied species included *S. kunthianum*, *S. colais*, *S. suaveolens*, *S. chelonoides*, *S. personatum*, *S. tetragonum* and *S. acuminatissimum*.

3.1. Antimicrobial Activity

Antimicrobial screening is one of the most crucial fields among other bioactivities. The antimicrobial activity of genus *Stereospermum* has been previously reported from different parts of the plant against multiple microbes' types and strains including Gram-positive and Gram-negative bacteria as well as fungi. The antimicrobial activity of *Stereospermum* species is summarized in Table 3 [38-45].

S. kunthianum was among the most studied species with different extraction methods and tested doses. A recent study reported by Kothai [38] showed synergistic activity upon combination of *S. kunthianum*'s ethanolic stem extract (100mg/mL) with brown honey and white honey at 1:1 ratio against *Streptococcus pyogenes* (ATCC19615). There was also synergistic effect observed when the ethanol extract was combined with honey (brown or white) and cinnamon at 1:1:1 ratio but antagonistically when combined with cinnamon only at 1:1 ratio. However, previous report of the ethanolic extract [39] showed less activity with higher value of

Minimum Inhibitory Concentration (MIC) when tested alone against the same strain which was 25 mg/mL as compared to the recent study with MIC value of the combination less than 1 mg/mL [38]. This observation suggested that the antimicrobial activity can be increased upon selective combination of *S. kunthianum* extract with brown and white honey.

Meanwhile, a study done by Tor-Anyiin and Anyam [9] investigated the activity of *S. kunthianum*'s stembark extract on different strains of bacteria which were *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. The finding demonstrated a weak inhibition against tested strains at 150 mg/mL by ethanolic crude extract and ethyl acetate fraction. According to a report by Van Vuuren and Viljoen [40], antimicrobial activity of water extract from *S. kunthianum*'s stem was observed in the MIC test against two yeasts, namely *Candida albicans* and *Cryptococcus neoformans*, and six bacterial strains viz., *Bacillus cereus*, *S. mutans*, *Lactobacillus acidophilus*, *S. aureus*, *Enterococcus faecalis* and *Klebsiella pneumonia* except against *E. coli*. Nevertheless, in a previous report by Adamu and colleagues [41], water extract of *S. kunthianum*'s bark (200 mg/mL) had demonstrated weak inhibition activity in an agar well diffusion assay against *E. coli*, *S. aureus* and *Proteus mirabilis*.

An experimental result obtained from the leaves of *S. kunthianum* however demonstrated good antimicrobial activity against wide spectrum of bacteria such as the antibacterial activity of methanol extract against *E. faecalis*, *S. aureus*, *K. pneumoniae*, *P. mirabilis*, *E. coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, and *Enterobacter aerogenes* at 100 mg/mL with the range of inhibition zone between 19-27 mm [7]. Petroleum ether extract of *S. kunthianum*'s leaves also showed significant antibacterial activity (9-35 mm) against clinical isolates viz., *P. aeruginosa*, *S. aureus*, *E. coli*, *Salmonella* spp., *Aeromonas hydrophila* and *Klebsiella* spp at 30 mg/mL [5].

Haque *et al.* reported that stembark of *S. chelonoides* was fractionated into *n*-hexane and chloroform fractions and tested their activity. At concentration of 250 µg/disc, both fractions were active (12-21 mm) against Gram-positive *B. cereus*, *B. megaterium*, *B. subtilis*, *S. aureus*, *Sarcina lutea*, Gram-negative *E. coli*, *P. aeruginosa*, *S. paratyphi*, *S. typhi*, *S. boydii*, *S. dysenteriae*, *Vibrio mimicus*, *V. parahemolyticus* and fungi *C. albicans*, *Aspergillus niger* and *Sacharomyces cerevaceae* [35]. Years later, a study was conducted with the different method of extraction and tested against the same strains, but using the stembark from different species which was *S. personatum*. In this study, methanol crude extract, petroleum ether (PE), carbon tetrachloride (CT), and chloroform (CF) fractions were tested against selected strains at 400 µg/disc whereby PE and CF fractions were active against all tested strains (8-13 mm) [42]. Overall, the active fractions of *S. chelonoides* were observed to have stronger antimicrobial activity especially against fungal strains even using lower concentration compared with the active fractions of *S. personatum*.

Successive extraction of *S. acuminatissimum*'s leaves was performed to obtain hexane, ethyl acetate, methanol and water extract. An antimicrobial screening test done using agar dilution streak technique demonstrated activity at concentration

Table 2. Extraction and isolation of compounds from genus *Stereospermum*.

Species	Extraction Method	Plant's Part	Isolated Compounds	References
<i>S. suaveolens</i>	Decoction	Root	Cycloolivil, lapachol and β -sitosterol	[21]
	Maceration	Leave and bark	Fridelin (1), β -sitosterone (stigmast-5-en-3-one) (2), stigmasterol (3), 3,4-dimethoxyciscaffeic acid (4), 3 β -friedelanol (5), β -amyrone (6) and glyceryl tricaprata (7).	[22]
<i>S. colais</i>	Cold Extraction	Root	β -sitosterol (1), 2-(4'-hydroxyphenyl) ethyl undecanoate (2), 2-(4'-hydroxyphenyl)ethyl pentadecanoate (3), 5 α -ergosta-7,22-dien-3 β -ol (4), ursolic acid (5), lapachol (6), and pinosresinol (7).	[23]
<i>S. acuminatissimum</i>	Maceration	Stembark	1,3,7-trimethylguanin-1/3-ium (1), 3,7-dimethylguanin-1/3-ium (2), 2-(4-hydroxyphenyl)ethyl hentriacontanoate (3), sterequinones A, F, and H (4, 5, 6), zenkequinones A-B (7, 8), p-coumaric acid (9), methyl caffeate (10), caffeic acid (11), psilalic acid (12), syringaldehyde (13), norviburtinal (14), specioside (15), verminoside (16), tyrosol (17), eutigoside A (18), ellagic acid (19), atranorin (20) and ursolic acid (21).	[24]
	Percolation	Stembark	2-(4'-hydroxyphenyl)ethyl dotriacontanoate (1), octacosan-1,28-dioldiferulate and triacontan-1,30-dioldiferulate (2), ursolic acid (3), pomolic acid (4), quinovic acid (5), oleanolic acid (6), (+)-cycloolivil (7), paulownin (8), methyl trans-ferulate (9), coniferaldehyde (10), (E)-methyl 3-(4'-hydroxyphenyl)acrylate (11), 2-(4'-hydroxyphenyl)ethyl undecanoate (12), 2-(4'-hydroxyphenyl)ethyl nonacosanoate (13), 2-methoxy-4-[3',3'',4'',5''-trimethoxyphenyl]allyloxymethyl]phenol (14), pinnatal (15), stereochoenol B (16), sterekunthal B (17), sterequinone B (18), sterequinone F (19), sterequinone H (20), sterequinone A (21), sterequinone E (22), zenkequinone B (23), zenkequinone A (24), sterequinone C (25) and norviburtinal (26).	[25]
<i>S. kunthianum</i>	Maceration	Stembark	Stereospermiside, Stereostin, (3, 4-dihydroxyphenyl)-ethyl-O- α -rhamnopyranosyl (1 \rightarrow 3)-4-O cinnamoyl- β -D-glucopyranoside	[26]
	Maceration	Root bark	Sterekunthals A and B, pyranokunthones A and B, anthrakunthone, pinnatal	[27]
	Soxhlet	Leave	15 α hydroxyolean - 12-en-3-one	[28]
<i>S. cylindricum</i>	Decoction	Leave and branch	(+)-cycloolivil (1), (+)-cycloolivil 6-O- β -D-glucopyranoside (2), (+)-cycloolivil 4'-O- β -D-glucopyranoside (3), (-)-olivil (4), (-)-olivil 4-O- β -D-glucopyranoside (5), (-)-olivil 4'-O-p-glucopyranoside (6), vanilloside (7), decaffeoyl-verbascoside (8), isoverbascoside (9), 3,4,5-trimethoxyphenyl 1-O- β -D-xylopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (10), ajugol (12), verminoside (13), specioside (14), stereospermiside (15)	[29]
<i>S. chelonoides</i>	Maceration	Stembark	Stereochoenols A and B, sterekunthal B, and sterequinone C	[30]
<i>S. personatum</i>	Soxhlet	Stembark	Sterequinone A, B, C, D and E, Sterekunthal B	[31]

of 2.0 mg/ml by hexane against *B. subtilis*, *S. aureus* and *S. typhi* and methanol extract against *P. aeruginosa* and *S. aureus* only [6]. According to Sob et al. [25], twenty-five compounds were isolated from this species but from different part of the plant which was stembark. The MIC test showed various degree of antifungal activity (25-150 μ g/ml) against different strain of *Candida* such as *C. albicans* ATCC 24433, *C. albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

The antimicrobial activities of two new anthraquinones, Zenkequinones A and B isolated from *S. zenkeri* stembark were investigated along with two known compounds (sterequinone F, p-coumaric acid) against *S. aureus*, *B. subtilis*, *B. megaterium*, *E. coli*, *P. aeruginosa*, and *P. vulgaris*. *P. aeruginosa* was the most sensitive strain with the lowest MIC value of Zenkequinones B, 9.50 μ g/mL [43]. As for *S. colais*, n-hexane, chloroform, ethyl acetate, ethanol and

water extract of the leaves were tested on multiple strain of bacteria as well as fungi. All extracts were active (8-40 mm) against tested bacteria strain viz., *Coagulase-negative staphylococci*, *Enterococci*, *S. aureus*, and *Acinetobacter*, *Citrobacter*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi* and *S. paratyphi A*. However, for antifungal activity, only ethyl acetate, aqueous and n-hexane extract were active against *A. flavus*, *A. fumigates*, *A. niger* and *C. albicans* [44].

Overall, *Stereospermum* genus possesses broad-spectrum antimicrobial activity which includes Gram-positive, Gram-negative bacteria and fungi as well. All cited literature in this review on antimicrobial activity of *Stereospermum* genus has been reported either from the stembark or the leaves. In terms of species, *S. kunthianum* inhibited wider range of pathogens compared to another species. Stembark extracts from various species inhibited at least 27 different pathogens, while leaf extracts inhibited the growth of at least

Table 3. Antimicrobial activity of genus *Stereospermum*.

Plant name	Plant's part	Types of extract	Microorganism	Reference
<i>S. kunthianum</i>	Leaf	Petroleum ether by soxhlet	Gram positive <i>S. aureus</i> Gram-negative <i>P. aeruginosa, E. coli, Salmonella</i> spp, <i>A. hydrophila, Klebsiella</i> spp	[5]
	Leaf	Hexane, ethyl acetate, methanol and water by successive extraction	Gram positive <i>S. aureus, B. subtilis</i> Gram-negative <i>P. aeruginosa, S. typhi, E. coli</i> Fungi <i>C. albicans</i>	[6]
	Leaf	Methanol	Gram positive <i>S. aureus, E. faecalis</i> Gram negative <i>K. pneumoniae, P. mirabilis, E. coli, A. baumannii, P. aeruginosa, C. freundii, E. aerogenes</i>	[7]
	Stembark	Ethanol & ethyl acetate	Gram positive <i>S. aureus</i> Gram negative <i>E. coli, S. typhi</i>	[9]
	Stembark	Ethanol	<i>S. pyogenes</i>	[38-39]
	Stem	Methanol/ dichloromethane (1:1) & water	Gram positive bacteria <i>B. cereus, S. mutans, L. acidophilus</i> and <i>S. aureus</i> Gram negative bacteria <i>E. coli</i> (inactive for water extract) and <i>K. pneumonia</i> Fungi <i>C. albicans, C. neoformans.</i>	[40]
	Bark	Aqueous	Gram positive <i>S. aureus</i> Gram-negative <i>P. mirabilis, E. coli</i>	[41]
<i>S. chelonoides</i>	Stembark	<i>n</i> -hexane & chloroform fractions	Gram positive <i>B. cereus, B. megaterium, B. subtilis, S. aureus, Sarcina lutea</i> Gram-negative <i>E. coli, P. aeruginosa, S. paratyphi, S. typhi, Shigella boydii, S. dysenteriae, Vibrio mimicus, V. parahemolyticus</i> Fungi <i>C. albicans, A. niger, S. cerevacaee.</i>	[42]
<i>S. personatum</i>	Stembark	PESF - Pet ether soluble fraction of methanolic extract, CTSF - Carbon tetrachloride soluble fraction of methanolic extract, CFSF - Chloroform soluble fraction of methanolic extract MSF - Methanolic extract	Gram positive <i>B. cereus</i> (except CTSF), <i>B. megaterium, B. subtilis, S. aureus, S. lutea</i> Gram negative <i>E. coli, P. aeruginosa, S. paratyphi S. typhi, S. boydii, S. dysenteriae</i> (except MSF), <i>V. mimicus</i> (except MSF), <i>V. parahemolyticus</i> Fungi <i>C. albicans, A. niger, S. cerevacaee.</i>	[43]
<i>S. acuminatis-simum</i>	Stembark	Isolation of 18 active compounds	Fungi <i>C. albicans</i> ATCC 24433 & ATCC 90028, <i>C. glabrata</i> ATCC 90030, <i>C. krusei</i> ATCC 6258, <i>C. parapsilosis</i> ATCC 22019.	[25]

(Table 3) Contd....

Plant name	Plant's part	Types of extract	Microorganism	Reference
<i>S. zenkeri</i>	Stembark	Isolation of two anthraquinones, zenkequinones A and B	Gram positive <i>B. subtilis</i> , <i>B. megaterium</i> Gram negative <i>E. coli</i> , <i>P. aeruginosa</i> , <i>P. vulgaris</i> .	[44]
<i>S. colais</i>	Leaf	Successive solvent extracts viz., <i>n</i> -hexane, chloroform, EtOAc, EtOH and water	Gram positive <i>Coagulase-negative staphylococci</i> , <i>Enterococci</i> , <i>S. aureus</i> Gram negative <i>Acinetobacter</i> , <i>Citrobacter</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aureginosa</i> , <i>S. typhi</i> and <i>S. paratyphi</i> . Fungi <i>A. flavus</i> (EtOAc, aqueous, <i>n</i> -hexane), <i>A. fumigates</i> , <i>A. niger</i> (EtOAc, EtOH, aqueous, <i>n</i> -hexane), <i>C. albicans</i> (Chloroform, EtOH, ethyl acetate, aqueous, <i>n</i> -hexane).	[45]

17 different pathogens. In terms of extraction solvents, hexane, petroleum ether, ethyl acetate, dichloromethane, chloroform, ethanol, methanol, carbon tetrachloride and water have all been used. However, in order to facilitate the isolation process, successive solvent extraction systems are recommended where solvent polarity increase from non-polar to polar [45].

In future research, certain criteria of anti-infective assay must be used including endpoint value in order to avoid false positive results. For any anti-infective assay, the endpoint for IC₅₀ values of crude should be below than 100 µg/mL or 25 µM for pure compound [45]. Broad screening on chemical constituents that are responsible for the antimicrobial activity with characterization of active constituents should be done as well. Mechanism of antimicrobial activity of the extract and the specific target in bacteria and fungi's essential components such as cell membrane, cell wall, nucleic acid and protein synthesis must be investigated as well.

3.2. Antioxidant Activity

An antioxidant agent can be defined as a substance capable of inhibiting or delaying the oxidation of another molecule [46]. Upon the discovery of the importance of this agent, several methods were established to evaluate the antioxidant activity. Examples of such methods are DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), 2,20-azinobis [3-ethylbenzoline-6-sulphonate] (ABTS), lipid peroxidation assay, total antioxidant capacity, hydroxyl radical and nitric oxide scavenging assay. Different degrees of antioxidant activity and phytochemical profiles documented from various types of extracts from *Stereospermum* genus is summarized in the following discussion.

According to Compaore *et al.* [47], an aqueous acetone extract of *S. kunthianum* demonstrated a high antioxidant activity using DPPH free radical scavenging, FRAP and ABTS methods. All the data collected for DPPH, FRAP and ABTS tests showed significant antioxidant activities which were 11.33±µg/ml (IC₅₀), 1.20±0.01 mmol/AEEg and 0.62±0.4 mmol/AEEg, respectively. The antioxidant activity was attributed to the presence of phenolics and flavonoids content in *S. kunthianum*. The aqueous acetone extract was

also capable of inhibiting xanthine oxidase activity which can reduce the production of hydrogen peroxide and superoxide anion. An earlier study on *S. personatum* had similar result whereby acetone extract of the stembark exhibited strong free-radical scavenging and moderate xanthine oxidase inhibition activity. In this study, the isolated iridoids and lignans were also reported to possess antioxidant and xanthine oxidase inhibition activity [48].

In a recent study done by Latha *et al.* [13], DPPH and nitric oxide radical scavenging tests were performed in order to compare the antioxidant activity of both *S. colais* (SC) and *S. suaveolens* (SS) root extracts. Based on the percentage of inhibition, ethanolic extract of *S. suaveolens* possessed stronger activity than *S. colais* in both tests in a dose-dependent manner. The difference in antioxidant efficacy could be due to the presence of terpenoids, steroids and anthraquinones in the ethanolic extract of *S. suaveolens*. A study was also done on *S. colais* using different extracts of leaves, namely as *n*-hexane, chloroform, ethyl acetate, ethanol and aqueous extracts. The chloroform and ethanol extracts were observed to give the best antioxidant activity with an IC₅₀ value of 36 µg/ml and 42 µg/ml, respectively. This observation was well correlated with its significant wound healing activity of both chloroform and ethanol extracts which further explained the role of antioxidant agent in the acceleration of wound healing process [49].

Profound antioxidant activity was also displayed by methanol extract of bark and leaf of *S. chelonoides*. The best activity was showed by the bark's extract whereby the parameters of the test included inhibition of DPPH radicals (IC₅₀ 53.99±3.25 µg/mL), ferric reducing power and total antioxidant capacity. These activities were attributed to the higher contents of phenol and flavonoid in the bark extract [16]. Previous study was also done on the methanol extract of *S. suaveolens*'s stembark which showed significant IC₅₀ value of lipid peroxidation inhibition activity, scavenging activity of DPPH, nitric oxide and hydroxyl radical at 8.51 µg/mL, 8.59 µg/mL, 5.92 µg/mL and 9.37 ppm, respectively [50].

Anti-ulcer and gastroprotective activity might be mediated by the presence of antioxidant agents. Based on a study

reported by Muchandi and Chandrashekhar [51], methanol stem bark extract of *S. suaveolens* was observed to give significant dose-dependent decrease of all ulcerogenic parameters such as volume of acid secretion, pH, free acidity, total acidity and ulcer index. As antioxidant is known for its protection against cellular damage, this study suggested that antioxidant agents might be responsible for the gastroprotective activity observed in pylorus-ligated gastric ulcer of tested rats. This statement was in line with the previous finding of *S. suaveolens*'s antioxidant activity [50].

The antioxidant activity had been reported on different parts of *Stereospermum* species especially stem bark, with varying polarity of solvents and using various assays. Among reviewed species, *S. suaveolens*'s seemed to possess the highest antioxidant activity. Nevertheless, different studies investigated different species, plant parts and solvents, making comparisons inaccurate. Stem bark, root and leaf had all shown antioxidant activity; however, stem bark seemed to be the most promising plant part. Methanol seemed to be the most preferable solvent which might be due to the good solvation of antioxidant molecules within this solvent [52]. It was also noted that the antioxidant activity might lead to the observation of other, closely related, bioactivities such as wound healing, anti-ulcer and gastroprotective activities. Sometimes activity may show in the *in vitro* but not in the *in vivo* because of the drug bioavailability. Therefore, future studies on animals should be done to evaluate the bioavailability of the drug, oxidation products or biomarkers of oxidative stress to protein, lipid and DNA.

3.3. Anti-Diabetic Activity

Diabetes mellitus (DM) is a metabolic disease manifested by hyperglycemic condition as a result of insulin secretion defect, inefficient of insulin action or combination of them [53]. Anti-diabetic activity of plants has offered promising alternative for diabetes treatments and gained the attention of scientist worldwide. Inhibition of glucose absorption across epithelial cells of intestine is among the main principal of blood-glucose management.

Based on an anti-diabetic study of *S. colais*'s leaf extracts, different extracts such as *n*-hexane, chloroform, ethyl acetate, ethanol and aqueous extracts had been tested *in vitro* in order to evaluate the movement of glucose across dialysis membrane. It was found that ethanol extract gave the most effective inhibition of glucose movement at concentration of 50 g/L [37]. A study on isolated compounds from *S. colais*'s root was also reported on their α -glucosidase and protein glycation inhibitory activities which were potential for the treatment of type 2 diabetes. Among the active compounds with the inhibition activities were ursolic acid, lapa-chol and pinoresinol [54].

Furthermore, a recent study reported a remarkable anti-hyperglycemic activity exhibited by water extract of *S. tetragonum* (50 mg/kg) in fed rats but interestingly not in fasted rats. The root part of this plant reduced more serum glucose than the aerial part [55]. Active fractions of *S. tetragonum*'s root were also reported to have significant anti-diabetic activity in streptozotocin (STZ)-induced type-2 DM. Serum glucose level was reduced and all the parameters tested such as body weight, liver glycogen, lipid peroxide,

triglycerides, cholesterol, vitamin C and glutathione were restored to the normal value at 25 mg/kg. Insulin release was not affected by the administration of active fraction and appeared to inhibit the absorption of glucose from intestinal tract. The best anti-hyperglycemic activity was showed by two isolated compounds (2 mg/kg) viz. iridoid glycoside and a naphthoquinone derivative [56]. Similar observations of anti-hyperglycemic and anti-DM activity were obtained in a previous study done on the active fractions (25 mg/kg) of *S. tetragonum*'s root in alloxan induced diabetic rats [57].

Nephroprotective effect of *S. suaveolens*'s bark by ethyl acetate fraction of ethanol extract was reported in the *in vivo* study of STZ-induced diabetic rats. Elevated fasting blood glucose was significantly reduced in STZ-induced diabetic rats at 200 and 400 mg/kg. Glutathione level and serum renal markers such as creatinine, urea, uric acid and total proteins were restored to normal value. Lipid peroxidation of kidney tissue was also protected by the extract [14]. In addition, anti-hyperlipidemic activity was also exhibited by the ethyl acetate fraction of ethanol extract from *S. suaveolens*'s bark. Significant reduction in the serum total cholesterol, the triglycerides, LDL and increase in HDL level of STZ-induced diabetic rats was observed at 200 and 400 mg/kg [58].

It is clear that *Stereospermum* species is a good candidate for further studies on the anti-diabetic activity. This was proven using different animal models such as alloxan and STZ induced diabetic rats as well as several assays such as glucose movement across dialysis membrane, α -glucosidase and protein glycation inhibition, nephroprotective effect and many more. Anti-diabetic activity of *Stereospermum* genus has been reported from various species such as *S. colais*, *S. tetragonum* and *S. suaveolens* as well as from various plant parts such as leaves, bark and root, however, root part might be the best source of anti-diabetic compounds. Roots are constantly under attacks from insect or microorganism present in the soil, which contribute to the production of various active metabolites in the root [59]. In terms of solvents, several solvents such as water, ethanol and ethyl acetate had all shown anti-diabetic activity, with the latter being the most reported.

3.4. Anti-Inflammatory and Analgesic Activity

Anti-inflammatory and analgesic activities of *Stereospermum* genus from previous studies was reported using different tests and methods. According to Balasubramanian *et al.* [60], ethanol extract of *S. suaveolens*'s bark exhibited significant anti-inflammatory activity on test rat at a concentration of 400 mg/kg body weight. This study found out that maximum inhibition of edema induced by carrageenan, dextran, and histamine in rat paw model was observed along with the reduction of granuloma weight in cotton pellet-induced granuloma model.

Stereostin, stereospermin and stereospermidine that were previously isolated from the stem bark of *S. kunthianum* [26] had been reported to exhibit significant anti-inflammatory and analgesic activity at 20 mg/kg. This study used carrageenan-induced pain (Randall-Selitto) and formalin induced pain tests. All the compounds showed significant increase in the threshold pain of rats whereby inhibition of

inflammatory activity might be responsible for the reduction of pain [61]. This study was in agreement with a study done on an aqueous extract of *S. kunthianum*'s stem bark which significantly inhibited the inflammatory action on rats at 400 mg/kg using the carrageenan-induced paw edema, leucocytes migration and granuloma air pouch test [62]. There was also a study reported on the analgesic activity of the aqueous extract indicated by inhibition of abdominal writhes, inhibition of both phases in formalin pain test in mice, elevation of pain threshold and tail flick latency in test rat [34]. Apart from *S. kunthianum*, analgesic activity was also reported from fractions of *S. colais*'s stem bark in mouse writhing and formalin test. The analgesic activity was observed in both central and peripheral mechanisms at concentration range of 150 - 450 mg/kg [63].

Study on anti-inflammatory and analgesic activities from other *Stereospermum* species such as *S. colais*, *S. tetragonum*, *S. suaveolens*, *S. acuminatissimum* and *S. chelonoides* might need to be done in future to investigate their therapeutic potential. Moreover, exact mechanisms behind the reported anti-inflammatory and analgesic activities of *Stereospermum* genus along with toxicological characterization of an acceptable oral dose for human consumption are also needed so that new medicinal products such as pain-killer and anti-inflammatory agents can be commercialized.

3.5 Antidiarrheal Activity

Ching and his colleagues [33] also conducted antidiarrheal tests on the aqueous extract of *S. kunthianum*'s stem bark. Intestinal transit time, castor oil-induced diarrhea of mice model, accumulation of intestinal fluid and gastric emptying effect were tested. The observations of this study included inhibition of bowel transit in castor-induced mice, reduction of the normal intestinal transit of rat in phenol red meal test without gastric emptying effect, delay in defaecation onset, and decreased in the number and weight of wet stool with the most effective concentration at 200 mg/kg. A year later, further study was conducted on the active fractions of its extract in a castor oil-induced diarrhea of mice model. Three methanol fractions showed various degrees of anti-diarrheal activity at concentrations range from 100 to 400 mg/kg [64].

Based on previous study of the antidiarrheal activity, some secondary metabolites were suggested to be responsible for this activity such as tannin [65], flavonoids and saponins [66]. This statement correlated with the detection of tannin, flavonoids and saponins in the *S. kunthianum* extract. It was not surprising that *Stereospermum* genus exhibited antidiarrheal activity since several studies had been reported on their antibacterial activity against common diarrheal causative microorganisms such as *E. coli*, *S. typhi*, *S. boydii*, *S. dysenteriae* and *V. mimicus* as discussed previously in section 3.1. This pharmaceutical property of the genus may provide an alternative to diarrheal remedy which needs to be supported by further studies on their effectiveness and possible side effects.

4. TOXICITY STUDY

Toxicity test are evaluated by different degree of concentration which can be categorized into No Observable Effect

Concentration (NOEC), Lowest Observable Effect Concentration (LOEC) and Maximum Allowable Toxicant Concentration (MATC). The type of toxicity test (acute or chronic) is classified based on the treatment period upon exposure. The methodology is performed according to the established standard by few organizations such as American Society for Testing and Materials (ASTM), Organization for Economic Cooperation and Materials (OECD) and National Toxicology Program (NTP) [67].

Stereospermum genus was reported to exhibit no mortality or adverse effect upon the acute toxicity tests on rats and mice. Based on a study by Ching *et al.* [34] on the aqueous extract of *S. kunthianum*'s stem bark, no death of rats and mice tested were recorded even at the highest dose of 800 mg/kg in 14 days treatment period. Methanol extract of *S. suaveolens*'s bark also caused no mortality when the mice were treated with up to 5000 mg/kg overnight [68]. A median Lethal Dose (LD50) value that equal to or more than 2000 mg/kg is considered as safe for a drug to be used according to OECD guidelines. Another toxicity study was done on *S. suaveolens* fractions such as petroleum ether, chloroform, ethyl acetate and aqueous fractions by oral administration on male Swiss albino mice for three days with no mortality recorded even at the highest dose of 3200 mg/kg [69].

Acute and sub-acute toxicity test were also conducted on the active fraction of *S. tetragonum*'s root. In 14 days of oral administration, the active fractions caused no symptoms of toxicity or mortality in albino mice at dose range of 500 - 2000 mg/kg. Zero mortality was also reported in 28 days sub-acute toxicity at 100, 200 and 400 mg/kg. The histopathology of vital organs of the 400 mg/kg treatment showed no abnormality compared to untreated group which suggested that active fractions were safe up to 400 mg/kg [70]. In sum, previous studies conducted on *S. kunthianum*, *S. suaveolens* and *S. tetragonum* showed no sign of toxicity in acute and sub-acute tests. However, many more toxicity tests are remained to be studied such as the toxicity effect in long term period on either reproductive ability or genetic modification.

5. FUTURE STUDY

The current findings of *Stereospermum* genus have raised many questions that need further investigations. Considerably more studies need to be undertaken especially on the undiscovered species such as *S. fimbriatum*. Phytochemical screening on *S. fimbriatum* should be done in order to evaluate its bioactive constituents. Future study on the extraction of *S. fimbriatum*'s phytochemical constituents will be a great help to obtain bioactive compounds. Conventional methods such as soxhlet, maceration, percolation, and decoction extraction can be used. Methanol, ethanol, chloroform, petroleum ether, ethyl acetate, and water solvent are among the most common solvent for extraction of potent bioactive compounds. Moreover, non-conventional methods such as supercritical fluid extraction (SFE), subcritical fluid extraction, ultrasonic extraction (UE) and microwave assisted extraction (MA) may also offer better option in the extraction as they are less time-consuming, reduce solvent waste, environmental friendly and safer to researchers. Among these

non-conventional methods, SFE offers a green alternative to toxic organic solvent by using nontoxic carbon dioxide (CO₂). Temperature, pressure, flow rate, time and percentage of co-solvent can all be changed to the sample type and target compounds [71]. The optimization process increases the extraction efficiency which at the same time minimizes the solvent consumption and waste produced [72]. It is suggested future studies look into isolation of potential active compounds from *S. fimbriatum* that might yield novel bioactive compounds.

CONCLUSION

The traditional uses and therapeutic properties of *Stereospermum* genus are reflected and justified by their phytochemical constituents. The data presented in this review shows that *Stereospermum* genus possesses various classes of phytochemical constituents in various plant parts which have been linked to its therapeutic and biological properties. However, these biological properties depend on which extraction material and methods are used.

To date, there is very little information available on *S. fimbriatum*. Based on the information compiled herein on the bioactivities of *Stereospermum* genus, it is anticipated that *S. fimbriatum* might possess remarkable pharmacological activities and could be a source of antimicrobial, antioxidant, anti-diabetic, anti-inflammatory and anti-diarrheal agents with little or no adverse effects. Based on Wiart [73], this plant species has the potential to be a source for new drugs and should be promoted for future research considering the known medicinal values as compared to other undiscovered species such as *S. randrianaivoi* and *S. gentryi* with unknown medicinal value [74].

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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