Review Article

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Traditional Uses, Pharmacology, Toxicology and Chemical Constituents of an Aphrodisiac Plant, *Smilax myosotiflora*: A Systematic Review

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ABSTRACT

Smilax myosotiflora is one of the well-known plants in Malaysian folk medicines for its aphrodisiac effect. The leaves and fruits were also claimed able to treat syphilis and rheumatism. This article aims to systematically provide an overview on the traditional uses, pharmacology, toxicology, and chemical constituents of *S. myosotiflora*. The e-searching on previous literature of the plant was conducted from its inception to the end 2021 according to the study's criteria. The development of this review was based on the Cochrane Collaboration framework and PRISMA guidelines. As the result, it was found that over half of 41 eligible articles have discussed on its aphrodisiac attribution while others reported on the synergistic, sex reversal, antioxidant, anthelmintic activities and so forth. Two stilbenoid compounds are successfully isolated from the plant; trans-resveratrol and trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene. Other than the detection of aurones and phytosterols, *S. myosotiflora* was delineated to be high in alkaloids, saponins and flavonoids. This systematic review can be a platform for other researchers to explore more on *S. myosotiflora* as it still has more pharmacology potential to be discovered. Further studies are required prior to the development of *S. myosotiflora*-based drugs for medicinal usage in the future.

Keywords: *Smilax myosotiflora;* traditional medicine; aphrodisiac; pharmacology; toxicology; chemical constituent.

1. Introduction

Mental stress, unhealthy lifestyle, chronic diseases and drug toxicity are among the multiple factors that can cause to the lack of male sexual function. Current findings also showed that the COVID-19 virus may now become a new threat to the problem [1–3]. Inability to perform in an intimate event may create huge problems in the future and socially, physiologically affects the man and his partner [4]. Patients may face depression, anxiety, low self-esteem disorders, and hardly encountered with physical symptoms [4,5]. This also indicates that they may have male sexual dysfunction (MSD) problem, one of the most common health threats other than heart and diabetes diseases among men [6]. Due to that, masses treatments have been developed in regard to the consistent growth of the cases. Unfortunately, many options are very high cost, not easily accessible and associated with numerous serious side effects such as penis pain, urethral bleeding and infections. Various synthetic drugs for instance, sildenafil (Viagra), papaverine, alprostadil, vardenafil or tadalafil are also available to overcome the problems. However, some of the drawbacks of the drugs including cost-consuming and serious adverse effects are yet unavoidable. Patients with certain chronic diseases such as cardiovascular associated-coronary heart or angina are not permitted to take the drugs due to the severe side effects that may cause to the organs system failure or death. Therefore, natural supplement from aphrodisiac plants is preferred due to their significant potency, lower side effects, wider availability, and low cost.

In Malaysia, the indigenous communities have orally practiced *Smilax myosotiflora* A. DC. as one of their libido enhancers. *S. myosotiflora* is a creeper plant that wildly grows in the forests of Peninsular Malaysia, southern Thailand and throughout the tropical climate regions in Southeast Asia (SEA) [7,8]. It has been seen as a promising local aphrodisiac drug after its numerous significant findings in pharmacology, endocrinology, and toxicology besides of other significant attributions in other important fields. This present systematic review aims to literate the published scientific evidence in order to gain a better comprehension of the plant in pharmacology, toxicology and other critical fields besides discussing its traditional and current local practice. The present review will not only describe on its aphrodisiac attitude but also other reported pharmacology interests too. To the best of our knowledge, no meticulous review of *S. myosotiflora* plant is available and has been reported so far.

2. Materials and Methods

This systematic review was conducted based on the Cochrane Collaboration framework guidelines and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. The online literature search was performed from its inception till the end of 2021 through several databases including Nature, Web of Science, SpringerLink, ScienceDirect, PubMed, Google Scholar, EBSCO, and Mendeley using the keywords but not limited to '*Smilax myosotiflora*', 'Smilax AND myosotiflora' and '*S. myosotiflora'*. The article screening was purportedly to select English articles which contained the corresponding keywords whether in the title, abstract or body text. Further review of all relevant articles was carried out and retrieved according to the year of publication, the objective of the study and its outcomes. Duplication, ineligible data and all nonverified sources of information were excluded. Eligible data of *S. myosotiflora* were summarized in the tables accordingly. All chemical structures in this review were drawn using ChemSpace online.

3. Results and Discussion

The workflow of *S. myosotiflora* literature search was described in Figure 1. Out of 188 available articles retrieved from the five databases; SpringerLink, ScienceDirect, Google Scholar, Mendeley and PubMed, there were only 41 have met the criteria and were further discussed. The rest articles were discarded for duplications (68), unrelated data (74) and nonverified sources (5). Overall, the published studies on the *S. myosotiflora* were in scope of traditional uses, pharmacology and toxicology activities and phytochemicals profile. In addition, researchers have also reported on preservation and conservation efforts toward the *S. myosotiflora* plant.

3.1 Botanical Data

The taxonomy of *S. myosotiflora* originates from the Kingdom of Plantae, division of Angiosperms, class of Monocots, order of Liliales, family of Smilacaceae,

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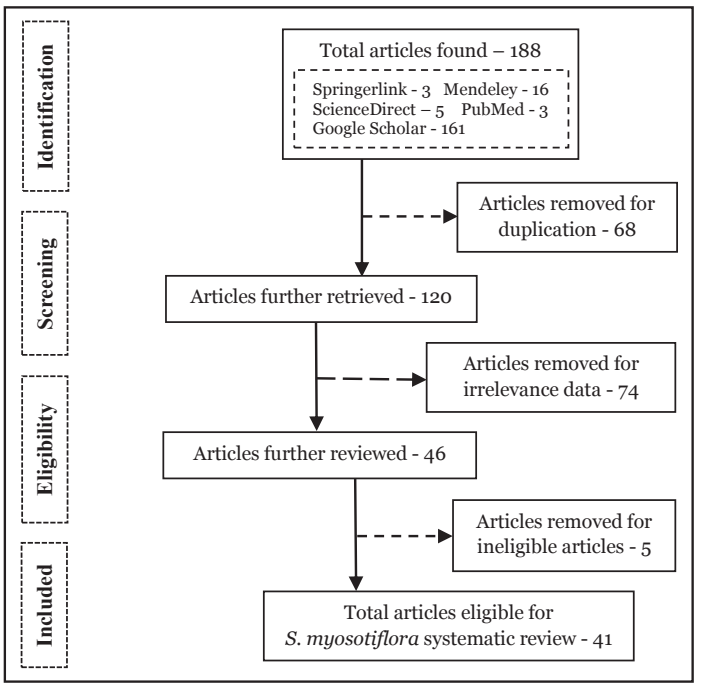

Figure 1. PRISMA flow diagram explains the development of the systematic review on *S. myosotiflora*.

and genus of *Smilax.* The monocotyledon family of Smilacaceae is a greenbrier family and the second largest family in Liliales with more than 750 species recorded in two generas; *Smilax* and *Heterosmilax*. Smilax is a genus of about 300–350 species and can be found all over tropical and subtropical regions. The *Smilax* plants will normally grow as shrubs, forming dense impenetrable thickets and growing under big trees. The hooked thorns allow the plant to hang onto and scramble over soil and surface. Other *Smilax* species which can be found in the SEA countries are *S. blumei* A.DC., *S. glabra* Roxb., *S. aspera* L., *S. leucophylla* Blume, and *S. hirtellicaulis* C.Y.Wu & C.Chen ex P.Li [8,9].

S. myosotiflora, the 'horny little devil', is an evergreen herbaceous creeper plant with other vernacular names, for instance, 'ubi jaga', 'ubi besi', 'akar dedingin' or 'itah besi' among Malaysian and Indonesian or 'Khao-Yen Bai Bang' in Thai. The plant is widely distributed in the SEA regions, for instance, western Malaysia, southern Thailand, Sumatera, Burma, and Indo-China [7,8]. To grow as a mature plant, *S. myosotiflora* only requires shelters under big trees, moist soil with pH 5-6, rich in humus and nutrients, as well as good drainage in lowlands or downhills [7,10]. The leaves are heart-shaped and deciduous with 4–30 cm long while the tubers are dark brown rough surface, irregular round shape covered with the hairy roots and slightly sweet in odor (Figure 2).

3.2 Traditional and Current Local Uses

S. myosotiflora is one of the most popular traditional remedies as a sexual booster among locals and

Figure 2. The *S. myosotiflora* tubers after the cleaning process and their hairy roots were cut off. The tubers were reported as the most functional part of the plant which mostly claimed for aphrodisiac activity (Tables 1, 2 & 3).

aboriginal tribes in Malaysia. Based on the previous studies which conducted using structured or semi-structured questionnaires and face-to-face interviews among the traditional healers and the villagers, there were several ways on how *S. myosotiflora* was used by the communities (Table 1). The tubers were the most documented plant part of *S. myosotiflora* consumed by the societies with mostly for the men health's benefits. It was reported that *S. myosotiflora*'s tubers were able to increase the production of testosterone in elderly men, improve sperm production and its viscosity, vitality and sexual strength [11]. For women, though its usage may be less common than the men, it was said able to restore vitality and libido, firming the vagina, especially after delivery, and arresting vaginal discharge [12].

According to the Table 1, the tubers of *S. myosotiflora* has also been used to treat diabetic problem [13,14]. Concurrently, its stipules, leaves, and fruits were consumed internally to treat rheumatism and syphilis [10,15]. However, till to date, no clinical finding was made to prove the calls. In traditional way, *S. myosotiflora* decoction was prepared by boiling the tubers solely or mixing with other medicinal plants such as *Eurycoma longifolia* Jack roots, *Labisa pumila* (Blume) flowers, *Quercus infectoria* Oliv. fruits, *Parameria polyneura* Hook. f. leaves or honey in order to get the synergistic effect from the plant or to maximize the efficacy and add more value to the drink [12,16,17]. The tonic would be taken orally in regular basis, once or twice a day or when necessary. Meanwhile, in some native committees, the *S. myosotiflora* is part of a legend cultural practice where the tubers become the insertion chips for the betel nut chewing [10,11,15].

Today, due to continuous demands for *S. myosotiflora* as a heritage herbal supplement which was more likely encouraged by the cultural belief, natural source, and good testimonial, over 100 *S. myosotiflora-based* products are now available in the Malaysian markets [22]. These included the illegal products containing a reproductive toxicant metal, mercury, at an excessive amount between 0.63-0.73 ppm and lead at the range of 10.23-23.05 ppm [22,23]. According to the Malaysian National Drug Control Authority (DCA) guidelines for the quality requirement of traditional medicines, the content of mercury in the food product should be lesser than 0.5 ppm and lower than 10 ppm for lead [22,23]. The researchers have also uncovered that 15% of the marketed *S. myosotiflora* herbal products were not registered under the DCA [22]. The dispensation of *S. myosotiflora* herbal products consisting of an exaggerated amount of mercury and/or lead will cause more adverse effects to human health rather than the benefits of the natural ingredient itself. Some of the potential risks of mercury or lead exposure were vital organs damage, paralysis, erectile dysfunction and food poisoning [24].

Table 1. Reported studies on traditional uses of *S. myosotiflora*.

Ethnic	Location	Part used	Preparation	Intake	Purpose	References
Mah Meri & Temuan	JAKOA, Selangor* (3°09'32.6"N, 101°42'32.8"E)	Tuber	Decoction	Drink	Diabetes	[13]
Malay	Bangi, Selangor* (2°54'03.7"N, 101°46'55.5"E)	Tuber	Decoction	Drink	Diabetes	[14]
Jah Hut	Kampung Keboi, Pahang (3°52'26.5"N, 102°19'49.7"E)	Tuber	Decoction	Drink	Virility, back pain	[16]
Temiar	Pos Pasik, Kelantan (5°12'59.4"N, 101°45'46.2"E)	Tuber	Decoction	Drink	Male energy booster	[17]
Jahai	Belum, Perak (5°40'51.1"N, 101°23'57.0"E)	Tuber	Raw	Eat	Male health tonic	[18]
Temiar	Post Brooke, Kelantan (4°40'30.7"N, 101°29'13.1"E)	Tuber	Raw	Eat	Male aphrodisiac	[19]
Temiar	Gua Musang, Kelantan* (4°52'N, 101°58'E)	Tuber	Decoction	Drink	Male aphrodisiac, energy drink	[20]
Kensiu	Baling, Kedah* (5°49'12.1"N, 100°56'20.2"E)	Tuber	Cooked	Eat	ns	[21]

* Abbreviations: - Study was performed in scattered places, the coordinate in the table represents one location of the study; JAKOA - Jabatan Kemajuan Orang Asli (Malaysian authorized department responsible for aboriginal community's affairs); ns - not stated in the study.

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The *S. myosotiflora*-ready products were prepared through extraction/decoction or freeze/spray-dried method and were mixed with other herbs as an additive to teas and coffees or were developed in capsules as a health supplement. The products have been extrapolated able to provide a wider range of benefits, not only to improve sexual function but also able to refine overall health and energy, improve nerve system and blood circulations, and reduce minor pain in separated male and female formulations. Meanwhile, due to its great demand and limited supply, the price of *S. myosotiflora* tubers was found to be higher than *E. longifolia* with 65-85 to 20-25 USD/kg accordingly. Therefore, this has generated an opportunity for the native tribe to trade the plant as one of their incomes [21].

3.3 Pharmacological Activities

Some people do not believe in love potions or aphrodisiacs but for centuries, countless numbers of men consumed them, merely based on the cultural beliefs with the hope to improve their intimacy. Ethnobotanical surveys have indicated a large number of plants which traditionally used as aphrodisiacs now are getting more scientifically validated. As a result, more scientific data has proved that the consumption of aphrodisiac plants is able to improve the MSD problems in general. The aphrodisiac plants can act in different modes either by increasing the libido, potency, sexual pleasure or helping to increase the intensity in the lovemaking [25–27]. Tables 2 and 3 showed the scientific reports on the *in vivo* and *in vitro* pharmacological activities, their works undertaken, and the important outcomes of the horny little devil which have been documented thus far. It is noticeable, tubers of *S. myosotiflora* as a male aphrodisiac substance were the most covered in earlier studies. Other significant pharmacology activities derived from the reported findings were antioxidant, sex reversal, anthelmintic, and synergistic activities.

Previous studies have displayed potential aphrodisiac properties in animal models using the tubers of *S. myosotiflora* (Table 2). The optimum dose of *S. myosotiflora* as an aphrodisiac was found to be between 400-800 mg/kg where aqueous and methanol were the most active forms [28–30]. All experiments in the Table 2 were performed through force oral administration. At all doses (200, 400, and 800 mg/kg) of *S. myosotiflora* methanol extract (SMME), the extract has significantly stimulated sexual behaviors in rats by increased the penile erection index (PEI) [28], reduced mount and intromission latency, and improved copulatory rate [30]. Furthermore, rats that received 400 mg/kg of SMME already showed a marked improvement in the number of intromission and epididymal sperm count by 18% [29,33]. Further results also revealed that the male rats treated with 800 mg/kg of the SMME were found to significantly reduce ejaculation latency, and inter-intromission interval, and increase the ejaculation frequency [30].

The modulations of erectile function are implicated by a few factors including hormonal aspect where one of the most necessary hormones involved in the erection occurrence is testosterone. Testosterone is a metabolic hormone that plays crucial physiological roles in the body. It exerts many functional tasks in tissues and organs, for instances, regulating energy metabolism, bone mass, nitrogen retention, muscle growth, and controlling adipogenesis and modulating male reproductive and sexual function [29,30]. A study proved that the administration of *S. myosotiflora* at 800 mg/kg has risen the testosterone level in male without disturbing their spermatogenesis stages [29]. Moreover, the combination of the plant with progesterone blocker drug, mifepristone, able to boost the testosterone level within a short period [34]. With a sufficient dosage regimen, *S. myosotiflora* manifested to improve the testosterone level, sexual behaviors, and reproductive function of the male rats [28–31].

Table 3 illustrated previous reported findings on pharmacological activities of *S. myosotiflora* done *in vitro*. Apparently, the aphrodisiac activity can also be investigated *in vitro* through biochemical or hormonal methods, for instance, by the determination of testicular and serum cholesterol, neuronal nitric oxide synthase or androgen receptor protein assay. Elsewhere, the *S. myosotiflora* tubers were found to have a comparable peak of protein to *E. longifolia*, *Rafflesia* sp. and *Labisia pumila* (Blume) Fern.-Vill. which are responsible to increase the expression of testosterone levels in the Leydig cells, the 4.3kDa peptide [38]. The finding was relatable to the *in vivo* test where the intake of the plant was able to significantly elevate the level of testosterone in male rats [29]. Although studies of *S. myosotiflora* showed positive effects on sexual enhancement, more research is still necessary to understand the mechanism of action of the activity. The need for clinical trials is

Table 2. Reported studies on pharmacological activities of *S. myosotiflora* done through *in vivo* experiments.

Pharmacology activities	Part used	Objective of study	Extract	Dosage regimen	Significant result	Remarks	Reference
Aphrodisiac	Tuber	To assess PEI & homosexual mounting in the absence of female rats	Chloroform, methanol, aqueous & butanol	200, 400, 800 mg/ kg in 60 days	PEI increased and no homosexual mounting in all dosages	Aqueous extract was the most aphrodisiac active extract	[28]
Aphrodisiac	Tuber	To investigate the effects on sexual hormone levels and testicular histology in male rats.	Methanol	200, 400, 800 mg/ kg in 60 days	Testosterone level increased in 800mg/kg group. Normal testicular histology in all groups	No disruption in any spermatogenesis stages.	[29]
Aphrodisiac	Tuber	To study the expression of fertility and sexual behavior on sexually experienced rats	Methanol	200, 400, 800 mg/ kg in 60 days	All dosages reduced mount and intromission latency but improved copulatory rate	Ejaculation latency and inter-intromission interval reduced but the frequency increased in 800mg/ kg group	[30]
Endocrine	Tuber	To compare the effects of different doses in 11β-HSD OA, plasma levels of testosterone and estradiol on corticosterone-treated rats	Aqueous	8 and 16 mg/kg in seven days	*S.myosotiflora* counteracted in testicular 11β-HSD OA, plasma testosterone and estradiol levels	8mg/kg demonstrated more marked effect on testosterone and estradiol levels	[35]
Endocrine	Tuber	To explore the effects on corticosterone plasma levels in male rats	Aqueous	8 mg/ kg in 14 days	Corticosterone plasma levels increased in day 2, 3 and 7	Corticosterone plasma levels dropped to normal by day 14	[36]
Endocrine	Tuber	To describe the effects of testicular 11β-HSD OA and plasma levels of corticosterone and testosterone	Aqueous	8 mg/kg in seven days	Corticosterone plasma increased but testosterone decreased	Combination of *S. myosotiflora* and mifepristone reversed the effect of main result	[34]
Aphrodisiac	Leaves	To investigate on sperm quality, histology of the testis and pregnancy rate after mating with fertile female rats	Aqueous	150 mg/ kg in 28 days	No significant result observed in all parameters	Normal histological characteristics of testis in treated group	[37]

Abbreviations: PEI - Penile erection index; 11β-HSD OA - 11β-hydroxysteroid dehydrogenase oxidative activity.

also required to prove the effectiveness and safety of *S. myosotiflora*'s aphrodisiacs compounds for human use.

Meanwhile, due to arising incidences in antibiotic resistance, scientists are now investigating the synergistic effect from the combination of the plant-derived active compounds and conventional antibiotics as one of the ways to combat the problem. Recently, many plant-derived active constituents were reported to have the respective effect when they were applied concurrently with the standard drugs [39–41]. Synergistic effect is a cumulative outcome from two or more active constituents which produces a larger effect in similar or related mechanism. The study of Chyang et al. (2018) [42] has given a new perspective on the synergistic potential of the *S. myosotiflora* plant when they found that the plant creates wider inhibition in the microorganisms of *Bacillus subtilis*, *Salmonella typhi*, and *Candida albicans* growths when treating with antibiotics gentamicin/ketoconazole. The study was executed using the disc diffusion method. However, to date, no data on solely antibacterial activity of *S. myosotiflora* was documented. Hence, more studies on the *S. myosotiflora* related activity should be furthered to solidify such findings.

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The antiproliferative activity is a complement consequence of bioactivity which leads to a stimulation of apoptosis in the cancer cells. There are a number of plants that have been documented to naturally consist of bioactive compounds for the prevention or treatment of the distinctive cancer diseases for examples *Vaccinium macrocarpon* Aiton. [43], *Passiflora foetida* L. [44], *Lawsonia inermis* L. [45] and *Chromolaena odorata* (L.) R.M.King & H.Rob. [46]. For acting as a potent anticancer agent, the median inhibition concentration (IC_{50}) value of the active substance against the cancer cells has to be lower than 20 µg/mL. Dasuki et al. (2012) [47] and Ng et al. (2010) [48] reported that *S. myosotiflora* has no acute cytotoxic attribution on the dysplasia cells as its IC_{50} was higher than 99 µg/mL. *S. myosotiflora* possibly was lack of an active compound as an antiproliferative agent to the cancer cells, thus, *S. myosotiflora* may not be an anti-cancer agent. Through Ng et al. (2010) [48], *S. myosotiflora* plant may also not a potential for anti-angiogenesis agent too as no effective metastasis inhibition of the blood vessel occurred.

The *S. myosotiflora* plant was evidently effective in many therapeutic purposes not only for human beings but also to the animals. *Haemonchus contortus* is one of the harmful livestock gastrointestinal parasites which responsible for the weight loss, anemia, unthriftiness, diarrhea, and death to the infected. Antecedently, the use of *S. myosotiflora* as an anthelmintic agent has never been recorded nor reported in old medicinal practice. Nevertheless, the scientific data had shown that 100% mortality of the parasitic worm was recorded at the concentration 5 mg/mL of SMME [49], thus providing scientific evidence that *S. myosotiflora* can be a natural promising anthelmintic agent to be utilized in the agriculture sector. The aqueous extract of *S. myosotiflora* was also used as a natural inducer for sex reversal in the aquaculture industry using the brine shrimp as the model [50]. Results showed that the extract able to produce an average of 98.3% of male shrimp after 21 days of intervention in all concentrations. Producing more male fish will allow higher demand among consumers whilst lower costs production are required. Further investigations are suggested to validate the findings on *S. myosotiflora* as anthelmintic and transexual agents.

The antioxidant spectrophotometric method such as 2,2-diphenyl-1-picryl-hydrazyl (DPPH) is widely established among researchers to study the scavenging activity from plants. Study has proved that *S. myosotiflora* was high with antioxidant compounds where the DPPH radical scavenging assay was 75.8% [47]. The activity was aligned to the high total phenolic content in the *S. myosotiflora* extract where the constituents would act to scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxyl that can cause to degenerative diseases [51]. Meanwhile, the dietary use of *S. myosotiflora* can be an alternative for the prevention and/or treatment of gout or hyperuricemia as the activity of xanthine/xanthine oxidase (X/XOD) superoxide scavenging activity was 79.24% with the total phenolic compound (TPC) was 654.83 mg of GAE/100g [47]. Study also showed that the XO inhibition activity could be linked to the content of phenolic compounds present in the plant/extract [52]. The presence of the antioxidant compounds in the plant also help to improve the spermatogenesis pathway and promote the synthesis of steroid hormones in male reproductive systems [53–55]. Additional research and clinical study are encouraged to prove these breakthrough findings in the future.

Researchers are now also anticipating the possibility to conserve the diversity of *S. myosotiflora* where several initiatives have been conducted to succumb to the depletion of the plant. In order to ensure the sustainability of the *S. myosotiflora*'s habitat and cater to its continuous demand from the industry, researchers are now looking for various conservation plans to conserve it. Using the tissue culture technique, a medium with 2.0 mg/L Benzylaminopurine (BAP) plant growth regulator was able to bring out the highest number of shoots and stipule compared to the other amounts of BAP; 0.25, 0.5 and 1.0 mg/L [56]. While another study demonstrated that nodal was an ideal starting segment for *S. myosotiflora in vitro* culture [57]. According to the team, MS supplemented with 2.5 mg/L BAP and deduced to 1.0 mg/L would be the best induction medium to get the highest shoot multiplication rate. On the other hand, in order to secure the *S. myosotiflora* genetic resources, the hexaploid microsatellite markers were developed for effective future *in situ* and *ex situ* conservation programs [58]. A Malaysian governing body, Forest Research Institute Malaysia (FRIM) has also taken an initiative to establish a germplasm bank to conserve the aborigine medicinal knowledge and plants including the *S. myosotiflora* [59].

3.4 Toxicological Studies

Toxicology studies on natural medicinal plants are becoming equally important nowadays to ascertain their safety profile since many unsafe and fatal side effects have been reported worldwide. The plant extract which scientifically produces adverse effects through experimental studies may also act similarly to humans. It is intolerably unaccepted for human consumption if the plant is ultimately effective for therapeutic events while causing the detrimental effect at the same time. The histopathological evaluation of testes is one of the persuasive ways to assess the safety effects of a compound on the male reproductive system. Even though *S. myosotiflora* was consumed considerably at a high dose, 800 mg/kg on daily basis for 70 days, no toxicity effect was found in the sperm morphology, reproductive organs, liver and kidney of the male rats [31]. With no abnormalities found in the testes, the ingestion of SMME was considerably safe provided further histopathology assessments on other organs of the male reproductive system were also performed.

Relative to other areas of toxicology, the reproductive field has never been less important as it provides the evidence of deterioration in male or female fertility. A study of reproductive toxicology on SMME had been performed by Ahmad et al. [60] and they have reported that the methanol extract of *S. myosotiflora* did not cause any impairment on sexual desire or the pregnancy outcome and the mothers. The extract also did not cause any malformation in the fetuses and the implantation of the blastocysts was within the normal range [60]. Therefore, based on Hilmi et al. [31] and Ahmad et al. [60], it can be summarized that *S. myosotiflora* tubers was safely used to enhance fertility and stimulate male sexual behaviors in male rats.

Brine shrimp lethality test (BSLT) is a rapid, economical, and reliable method which have been used extensively in the past 30 years to evaluate the toxicological properties of plant extracts, heavy metal, metal ions, dental materials, nanoparticles or marine natural products using the brine shrimp [61]. Through the test, any cytotoxic signs to the shrimp caused by the substance can be a potential compound to be used to inhibit the malignant cells. The sample assayed might have an active anti-proliferation agent due to its ability to kill the brine shrimp. Osman et al. (2001) [62] and Wan et al. (2016) [29] had performed the BSLT on *S. myosotiflora* to investigate the cytotoxicity profile of the plant. As the result, the LC_{50} values of the plant extracts were higher than 1000 mg/mL demonstrating that *S. myosotiflora* was non-toxic and safe to the biological model, the brine shrimp. In the *S. myosotiflora* cases, the BSLT finding considerably aligned with the antiproliferative studies done elsewhere where they found that *S. myosotiflora* caused no inhibition effect on the cancer cells tested [47,48].

3.5 Chemical Constituents

Determining phytochemical constituents in the plant will allow researchers to get a hint on the association of the respective pharmacological activities with the chemical attitude. Several studies have been carried out through the chemical analyses and structural compound determination in the *S. myosotiflora* plant particularly from the tubers in order to find out the active compounds relatable to various pharmacological activities. A previous study has reported that *S. myosotiflora* tubers contained the most frequent phytosterols compounds or sterols in nature namely sitosterol, campesterol, and stigmasterol [63] (Figure 3). The chemical structures of sterols are similar to cholesterol but differ in their side-chain configurations. Studies showed that by taking adequate food sources enriched with the phytosterols including sitosterol, campesterol, and stigmasterol, can significantly lower the LDL-cholesterol absorption in the body [64–66].

Many vegetables and herbs such as alfalfa, licorice, artichoke, and garlic have been regarded as cholesterol-lowering agents in the human diet since long ago. Ergo, this horny little devil plant may be another natural potent hypocholesterolemia agent that can be tested on its efficiency of the activity and considered for benefits to humans. To the plant itself, sitosterol is involved in cellulose synthesis and sometimes would be converted to stigmasterol due to the plant's response to environmental stimuli and stress compensation. While campesterol plays a crucial role as a precursor to brassinosteroids, a plant steroid hormone that implicates in growth and development of the plant.

Furthermore, the methanol extract of *S. myosotiflora* tubers conceived of secondary metabolites such as alkaloids, saponins, flavonoids, tannins, and cou-

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Table 3. Reported studies on pharmacological activities of *S. myosotiflora* done through *in vitro* experiments.

Pharmacological activities	Part used	Objectives of study	Type of extract	Concentration used	Significant result	Remarks	Reference
Aphrodisiac	Tuber	To detect the presence of 4.3 kDa aphrodisiac protein marker using SELDI-MS	Ethanol	0.5 mg/mL	4.3 kDa protein was detected	The marker was isolated from E. longifolia, suggested they may have similar mechanism of aphrodisiac effect	[38]
Synergistic	Tuber	To assess the efficacy of antibacterial and antifungal activities when combined with antibiotics using well diffusion method	Methanol	25 & 50 µg/µL	Positively inhibited B. subtilis, S. typhi and C. albicans growths	SMME showed synergistic effect when combined with antibiotics drugs	[42]
Antiproliferative	Tuber	To screen the antiproliferative activity on cell lines* through methylene blue assay	Methanol	0.39-99 µg/ml	No significant antiproliferative activity	Cell viability remained constant at all concentrations after 72 hours.	[47]
Antioxidant	Tuber	To evaluate antioxidant activities through X/XOD and DPPH assays	Methanol	50 mg/mL	Activities of X/XOD and DPPH were 79.24 and 75.84%	TPC was 654.83 mg of GAE/100g	[47]
Antiproliferative	Tuber	To evaluate cytotoxicity activity on cells lines** via MTT assay	Methanol	100 µg/mL	No significant cytotoxic activity	IC_{50} values was more than 200 µg/mL	[48]
Anti-angiogenic	Tuber	To screen the anti-angiogenic property using rat aortic ring assay	Methanol	100 µg/mL	No significant blood vessel inhibition	*S. myosotiflora* may not have potential anti-angiogenic properties	[48]
Anthelmintic	Leaves	To determine the anthelmintic activity against third-stage H. contortus larvae parasites in goats	Methanol	1-5 mg/mL treated for 24, 48, 72, 96 and 120 hours	Parasites was 100% killed at 5mg/mL in all intervention times	*S. myosotiflora* has potential to be an anthelmintic to nematode parasite	[49]
Aquaculture	Tuber	To test *S. myosotiflora* for masculinity effect using A. salina	Aqueous	0.2-1.0 g/L in 21 days	Higher growth rate and lower mortality of nauplii as concentrations of extract increased	Means of male nauplii produced was 98.28% in all concentrations	[50]

Abbreviations: BSLT - Brine shrimp lethality test; ns - Not stated in the study; ppm - part per million; LC_{50} - Median lethality concentration, SELDI-MS - Surface enhance laser desorption ionization mass spectrometer; *E. longifolia* - *Eurycoma longifolia*; *H. contortus* - *Haemonchus contortus*; * - Study done on cervical cancer (HeLa), liver cancer (HepG2), colon cancer (HCT), breast cancer (MCF-7), osteosarcoma (MG-63), ovarian cancer (Caov3) and non-cancerous kidney (Vero) cell lines; MTT - 3-(4,5- dimethylthiazol- 2-yl)-2,5- diphenyltetrazolium bromide; IC_{50} - Median inhibition concentration; ** - Study done on human colon carcinoma (HCT-116), human colon normal (CCD-18Co), human hepatocellular carcinoma (HepG2), human non-small cell lung adenocarcinoma (NCI H23) and human breast ductal carcinoma (T-47D) cell lines; X/XOD - 2, xanthine/xanthine oxidase; DPPH – 2,2-diphenyl-1-picrylhydrazyl; TPC - Total phenolic content; SMME – *S. myosotiflora* methanol extract; *A. salina* - *Artemia salina*; *B. subtilis* - *Bacillus subtilis*, *S. typhi* - *Salmonella typhi*; *C. albicans* - *Candida albicans.*

marins [47] (Figure 4). Those phytocompounds were discovered to be associated with aphrodisiac, antimicrobial, anthelmintic, and antioxidant activities and can also be found in other aphrodisiac plants such as *Hibiscus macranthus* Hochst. [67], *Mondia whitei* (Hook.f.) Skeels [68], *Sesamum radiatum* Thonn. ex Hornem. [69] and *Vanda tessellate* L.H.Zou & Z.J.Liu [26]. The *S. myosotiflora* roots or tubers were also described to have a remarkable amount of aurones which are known to be useful in the treatment of prostatomegaly, masculinism, breast cancer, and endometriosis according to a patent by George et al.

Table 4. Reported toxicological studies on *S. myosotiflora.*

Experiment type	Part used	Objective of study	Extract form	Dosage regimen	Significant result	Remarks	Reference
In vivo	Tuber	To evaluate toxicity effects on vital organs and reproductive system of male rats	Methanol	200, 400, 800 mg/kg in 70 days	No changes in sperm morphology, general behaviors and weight of body and internal organs	400 and 800 mg/kg statistically improved the epididymal sperm count by 18%	[31]
In vivo	Tuber	To determine the effects of paternal consumption on the pregnancy outcome	Methanol	200, 400, 800 mg/kg in 60 days	No detrimental effect on pregnancy outcome	No effect on reproductive parameters, preimplantation and post implantation loss	[60]
In vitro	Tuber	To investigate cytotoxicity effect of SMME through BSLT	Methanol	7.8-1000 mg/mL	LC_{50} of the extract was more than 1000 mg/mL	No mortality found in all concentration	[29]
In vitro	Tuber	To test for toxicity of extracts through BSLT	Petroleum ether, ethyl acetate & methanol	ns	LC_{50} of the extracts were between 2900-4800 ppm	Relatively non-toxic to shrimps	[62]

Abbreviations: SMME – *S. myosotiflora* methanol extract; BSLT – Brine shrimp lethality test; LC_{50} – Median lethality concentration.

(2010) [12]. The researchers have conducted high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) techniques to detect the compounds in the plant. The compounds belong to the family of flavonoids and are systematically known as 2-benzylidene-1-benzofuran-3-one.

Figure 5 shows aurone compounds distinguished from *S. myosotiflora* tubers; 2',4,4',6-tetrahydroxyaurone, and 4,4',6-trihydroxyaurone. The 2',4,4',6-tetrahydroxyaurone ($C_{15}H_{10}O_6$) compound was identified at 10.07 retention time (rt) in the LC-MS whereby the latter (formula molecule: $C_{15}H_{10}O_5$) was one of the best tyrosinase inhibitors without toxic effects [70]. Likewise, 4,4',6-trihydroxy aurone has also been isolated in other *Smilax* species, the *Smilax bracteate* [71].

In contrast, there are two natural polyphenolic stilbenoid compounds been isolated from *S. myosotiflora* through the ethyl acetate extract using silica gel and Sephadex LH-20 column chromatography namely as trans-resveratrol (trans-3,4',5-trihydroxystilbene) and its derivative, trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene [72] (Figure 6). Stilbenoids are classified as phytoalexins, the antimicrobial compounds that existed *de novo* in plants to protect against fungal infections and toxins. While the trans-resveratrol and the derivatives are synthesized by many plants exhibit anticancer, anti-viral, antioxidant, and anti-inflammatory properties. Based on the previous pharmacology activities and this finding, the *S. myosotiflora* ethyl acetate extract may incline a unique character as a promising candidate for respective pharmacology activities. Future evaluations should be performed to investigate any potent bioactivity related to the compounds.

Although a large number of *S. myosotiflora*-based products are now available in the market, approximately only little discoveries on the phytochemical entities have been done on the plant. Therefore, more studies on the related or new biochemical compounds and their bioactivities should be performed and characterized from this multifunction botanical plant. Studies on the isolation and characterization of the phytoconstituents will provide a new insight in the particular mechanism of action on the respective bioactivities of the plant.

4. Conclusions

S. myosotiflora is a well-known vitalizer in improving sexual satisfaction, especially among local and na-

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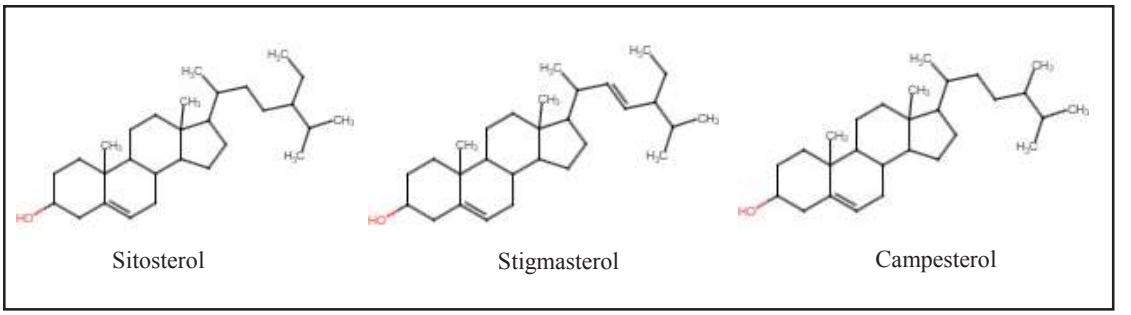

Figure 3. The 2-dimensional (2D) structures of sitosterol, campesterol and stigmasterol.

tive communities in Malaysia. It is a promising drug source which should enter the world market by scientific-evidence-based for its therapeutic effects and as an alternative treatment for many critical health issues such as the MSD. The animal studies have indicated its effectiveness in the stimulation of penile erection, positive orientation towards sexual behaviors pattern, altering related venereal hormones, and enhanced blood flow for smooth muscles of corpus cavernosum relaxation with no harmful effects. This review has also enlightened other therapeutic effects of the plant as a synergistic, antioxidant, anti-enterobacterial, enzyme inhibitory, and anthelmintic agents. However, the activities of other crucial ailments such as coronavirus, antidiabetes, anti-HIV, anti-inflammatory, anti-stress, and anticonvulsant should be executed to ensure a greater chance of advancing the *S. myosotiflora* plant and *S. myosotiflora*-based products for a clinical and marketable drug.

Limited chemical profile evidence but emerging data presented above makes this plant a proficient option to further investigation on its biochemical property and at molecular level. Therefore, several works are currently under progress in our laboratory to identify the active components which responsible for inducing male sexual function and to establish a possible explanation on the mechanism of action in penile erection induction. In addition, more evidence pertaining to the nutritional values, therapeutic efficacy, and safety are required to establish proper medical

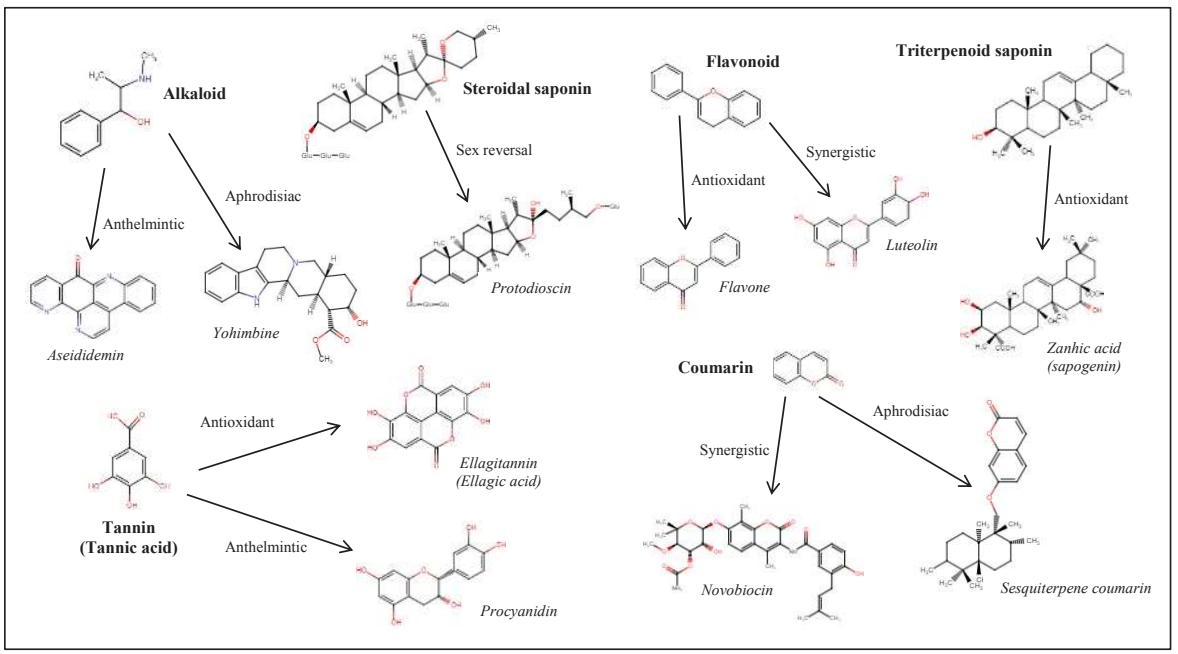

Figure 4. Secondary metabolite classes detected in *S. myosotiflora* with the examples of bioactive compounds related to reported bioactivities in the plant.

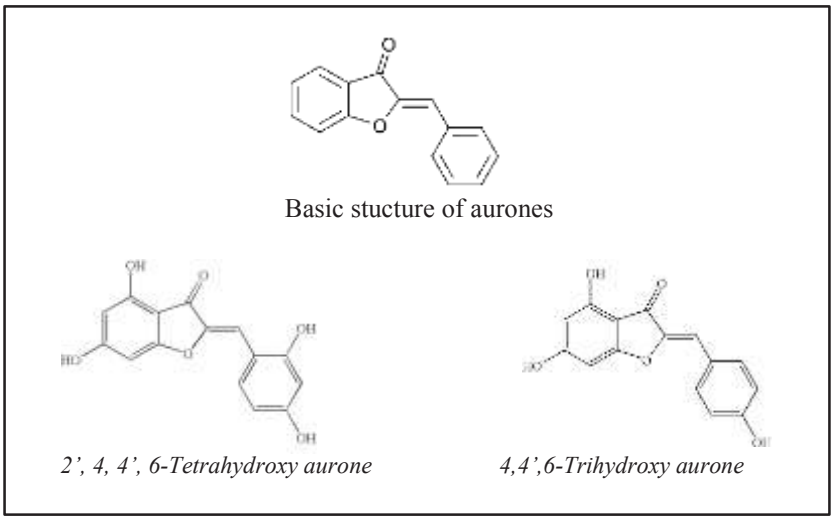

Figure 5. Aurone in basic form and its derivatives found in the *S. myosotiflora* tubers

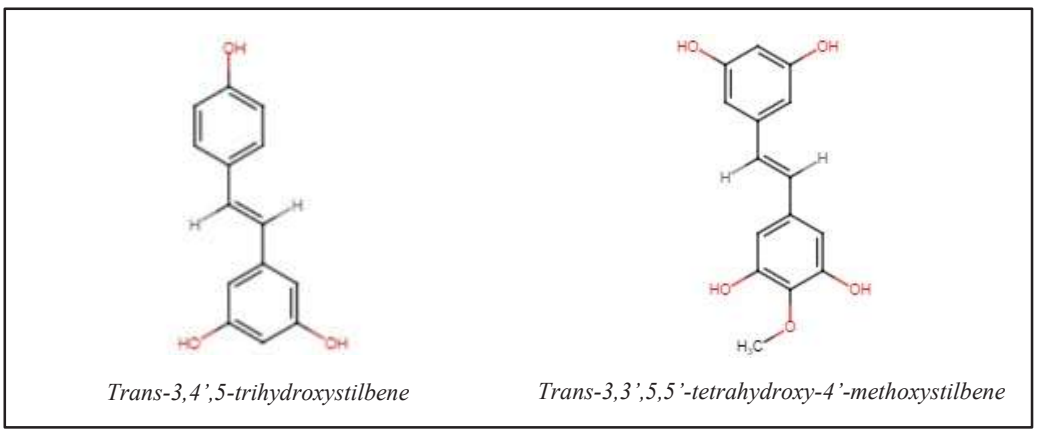

Figure 6. The 2D structures of resveratrol or trans-3,4',5-trihydroxystilbene and trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene isolated from *S. myosotiflora* tubers

recommendations for *S. myosotiflora*'s safe use and to conserve this valuable medicinal plant for the health benefit of future generations. Scientific validation and supporting evidence are pre-requisite in exploring the potential, efficacy, safety, and toxicity of the plants besides for commercial exploitation and narrowing the international knowledge barrier.

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Statement of Contribution of Researchers

Concept – Mohd Dasuki Sul'ain, Rasmaizatul Akma Rosdi; Design – Wan Rosli Wan Ishak, Rasmaizatul Akma Rosdi; Supervision – Mohd Dasuki Sul'ain, Deny Susanti Darnis, Wan Rosli Wan Ishak; Resources and materials – Mohd Dasuki Sul'ain, Rasmaizatul Akma Rosdi; Data Collection and/or processing – Rasmaizatul Akma Rosdi; Analysis and/or Interpretation – Rasmaizatul Akma Rosdi; Literature Search – Mohd Dasuki Sul'ain, Rasmaizatul Akma Rosdi; Writing – Rasmaizatul Akma Rosdi; Critical Reviews – Mohd Dasuki Sul'ain, Deny Susanti Darnis, Wan Rosli Wan Ishak.

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Conflict of Interest

All authors have their responsibility for contributing to their parts on the article and no conflict of interest to disclose.

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