

Ethnopharmacological and Network Pharmacology Insights into Indonesian Native Plants for Erectile Dysfunction: Focus on *Eurycoma longifolia*-Mediated Testosterone Biosynthesis Pathways

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ABSTRACT

Rates of Erectile Dysfunction (ED) continue to rise each year. Synthetic pharmacological agents used for ED frequently produce unwanted reactions, and their therapeutic performance is limited. Within Indonesia, several native plants have traditionally been used to manage ED symptoms. This review seeks to compile updated scientific data on Indonesian medicinal plants with potential efficacy against ED and to clarify their molecular actions using a systematic review and a Pharmacological Network framework. A total of 12 candidate plant species widely employed by Indonesian ethnic groups for treating ED were identified. Literature searches were performed in PubMed, Scopus, and Springer without restricting publication date. Article selection followed the PRISMA workflow. Compound-related gene targets were identified via GeneCards, while disease-associated genes were obtained from DisGeNET. Network features were analyzed using Cytoscape 3.10, and STRING 12.0 supported the creation of Protein–Protein Interaction maps. Subsequent GO and KEGG enrichment analyses were completed using SRplot. The systematic review ultimately included 12 publications matching the eligibility standards. Pharmacology Network evaluation showed that constituents of *Eurycoma longifolia*—particularly stigmaterol, eurycomanone, and eurycomalactone—interact with 13 ED-related genes: BCL2, AKT1, SOAT1, PCSK9, ACHE, BDNF-AS, TMX2-CTNND1, GSK3B, LINCO1672, TP53, H19, HIF1A, and IL1B. These genes participate in pathways linked to steroid hormone biosynthesis, a key process in testosterone formation. Thus, *Eurycoma longifolia* emerges as a strong phytopharmaceutical candidate for improving sexual function.

Keywords: Aphrodisiacs, Erectile dysfunction, Eurycomanone, Stigmaterol, Testosterone

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Introduction

Sexual dysfunction is clinically defined as an alteration in one or more phases of the sexual response cycle—desire, arousal, orgasm, or pain—or discomfort experienced during intercourse. It manifests as difficulty engaging in or deriving satisfaction from sexual activity, generating marked distress and reducing sexual and general well-being in both sexes [1, 2]. Erectile dysfunction (ED), commonly termed male impotence, describes a persistent incapacity to obtain and maintain an erection adequate for mutually satisfying intercourse. ED forms a major subset of male sexual disorders and is often used as an indicator of broader sexual health concerns because it reflects interacting physical, emotional, and social factors [3]. Beyond diminishing sexual performance, ED may contribute to infertility, reduced quality of life, and signs of early aging [4]. Worldwide prevalence estimates range between 3 % and 76.5 % [5]. Etiologies include psychological components (anxiety, depressive symptoms, stress, intimacy fears, neuropathic issues), as well as physiological origins such as stroke, neurological trauma, degenerative diseases like Alzheimer's or Parkinson's, chronic illnesses (diabetes, hypertension), vascular abnormalities, atherosclerosis, phimosis, Peyronie's disease, lifestyle habits such as smoking or chronic alcohol use, hormonal decline with age, and systemic organ disorders involving the heart, kidneys, liver, lungs, or

malignancy [6, 7]. Prior investigations have also suggested links between prostate or kidney conditions, renal impairment, and elevated ED risk [8].

At the molecular level, erectile dysfunction arises from disruptions in several key processes, most notably the nitric oxide (NO)–cyclic GMP (cGMP) signaling cascade. Under normal circumstances, NO generated by nitric oxide synthase (NOS) is released from neuronal and endothelial sources during sexual stimulation. NO activates guanylate cyclase in smooth muscle cells, elevating cGMP. This sequence produces relaxation of corpus cavernosum smooth muscle, enhanced arterial inflow, and restricted venous return—together sustaining an erection. In ED, NO synthesis may decline due to endothelial injury or neural impairment. Additionally, heightened activity of phosphodiesterase-5 (PDE-5) accelerates cGMP degradation, further suppressing erectile function [9–11].

Oxidative stress—marked by excessive reactive oxygen species (ROS)—injures endothelial tissues and lowers NO bioavailability, diminishing vasodilation and contributing to ED [12]. Chronic disorders, including diabetes and hypertension, elevate pro-inflammatory cytokines such as TNF- α and IL-6, which aggravate penile tissue damage and induce fibrotic changes, reducing tissue flexibility and hampering erectile capability. These mechanisms are particularly evident in conditions like Peyronie's disease and long-term diabetes [11, 12].

Presently, the mainstay pharmacologic treatment for ED utilizes PDE-5 inhibitors such as sildenafil, vardenafil, and tadalafil. These agents act by preventing cGMP breakdown in penile smooth muscle cells, thereby sustaining concentrations of this essential second messenger [13]. Sildenafil citrate, for example, has shown positive effects on penile blood dynamics [14]. Nonetheless, these medications are limited by inconsistent effectiveness, undesirable adverse effects, and contraindications for individuals with particular health issues [15].

The investigation of natural agents with possible aphrodisiac effects or therapeutic value for erectile dysfunction (ED) offers an important alternative for overcoming the adverse reactions and limited performance of current ED medications [16]. Indonesia, known for its vast botanical resources and diverse cultural traditions, harbors a substantial collection of plant-based remedies relevant to ED management. Indonesian medicinal plants encompass species with therapeutic properties that naturally grow throughout the archipelago. Their use has developed through long-standing traditional knowledge, especially ethnomedicine and community practices. Earlier ethnomedicinal surveys documented 204 plant species spanning 78 families for alleviating ED. The five families most often represented were Zingiberaceae, Euphorbiaceae, Arecaceae, Fabaceae, and Rubiaceae. Frequently cited species included *Imperata cylindrica* (19 ethnic groups), *Zingiber officinale* Roscoe (17 ethnic groups), *Areca catechu* L. (14 ethnic groups), *Eurycoma longifolia* Jack. (10 ethnic groups), *Piper nigrum* L. (9 ethnic groups), *Pimpinella pruatjan* (5 ethnic groups), *Panax ginseng* (4 ethnic groups), *Kaempferia galanga* (4 ethnic groups), *Talinum paniculatum* (4 ethnic groups), *Luvunga sarmentosa* (4 ethnic groups), *Piper retrofractum* (3 ethnic groups), and *Mimosa pudica* (3 ethnic groups) [17, 18]. Yet, despite this extensive documentation, a systematic review examining the ED-related potential of these Indonesian botanicals has not previously been published.

Notably, neither narrative reviews nor systematic evaluations have focused on native Indonesian plants with potential benefits for ED. Likewise, the molecular pathways and gene targets associated with the active compounds in these plants—mechanisms that may be critical for ED therapy—remain poorly explored. This review, therefore, addresses these gaps by applying a pharmacological network framework to forecast molecular actions, identify key gene targets, and map biological pathways relevant to ED management.

Within the broader objective of improving treatments for sexual dysfunction, network-based pharmacological analysis serves as a key methodology for identifying mechanisms of action and determining how specific plant-derived compounds influence biological systems governing sexual responses. This analytical approach centers on protein–protein interactions, clarifying how bioactive constituents connect with regulatory components that modulate sexual function.

The primary goal of this work is to systematically examine the existing research on Indonesian medicinal plants with therapeutic promise for ED. Following this, we aim to characterize up-to-date insights into their bioactive constituents and mechanisms by applying a detailed pharmacological network analysis.

Materials and Methods

Systematic review

Data sources and search strategy

This systematic review followed the PRISMA reporting guidelines. Searches were conducted in PubMed, Scopus, and Springer Link up to June 30, 2023. Plant selection was informed by earlier investigations of ethnomedicinal practices and community knowledge across 34 provinces and 405 ethnic groups. Of the 204 plant species identified as having relevance to ED, 12 were most commonly used among Indonesian ethnic communities: *Imperata cylindrica* (19 ethnic groups), *Zingiber officinale* Roscoe (17 ethnic groups), *Areca catechu* L. (14 ethnic groups), *Eurycoma longifolia* Jack. (10 ethnic groups), *Piper nigrum* L. (9 ethnic groups), *Pimpinella pruatjan* (5 ethnic groups), *Panax ginseng* (4 ethnic groups), *Kaempferia galanga* (4 ethnic groups), *Talinum paniculatum* (4 ethnic groups), *Luvunga sarmentosa* (4 ethnic groups), *Piper retrofractum* (3 ethnic groups), and *Mimosa pudica* (3 ethnic groups) [17, 18]. Consequently, the literature search employed these plant names—*Eurycoma longifolia*, *Imperata cylindrica*, *Zingiber officinale*, *Areca catechu*, *Piper nigrum*, *Panax ginseng*, *Pimpinella pruatjan*, *Kaempferia galanga*, *Piper retrofractum*, *Mimosa pudica*, *Lavanga sarmentosa*, and *Talinum paniculatum*—combined with “aphrodisiac” and “Erectile Dysfunction (ED).” Articles categorized as “Toxicity,” “Review,” or “Systematic Review” were excluded.

From these 12 plants, those supported by robust scientific evidence and containing compounds implicated in modulating ED-related molecular mechanisms were prioritized. No temporal limits were set, although only English-language studies were included. Reference lists of retrieved articles were also screened. When essential information was missing, corresponding authors were contacted to obtain additional data.

Study selection and eligibility criteria

All identified records were transferred into reference-management software for screening. Two reviewers independently examined titles and abstracts. Repeated discussions ensured agreement on operational definitions and data extraction procedures. Any uncertainties or disputes were resolved through consultation with a third reviewer. Eligibility was determined using the PICO framework shown in **Table 1**.

Articles passing initial screening underwent full-text review. Assessments from both reviewers were combined. Critical appraisal of included studies was completed using the Joanna Briggs Institute Critical Appraisal Tool appropriate for cohort, cross-sectional, and quasi-experimental designs. When disagreements or unclear points arose, team meetings were held. Extracted information was recorded and structured in tabular form [19].

Table 1. PICOS criteria for inclusion of studies.

Parameter	Inclusion Criteria
Population	Human subjects and animal models
Intervention	Any form of preparation derived from traditional Indonesian medicinal plants (e.g., extracts, infusions, decoctions, pounded herbs, etc.) demonstrating activity against erectile dysfunction
Comparator	Standard/reference erectile dysfunction drugs (e.g., sildenafil) or placebo/vehicle administered under comparable conditions
Outcome	Measurable therapeutic effects on erectile dysfunction in humans or animal models
Study Design	All types of experimental/laboratory studies (in vivo, in vitro, or clinical experimental designs)

Pharmacology network

Collection and screening of target proteins

To determine the genes linked to erectile dysfunction (ED), this study utilized the DisGeNET platform (<https://www.disgenet.org/>).

From DisGeNET, 256 genes associated with ED (disease code C0242350) were retrieved. Target genes corresponding to compounds present in *Eurycoma longifolia* were subsequently identified using the GeneCards database (<https://www.genecards.org/>).

A total of 11 compounds—Eurycomanone, 13 α ,21-Dihydroeurycomanone, Stigmasterol, Trans-coniferyl aldehyde, Scopoletin, Eurycomalactone, 6 α -Hydroxy-eurycomalactone, Eurycomanone, Eurycomanol,

Eurycomanol-2-O- β -D-glucopyranoside, and 9-Hydroxycanthin-6-one—were screened for gene targets. Only three compounds (Eurycomanone, Stigmasterol, and Eurycomalactone) had gene targets documented in GeneCards. Together, these three compounds were linked to 88 genes.

A Venn diagram comparison (<https://bioinfogp.cnb.csic.es/tools/venny/>) was then conducted to identify the shared genes between the 256 ED-related genes and the 88 compound-related genes.

The compound–gene–disease network was generated using Cytoscape version 3.10 [20].

Construction of protein–protein interaction (PPI) network and enrichment analysis

Genes that overlapped between the active compounds and ED were used for subsequent analysis via the STRING database version 12.0 (<https://string-db.org/>). A Protein–Protein Interaction (PPI) network was built using these shared genes, applying a minimum confidence interaction score of 0.400.

The PPI network supported functional examination of biological activity through Gene Ontology (GO) annotations and KEGG pathway enrichment, highlighting signaling processes relevant to ED. The processed results were visualized using SRplot (<http://www.bioinformatics.com.cn/srplot>) [21].

Results and Discussion

Results of systematic review

Indonesian medicinal plants with potential as remedies for ED

Screening procedures followed the PRISMA guidelines (**Figure 1**). The initial search retrieved 248 articles, which were refined by eliminating 50 duplicates. The remaining 198 titles and abstracts were assessed, and 150 were excluded for not meeting the study criteria.

Out of 48 full-text articles reviewed for relevance, 36 did not satisfy inclusion requirements. Consequently, 12 articles were finalized for analysis.

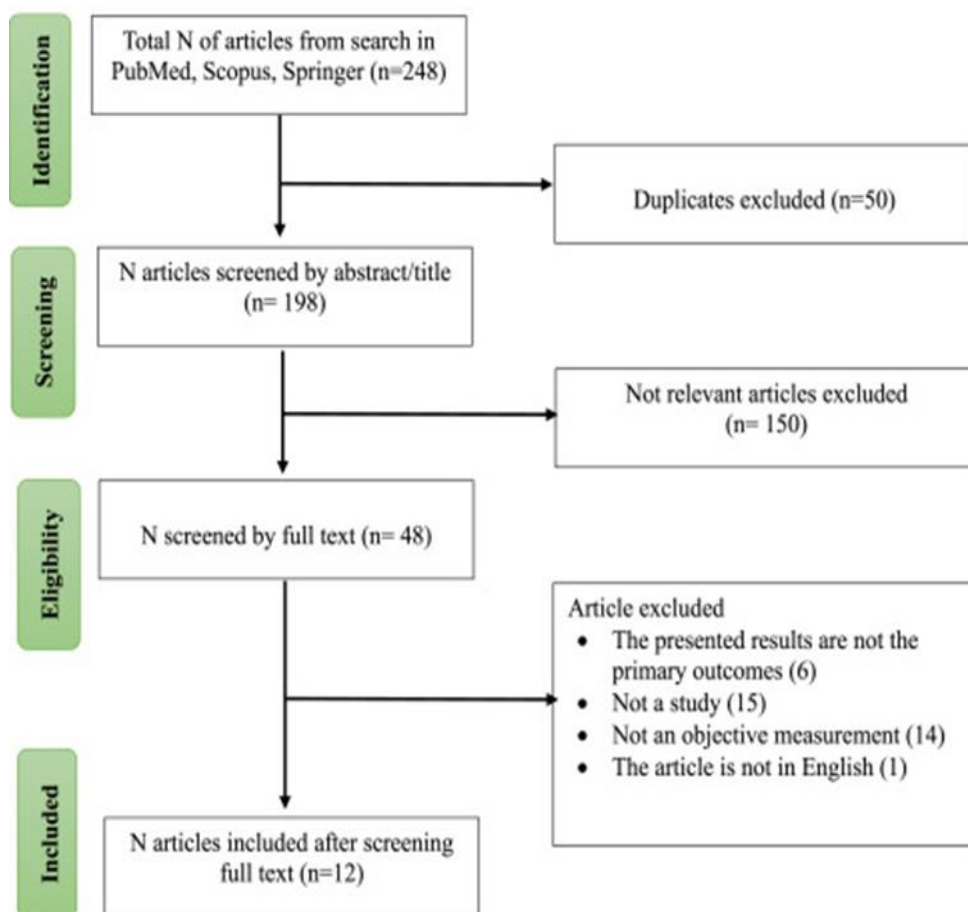


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart.

Traditional uses, parts used, dosage form, and compound class

Among the plant forms traditionally used in Indonesian remedies for sexual dysfunction, herbs (36%) were most common, followed by rhizomes (15.38%), shrubs (15.38%), climbers (7.6%), and grasses (7.69%) (**Table 2 and Figure 2**). These results are consistent with earlier reports showing that herbaceous plants are widely used in traditional medicine owing to their abundance and accessibility [22].

Frequently utilized plant parts included roots (45.45%), rhizomes (25.0%), seeds (16.67%), herbs (8.33%), and fruits (8.33%). Prior studies have also identified roots as the primary plant material used by Indonesian healers for managing sexual disorders, possibly due to the belief that roots contain the most potent therapeutic constituents. In addition, roots often contain higher concentrations of bioactive metabolites [23, 24].

In terms of preparation methods for aphrodisiac and ED-related remedies, decoction (46.15%) was the most frequently employed technique, followed by infusion (38.6%) and pounding (15.38%) (**Table 1 and Figure 3**). Decoction, which involves boiling plant material until the volume is reduced to about one-quarter, is widely recognized across cultures as a dominant traditional preparation method [25, 26]. Unlike decoction, infusion does not involve boiling, enabling better preservation of volatile or heat-sensitive constituents.

In Indonesian traditional medicine, pounding—with the resultant mixture known as *jamu*—is also a common approach, wherein crushed plant material is blended with warm water and consumed [27, 28].

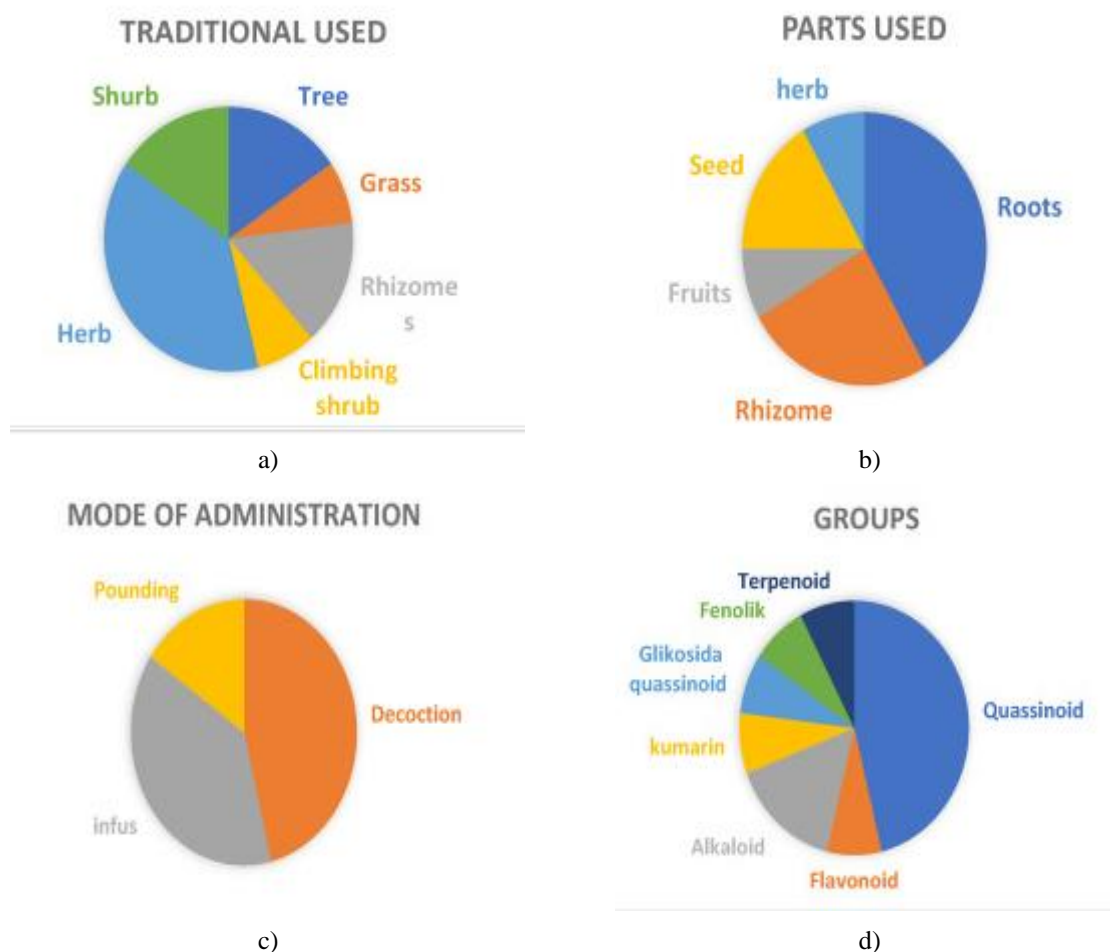


Figure 2. Traditional use, plant parts, dosage forms, and compound classes of Indonesian medicinal plants for ED.

Table 2. Native Indonesian Medicinal Plants with Efficacy as Remedies for ED: Traditional Uses, Parts Utilized, Administration Modes, Toxicity, and Scientific Evidence.

Plant Name	Family	Habit / Form	Part(s) Used	Preparation / Administration	Reported Toxicity	Scientific Evidence / Validation
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Eurycoma longifolia	Simaroubaceae	Tree	Roots	Infusion	No toxicity [29]	Root powder increases sexual performance via elevated testosterone levels in impotence animal models [29]
Eurycoma longifolia	Simaroubaceae	Tree	Roots	Infusion	No toxicity [29]	Butanol, methanol, water, and chloroform fractions (800 mg/kg) significantly increase levator ani muscle weight ($p < 0.05$) in animal models [30]
Eurycoma longifolia	Simaroubaceae	Tree	Roots	Infusion	No toxicity [29]	Water extract and isolates improve sexual dysfunction through Rho-kinase II inhibition [31]
Eurycoma longifolia	Simaroubaceae	Tree	Roots	Infusion	No toxicity [29]	9-Hydroxycanthin-6-one identified as active aphrodisiac compound that relaxes corpus cavernosum and seminal vesicles [31]
Eurycoma longifolia	Simaroubaceae	Tree	Roots	Infusion	No toxicity [29]	Water extract produces dose-dependent enhancement of sexual performance in animal models [30]
Eurycoma longifolia	Simaroubaceae	Tree	Roots	Infusion	No toxicity [29]	Water extract increases libido in sexually experienced male rats [32]
Eurycoma longifolia	Simaroubaceae	Tree	Roots	Infusion	No toxicity [29]	Water extract effective as a supplement for managing late-onset hypogonadism (LOH) and hypogonadism symptoms in patients [33]
Imperata cylindrica	Poaceae	Grass	Rhizome	Decoction	No toxicity [34]	No scientific validation records available
Zingiber officinale	Zingiberaceae	Rhizomatous herb	Rhizome	Decoction	No toxicity [35, 36]	Extract significantly increases sperm motility and count in epididymis and vas deferens without sperm toxicity [36]
Areca catechu	Arecaceae	Tree	Fruits	Decoction	No toxicity [37]	No scientific validation records available
Piper nigrum	Piperaceae	Woody climber	Seeds	Pounding	No toxicity [38]	No scientific validation records available
Panax ginseng	Araliaceae	Herb	Roots	Infusion	No toxicity [39]	Infusion significantly improves sexual experience in male volunteers with no side effects [39]
Panax ginseng	Araliaceae	Herb	Roots	Infusion	No toxicity [40]	Significantly improves erectile dysfunction in male patients [40]
Pimpinella pruatjan	Apiaceae	Herb	Roots	Decoction	No toxicity [41]	Extract increases nNOS expression in NANC nerve fibers of corpus cavernosum, promoting smooth muscle relaxation and erection [42]

Kaempferia galanga	Zingiberaceae	Rhizomatous herb	Rhizome	Pounding	No toxicity [43]	No scientific validation records available
Piper retrofractum	Piperaceae	Shrub	Seeds	Infusion	No toxicity [44]	No scientific validation records available
Mimosa pudica	Fabaceae	Herb	Whole herb	Infusion	No toxicity [45]	Demonstrates protective and restorative effects on testicular tissue in cadmium-treated rats [46]
Lavanga sarmentosa	Rutaceae	Shrub	Roots	Decoction	No records	No scientific validation records available
Talinum paniculatum	Portulacaceae	Herb	Roots	Decoction	No toxicity [47]	No scientific validation records available

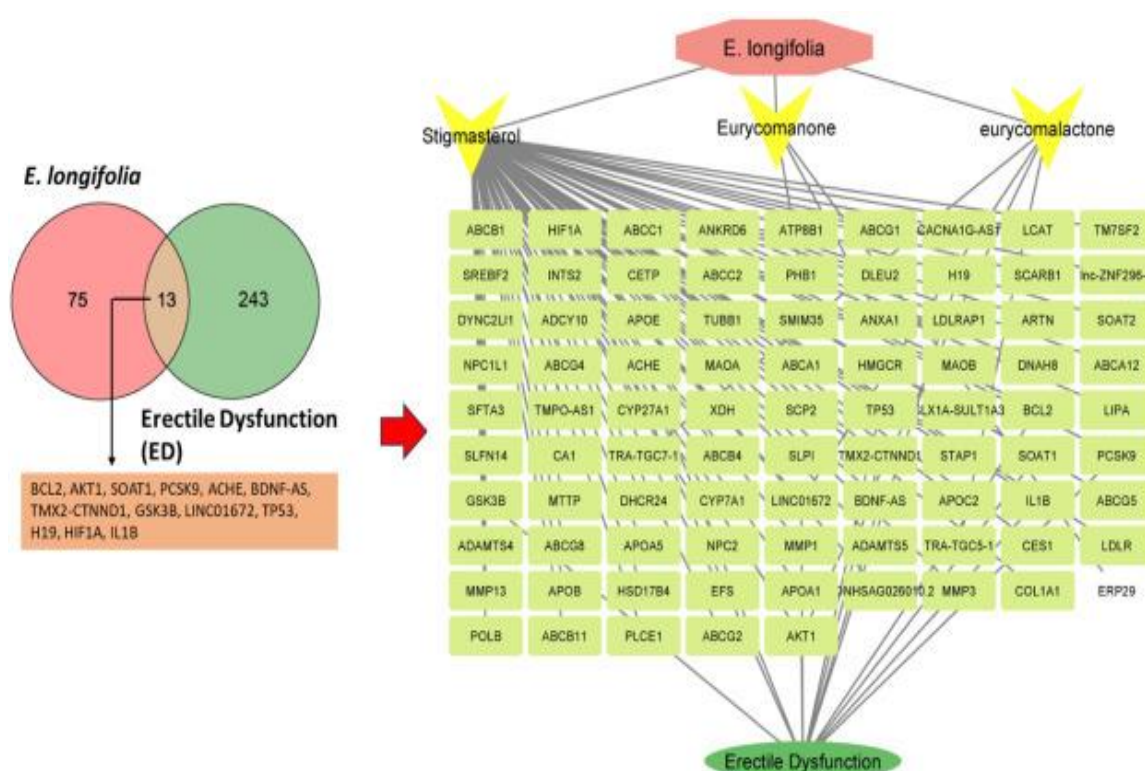


Figure 3. Venn diagram and Cytoscape of *E. longifolia* compounds (stigmasterol, eurycomanone, Eurycomalacton).

Compounds and pharmacological activities

A literature search on scientific reports related to plants traditionally used for managing sexual dysfunction (**Table 3**) showed that couassinoids were the most commonly highlighted constituents with aphrodisiac potential, followed by alkaloids and flavonoids. Additional information is presented in **Table 3 and Figure 2**.

A systematic review examining 12 indigenous Indonesian medicinal species identified two plants that contain active constituents with pharmacological actions relevant to treating sexual dysfunction: *Eurycoma longifolia* and *Panax ginseng*. As indicated in **Table 2**, *E. longifolia* contains multiple bioactive components with diverse target interactions linked to ED. To clarify how these constituents influence molecular pathways associated with ED improvement, *E. longifolia* was selected for further analysis. The network-based pharmacological strategy used here was intended to provide an integrated perspective on how its compounds collectively contribute to therapeutic effects in ED.

Table 3. Active constituents in aphrodisiac and erectile dysfunction–related plants and their associated molecular mechanisms.

Plant Name	Chemical Compound	Chemical Class	Reported Pharmacological Effect	Reference
<i>Eurycoma longifolia</i>	Eurycomanone	Quassinoid	Increases testosterone production	[23]
<i>Eurycoma longifolia</i>	13 α ,21-Dihydroeurycomanone	Quassinoid	Improves spermatogenesis	–
<i>Eurycoma longifolia</i>	Stigmasterol	Phytosterol (steroid)	Inhibits Rho-Kinase II	[48]
<i>Eurycoma longifolia</i>	trans-Coniferyl aldehyde	Phenylpropanoid/Alkaloid	Inhibits Rho-Kinase II	–
<i>Eurycoma longifolia</i>	Scopoletin	Coumarin	Inhibits Rho-Kinase II	–
<i>Eurycoma longifolia</i>	Eurycomalactone	Quassinoid	Inhibits Rho-Kinase II	–
<i>Eurycoma longifolia</i>	6 α -Hydroxyeurycomalactone	Quassinoid	Inhibits Rho-Kinase II	–
<i>Eurycoma longifolia</i>	Eurycomanone	Quassinoid	Inhibits Rho-Kinase II	–
<i>Eurycoma longifolia</i>	Eurycomanol	Quassinoid	Inhibits Rho-Kinase II	–
<i>Eurycoma longifolia</i>	Eurycomanol-2-O- β -D-glucopyranoside	Quassinoid glycoside	Inhibits Rho-Kinase II	–
<i>Eurycoma longifolia</i>	9-Hydroxycanthin-6-one	β -Carboline alkaloid	Reduces muscle tone of corpus cavernosum and/or seminal vesicles	[31]
<i>Panax ginseng</i>	Ginsenosides	Triterpenoid saponins	Increases nitric oxide synthase (NOS) activity and improves penile blood flow	[40]
<i>Panax ginseng</i>	Ginsenosides	Triterpenoid saponins	Stimulates hypothalamic–pituitary–adrenal axis, elevating plasma corticotropin and corticosteroids	[40]

Network pharmacology

Gene targets of compounds in *E. longifolia* for ED treatment

Network analysis of compound–target relationships revealed that 11 constituents from *E. longifolia* correspond to 88 predicted gene targets. Using DisGeNET, 256 genes associated with ED were identified. A Venn diagram comparison demonstrated 13 shared targets between the plant’s active compounds and ED-related genes (**Figure 3**). These overlapping genes are BCL2, AKT1, SOAT1, PCSK9, ACHE, BDNF-AS, TMX2-CTNND1, GSK3B, LINCO1672, TP53, H19, HIF1A, and IL1B.

Within the pharmacological network, the topology reflects connections between compound nodes and the corresponding regulatory edges. **Figure 3** indicates the presence of 3 compounds interacting with 86 protein nodes and forming 468 edges. Each node signifies a protein, while each edge denotes an interaction such as binding, expression modulation, or other regulatory events.

Protein–protein interaction

Figure 4a displays the protein–protein interaction (PPI) profile of the potential targets linked to *E. longifolia* constituents in the context of ED. These PPIs participate in biological functions such as GO:0042632 Cholesterol homeostasis, GO:0010876 Lipid localization, GO:0015850 Hydroxy organic compound transport, GO:0055088 Lipid homeostasis, GO:0006869 Lipid transport, GO:0030301 Cholesterol transport, GO:0015918 Sterol

transport, GO:0008202 Steroid metabolic process, GO:0016125 Sterol metabolic process, and GO:0008203 Cholesterol metabolic process (**Figure 4b**).

Beyond Gene Ontology insights, the PPIs also map to KEGG pathways, including hsa04979 Cholesterol metabolism and hsa00100 Steroid biosynthesis, which influence cholesterol and steroid formation [23]. These results outline how *E. longifolia* constituents may modulate molecular events related to ED through complex PPI networks and their associated pathways.

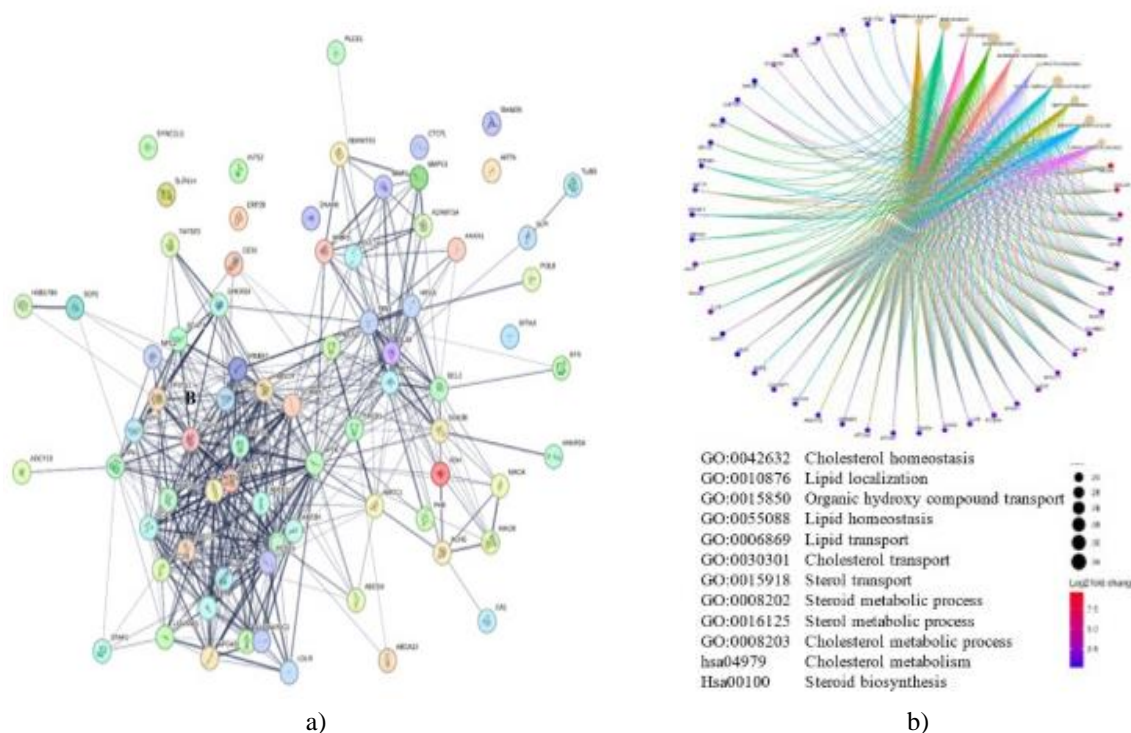


Figure 4. Protein–Protein Interactions of *Eurycoma longifolia* compounds involved in ED-related biological processes. The compounds include stigmasterol, eurycomanone, and eurycomalacton.

a) The PPI network of all targets shows 86 nodes and 468 edges.

b) Potential ED-related pathways, number of involved targets, and interaction relevance.

Enrichment analysis of gene ontology and KEGG pathway

Gene ontology enrichment demonstrated that *E. longifolia* constituents affect several ED-related biological processes (**Figure 5a**). Among them, the steroid metabolic process included 30 gene targets, while lipid transport and lipid localization each involved 34 targets. Sterol transport also showed notable activity, with 26 targets. Concerning cellular components, the most enriched categories were the apical cell region, plasma lipoprotein particles, and protein–lipid complexes. In terms of molecular functions, lipid transporter activity was the most prominent enrichment, followed by cholesterol transport and sterol transport (**Figure 5b**). This analysis indicated that stigmasterol, eurycomanone, and eurycomalacton interact with the KEGG hsa00100 pathway associated with steroid biosynthesis (**Figure 6**). This pathway is essential for generating testosterone, beginning with cholesterol mobilization and proceeding through multiple enzymatic steps that ultimately yield steroid hormones, including testosterone.

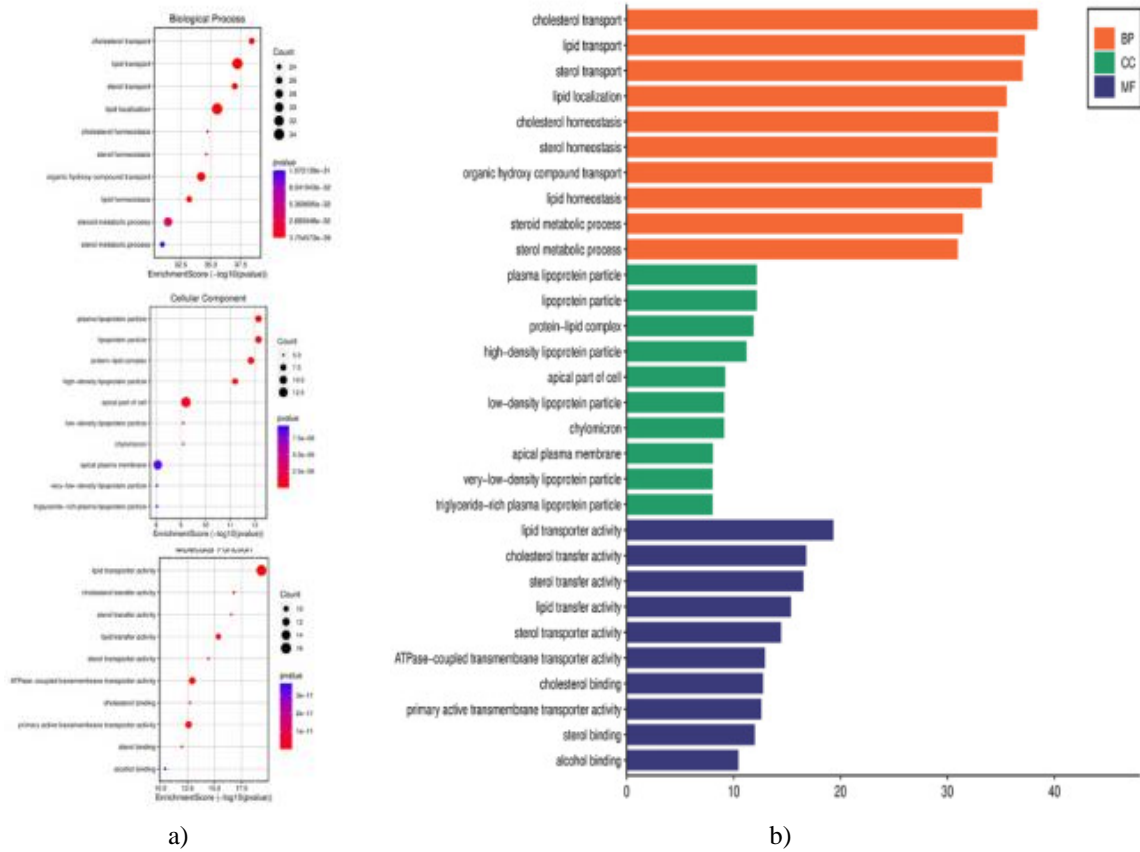


Figure 5. Enrichment Analysis of Gene Ontology and KEGG Pathway; a) The enrichment bubble chart shows the participation of 32 genes in the steroid metabolic biological process. b) GO enrichment bar chart presenting Biological Processes, Cellular Components, and Molecular Functions.

AKT1, SOAT1, PCSK9, ACHE, BDNF-AS, TMX2-CTNND1, GSK3B, LINCO1672, TP53, H19, HIF1A, and IL1B.

Protein–Protein Interaction (PPI) evaluation further highlighted 10 biological pathways strongly associated with ED, including GO:0042632 Cholesterol homeostasis, GO:0030301 Cholesterol transport, GO:0015918 Sterol transport, GO:0008202 Steroid metabolic process, GO:0016125 Sterol metabolic process, and GO:0008203 Cholesterol metabolic process. When these processes are examined in relation to the steroid biosynthesis pathway and its involvement in testosterone production, they demonstrate a connection to spermatogenesis, which may explain their relevance to ED treatment (**Figure 6**). Steroid biosynthesis consists of sequential biochemical reactions that generate steroid hormones—among them, testosterone [51]. Cholesterol and other sterols act as essential precursors in this pathway and are important for maintaining sexual and reproductive physiology [52]. Since testosterone is a major androgen influencing erectile function and fertility [33], disturbances in its synthesis or precursor availability can contribute to ED.

Earlier research indicates that Eurycomanone may elevate testosterone levels [53]. Extracts from the roots of *Eurycoma longifolia*, as well as purified Eurycomanone, have been shown to significantly boost testosterone production in rat models, likely through modulation of reproductive hormone systems [29].

Our results further support the role of Eurycomanone in the steroid biosynthesis pathway, a key metabolic route responsible for generating steroidal hormones such as testosterone (**Figure 6**). Increased testosterone output is closely associated with enhanced spermatogenesis, the series of events leading to the formation and maturation of sperm cells within the testes. This hormone regulates several phases of the process, including spermatogonium proliferation, spermatid differentiation, and final sperm maturation [54, 55].

Additionally, compounds within the quassinoid group—especially 13 α ,21-Dihydroeurycomanone—have been reported to promote spermatogenesis. Evidence suggests that Eurycomanone and 13 α ,21-Dihydroeurycomanone may act synergistically, since whole-plant fractions often demonstrate stronger biological activity than isolated constituents. The proposed mechanism linking testosterone enhancement to improved spermatogenesis involves increased LH stimulation of testosterone production. Testosterone, in turn, modulates gene expression in spermatogenic cell populations. By elevating testosterone concentrations, quassinoid compounds may influence the genetic regulatory networks governing spermatogenesis, thereby promoting the expansion and differentiation of spermatogonium cells and ultimately raising sperm output [56, 57].

In addition, *Eurycoma longifolia* contains several bioactive molecules, including stigmaterol, trans-coniferyl aldehyde, scopoletin, eurycomalactone, 6 α -hydroxyeurycomalactone, eurycomanone, and eurycomanol. These constituents have been documented to suppress the activity of Rho-Kinase II (ROCK-II). ROCK-II is an enzyme that governs various cellular processes and contributes to structural regulation within vascular tissues. In relation to erectile dysfunction, ROCK-II is central to controlling smooth muscle contraction in penile vasculature and influences the hemodynamic changes required to initiate and maintain an erection [47].

Under ED conditions, elevated ROCK-II function may intensify smooth muscle tension, interfere with nitric oxide (NO) pathways, and promote aberrant vascular remodeling. These disturbances hinder proper penile blood flow. Therefore, ROCK-II inhibition has been proposed as a therapeutic approach, as reducing its activity may promote vascular relaxation and improve circulation to the penile tissue [58, 59].

The significant effects of *Eurycoma longifolia* on sexual disorders have also been linked to its antioxidant and anti-inflammatory properties. Oxidative stress plays a substantial role in ED by contributing to endothelial impairment and disrupting NO signaling—both vital for normal erectile function. Reactive oxygen species (ROS) can react with NO to generate peroxynitrite, lowering available NO required for vasodilation [60]. Additionally, ROS may damage neuronal pathways and impair neuronal nitric oxide synthase (nNOS) function, reducing NO release during sexual arousal [61]. Decreasing NO levels leads to vascular dysfunction, a primary mechanism underlying ED [12, 62]. Antioxidant compounds help counter these effects by neutralizing free radicals, preserving NO availability, and suppressing inflammatory mediators, thereby offering protection to endothelial tissue [63].

Eurycomalactone (EL), a major constituent of *Eurycoma longifolia*, acts as a strong NF- κ B inhibitor [64]. Both EL and extracts prepared with ethanol may provide benefits in disorders involving inflammatory signaling, including sexual dysfunction [65]. Elevated aldosterone levels have been linked to severe ED due to their ability to increase inflammatory cytokines and trigger NF- κ B activation within the penile corpus cavernosum [66, 67]. Persistent NF- κ B activity and chronic inflammation in testicular tissue may also contribute to testicular impairment through age-related rises in COX-2 and pro-inflammatory cytokines [68, 69].

Moreover, 9-hydroxycanthin-6-one, another component present in *Eurycoma longifolia*, has been reported to possess activity relevant to ED management. Its proposed action involves reducing the contractile tone of the corpus cavernosum and/or the seminal vesicles (SV) [31]. This effect is thought to occur through inhibition of phosphodiesterase type 5 (PDE5), an enzyme responsible for degrading cyclic guanosine monophosphate (cGMP)—a molecule crucial for smooth muscle relaxation in both corpus cavernosum and SV tissues. By blocking PDE5, the compound maintains elevated cGMP concentrations, promoting smooth muscle relaxation and enhancing penile blood flow [5, 13, 70].

Limitation

This review highlights that traditional claims do not always align with scientific validation. Among the 12 plants surveyed, 7 lacked adequate supporting evidence in the literature. Only 2 species, *Eurycoma longifolia* and *Panax ginseng*, were clearly reported to contain compounds relevant to sexual dysfunction mechanisms. Additionally, all mechanistic predictions in this work rely solely on bioinformatic interpretation. Experimental confirmation through *in vitro* and *in vivo* approaches will be required to substantiate these findings.

Conclusion

This systematic investigation shows that from 12 reviewed articles concerning erectile dysfunction (ED), *Eurycoma longifolia* and *Panax ginseng* were the plants with the strongest scientific support. Using network pharmacology, we identified three key compounds in *E. longifolia*—stigmaterol, eurycomanone, and eurycomalactone—associated with 13 ED-related genes: BCL2, AKT1, SOAT1, PCSK9, ACHE, BDNF-AS, TMX2-CTNND1, GSK3B, LINCO1672, TP53, H19, HIF1A, and IL1B. These genes participate in processes central to steroid hormone production, including GO:0030301 Cholesterol transport, GO:0015918 Sterol transport, GO:0008202 Steroid metabolic process, GO:0016125 Sterol metabolic process, and the KEGG hsa00100 steroid biosynthesis pathway. This pathway underpins testosterone formation, which is essential for reproductive and erectile physiology. As such, *Eurycoma longifolia* shows strong potential as a phytopharmaceutical candidate for managing sexual dysfunction.

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