



Ethnobotany, Phytochemistry, Pharmacology, and Toxicology of *Cycas revoluta* Thunb.: An Updated Review

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Abstract: *Cycas revoluta* Thunb. (sago palm), a cycad native to southern Japan, is widely cultivated and has a long history of ethnobotanical use, including as a famine food and in traditional medicine, but is also well known for its pronounced toxicity to humans and animals. This review critically synthesizes literature published between 1958-2025 on the ethnobotany, phytochemistry, pharmacology, and toxicology of *C. revoluta* from ScienceDirect, PubMed, Scopus, and Google Scholar. Presented as a structured narrative review, the study highlights diverse phytochemicals, including biflavonoids, cycasin, β -N-methylamino-L-alanine (BMAA), and reported antimicrobial peptides, noting variability in compound verification and reproducibility. Reported pharmacological activities are derived mainly from in vitro and preclinical studies, with limited in vivo validation and no established clinical relevance, whereas toxicological evidence for neurotoxicity, genotoxicity, and hepatotoxicity is robust. This imbalance underscores the need for caution in extrapolating therapeutic potential. Key gaps include inadequate extract standardization, limited bioavailability and safety data, and overreliance on single-study findings. Future research should prioritize rigorous toxicological assessment and reproducible validation before any translational application is considered. In addition, the review emphasizes the importance of distinguishing traditional knowledge from experimentally validated evidence and separating descriptive phytochemical inventories from mechanistic and translational insights. Particular attention is given to methodological limitations, including inconsistent extraction protocols, insufficient structural confirmation of reported compounds, and the frequent absence of dose-response, pharmacokinetic, and long-term safety evaluations. By adopting a critical and balanced perspective, this review aims to guide future studies toward scientifically rigorous, ethically responsible, and clinically relevant research directions.

Introduction

Cycas revoluta Thunb., commonly known as the Japanese sago palm or king sago, is an ancient gymnosperm belonging to the family Cycadaceae (1-30). Endemic to the Ryukyu Islands of southern Japan, it is now widely cultivated as an ornamental plant across tropical, subtropical, and temperate regions due to its resilience and aesthetic appeal (10-12). Its tolerance to diverse habitats and soil types has facilitated its global distribution (4-9). In this review, these ecological characteristics are considered only in terms of their influence on human exposure, utilization, and associated health and environmental risks.

Historically, *C. revoluta* has occupied a complex and often contradictory role in human societies, functioning both as an emergency food source during periods of famine and as a traditional medicinal resource. In times of food scarcity,



Figure 1. *Cycas revoluta* plant.

starch derived primarily from seeds or stems—was consumed only after extensive detoxification processes, reflecting long-standing empirical awareness of the plant's inherent toxicity. At the same time, ethnobotanical records document the medicinal use of various plant parts within specific cultural and geographical contexts, underscoring the plant's dual significance as both a potentially hazardous and a culturally valuable biological resource. Leaves have been used for gastrointestinal complaints such as vomiting and flatulence, fruits for conditions including cough and hypertension, and preparations from the whole plant for ailments such as paralysis, snakebite, and nephritic pain (13-17). These ethnomedicinal practices are culturally specific and heterogeneous, and they are largely supported by traditional knowledge rather than systematic clinical or epidemiological evidence. Information regarding the prevalence, consistency, and therapeutic reliability of these uses remains limited.

Although traditional medicinal claims are often cited as indicators of pharmacological potential, concordance between ethnobotanical use and experimentally validated bioactivity cannot be assumed. In the case of *C. revoluta*, many reported folk applications coexist with substantial toxicological evidence, highlighting a frequent disconnect between traditional practices and modern biomedical validation. This disconnect is particularly significant because the same plant tissues employed in traditional remedies contain compounds now recognized as potent neurotoxins and genotoxins. Consequently, ethnomedicinal relevance must be interpreted cautiously and within a framework that explicitly considers risk.

Phytochemical investigations of *C. revoluta* have identified a diverse array of secondary metabolites, including biflavonoids, terpenoids, sterols, neurotoxic glycosides such as cycasin, and non-protein amino acids such as β -N-methylamino-L-alanine (BMAA) [15,17-21]. On the basis of these constituents, several biological activities—most notably antimicrobial, antioxidant, anticancer, cytotoxic, and antileishmanial effects—have been reported, primarily from *in vitro* assays and limited preclinical studies (15, 17-21). While these findings demonstrate biochemical activity, they do not establish therapeutic efficacy, safety, or translational relevance. In contrast, the toxicological properties of *C. revoluta*, including neurotoxicity, hepatotoxicity, and genotoxicity, are supported by consistent experimental evidence and documented cases of animal and human poisoning (22-23).

This pronounced imbalance between speculative pharmacological promise and well-established toxicity represents a central challenge in evaluating *C. revoluta* as a potential source of therapeutic agents. Any assessment of its medicinal prospects must therefore be grounded in a critical appraisal of evidence quality, reproducibility, and safety rather than a descriptive accumulation of reported activities.

Accordingly, the aim of this review is not to merely catalogue the ethnobotanical uses or reported bioactivities of *C. revoluta*, but to critically evaluate the strength and limitations of existing evidence across ethnobotany, phytochemistry, pharmacology, and toxicology. Particular emphasis is placed on distinguishing traditional knowledge from experimentally validated findings, separating *in vitro* observations from *in vivo* relevance, and explicitly weighing reported pharmacological activities against documented toxic risks. By adopting this focused and critical approach, the review seeks to clarify realistic research priorities and to

provide a balanced scientific framework for future investigations involving *C. revoluta* and related cycad species.

Methodology

A structured literature search was conducted using ScienceDirect, PubMed, Scopus, and Google Scholar to identify studies on the ethnobotany, phytochemistry, pharmacology, and toxicology of *Cycas revoluta* Thunb., covering publications available up to [September 2025]. Search terms included “*Cycas revoluta*” combined with “ethnobotany,” “phytochemistry,” “pharmacology,” “bioactivity,” “toxicity,” “cycasin,” and “BMAA” using Boolean operators. Only English-language articles were considered. Studies were included if they reported traditional uses, chemical characterization, biological activity, or toxicological effects of *C. revoluta*, and excluded if species identification was unclear, methodological detail was insufficient, or data were duplicated. Patents and conference proceedings were screened but interpreted cautiously due to lower evidentiary weight. Articles were categorized as ethnobotanical, phytochemical, pharmacological, or toxicological, and primary studies were distinguished from secondary reviews. Evidence quality was assessed qualitatively based on study design, reproducibility, and relevance, with greater weight assigned to *in vivo* and toxicological data. This review represents a structured narrative and critical synthesis rather than a formal systematic review; therefore, no PRISMA flow diagram or quantitative study count is provided.

Ethnobotanical Uses of different plant parts of *C. revoluta*

The ethnobotanical applications of *C. revoluta* are extensive and multifaceted, primarily documented in Japan, India, the Philippines, and Indonesia.

Leaves and whole plant

Leaves of the plant are widely used in medicine, food, and household applications. Medicinally, tender leaves are boiled to prepare a decoction for treating flatulence and vomiting (24-25). Tinctures made from leaves are effective against estrogen-dependent carcinoma, hepatoma, and cancer (26). Leaves are also used for treating skin diseases (27-28) and possess astringent and diuretic activity (24). As a food source, young leaves are boiled and consumed as vegetables for nutrition (27-28). In household uses, whole leaves are utilized for decorations in funeral wreaths, church festivals, and marriages (29). Strong, leathery leaves are employed in making baskets, brooms, and for thatching huts, while fibers are used for twines, ropes, and cloths (29). Additionally, leaves are applied as manure in mushroom cultivation (30). The leaves and whole plant is valued in traditional medicine. It is used for treating snake bites, paralysis, swollen glands, nephritic pain, and also as a stomach purifier (25, 26).

Seeds

Seeds hold both medicinal and nutritional importance. Medicinally, dried and powdered seeds in flour form are used to treat piles (29). Extracts from seeds are used for tumor diagnosis (31). Powdered seeds serve in managing hypertension, musculoskeletal disorders, gastrointestinal distress, cough, and amenorrhea (24). Seeds also act as

expectorants and anti-rheumatic agents (32). They are reported in the treatment of certain tumors (31), for reducing high blood pressure, rheumatism, headache, bone pain, and congestion (26), and for colon cancer treatment [33]. Furthermore, seeds are applied in managing liver disease and AIDS, as well as promoting hair growth and preventing premature greying of hair (34-37). As food, seeds are extensively processed (washed and fermented) to remove toxins and then used in flour for bread, sweets, and steam cakes, particularly as famine food (38-39). Detoxified seeds are also made into noodles and confectionaries as a starch source (38-39). A fermented mixture of powdered seeds with brown rice produces "sotetsu miso" or "date miso" (38-39). Processed seeds may also be consumed raw or cooked for nutrition (38-39).

Stem

The stem provides both nutritional and medicinal uses. For food, stems are cut and the pith and cortical cells are chopped, ground, and detoxified to produce sago, which serves as a major starch source (29). Medicinally, crushed seeds or megasporophyll and bark are mixed with coconut oil to form a paste used as an ointment for wounds and sores (29). Stems also provide fibers that are traditionally employed for making cloth and rope.

Roots

Roots are used both for food and cultural purposes. When processed by washing, they are consumed as a nutritional source. Culturally, roots are sometimes buried near houses to protect against lightning (29).

Central Pith

The central pith is an important food and drink source. When chopped, ground, and washed to remove toxins, it is processed into sago grains, serving as a famine food (29). Processed pith is also used for preparing bread, which is often wrapped and buried to ferment (29). Additionally, the pith is fermented into wine, used as an alcoholic beverage (31).

Cone and Pollen Grain

Dry cones are crushed into powder and used medicinally to treat painful urination (40). Pollen grains are recognized for their medicinal properties and are reported to provide various health benefits (31).

Phytochemistry of *C. revoluta*

A diverse range of flavonoids has been isolated from the leaves of *Cycas revoluta*. Naringenin, flavone A, and amentoflavone were reported using methylene chloride and ethyl acetate extracts (32). From distilled water extracts, 2,3-dihydroamentoflavone and 2,3-dihydrohinokiflavone were identified (41), while ethanol (95%) extracts yielded amentoflavone, 5,6,7,8,3',4'-hexamethoxyflavone, and 5,6,7,8,4'-pentamethoxyflavone (41, 42). Chloroform extracts provided several flavonoids, including 7,3'-dihydroisoflavones, naringenin, 2,3-dihydrohinokiflavone, amentoflavone, (2S, 2''S)-2,3,2'',3''-tetrahydroamentoflavone, 2,3-dihydroamentoflavone, podocarpusflavone A, (2S)-2,3-dihydroisocryptomerin, and (2S, 2''S)-2,3,2'',3''-tetrahydrohinokiflavone (10,42). Hinokiflavone was identified in ethyl acetate extracts (10), while 2,3-dihydro-4'-O-methylamentoflavone was recorded from chloroform extracts

(43). Several flavonoid glycosides have been reported from the leaves of *C. revoluta*. Amentoflavone-4'-O- α -D-glucopyranoside, neohesperdin, and rutin were isolated using ethyl acetate, n-butanol, and distilled water, respectively (32). Additional compounds include (2S)-1-(2, 3)-dihydro-1-7-O- β -D-glucopyranosylamentoflavone, (2S)-1-(2, 3)-dihydro-1-7,11-7-di-O- β -D-glucopyranosylamentoflavone, prunin, and vitexin-2''-rhamnoside, all extracted from leaves with ethyl acetate (10). Seeds of *C. revoluta* contain a wide range of non-protein amino acids. Compounds such as 3-[3'-amino-indenyl-2']-alanine, 6-N-oxalyl-ornithine, β -N-methylamino-L-alanine, pipecolic acid, N-methylaspartic acid, c-acetamidocaproic acid, and 2,4-diaminobutyric acid were detected in ethanol extracts (45). Further analysis revealed N-[glycylalanyl-11-thio]-5-one-pipecolic acid and α -aminoadipic acid in seeds extracted with 80% ethanol (45). Other amino acids including β -alanine, α -aminobutyric acid, β -aminobutyric acid, γ -aminobutyric acid, citrulline, and hydroxyarginine were recorded from ethanol extracts (46). Additionally, 2-amino-3-(methylamino)propanoic acid (BMAA) was reported in ethanol seed extracts (54). Azoxy glycosides represent a major class of secondary metabolites in *C. revoluta*. O- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-[O- β -D-xylopyranosyl-(1 $\rightarrow$ 6)]-O- β -D-glucopyranosyloxyazoxymethane was isolated from leaves in ethanol extracts (49). Cycasin (β -D-glucosyloxyazoxymethane) was identified in seeds using ethanol and petroleum ether (48). Other derivatives such as β laminaribiosyloxyazoxymethane, β -gentibiosyl oxyazoxy methane, O- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-O- β -D-glucopyranosyloxy azoxy methane, O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-O- β -D-glucopyranosyloxy azoxy methane, and O- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-O- β -D-glucopyranosyloxy azoxy methane were detected in seeds (49, 50, 51). Another compound, O- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-[β -D-glucopyranosyl-(1 $\rightarrow$ 6)]-O- β -D-glucopyranosyloxyazoxymethane, was reported from seeds extracted with distilled water (49). Furthermore, 6-O- β -D-xylosyl- β -D-glucosyloxyazoxymethane was obtained from petroleum ether extracts of seeds (51).

Fatty acid profiling of *C. revoluta* revealed several compounds from leaves. These include hexadecanoic acid methyl ester (methyl palmitate) and 9-octadecanoic acid-(Z)-methyl ester (methyl oleate), both obtained using petroleum ether (24). Other fatty acids such as linolenic acid, oleic acid, linoleic acid, palmitic acid, and stearic acid were identified without specified solvents (43). Additionally, 4',4'-dimethylloxazoline was detected in pollen grains extracted with chloroform (53).

Benzenoid compounds such as estragole, anethole, and safrole have been identified in *C. revoluta*. These were reported from leaves and cones using diethyl ether extracts (24). Terpenes including γ -terpinene and phellandrene were isolated from the leaves of *C. revoluta* using diethyl ether as solvent (24). The terpenoid profile of *C. revoluta* includes vomifoliol, phytol, and β -amyrin. Vomifoliol was isolated from chloroform extracts (10), phytol from petroleum ether extracts (24), and β -amyrin from leaves in petroleum ether (13). Sterols and related compounds are also present in *C. revoluta*. β -sitosterol was reported from leaves extracted with petroleum ether (13), while ecdysteroid hormones were identified in ethanol extracts (21). In addition, methyl salicylate was detected in leaves using petroleum ether (24). Lignans and phenolic acids from *C. revoluta* include

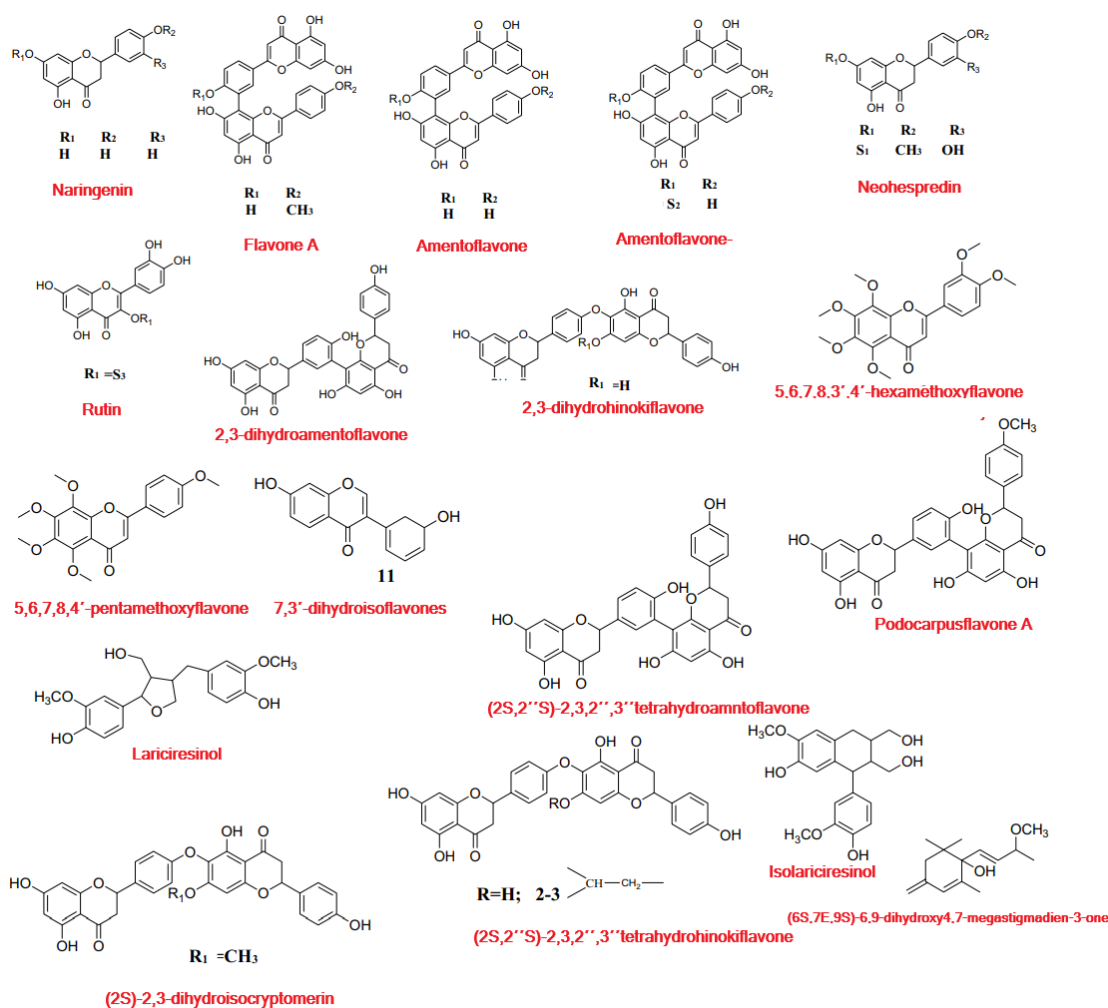


Figure 2. Structure of some important phytochemicals isolated from *C. revoluta* (17,43).

lariciresinol, isolariciresinol, and protocatechuic acid. All three compounds were recorded from chloroform extracts of leaves (10).

Phytochemical studies of *Cycas revoluta* report diverse secondary metabolites, including biflavonoids, glycosides, non-protein amino acids, terpenoids, and sterols. However, these findings are often presented as compound inventories with limited critical evaluation of analytical rigor, and several reports rely on preliminary chromatographic or spectroscopic identification without comprehensive structural confirmation or cross-laboratory validation. As a result, the reproducibility and authenticity of some reported constituents remain uncertain.

Interpretation is further complicated by solvent-specific extraction studies, which frequently lack data on extraction yields, batch variability, and standardization as presented in **Figure 2-4**. In addition, the relevance of organic solvent extracts to traditional aqueous or crude preparations is seldom addressed, limiting their ethnopharmacological significance.

Pharmaceutical significances of *C. revoluta* Antimicrobial activities

Extracts and isolated compounds from various parts of *C. revoluta* have demonstrated a range of antimicrobial activities against both human and plant pathogens, as

evaluated by several research groups using different methodologies as summarized in **Figure 5**. Specific biflavonoids, namely compounds ((2S,2''S)-2,3,2'',3'''-tetrahydro-4'-O-methylamentoflavone), ((2S,2''S)-2,3,2'',3'''-tetrahydro-4',4'''-di-O-methylamentoflavone), and (2,3-Dihydroamentoflavone), exhibited moderate antibacterial activity(10). These compounds were effective against *Staphylococcus aureus* (with IC₅₀ values of 9.6, 3.8, and 8.2 μM, respectively) and methicillin-resistant *S. aureus* (MRSA) (with IC₅₀ values of 12.5, 5.9, and 11.5 μM, respectively). In contrast, other tested biflavonoids like Amentoflavone and Hinokiflavone showed no significant antifungal, antimalarial, or antileishmanial activity (10). Furthermore, peptides isolated from seeds have shown potent effects. A small peptide (Cr-ACP1) and its acetylated derivative (Cr-AcACP1) from a methanolic seed extract were active against human pathogenic bacteria including *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus epidermidis*, with Cr-ACP1 being more effective(15). Purified antimicrobial peptides (Cy-AMP1, Cy-AMP2, Cy-AMP3) from seeds, where Cy-AMP1 and Cy-AMP2 inhibited the growth of plant pathogenic fungi (*Fusarium oxysporum*, *Geotrichum candidum*) and bacteria (*Clavibacter michiganensis*, *Curtobacterium flaccumfaciens*, *Agrobacterium radiobacter*, *A. rhizogenes*, *Erwinia carotovora*), while Cy-AMP3 showed weaker activity.(18).

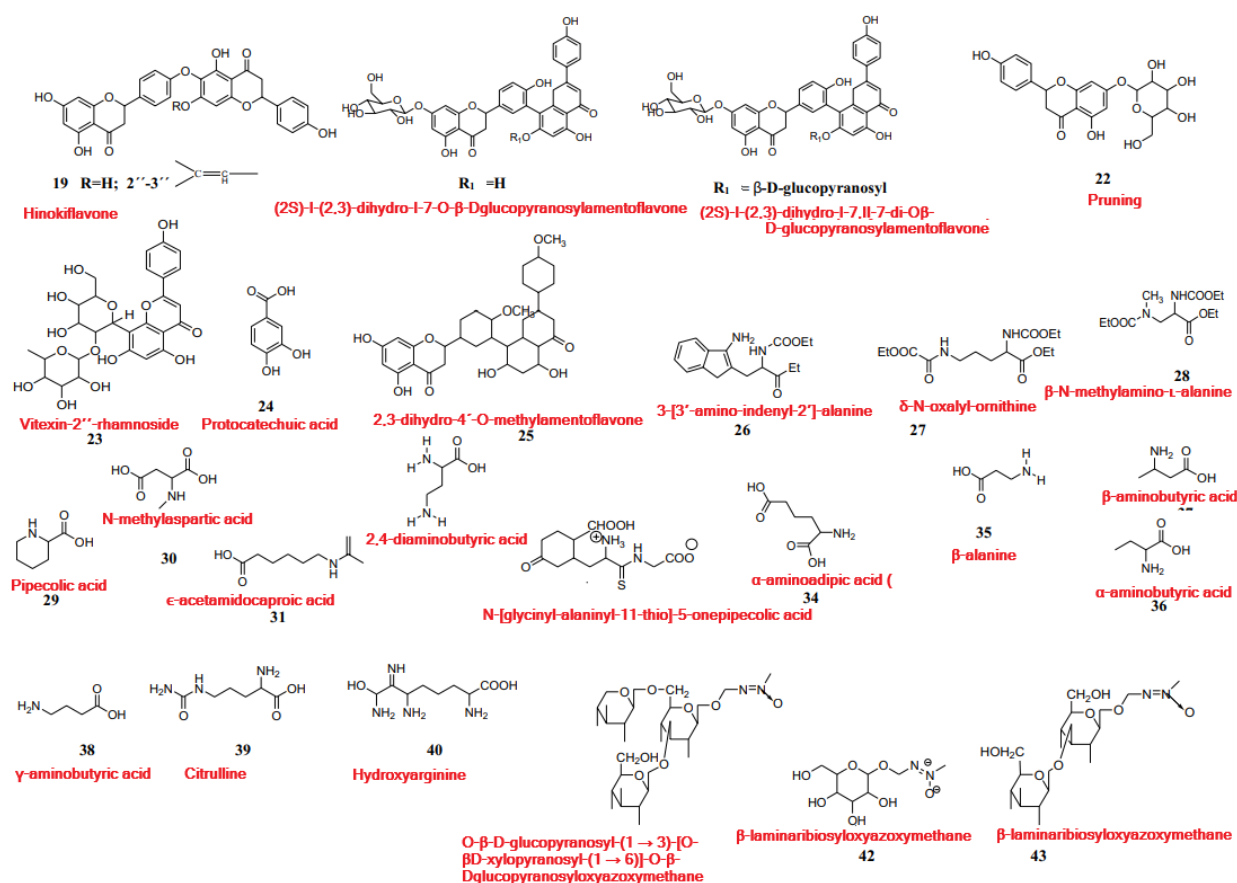


Figure 3. Structure of some important phytochemicals isolated from *C. revoluta*(17,43).

The efficacy of crude extracts varies significantly with the solvent used and the plant part. Ethanolic extracts of leaves exhibited potent and similar activity against *E. coli* and *Staphylococcus aureus* [56]. Chloroform extracts of female cones demonstrated strong, broad-spectrum antimicrobial activity against human pathogens including *E. coli*, *Lactobacillus plantarum*, *Micrococcus luteus*, *Salmonella abony*, *Candida albicans*, *Aspergillus niger*, and methicillin-resistant *Staphylococcus aureus* strains (*S. aureus* 101 and 102), outperforming chloroform extracts of leaves [29]. Methanolic extracts of leaves have consistently shown good antibacterial results. Methanolic extracts of leaves have consistently shown good antibacterial results against several pathogens like *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *S. aureus*. [58]. Similarly, a methanolic extract of ovules showed strong antibacterial activity against *E. coli*, *S. aureus*, and *B. subtilis* [59]. Hydro-alcoholic extracts of leaves showed potent activity against *E. coli*, *K. pneumoniae*, *Streptococcus pyogenes*, and *Saccharomyces cerevisiae* but were inactive against *Lactococcus sp.*, *Aspergillus niger*, and *Candida albicans*. Chloroform extracts of leaves showed a narrower spectrum, acting only against *E. coli* and *S. cerevisiae* [6]. Chloroform extracts of female cones demonstrated strong, broad-spectrum antimicrobial activity against human pathogens, outperforming chloroform extracts of leaves [29]. Methanolic extracts of leaves have consistently shown good antibacterial results against several pathogens [58], and a methanolic extract of ovules showed strong antibacterial activity [59]. Hydro-alcoholic extracts of

leaves showed potent activity against some organisms but were inactive against others, while chloroform extracts of leaves showed a narrower spectrum [6]. Reported antimicrobial, antioxidant, and anticancer activities of *C. revoluta* extracts and compounds are based predominantly on *in vitro* assays, typically expressed as IC_{50} values, which demonstrate biological activity but offer limited insight into *in vivo* pharmacological relevance. Key parameters such as bioavailability, metabolic stability, selectivity toward diseased versus normal cells, and dose-dependent toxicity are rarely assessed.

Cytotoxic and Anti-Cancer Properties of *C. revoluta* Extracts and Compounds

The cytotoxic potential of *C. revoluta* leaf extracts has been evaluated against various cancer cell lines. Ethyl acetate extracts of leaves have shown specific activity. According to [32], flavonoid compounds (4, 5) from this extract demonstrated cytotoxic activity against MCF-7 (breast cancer) and HepG2 (liver cancer) cell lines, using Doxorubicin as a standard. Further supporting this, [10] reported that two flavonoid compounds (21, 22) from an ethyl acetate leaf extract showed weak cytotoxicity against HepG2 cells ($IC_{50} = 207.6 \mu\text{g/mL}$) and moderate activity against RAW 264.7 murine macrophage cells ($IC_{50} = 160.8 \mu\text{g/mL}$), as determined by the MTT assay. A more comprehensive study by [13] analyzed a methanolic leaf extract and its subsequent fractions. The ethyl acetate fraction exhibited the highest inhibitory activity (69.06%) against the MCF-7 breast cancer cell line, followed by n

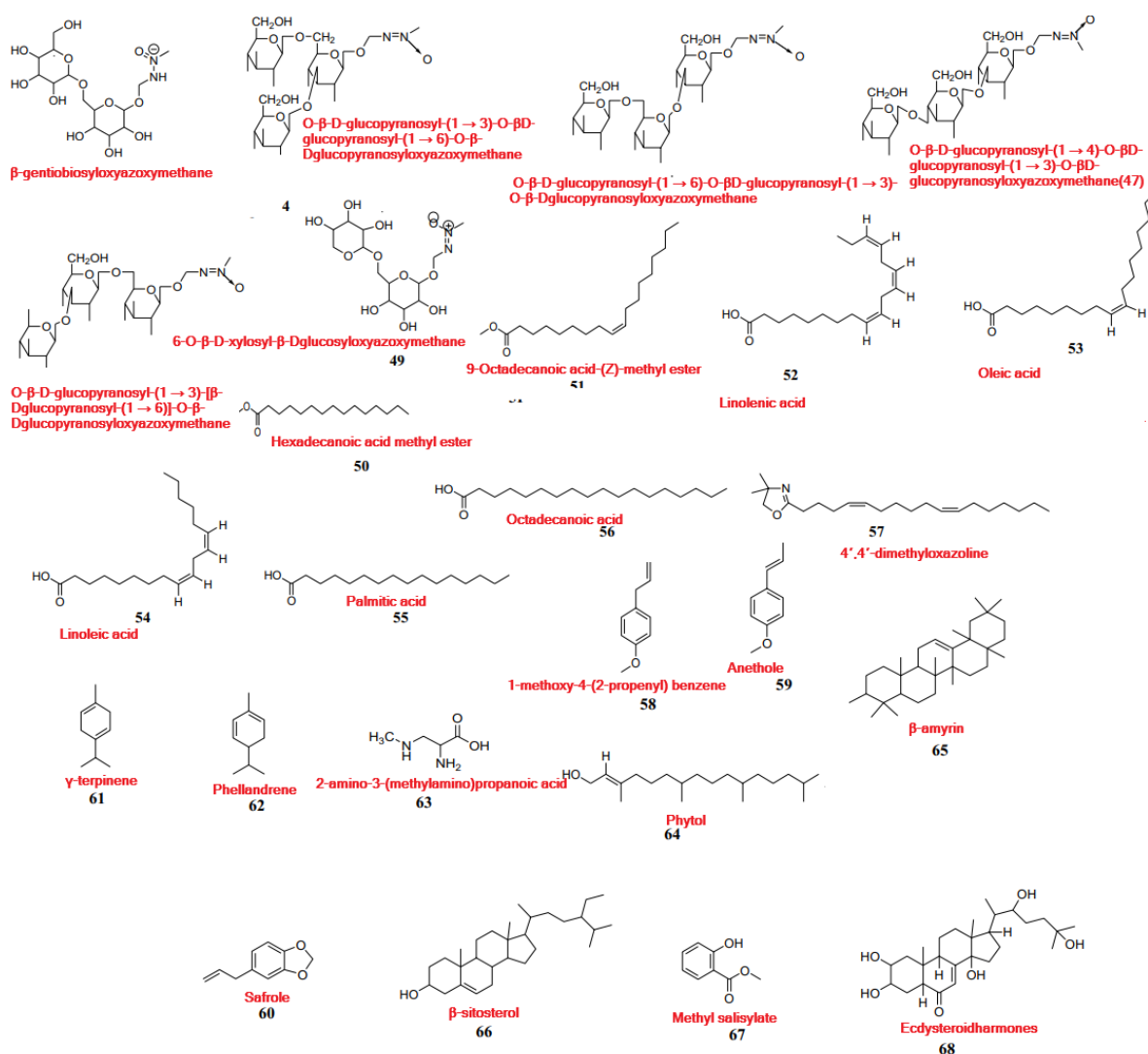


Figure 4. Structure of some important phytochemicals isolated from *C. revoluta*(17,43).

butanol and methylene chloride fractions. Against the HepG2 liver cancer cell line, the n-butanol fraction was most active (68.4% inhibition), followed by petroleum ether and methylene chloride fractions. Specific isolated compounds have shown remarkable potency. Neohesperidine, Amentoflavone, and Amentoflavone-4''-O- α -D-glucopyranoside were isolated from *C. revoluta* and demonstrated strong cytotoxic activity against the MCF-7 cell line, with IC_{50} values of 4.73, 18.7, and 6.12 μ g/mL, respectively (60). This activity is significant, especially for Neohesperidine, as it is comparable to the standard chemotherapeutic drug Doxorubicin (IC_{50} = 4.13 μ g/mL). The therapeutic potential extends beyond leaves. The methanolic extract of *C. revoluta* cones was evaluated for anti-colon cancer properties (61). Using a suite of assays (Cell Viability, Colony Formation, ROS Determination, Flow Cytometry, etc.), the extract demonstrated significant activity by reducing proliferation and inducing apoptosis in colon cancer cells (HCT-8 line), with an IC_{50} value of 500 ± 1.09 μ g/mL. Recent investigations have delved into the mechanisms behind this efficacy. The n-butanol fraction of the root extract showed notable anti-proliferative effects against A549 human lung adenocarcinoma cells (62). Research has particularly focused on biflavonoids from the leaves. Sotetsuflavone has been identified as a key anti-tumor agent against non-small-cell

lung cancer (NSCLC). Its robust activity works through multiple mechanisms, including inhibiting proliferation, inducing apoptosis via a ROS-mediated pathway, suppressing invasion and metastasis by reversing epithelial-mesenchymal transition (EMT), and modulating critical signaling pathways such as TNF- α /NF- κ B and PI3K/AKT (62). Systematic analyses confirm that leaf flavonoids (CRL) act as multi-target agents against lung adenocarcinoma, impacting oxidative stress and inflammatory immune response pathways by targeting key markers like FLT3, CCNA2, and MCL1 (63). Molecular studies show that specific biflavonoids, including amentoflavone and podocarpusflavone A, exhibit strong binding stability with targets such as the GABRG2 ion channel, suggesting a novel mechanism linked to synaptic regulation (64). The promise of *C. revoluta* extends to gastric cancer. A natural extract has been shown to significantly inhibit growth, migration, and invasion of gastric cancer cells. Crucially, it enhanced the sensitivity of these cells to the chemotherapy drug 5-Fluorouracil (5-Fu), promoting apoptosis and reducing the expression of drug-resistance-related proteins p-AKT and mTOR. This indicates a potent chemosensitization effect via the AKT-mTOR pathway (65).

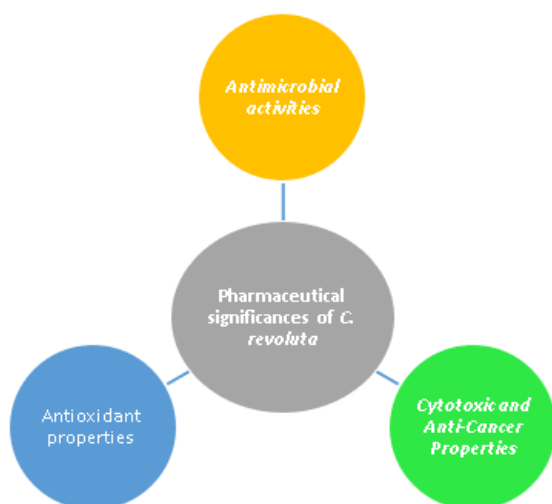


Figure 5. Pharmaceutical significances of *C. revoluta*.

Antioxidant properties:

In vitro antioxidant activities of *C. revoluta* leaves have been widely investigated using different solvent extracts and assays. Hydroalcoholic and chloroform extracts showed significant antioxidant activity when evaluated by superoxide anion radical scavenging, with the hydroalcoholic extract exhibiting better results (77.0%) compared to the chloroform extract (75.0%) (6). Methanol, ethanol, and ethyl acetate extracts were tested using the DPPH assay, all displaying potent antioxidant potential, with methanolic extract showing the strongest activity (110.25 µg/mL) (58). Isolated compounds such as neohesperidin, amentoflavone, and amentoflavone-4'-O-α-D-glucopyranoside demonstrated antioxidant activities nearly two- to four-fold higher than that of quercetin at 12.5 µg/mL in the DPPH assay (60). Similarly, flavonoids isolated from the ethyl acetate extract of leaves also exhibited marked activity by the DPPH radical scavenging method (32).

Further comparative studies on different plant parts revealed that chloroformic leaf extract (IC₅₀ = 15.00 µg/mL) and hydroalcoholic female cone extract (IC₅₀ = 17.00 µg/mL) showed notable antioxidant activity, while chloroformic female cone (IC₅₀ = 12.00 µg/mL) and hydroalcoholic leaf extract (IC₅₀ = 15.00 µg/mL) also demonstrated strong scavenging potential, with ascorbic acid used as the control (44). Overall, methanolic and hydroalcoholic extracts, along with specific flavonoid constituents, appear to contribute significantly to the antioxidant efficacy of *C. revoluta* leaves. Proposed mechanisms of action, including pathway modulation, enzyme inhibition, and receptor interactions, are frequently inferred from computational docking or isolated studies and lack validation through functional assays, genetic approaches, or *in vivo* models. Consequently, these mechanistic claims remain speculative and should be interpreted with caution.

Toxicology and Safety Considerations

The therapeutic potential of *C. revoluta* is inextricably linked to its toxicity. Improperly processed plant material, particularly seeds, can cause severe poisoning. Cycad plants contain several toxins responsible for poisoning in both humans and animals (66). These include azoglycosides like macrozamin, neocycasin, and cycasin as shown in Figure 6.

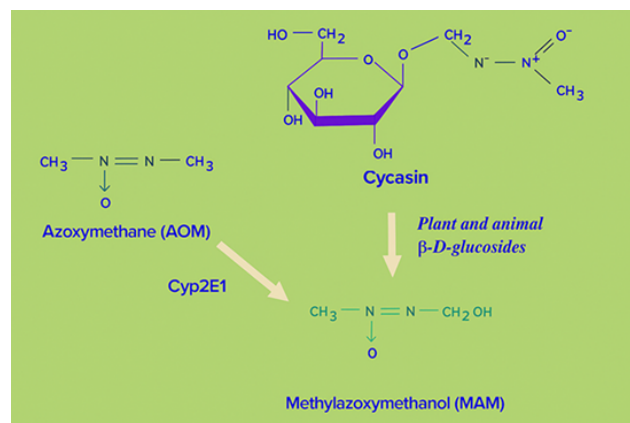


Figure 6. Structures of the cycad genotoxins cycasin, MAM, and the related compound azoxymethane (AOM).

When metabolized by β-glucosidase, cycasin is converted into methylazoxymethanol, a compound known to be hepatotoxic, carcinogenic, and teratogenic. Other significant toxins are the neurotoxic amino acid beta-N-methylamino-L-alanine (BMAA) and a high molecular weight compound that has yet to be fully identified (67-69). While present throughout the plant, the highest concentrations of these toxins are found in the seeds (68). Ingestion of even one or two seeds can be fatal to dogs (69). Clinical signs of poisoning, which typically manifest within a few h, are primarily gastrointestinal (e.g., bloody diarrhea or constipation) and neurological. Liver damage is also common, presenting as altered hepatic enzymes, hypoproteinemia, hypoglycemia, and thrombocytopenia (68). Cycad poisoning has a high mortality rate, approximately 30%, with dogs appearing to be the most susceptible species (69). Documented cases in Europe include reports from Italy and Sweden (70) (71). In 2008, the Swedish Poisons Information Center received three inquiries concerning *Cycas revoluta* poisoning in dogs; two exhibited typical gastrointestinal symptoms, and a third developed severe disseminated intravascular coagulation (DIC) and was euthanized (72)(73).

The azoxyglycoside cycasin is converted to MAM by β-glucosidases, while azoxymethane is converted to MAM by mixed function oxidases (e.g., P450 Cyp2E1) (74) (Kisby et al., 2011). A key neurotoxin, L-BMAA, is an unusual amino acid associated with Western Pacific Amyotrophic Lateral Sclerosis-Parkinsonism Dementia Complex (ALS-PDC). Its origin, whether produced by the cycad itself or by symbiotic cyanobacteria, is debated. Notably, cyanobacteria also produce genotoxins that can cause DNA damage in human and rodent cells (75). Research has established that L-BMAA is an excitotoxin, with acute neurotoxic effects that can be reduced by glutamate receptor antagonists. Multiple studies have demonstrated its impact on both ionotropic and metabotropic glutamate receptors (76-78). BMAA can block these receptors (79), leading to neurotoxicity in insects (80-81) and mammals (82), and causing developmental issues in plants like *Arabidopsis thaliana* (83). However, some research suggests the levels of BMAA in cycad leaves may not be acutely toxic to insects (84). In livestock, cycad consumption is known to cause two main syndromes: one affecting the liver and gastrointestinal tract, and a neurotoxic disorder that induces ataxia [84]. Human exposure has been linked to numerous cases of gastroenteritis. Furthermore, a paralytic/ataxic syndrome

(ALS-PDC) has been observed in tropical populations with long-term consumption of these plants (85-88). The primary toxic agent in *C. revoluta* is cycasin (methylazoxy methanol b-D-glycoside), a compound considered unique to cycads and found across all Cycadales genera (10). Related compounds include neocycasins A, B, C, and E (80). These are all glycosides that share methylazoxymethanol (MAM) as their aglycone, and they are responsible for the characteristic gastrointestinal and hepatic toxicity (86).

Of the toxins implicated in Amyotrophic Lateral Sclerosis - Parkinsonism Dementia Complex. ALS-PDC, methylazoxymethanol (MAM), the genotoxic metabolite of cycasin, is increasingly considered a more significant etiologic agent than L-BMAA. This focus is supported by the fact that cycasin is present in cycad seeds in much larger quantities (up to 4% w/w) than L-BMAA, resulting in higher doses upon consumption (89). Furthermore, a striking correlation exists between the cycasin content of cycad flour and the historical age-adjusted incidence of ALS and PD on Guam (91, 92). The potency of this compound is evidenced by MAM's established role as a developmental neurotoxicant and by the fact that cycasin induces cycadism, a neuromuscular disorder in grazing animals [93]. MAM is released upon the enzymatic hydrolysis of various azoxyglycosides found in cycads, all of which share this common genotoxic mechanism (94). In contrast to the pharmacological literature, toxicological studies on *C. revoluta* show greater methodological rigor and consistency, with compounds such as cycasin and β -N-methylamino-L-alanine (BMAA) clearly associated with neurotoxic, genotoxic, and hepatotoxic effects in animal models and human exposures. This evidentiary imbalance underscores that toxicological risks are well established, whereas therapeutic benefits remain largely exploratory.

Future research scope

Based on this review future research on *Cycas revoluta* should focus on four major directions. First, toxin removal and purification strategies must be optimized to isolate beneficial compounds such as biflavonoids and antimicrobial peptides (AMPs) while ensuring the complete elimination of toxic constituents like cycasin and BMAA. Second, mechanistic studies integrating genomics, transcriptomics, proteomics, and metabolomics are essential to unravel the precise molecular pathways through which these bioactive molecules exert their effects. Third, structure-activity relationship studies should be employed to design and synthesize novel analogs of biflavonoids and AMPs, aiming to improve efficacy, enhance stability, and minimize off-target interactions. Finally, comprehensive preclinical and clinical studies are required to establish the safety, pharmacological profile, and therapeutic potential of these purified extracts or compounds, thereby advancing their development into viable medicinal agents.

Conclusion

This review highlights a clear imbalance between the reported pharmacological activities of *Cycas revoluta* Thunb. and the strength of supporting evidence. While diverse phytochemicals, including biflavonoids, have been identified, most claimed antimicrobial, antioxidant, and anticancer effects are based largely on *in vitro* assays and limited preclinical studies, and therefore lack translational validity. In contrast, the toxicological profile of *C. revoluta*,

particularly the well-documented effects of cycasin and β -N-methylamino-L-alanine (BMAA), is robust and supported by consistent experimental and clinical observations.

Given this disparity, therapeutic development from *C. revoluta* remains highly constrained by safety, regulatory, and ethical barriers. Future research should prioritize rigorous toxicological risk assessment, reproducible phytochemical standardization, and validation of non-toxic constituents using appropriate *in vivo* models, rather than speculative extrapolation of bioactivity. Conservation considerations are also critical, as *C. revoluta* is a slow-growing cycad, and unsustainable harvesting could exacerbate ecological risks. Overall, the available evidence supports a cautious, critically limited view of the therapeutic potential of *C. revoluta*, emphasizing scientific restraint over aspirational interpretation.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

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The data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Additional Information

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