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Review

A Review of Extraction Techniques of Bioactive Compounds and Pharmacological Properties of Guam's Invasive Vine—*Antigonon leptopus*

Christel Kei U. Valerio and Sahena Ferdosh *

Western Pacific Tropical Research Center, University of Guam, Mangilao, GU 96923, USA
* Correspondence: ferdoshs@triton.uog.edu; Tel.: +671-735-2144

Abstract: *Antigonon leptopus* Hook. & Arn., commonly known as the chain of love, is a fast-growing leafy vine characterized by its pink or white heart-shaped flowers and is considered among the most invasive vine species in Guam. In Guam, the vine is considered to be a weed, but worldwide it is utilized in different folk medicine practices, such as for alleviating colds and tending to wounds. As a resource, *A. leptopus* is underutilized in Guam, prompting the search for possible pharmacological properties. *A. leptopus* contains a wide range of phytochemicals, including alkaloids, terpenoids, flavonoids, phenols, tannins, saponins, glycosides, and amino acids, which have been found to have many bioactive properties, including antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticancer activities. Extraction methods varied according to specific research objectives, but overall, the most common methods involved were maceration, Soxhlet extraction, and hot extraction techniques. The application of green extraction methods, such as the use of supercritical CO_2, is currently lacking for this species. *A. leptopus* may serve as a promising source of bioactive compounds for the pharmaceutical and nutraceutical industries.

Keywords: *Antigonon leptopus*; ethnobotanical uses; bioactive compounds; pharmacological properties; extraction methods

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1. Introduction

Antigonon leptopus has several common names that are used worldwide, such as the chain of love, coral vine, Mexican creeper, and more [1,2]. In Guam, it is known as kadena in Chamorro. Originally imported to Guam in 1905 for its ornamental properties, *A. leptopus* has since spread throughout the island and threatens plant biodiversity as its smothering vines outcompete endemic plants growing beneath it [3,4]. A survey reported that an estimated 6.16% of Guam is covered by this species, which is estimated to be 8236.52 acres [5]. Thus, it is reported as the fourth worst weed on Guam, becoming one of Guam's most invasive vines that can commonly be seen on roadsides. A USDA NRCS report states that in the Pacific islands, it completely smothers other plants in the wet season [6], out-competing understory plants. Moreover, dry leaves that drop during the dry season provide fuel for wildfires, gaining it a PIER Risk Assessment score of 19, meaning that it is a high-risk species.

As an invasive plant, the fast-growing vine affects native flora as it climbs to the top of the canopy, covering trees and blocking sunlight from reaching plants in the understory, thus killing them [7]. Figure 1 depicts the worldwide distribution of the vines. There is no conclusive way to manage the plant, as physical, chemical, and biological management methods have been ineffective in controlling and removing it. Even if the vine is cut, it

will resprout unless the underground tubers are entirely removed. Spraying the vine with chemicals had inconclusive effects, and there are currently no known biological agents to combat the vine [6].

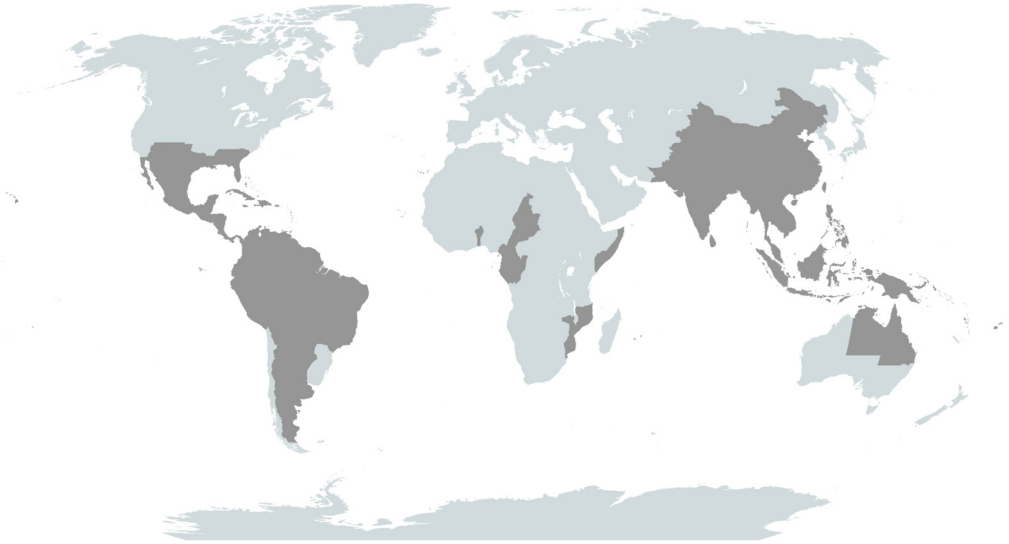

Figure 1. Distribution map of *A. leptopus*.

Other parts of the world face the same issues with these prolific vines but have also found ways to utilize the plant. From being used as an ingredient in local cuisine in Thailand [8] to its use as a remedy in folk medicine to cure common colds in Jamaica [9], its varied ethnomedicinal uses worldwide have prompted several researchers to conduct studies on the many claimed properties of the plant. In this current review, a literature search was conducted to unveil the ethnobotanical uses of A. leptopus worldwide, the extraction of phytochemicals and their pharmacological properties, and the toxicity and safety of their uses.

2. Botanical Description and Ecology of *A. leptopus*

A. leptopus is described as a vine with tendrils and simple, ovate, usually undulate leaves [10], as seen in Figure 2. It is a vascular plant and a seeded dicotyledon belonging to the buckwheat family, Polygonaceae [6]. Additional taxonomical information is listed in Table 1. It alternates between flowering, fruiting, and vegetative phases [9]. The leaves of the vines are soft, narrowly heart-shaped, light green, and prominently veined with wavy margins, alternating along the stems and measuring 1–5 cm in length [11].

A. leptopus can be easily identified by its distinct white, red, or pink heart-shaped flowers that occur as a raceme, and its fruit is an achene [10,11]. Flowers typically have eight, sometimes ten, stamens, with four being 1 mm shorter than the others in an alternating pattern [9], as seen in Figures 2 and 3. These fast-growing vines grow from small underground brown tubers, reaching maximum heights of 9–12 m and crown widths of 6 m. However, the plant can also be propagated by seeds or trimmings [4,11].

Figure 2. *A. leptopus* vine.

Table 1. Taxonomic Classification for *Antigonon leptopus*.

Rank	Scientific Name and Common Name
Kingdom	Plantae—Plants
Subkingdom	Tracheobionta—Vascular plants
Superdivision	Spermatophyta—Seed plants
Division	Magnoliophyta—Flowering plants
Class	Magnoliopsida—Dicotyledons
Subclass	Caryophyllidae
Order	Polygonales
Family	Polygonaceae Juss.—Buckwheat family
Genus	*Antigonon* Endl.—Antigonon
Species	*Antigonon leptopus* Hook. & Arn.—Coral vine

Sources: Natural Resources Conservation Service, USDA [2].

Originating from Mexico, *A. leptopus* grows in a tropical or subtropical/monsoonal climate and prefers terrestrial habitats [4,11]. The vine tolerates drought by shedding its leaves and can quickly regrow after rain [6]. The plant is spread by animals and insects that eat the seeds and bring them to new locations. In addition, the lightweight seeds are easily carried and distributed by both wind and water, growing in most soil types [7]. *A. leptopus* does not need much sunlight to thrive and can produce flowers all year long, with enough rainfall, allowing the vine to thrive in tropical climates [9]. The *A. leptopus* flowers attract different pollinators as they can easily reach the nectar, and they are foraged upon by birds and bees [9,12].

Figure 3. Leaves and flowers (pink and white variant) of *A. leptopus.*

3. Ethnobotanical Uses of *A. leptopus*

The different parts of *A. leptopus* have varied uses from food to traditional medicines around the world, with some regions overlapping practices with others.

3.1. Marianas Islands

Since its introduction to Guam as an ornamental plant in 1905, the use of *A. leptopus* is not widely known on the island. More recently, traditional healers have been looking into its special properties as an herbal tea, as used historically by other parts of the world. Local business owners have also tried to use the vine in a modern way, putting the flowers into beauty products such as bath bombs and using the roots to dye clothing. However, it is said that in some tropical areas, the tuber is used as a source of starch [10].

3.2. Asia

In Thailand, all parts of the vine have traditional uses as an ingredient in food dishes [8]. The flowers are mixed into noodle dishes and omelets, while the leaves are coated with flour and fried [13]. Aerial parts of the plant, from the vines to the flowers, are boiled in hot water to create an herbal tea, which is used as a remedy for the common cold due to its antioxidant properties [14]. According to the authors of a previous study, some even claim that tea brewed from the leaves possesses antidiabetic properties, while tea brewed from the leaves lowers blood pressure [13].

Meanwhile, in different parts of India, *A. leptopus* is utilized in their traditional medicinal practices, with claims that is has various healing and medicinal properties [13]. According to the authors of a previous study, aerial parts of the plant are used to treat common colds, while the leaves are used to make a paste to alleviate and treat skin irritations, blisters, and even diseases. In the mountains of the Philippines, the Ifugao tribe uses *A. leptopus* to apply to open wounds to assist with proper closure and to reduce inflammation [15–17], while those in Iloilo use it to treat gastrointestinal disorders [13].

3.3. Americas

In a similar manner to Thailand, the people of Kingston, Jamaica, are also said to use *A. leptopus* tea to treat common colds. However, the plant material utilized and preparation

methods appeared to vary based on personal preference [9]. In addition, many reports claimed that hot tea is often traditionally used as a cure for coughs and to ease throat constriction. Vandebroek et al. [9] reported that the vine was used more commonly in urban areas as compared to rural areas, possibly due to the availability of the plant. In Trinidad and Tobago, the plant was used traditionally as a sort of treatment for diabetes and to low blood pressure [13]. Authors also previously reported the leaves of the vine being utilized in folk medicine for boils and swelling [18].

In parts of America, different parts of *A. leptopus* are said to be prepared and eaten by the native people. The tubers are eaten in Guatemala and are described as having an agreeable nutlike flavor [19]. Even the seeds can be roasted and consumed, prepared similarly as popcorn, within the indigenous communities of Baja California [20,21]. In Mexican folk medicine, the leaves and roots of *A. leptopus* have been used for the treatment of stomach aches [13]. In addition to being used as a medicinal plant, its name Cadena de amor or chain of love in English highlights the plant's symbolism of affection and emotional bonds. Thus, the beautiful flowering vines are often used in traditional celebrations to decorate for weddings and other festivities [22].

4. Major Bioactive Compounds of *A. leptopus*

Phytochemical screenings of different parts of *A. leptopus* revealed the plant to contain phenolic compounds, alkaloids, terpenoids, triterpenoids, terpenes, triterpenes, flavonoids, tannins, glycosides, saponins, carbohydrates, and more, as listed in Table 2. Preliminary screenings of a methanol root extract revealed that it contains steroids, flavonoids, tannins, alkaloids, and glycosides [23]. Carey et al. [24] reported similar constituents from methanol root extracts. Vanitha and Thangarasu [25] performed a GC–MS phytochemical analysis of the tubers and reported them to contain alkaloids, saponins, steroids, phenols, fatty acids, and flavonoids.. Hemke et al. [26] performed screenings of a water extract of the tubers, finding the presence of carbohydrates, tannins, saponins, and flavonoids.

Table 2. Bioactive compounds of the different parts of *A. leptopus*.

Part Used	Analytical Techniques	Phytochemicals	Bioactivities	Reference
Aerial parts	HPLC; H-NMR; C-NMR; TLC; GC–MS	Anthraquinone derivatives; phenolics; flavonoids; phenolic acids; coumarins; organic acids	Antimicrobial; antioxidant; LPO inhibitory; COX inhibitory; anti-inflammatory; anticoagulant; analgesic; antithrombin; antidiabetic; antidepressant	[14,27–29]
Flowers	Qualitative assays	Alkaloids; tannins; terpenoids; flavonoids; triterpenoids; volatile oils; carboxylic acids; glycosides; carbohydrates; phenolics; saponins; steroids; phytosterols; sapogenins	Antibacterial; antimicrobial	[30–33]
Flowers	RP-HPLC; qualitative assays	Phenolics; flavonoids; terpenoids; alkaloids; phytosterols; proteins	Antioxidant; antidiabetic; hypolipidemic; hypoglycemic	[8,34,35]
Flowers	HPLC–DAD	Phenolic acids; flavonoids	Antioxidant; antiproliferative activity	[36]

Table 2. *Cont.*

Part Used	Analytical Techniques	Phytochemicals	Bioactivities	Reference
Leaves	Qualitative assays	Phenolics; alkaloids; tannins; glycosides; coumarins; flavonoids; triterpenes	Antioxidant	[37–39]
Leaves	GC–MS; qualitative assays	Saponins; fixed oils; phenolics; tannins; flavonoids; alkaloids; amino acids; phlobatannins	Antibacterial	[40,41]
Leaves	Qualitative assays	Phenolics; flavonoids; tannins; carbohydrates; steroids	Antioxidant; hepatoprotective	[42]
Leaves	GC–MS	Phenolics; terpenes; hydrocarbons; quinazolines; coumarins; steroids	Anthelmintic	[43]
Leaves	HPLC; C-NMR; H-NMR; qualitative assays	Alkaloids; carbohydrates; glycosides; phenolics; tannins; saponins; flavonoids	Antioxidant; antidepressant; antihemolytic; MMP-9 inhibitory; xanthin oxidase inhibitory; antioxidant;	[16,17,44,45]
Leaves	GC–MS	Saponins; phenolics; tannins; flavonoids; alkaloids; fixed oils; amino acids		[25,46]
Tubers, roots and rhizomes	GC–MS	Carbohydrates; saponins; phenolics; fatty acid; volatile oils; steroids; Flavonoids; tannins; alkaloids; glycosides; triterpenoids	Analgesic; anti-inflammatory; hepatoprotective; antimicrobial; antifungal	[23–26,47,48]

In some studies, the aerial parts of *A. leptopus* as a whole are utilized in the experiment, thus making it impossible to determine the specific plant parts of the phytochemicals located. Vanisree et al. [14] studied a methanol extract of the aerial parts of *A. leptopus* and identified specific phenols. Through H-NMR and C-NMR analysis, the purified extract yielded ferulic acid, 4-hydroxycinnamic acid, quercetin-3-rhamnoside, kaempherol-3-glucoside, β-sitosterol-glucoside, and d-mannitol [14]. Isolation of a crude water extract from the aerial parts of *A. leptopus* was performed by preparative TLC and gave a phenolic compound with a COX-2 enzyme inhibitory activity of 90% inhibition at 25 µg/mL and reported an IC_{50} value of 9.7 µg/mL [28]. HPLC and NMR screenings of the aerial parts of *A. leptopus* identified several anthraquinone derivatives, specifically 1,8-dihydroxy6-(hydroxymethyl)-3-methoxy-2-pyrrolidinium anthraquinone; 1,8-dihydroxy-6-(methyl)-3-methoxy-2-pyrrolidinium anthraquinone; 1,8-dihydroxy-6-(hydroxymethyl)-3-methoxy2-piperidinium anthraquinone; and 1,8-dihydroxy-6-(methyl)-3- methoxy-2-piperidinium anthraquinone [27].

Rudhra and Venkatesan [30] studied the phyto-constituents of *A. leptopus* leaf extracts and confirmed that alkaloids, terpenoids, and flavonoids were highly present; tannins and triterpenoids were moderately present; and steroids, phenols, and saponin were present. Phytochemical screening was performed by various qualitative assays. The authors also reported that the total phenol content of the different leaf extracts ranged from 0.094 to 2.565 mg GAE/100 g extract with the ethanol extract displaying the highest total phenol content. Balasbrumani et al. [40] reported the presence of saponin, phenolic compounds, tannins, flavonoids, alkaloids, fixed oils, and amino acids from a leaf methanol extract. A study by Elhaj et al. [37] found that an ethanolic leaf extract of *A. leptopus* was rich in alkaloids, saponins, unsaturated sterols, and/or triterpenes, flavonoids, glycosides, and coumarins through phytochemical screenings of the extract.

A GC–MS analysis study revealed that the leaves of *A. leptopus* contained 1-tetradecene; 2-tert-butyl-4-isopropyl-5-methylphenol; Spiro[3.6]deca-5,7-diene-1-one; ë-Cadinene; Juniper camphor; 1-nonadecene; 10-azatricyclo[4.3.1.0(1,6)]deca-2,4-diene; 6-Hydroxy-4,7-dimethylcoumarin; Cholest-2-eno[2,3-b]naphthalene; Cholesta-5,7,9(11)-trien-3-ol, 4,4dimethyl-,(3á)-; 6-Chloro-2-[2-(4-methylphenyl)-1,1-diphenylethyl]-4H-3,1-benzoxazine-4-one; 4,5-Bis(p-bromophenoxy)-1,2-Dicyanobenzene; 4,4′-Isopropylidene-Bis-(2-Cyclohexylphenol); 2-Thioxo-2,3-dihydro-3,5-dimethyl-1,3,4-oxadiazole; 9,19-Cyclola nostane-3,7-diol; and 2-Chloro-1,4-bis(dibromomethyl) benzene [43]. In addition, a separate GC–MS analysis on *A. leptopus* leaf and tuber samples identified compounds such as glycerin, propane,1,1,3-triethoxy, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, methyl salicylate, 2-furancarboxaldehyde,5-(hydroxymethyl) dodecanoic acid, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl)ester, n-hexadianoic acid, oleic acid, 1,2-benzenedicarboxylic acid, and diisooctyl ester [25].

Ethanolic extracts of *A. leptopus* flowers were confirmed to contain a rich composition of phenolic acids and flavonoids after performing qualitative assays [8]. RP-HPLC analysis also identified polyphenols, including protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, and syringic acid, which are known to be efficient antioxidants, while the flavonoids identified were kaempferol, myricetin, apigenin, and rutin, which had the highest level in mg/g dry weight. Bolla and Bhogavalli et al. [31] confirmed the presence of volatile oils, carboxylic acids, glycosides, carbohydrates, and terpenes from *A. leptopus* flower extracts through various qualitative assays. Another phytochemical investigation on flower extracts confirmed the presence of flavonoids, phenols, phytosterols, carbohydrates, terpenoids, and proteins by qualitative analysis [34].

5. Major Pharmacological Properties of *A. leptopus* Extracts

Different parts of the plants had varying bioactivities, which may contribute to the previously discussed phytochemicals present. In vivo and in vitro studies were utilized to test the pharmacological properties of *A. leptopus*, revealing antioxidant, antibacterial, antimicrobial, antidiabetic, anti-inflammatory, and anticancer activities, as presented in Table 2 and in Figure 4.

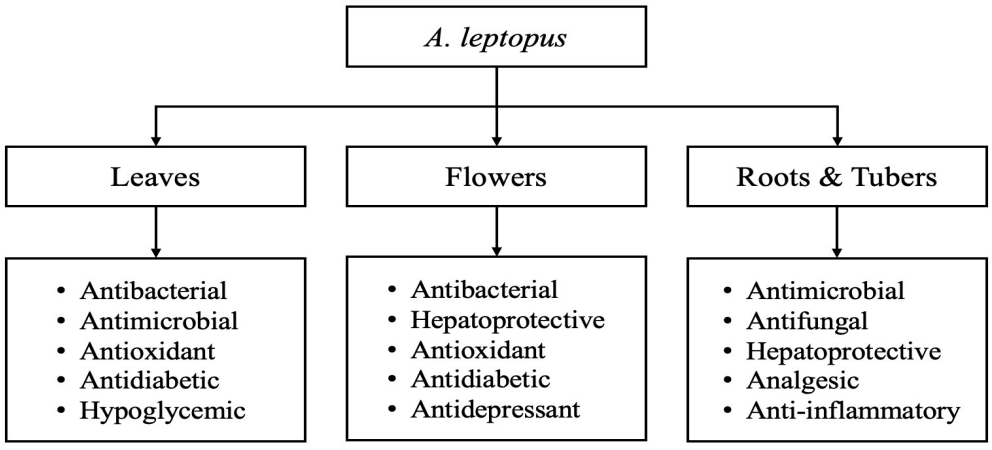

Figure 4. Plant components and main bioactive properties.

5.1. Antioxidant Activity

The majority of the studies utilized a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay known to be a standardized method to directly test for antioxidant activity. DPPH is a stable radical, typically prepared in methanol. Once reacted with an antioxidant, it is reduced and this causes a change in color, which can then be measured with a UV

spectrometer and analyzed for antioxidant activity. A DPPH assay of a methanol *A. leptopus* leaf extract gave an 89 ± 0.04 RSA % [37], while the findings of Kaisoon et al. [8], who tested a flower methanol extract, reported an 89.36 ± 3.51% inhibition. Kavatagimath et al. [34] performed a DPPH assay on ethanol flower extracts and reported the IC_{50} to be 439 µ/mL. The flower extracts appeared to have a higher IC_{50} compared to the standard butylated hydroxytoluene, which had an IC_{50} of 419 µg/mL, suggesting a relative antioxidant activity. Balasbrumani et al. [40] synthesized gold nanoparticles made from a leaf extract and compared its antioxidant activities to that of a crude leaf extract. By performing a DPPH assay they found that gold nanoparticles causes a higher degree of inhibition than the crude leaf extract, thus having more potent antioxidant properties. A study comparing the antioxidant capacities of eight different Thai edible flowers reported that *A. leptopus* had the highest ferric reducing antioxidant value at 62.0 mmol$FeSO_4$/g 100 dry weight [8].

5.2. Antibacterial Activity

The antibacterial activity of *A. leptopus* was tested using Kirby–Bauer's Disc diffusion method [30] with both Gram-positive and Gram-negative bacteria. This assay is widely used to determine the sensitivity or resistance certain bacteria has against a sample by measuring the inhibition of bacterial growth in a culture. The ethanol extract had the highest activity at 200 µg/mL, the methanol extract had moderate activity, and the acetone extract had the lowest activity. The results also showed that *A. leptopus* showcased higher inhibition against Gram-positive bacteria compared to Gram-negative bacteria. A separate test also utilizing the Kirby–Bauer's Disc diffusion method evaluated the antibacterial activities of *A. leptopus* leaf extracts made from varying solvents [41]. Leaf extracts made from chloroform and hexane were reported to show no activity against the bacteria studied. However, acetone and methanolic extracts did show activity, with methanolic extracts displaying higher activity compared to the acetone extracts. A study evaluating the inhibition of Gram-positive bacteria using *A. leptopus* flower extracts reported that both extracts had exhibited significant inhibition, comparable to the control using the drug streptomycin. The ethanol extract in particular was proven to extract a broader spectrum of compounds from the source material and displayed a better antibacterial effect [31].

5.3. Antimicrobial Activity

Olaoluwa et al. [27] isolated one flavonoid and four anthraquinone derivatives by gradient elution fractionation of the ethanol extract of the aerial parts of *A. leptopus* and identified the compounds through HPLC, C-NMR, and H-NMR analyses. Researchers then studied their antimicrobial activities against two Gram-positive bacteria and a fungus and found that the isolated compounds are active against *Bacillus subtilis* and *Staphylococcus aureus*, inhibiting its growth in the experiment. From *A. leptopus* flower extracts, researchers sought to synthesize silver, copper, and zinc nanoparticles, which were then tested for their antimicrobial activities against six human pathogens including *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus*, and *Candida albicans*. Substantial results were found to be dependent on the type of nanoparticles against *Candida albicans, E. Coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus* [33]. Furthermore, bioactive compounds of *A. leptopus* flower extracts displayed the presence of phenol, saponins, amino acids, steroids, phytosterols, triterpenoids, saponins, tannins, xanthoproteins, carboxylic acids, and coumarins, which were attributed to the antimicrobial properties it exhibits [32].

5.4. Antidiabetic Activity

Sujatha et al. [35] studied methanolic *A. leptopus* flower extract to determine the antidiabetic effect on rats with alloxan-induced diabetes. It was found that the extract had

antihyperglycemic effects with the best performance a dose of 200 mg/kg body weight, with effects comparable to those of a reference drug glibenclamide at a dose of 10 mg/kg body weight [35]. In another study, rats with alloxan-induced diabetes given ethanolic flower extracts exhibited a reduction in their fasting blood glucose level throughout the period of study [34]. Researchers suggested that secondary metabolites may be attributed to antioxidant activity, the protection and regeneration of B cells, increased insulin sensitivity, and optimum glucose utilization as mechanisms for its antidiabetic activity. Rani et al. [49] investigated the antidiabetic potential of *A. leptopus* leaf methanol extracts and testing showed that the extract resulted in significant oral glucose tolerance at a dose of 200 mg/kg body weight in rats with streptozotocin-induced diabetes. Different solvent fractions were then tested at different doses. Oral doses of 50 and 100 mg/kg body weight were reported to significantly reduce the fasting blood glucose levels of the diabetic rats, with the ethyl acetate fraction performing the best [49].

5.5. Anti-Inflammatory Activity

An in vivo study utilizing rats as a model demonstrated that the anti-inflammatory activity of *A. leptopus* root extract at doses of 200 and 400 mg/kg for carrageenan-induced paw inflammation was similar to that of diclofenac sodium, a commercial anti-inflammatory medication [23]. A similar study showed the effect of methanolic root extract given in different doses to rats with induced foot paw edema [24]. The researchers reported that significant inhibition occurred at all three doses. A separate study extracted and isolated alkaloids from the leaves of *A. leptopus* to test for its inhibitory effect on MMP-9 [17]. Alkaloids make up a vast group of natural organic compounds and are known to be effective anti-inflammatory agents through inhibiting the release of pro-inflammatory mediators [50]. The in vitro study reported that the extracts had a dose-dependent inhibitory activity on MMP-9 from concentrations ranging from 1000 µg/mL to 62.5 µg/mL with an IC_{50} of 115.8 µg/mL when compared against an NNGH standard [17]. Their findings aligned with the results of a previous study [30], which also identified alkaloids in the sample during phytochemical screenings, suggesting alkaloids are the source for *A leptopus's* anti-inflammatory activity. A recent in vitro study investigated the anti-inflammatory properties of ethanolic *A. leptopus* flower extracts through protein degradation inhibition and proteinase activity assays. Normally, proteinase activity plays a central role in the inflammatory response, which then promotes protein degradation. Researchers tested extract concentrations ranging from 2.0–10.0 mg/mL and reported a protein degradation inhibition of 7.40–68.69% and proteinase activity inhibition of 16.97–87.87% [51]. These findings add to the results of previous studies mentioned, suggesting that *A. leptopus* is able to inhibit inflammatory mechanisms, as exhibited by the dose-dependent results.

5.6. Anticancer Activity

Balasbrumani et al. [52] synthesized gold nanoparticles from the powdered leaf extract of *A. leptopus*. An MTT assay was performed to determine the cytotoxicity of the *A. leptopus* gold nanoparticles against a Michigan Cancer Foundation-7 (MCF-7) cell line. Results of the test show that the gold nanoparticles of *A. leptopus* exhibited moderate inhibition of the MCF-7 cells, as the percentage of growth of the MCF-7 cells decreased as the concentration of gold nanoparticles increased [52]. In a separate in vitro study with *A. leptopus* flower extracts that measured the inhibition of human cancer cell proliferation (AGS and BL-13 cell lines), the results showed that the extract had relatively high antiproliferative activities dependent on the dosage [36]. These studies suggest that *A. leptopus* can be a promising source for developing an anticancer drug.

6. Extraction Methodology of Bioactive Compounds from *A. leptopus*

Various factors affect the extraction of bioactive compounds from *A. leptopus,* including solvent types, sample-to-solvent ratios, temperatures, and extraction times. Common solvents used in the studies included water, methanol, ethanol, ethyl acetate, hexane, and chloroform. Resulting extracts were then prepared according to the analytical and biological activity tests.

6.1. Conventional Extraction Techniques

The main extraction methods used for the study of *A. leptopus* have included hot extraction, maceration, Soxhlet extraction, and sequential extractions.

6.1.1. Hot Extraction

In studies performed by Balasbrumani et al. [52], and Rudhra and Venkatesan [30], plants were extracted by mixing the dried powder with a solvent and boiling for different periods of time. In the study of Balasbrumani et al. [52], hot extraction of 8 g samples of leaf powder in 100 mL of deionized water lasted for 30 min, while the extraction performed by Rudhra and Venkatesan [30] of 50 mg of leaf powder using 250 mL of methanol, ethanol, and acetone was carried out over a period of 72 h. Vanisree et al. [28] extracted phenolic components in a manner analogous to tea preparation, where 50 mL of boiling water was poured directly onto 5 g of the dried aerial parts of the plant and was allowed to steep for 6 h. The resulting tea extract was then dried before testing.

6.1.2. Soxhlet Extraction

Pradhan and Bhatnagar [38] used a conventional method to perform hot extraction, which utilized a glassware called the Soxhlet extractor, named after its German inventor Franz von Soxhlet. About 20 g of *A. leptopus* leaf powder was uniformly packed into a thimble and extracted with 250 mL of solvents, starting from non-polar to polar solvents, including hexane, chloroform, ethyl acetate, and methanol [38]. Ranjan and Ahmad [53] used Soxhlet extraction to study the antimicrobial and anti-inflammatory properties of *A. leptopus*, where 10 g of powdered samples were extracted with methanol at 80 °C for three cycles over 12 h.

6.1.3. Maceration

Maceration is the most common extraction techniques used to extract the bioactive compounds from *A. leptopus.* Mamidipalli et al. [23] simply used coarse powder from the roots of *A. leptopus* and methanol to extract the compounds at room temperature. Meanwhile, in the study performed by Kaisoon et al. [36], they used a maceration technique to study the phenolic compounds and antioxidant activities of *A. leptopus* flowers. Ethanol was first mixed with the flower powder and was shaken at room temperature (25 °C) for 2 h before being centrifuged for 10 min. The supernatant was then collected and the extraction process was repeated two more times before evaporation [36]. According to Bolla and Bhogavalli [31], the flower powder was extracted using a mixture of ethanol and chloroform in a 1:10 sample-to-solvent ratio. The solvent and powder were placed in a stoppered flask for 48 h at room temperature (25 °C), shaking frequently during the first 6 h [31]. Shantaram and Bollapragada [39] used a similar 1:10 ratio of dried *A. leptopus* leaves and absolute methanol, but macerated for just 24 s with occasional stirring. While a number of the researchers who performed maceration did so for 1–3 days, Poosa and Vanapatla [42] extracted their leaf powder by macerating it in methanol for 7 days before concentrating it. A cold maceration method was used to study the in vitro cytotoxic activity of *A. leptopus* chloroform extract [54].

6.1.4. Sequential Extraction

To study different phytochemicals and components of the plant, some researchers performed successive extractions using various solvents such as methanol, ethyl acetate, deionized water, and more. Vanisree et al. [14] performed successive extractions on the aerial parts of *A. leptopus* with hexane, ethyl acetate, and methanol. Extracts were then subjected to silica-gel medium-pressure chromatography (MPLC) in order to collect various fractions for testing. Apaya et al. [16] first extracted the powder using methanol, which was then filtered and concentrated to create a crude methanol extract. Hexane and deionized water were then used to partition the crude extract and subsequent extraction of the aqueous layer was carried out using ethyl acetate.

6.2. Non-Conventional Extraction Techniques (Green Technology)

Non-conventional or green extraction technologies aim to efficiently obtain bioactive compounds from plants while minimizing the environmental impact, including the use of hazardous solvents and reducing energy usage. Specific studies on green extraction methods that have been applied to *A. leptopus* are limited.

6.2.1. Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE), a sustainable and environmentally friendly method, uses high frequency sounds to extract bioactive compounds from plant material. Fernández et al. [55] compared UAE with traditional extraction methods—maceration and percolation—to prepare 20% tinctures of *A. leptopus* and found that UAE was more efficient, yielding higher extract quantities in a shorter amount of time. UAE can also be combined with other extraction methods such as S-CO_2 allowing for a more effective extracting method. Compared to traditional methods, extraction times can be significantly reduced and extraction takes place at a lower temperature while still maximizing product yields [56,57]. Pereira et al. studied optimized UAE techniques for the extraction of phenolic compounds from *Camillia japonica,* testing variables such as power, time, and the solvent used. Their optimized method conditions were 62% amplitude, 8 min, and 39% acidified ethanol, which allowed them to obtain a 56% yield [58]. However, optimal extraction conditions are highly dependent on the sample composition.

6.2.2. Supercritical-Carbon-Dioxide (S-CO_2) Extraction

Supercritical carbon dioxide (S-CO_2) extraction is regarded as green technology, that utilizes CO_2 as a solvent at a temperature above 31 °C and pressure above 7.3 MPa to extract bioactive compounds from plant materials. Supercritical fluid extraction (SFE) is currently gaining attention due to its many advantages and sustainable applications over traditional extraction methods. The supercritical fluid properties, moderate temperature, and low pressure required for extraction allows for the selective and efficient separation of bioactive compounds while preserving its functional properties. Furthermore, the utilization of CO_2 with SFE technology to eliminate hazardous waste materials is currently being considered as a method to reduce CO_2 to reduce pollution and overall environmental impacts [56]. Specific studies on the application of S-CO_2 to *A. leptopus* are currently lacking; however, the technique has been successfully employed in extracting bioactive compounds from various other plant species [59,60]. However, the main drawback of SFE is its costly equipment, operating expenditures, and the requirement for specialized knowledge operate the equipment. These factors make this extraction method not as accessible as other methods. Additionally, SFE is not suitable for all extractions as some compounds may be difficult to extract while using supercritical fluids such as non-polar compounds [61].

6.2.3. Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) is another form of green technology that utilizes microwaves to stimulate liquid particles. The movement of the liquid then assists in making the extraction process more efficient. The benefits of MAE methods over traditional extraction methods include shorter extraction times, less solvent being required, and increased automation [57,60]. On the other hand, a difficulty faced with MAE is the possibility of non-uniform heating, which may affect the extractions. However, this issue can be avoided by taking proper precautions such as uniformly distributing the sample or utilizing proper mixtures [62]. Both MAE and UAE can be equally effective methods for extracting high-quality phenolic compounds, but the method should ultimately be chosen based on the sample composition and specific extraction objectives. More studies are required to determine an optimized *A. leptopus* extraction method as there is a lack of information and studies on the application of MAE techniques to *A. leptopus*. Nonetheless, its advantages have been showcased for other plants. Tambun et al. [57] tested different extraction methods on *Annona* muricata and reported that the MAE method produced the highest yield (33.98%) when compared to maceration and Soxhlet extraction methods, showing that MAE is a valuable technique worth researching [57].

7. Toxicity and Safety

Multiple in vitro and in vivo studies showed that *A. leptopus* had no significant mutagenic, toxic, or adverse effects on their model systems, as shown in Table 3, which summarizes the cytotoxic analysis of *A. leptopus*. Mamidipalli et al. [23] conducted an acute toxicity study on *A. leptopus* roots using Wistar rats and albino mice as models. During a 24 h period, the animals were given different doses with the highest dose level of 2000 mg/kg body weight, and none showed any signs of mortality or symptoms associated with toxicity [23]. Poosa and Vanapatla [42] performed a similar toxicity test on the *A. leptopus* leaves according to test dose guideline 423 of the Organization for Economic and Cultural Development. The results showed that *A. leptopus* did not cause any adverse effects or mortality in the rats up to a dosage of 2000 mg/kg, aligning with Mamidipalli's findings. Prajna et al. [63] studied the cytotoxic effects of a hexane fraction from a methanolic extract of *A. leptopus* flowers in vitro against a CHOK 1 cell line (Chinese hamster ovary cell line) and an A-549 cell line (human lung adenocarcinoma epithelial cell line) and reported there to be no cytotoxic effects, displaying the safety of the flowers. All components of the plant are edible and are consumed in Thai cuisine [8]. Overall, studies have shown that *A. leptopus* has no acute toxic effect.

Table 3. Cytotoxic analysis of different *A. leptopus* parts.

Part Used	Method	Toxicity	Reference
Roots	In vivo Acute toxicity study with methanol extract on albino mice	No cytotoxicity up to a dose of 2000 mg/kg B.W.	[23]
Flowers	In vivo Acute toxicity study with ethanol extract on albino mice	No cytotoxicity up to a dose of 2000 mg/kg B.W.	[34]
Flowers	In vitro MTT assay against CHOK 1 cell line and A-549 cell line	No cytotoxicity was observed	[63]

Table 3. *Cont.*

Part Used	Method	Toxicity	Reference
Leaves	In vivo Cytotoxicity brine shrimp assay	Mild dose-dependent activity	[38]
Leaves	In vivo Acute toxicity study with methanol extract on male Wistar albino rats	No cytotoxicity up to a dose of 2000 mg/kg B.W.	[42]
Leaves	In vitro Microculture tetrazolium assay with ethanol extract	No cytotoxicity observed	[37]

8. Conclusions

A. leptopus has been reported to be a highly invasive vine in Guam; however, around the world, it has been utilized as an herbal plant in folk medicine to treat common colds, stomach flus, skin irritations, and more. Its various ethnomedicinal uses have prompted researchers to investigate its phytochemical components and bioactivities. *A. leptopus* contains bioactive compounds, including polyphenols, flavonoids, terpenoids, alkaloids, glycosides, and tannins, which exhibit pharmacological properties such as antioxidant, antibacterial, antidiabetic, anti-inflammatory, and anticancer activities, without acute cytotoxic effects. Conventional methods are most commonly used for the extraction of bioactive compounds from *A. leptopus*; however, specific studies are lacking on the application of green extraction technology, including UAE, MAE, and S-CO_2 extraction methods. Biological studies and standardized extraction techniques are required for the isolation and characterization of specific components attributed to the pharmacological properties of *A. leptopus*. Currently, there is limited and outdated information on this species in the Marianas region despite its vast ecological impact. *A. leptopus* holds promise as a source of bioactive compounds for the nutraceutical industry. Future research should focus on analyzing and isolating its phytochemicals using green technologies to validate its potential for commercial applications within the region.

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