Review

Traditional Importance, Phytochemistry, Pharmacology, and Toxicological Attributes of the Promising Medicinal Herb Carissa spinarum L.

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Abstract: Carissa spinarum L. (Apocynaceae), commonly known as Garni or Jungli Karonda, has a rich history of use in indigenous traditional medicinal systems owing to its tremendous medicinal and nutritional benefits. The present review aims to discuss the traditional uses, ethnopharmacology, bioactive composition, toxicity analysis, and biotechnological applications of Carissa spinarum L. (CS) to identify the gap between current applications and research conducted on this plant. We collected the literature published before December 2022 on the phytochemical composition, pharmacological properties, and biotechnological applications of CS. Literature in English from scientific databases such as Google Scholar, PubMed, ScienceDirect, Springer, and Wiley, along with books on CS, was analyzed and summarized to prepare this review. The plant taxonomy was verified using the “World Flora Online” database. The in vitro and in vivo pharmacological studies on CS revealed its anthelmintic, anticonvulsant, anti-arthritic, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, hepatoprotective, vasorelaxant, antihypertensive, wound-healing, anti-venom, and antipyretic effects. Toxicological studies on CS also indicated the absence of any adverse effects even at high doses after oral administration. Although CS showed remarkable therapeutic activities against several diseases—such as diabetes, cancer, inflammation, and hepatitis B virus—there are several drawbacks in previous reports, including the lack of information on the drug dose, standards, controls, and mechanism of action of the extract or the phytocompounds responsible for its activity. Extensive research with proper in vivo or in vitro model systems is required to validate its reported activities.

Keywords: Carissa spinarum L.; Apocynaceae; phytochemistry; ethnopharmacology; toxicity analysis

1. Introduction

The demands for phytomedicines have frequently increased worldwide due to their safety and effectiveness compared to modern synthetic medicines, which are associated with major side effects [1]. There has been an increase in the popularity and acceptance of natural medicines in developing and developed countries over the last decade, as these herbal remedies are now available in drug stores, grocery stores, and supermarkets [2]. In India, the traditionally reported medicines in the Ayurvedic medicinal system are based on medicinal plants, reflecting a solid relationship between folk-employed healing practices, local diet practices, and natural remedies, which are region-specific [3]. Medicinal plants play a crucial role in nourishing the backbone of the traditional medicinal system. The World Health Organization (WHO) estimates that around 80% of the population of developing countries depends on traditional medicinal systems as their first option [4–6]. The WHO has highlighted the importance of traditional medicines—especially in developing countries—to meet the population’s healthcare needs [7].

The Himalayas are a rich source of unique flora that has been employed in various therapeutic systems worldwide for centuries. Around 20,000 medicinal plant species have
been reported from this region, about 500 plants have been mentioned in ancient literature, and 800 plants have been used in various indigenous medical systems [8,9]. India meets 80% of the world’s demand for Ayurvedic medication, 46% for Unani pharmaceuticals, and 33% for allopathic drugs [10,11]. The genus Carissa is a part of the Apocynaceae family, which contains 5 subspecies, 410 genera, and 5556 species. The Apocynaceae family is known as one of the enormous flowering plant families. The Carissa L. genus of the Apocynaceae was listed as having about 36 species [12]. Indian traditional medicine uses the plant Carissa spinarum L. (CS) to treat various illnesses. CS is widely distributed worldwide, including in China, Sri Lanka, India, Myanmar, tropical Africa, South Asia, Thailand, and Australia. CS has been explored for its pharmacological properties using crude extracts of the plant and their different fractions through various in vitro and in vivo methods. Almost all of the organs of CS—including the roots and root bark, leaves, ripe or unripe fruits, and stems and stem bark—are used for the treatment of several diseases, exhibiting antidiabetic [13], purgative, anticonvulsant [14], anti-arthritis [15], antimicrobial [16–18], anthelmintic [19,20], wound-healing [21], hepatoprotective [22–24], antipyretic [14], vasorelaxant [25], antioxidant [26–28], anti-inflammatory [29], and anticancer activities [30,31]. The present review aims to provide updated information on the traditional applications, phytochemical composition, pharmacological activities, and toxicity evaluation of CS. The review also shows the importance of CS fruits in the fields of food preservation and wine production.

2. Materials and Methods

The present review collected the literature published on the botanical classification, taxonomy, ethnobotany, phytochemistry, ethnopharmacology, and other biotechnological applications of C. spinarum before December 2022, via a variety of scientific search engines, including Google Scholar, ScienceDirect, Springer, PubMed, SciFinder, Wiley Online Library, and Taylor & Francis. The following keywords were used to search for relevant literature: “Carissa spinarum”, “botany of Carissa spinarum”, “secondary metabolites of Carissa spinarum”, “pharmacological activity of Carissa spinarum”, “biological activities of Carissa spinarum”, “safety of Carissa spinarum”, “traditional uses of Carissa spinarum”, and “toxicology of Carissa spinarum”.

3. Results

3.1. Botanical Description and Growth Conditions

C. spinarum is a thorny shrub with forked branches, hard wood, and bark ranging from light brown to green in color. The plant can grow up to 2–3 m in height. The plant has 3.2 cm long thorns and ovate leathery leaves that are 4.5 cm long and 2.5 cm broad, with pinnate reticulate venation, entire margins, and petioles 3 mm long. Its flowers are white, bisexual, complete, short-stalked, and have a pleasant smell [12,18]. The fruit is an ovoid berry that is 5–12 mm in length, 6 mm in diameter, green in color when unripe, and shining black when completely ripe [12]. The leaves and unripe fruit exude white latex when plucked from the stem (Figure 1). It grows from sea level to 600 m but can be found at altitudes up to 1800 m, e.g., in the Himalayas. It occurs on a wide range of soil types. In India, it grows wild in rocky soils and is grown as a hedge plant in dry, sandy, or rocky soils, as well as in Florida on sand or limestone.
Separations 2023, 10, x FOR PEER REVIEW 3 of 21

Figure 1. Morphology of CS in its natural habitat in Bankhandi village of the Kangra district, Himachal Pradesh, India (A). Branches of CS, showing the leaves and fruits (B). Part of a branch, with unripe fruits (C). Scientific classification of CS (D).

3.2. Traditional and Ethnomedicinal Importance

The use of CS has a very long history. Traditionally, the whole plant is used in India, Ethiopia, and other African countries for the treatment of venereal, respiratory, and gastrointestinal infections [32–35], fever, jaundice, hepatitis, cardiac diseases, diabetes, malaria, pneumonia [36], chronic joint pain [37], asthma, rheumatism, stomachache, cough, chest complaints, sickle-cell anemia, hypertension, kidney complications, diarrhea, worms, eye cataracts, gastric ulcers, and cancer [38]. It has also been used for treating microbial infections such as herpes, gonorrhea, syphilis, rabies, typhoid fever, jaundice, and polio, and as an antivenin for snake bites [39]. It has also been conventionally used to treat infertility and sexual problems such as asthenia and premature ejaculation [40] in males. It is also used as an ethnoveterinary medicine for the treatment of anaplasmosis [41]. The leaves of CS are also used as a mosquito repellent [35,42]. C. spinarum has been reportedly used to treat ulcers, muscle cramps, to stop bleeding after delivery, and to treat worm infestations in wounded animals [18].

3.3. Phytochemical Composition

Preliminary analysis of different parts of CS revealed the presence of various phytochemicals, such as saponins, alkaloids, tannins, flavonoids, glycosides, and sterols [16,43–45]. Physiochemical characterization of the leaves of CS revealed the presence of 14% total ash, 5.3% acid-insoluble ash, 6.6% water-insoluble ash, and 10.3% sulfated ash 10.3% [43]. The presence of minerals such as calcium (28–287 mg/100 g), iron (3–13 mg/100 g), and phosphorus (31–109 mg/100 g) in the fruits of CS was reported by Chauhan et al. [46], who also reported the nutritional and dietary composition of the fruits of CS; the fruits have 16–82% moisture content, 2–3% ash content, 18–62% carbohydrate content, 1–2% fat content, 2–3% protein content, 9–10% hemicellulose content, 12–15% cellulose content, and 3–4% lignin content. Several bioactive compounds have been reported from the leaves, stems, and roots of CS, which are responsible for its medicinal value. The presence of germacrane sesquiterpenes such as carenone (1), 3′-(4″-methoxy phenyl)-3′-oxo-propionyl hexadecanoate (2), germacenone (3), coniferaldehyde (4), (+)-pinoresinol (5), (-)-nortrachelogenin (6), (-)-carissanol (7), (-)-secosolariresinol (8), (-)-carinol (9), (-)-olivil (10), ethyl-3-hydroxy-3-(4″-methoxy-phenyl)-propionate (11), 1-(4″-methoxy-phenyl)-propene-1,3-diol (12), and 3-hydroxy-1-(4″-methoxy-phenyl)-propene-1-one (13) in chloroform stem extracts of CS was first reported by Rao et al. [26]. The presence of anticancer lignans such as scopoletin...
(14), nortrachelogenin (15), (-)-carissanol (7), (-)-carinol (9), (+)-cycloolivil (16), (+)-8-hydropyronesinol (17), (-)-olivil (10), (+)-secoisolariciresinol (8), (+)-pinoresinol (5), carissone (18), digitoxigenin, 3-β-D-digitoxigenin (19), and evomonoside (20) was reported from stem extracts of CS by Wangteeraprasert et al. [27].

The presence of ursolic acid (21) and betulic acid (22) in the leaves of CS was reported by Chanda et al. [47] and Feyissa and Melaku [16]. GC-MS analysis of hexane and aqueous leaf extracts of CS indicated the presence of several compounds, including hexadecanal (23), 2-[1-cyclohexenyl] cyclohexanone (24), phytol (25), squalene (26), vitamin E (27), hexadecamethylcyclosiloxane (28), 1-monolinoyleylglycerol trimethylsilyl ether (29), β-sitosterol (30), α-amyrin (31), lupeol (32), catechol (33), resorcinol (34), lup-20(29)-en-3-ol (35), n-benzyl-2-phenethylamine (36), 2-methyl-9-β-D-ribonolane (37), digitoxigenin, 3-β-D-digitoxigenin (19), and evomonoside (20), which are responsible for its medicinal properties [45]. Several compounds from the root bark of CS were also identified by Liu et al. [23,24]. In 2022, the n-hexane-soluble fraction of the smoke derived from the roots of CS showed the presence of 2,6-dimethoxyphenol (14.16%), 2-methoxyphenol (10.34%), and 2-hydroxyacetophenone (9.51%), while the methanol-soluble fraction showed the presence of 2,6-dimethoxyphenol (17.51%), 2-methoxyphenol (13.02%), and 2-hydroxyacetophenone (10.98%) using GC-MS analysis [48]. The various phytocompounds reported from different parts of CS using different techniques (such as mass spectrometry, NMR, HPLC, column chromatography, and GC-MS) are listed in Table 1, and the chemical structures of the compounds were drawn using ChemDraw software (Figures 2–4).

Figure 2. Phytocompounds identified by various reports in the stems of Carissa spinarum.
Table 1. List of compounds reported in different plant parts in different solvent fractions.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Name of the Phytocompounds</th>
<th>Method of Identification</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stems</td>
<td>Carenone (1), 3′-(4′′-methoxy phenyl)-3′-oxo-propionyl hexadecanoate (2), germacrenone (3), coniferaldehyde (4), (+)-pinoresinol (5), (-)-nortrachelogenin (6), (-)-carissanol (7), (-)-secoisolariciresinol (8), (-)-carinol (9), (-)-olivil (10), ethyl-3-hydroxy-3-(4′-methoxy-phenyl)-propionate (11), 1-(4′-methoxy-phenyl)-propane-1,3-diol (12), 3-hydroxy-1-(4′-methoxy-phenyl)-propene-1-one (13)</td>
<td>Mass spectrometry, $^1$H and $^{13}$C NMR</td>
<td>Rao et al. [26]</td>
</tr>
<tr>
<td>Stems</td>
<td>Scopoletin (14), nortrachelogenin (15), (-)-carissanol (7), (-)-carinol (9), (+)-cycoolivil (16), (+)-8-hydroxypinoresinol (17), (-)-olivil (10), (-)-secoisolariciresinol (8), (+)-pinoresinol (5), carissone (18), digitoxigenin, 3-o-β-D-digitoxilopyranoside (19), evomonoside (20)</td>
<td>UV–Vis spectroscopy, Micromass Q-TOF global tandem mass spectrometry, $^1$H and $^{13}$C NMR</td>
<td>Wangteeraprasert et al. [27]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Ursolic acid (21) and betulic acid (22)</td>
<td>TLC, RP-HPLC, column chromatography, $^1$H and $^{13}$C NMR</td>
<td>Feyissa and Melaku [16]; Chanda et al. [47]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Hexadecanal (23), 2-(1-cyclohexenyl) cyclohexanone (24), phytol (25), squalene (26), vitamin E (27), hexadecamethyloctasiloxane (28), 1-monolinoleoylglycerol trimethylsilylether (29), β-sitosterol (30), α-amyrin (31), lupeol (32), catechol (33), resorcinol (34), lup-20(29)-en-3-ol (35), n-benzyl-2-phenethylamine (36), 2-methyl-9-β-D-ribosyl hypoxanthine (37), paromomycin (38), 3-O-methyl-D-glucose (39)</td>
<td>GC-MS</td>
<td>Rao, and Anisha [45]</td>
</tr>
<tr>
<td>Root bark</td>
<td>3-O-vanilloylquinic acid (40), 3-O-syringolylquinic acid (41), 3,4-di-O-syringolylquinic acid (42), neochlorogenic acid (43), cryptochlorogenic acid (44), 3,4-dicaffeoylquinic acid (45), 3,5-dicaffeoylquinic acid (46), 4,5-dicaffeoylquinic acid (47), methyl 4,5-dicaffeoylquiniate (49), 4-O-cafeeyl-3-O-syringolylquinic acid (50), 4-O-cafeeyl-3-O-vanilloylquinic acid (51)</td>
<td>(+)-HR-ESI-MS spectrum, $^1$H and $^{13}$C NMR</td>
<td>Liu et al. [23]</td>
</tr>
<tr>
<td>Root bark</td>
<td>3x-O-β-D-glucopyranoside (53), (-)-lyoniresinol 3x-O-β-D-glucopyranoside (54), acetophenone-2-O-β-xylopyranosyl-(1-6)-O-β-glucopyranoside (55), erythro-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,3-diol (56), threo-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol (57), 3-carboxymethyl-benzoic acid (58), protocatechuic acid (59), vanillic acid (60)</td>
<td>HPLC–UV, HPLC–ESI–MS/MS, UV–Vis spectra, $^1$D NMR, 2D NMR spectra, LC-MS</td>
<td>Liu et al. [24]</td>
</tr>
</tbody>
</table>
Figure 3. Phytocompounds identified by various reports in CS leaves.
Figure 4. Cont.
Figure 4. Phytocompounds identified by various reports in CS roots.

3.4. Pharmacological Properties

Several pharmacological activities have been reported from different parts of CS. Some of these properties have been discussed as follows:

3.4.1. Anthelmintic Activity

Several studies were conducted to investigate the anthelmintic properties of CS. Harwansh et al. [19] studied the effects of different extracts of dried CS roots on adult *Pheretima posthuma*—an Indian earthworm—due to its structural and anatomical similarity to human intestinal helminthes. They found that methanolic extract (100 mg/mL) and chloroform extract (50 mg/mL and 100 mg/mL) had a potency almost equivalent to that of the standard drug piperazine citrate (10 mg/mL), and both solvent extracts showed similar paralysis time as well as death of the *P. posthuma*, indicating their efficacy against human intestinal helminthes. In another in vitro experiment, the anthelmintic effect of crude aqueous extracts of CS leaves on *H. contortus* was reported. Both aqueous extract of CS leaves (0.5 mg/mL) and the standard drug albendazole (0.25 µg/mL) were found to induce 100% egg hatch inhibition. In adult *H. contortus*, aqueous extracts of CS leaves (4 mg/mL) induced significant mortality of parasites (96.8%), as compared to albendazole (0.5 mg/kg), which showed 100% parasite mortality [49]. A study conducted by Jan et al. [20] on condensed tannins derived from CS leaves showed a protective role of CS against the pathogenic gastrointestinal nematode *H. contortus* in goats. The animals were fed with 1.96% condensed tannins (CT)-rich leaf meal mixture for 90 days; a group of animals with 0% CT in their feed were taken as controls. Significant improvements in hematological parameters were observed in terms of increased levels of hemoglobin, total protein and globulin, hematocrit, calcium, and glucose in nematode-infected animals. There were also no detrimental health effects of the CT-rich diet on the goats. However, there is also a
need to explore the anthelmintic properties of other parts of CS. Future studies could be conducted using different drug doses, model systems, and to explore the mechanism of action of CS against pathogenic helminthes.

3.4.2. Anticonvulsant Activity

CS also exhibits anticonvulsant activity, as reported by different scientists. Hedge et al. [14] reported an anticonvulsant effect of ethanolic extracts of CS roots. Three differently induced murine models—i.e., maximal electroshock-induced convulsion, picrotoxin-induced seizures, and pentylentetrazole (PTZ)-induced convulsion—were used to study the anticonvulsant effects of ethanolic extracts of CS roots at three different dose levels: 100 mg/kg, 200 mg/kg, and 400 mg/kg. Significant reductions in the latency of tonic seizures were observed with all three doses. It was found that the ethanolic extract of CS roots protected 50% and 62.5% of the total mice from PTZ-induced convulsions and 25% and 62.5% of the total mice from maximal electroshock-induced convulsions at CS root extract doses of 200 mg/kg and 400 mg/kg, respectively. Further studies need to be conducted to explore the anticonvulsant activity of this plant using different model systems. Moreover, studies could also be carried out to determine the optimal drug dose without any toxic effects, as well as the mechanism of action of CS.

3.4.3. Anti-Arthritic Activity

The anti-arthritic properties of ethanolic extracts of CS roots were studied by Hegde et al. [15] for the treatment of Freund’s-adjuvant-induced polyarthritis in rats, using phenylbutazone (100 mg/kg) as a standard drug. Arthritic mice were administered with ethanolic extracts of CS roots at different doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg. The results in non-established arthritis were 36.23%, 49.27%, and 66.66%, while in established arthritis they were 30.43%, 44.92%, and 57.97% reduction in edema of affected joints at the doses of 100, 200, and 400 mg/kg, respectively. The CS root extract was found to reduce the pain and swelling of the affected joints, while also reducing the development of secondary lesions. Further studies need to be conducted to explore the anti-arthritic activity of this plant using different model systems. More research could also be carried out to determine the optimal drug dose without any toxic effects, as well as the mechanism of action of CS.

3.4.4. Anti-Inflammatory Activity

Different parts of CS have been traditionally used to treat pain and inflammation. Beck and Namdeo [29] observed significant ($p < 0.01$) anti-inflammatory activity of different extracts of CS leaves at a dose of 200 mg/kg, which was comparable to that of analgin (30 mg/kg), in the treatment of formalin-induced edema in the hind paws of rats. The aqueous extracts of CS leaves showed the greatest inhibition percentage (17.04%) after the standard drug (33.87%), followed by chloroform extracts (6.93%), ethanolic extracts (6.93%), and petroleum ether extracts (4.76%). The anti-inflammatory potential of the crude root bark, dichloromethane (DCM) fraction, petroleum ether (PE) fraction, ethyl acetate (EA) fraction, and $n$-butanol (BUT) fraction was determined via COX-2 inhibition assay, using indomethacin (INM) as a positive control [24]. Among the crude extract and its fractions, the EA fraction (0.2 ± 0.0 µg/mL) exhibited the lowest IC$_{50}$ value, followed by the DCM (0.5 ± 0.0 µg/mL), BUT (0.2 ± 0.0 µg/mL), crude extract (0.2 ± 0.0 µg/mL), and PE (0.2 ± 0.0 µg/mL) fractions, whereas INM showed an IC$_{50}$ of 0.4 ± 0.1 µg/mL [24]. In future, the anti-inflammatory potential of CS could also be investigated using different model systems. Moreover, the mechanism of its anti-inflammatory activity and the optimal dose for its anti-inflammatory activity could be determined.

3.4.5. Antimicrobial Activity

Several studies have been reported the antimicrobial potential of various parts of CS. The antimicrobial activities shown by different extracts of CS are summarized in Table 2.
The methanolic extract of CS roots was evaluated for its antimicrobial activity against five pathogenic bacteria and fungi, with MIC values of $125 \pm 10 \mu g/mL$ (Escherichia coli), $512 \pm 43 \mu g/mL$ (Bacillus subtilis), $110 \pm 28 \mu g/mL$ (Staphylococcus aureus), $165 \pm 20 \mu g/mL$ (Streptococcus sp.), and $256 \pm 30 \mu g/mL$ (Aspergillus niger) [21]. Rubaka et al. [44] reported strong antibacterial activity of ethanolic and methanolic root extracts of CS against S. aureus and E. coli, with relative inhibition zone diameters of 66.97–70.13% and 54.66–57.24%, respectively. The PE extract of CS leaves displayed the lowest MIC value against S. aureus (312 $\mu g/mL$), while the ethanolic and methanolic root extracts of CS showed the lowest MIC (312 $\mu g/mL$) against E. coli. Kumar et al. [17] reported strong inhibition of the EA extract (25 mm) and PE extract of CS roots against E. coli (23 mm), of the EA extract (20 mm) and chloroform extract (CE) of CS roots (20 mm) against Proteus, PE (20 mm), and of the CE (20 mm) against MRSA. The PE and hexane extract of CS leaves showed strong inhibition against E. coli, Proteus, and MRSA, with zones of inhibition ranging between 16 and 30 mm. The modest antibacterial activity of the ethyl acetate extract of CS leaves against S. aureus was reported by Feyissa and Melaku [16], with a zone of inhibition of 15 mm at a 1.5 mg/mL concentration, which was due to the presence of ursolic and betulinic acids in the leaves of CS. Doshi et al. [50] showed the strong antimicrobial activity of a nanoemulsion prepared from CS fruits against S. aureus, B. subtilis, S. typhi, and E. coli, with MIC values ranging between 30 and 50 $\mu g/mL$. Tiruneh et al. [51] reported a relative antibacterial effect with varying zones of inhibition. However, the ME showed superior antibacterial activity compared with the DMSO, EA, and CE, possibly due to the different phytoconstituents in the different solvents. Saka et al. [52] reported the antibacterial activity of ZnO nanoparticles from fresh leaves of CS against Staphylococcus aureus and Bacillus, while AgNO$_3$ nanoparticles prepared from the leaves of CS were found to have strong antifungal activity against Sporisorium scitamineum [53]. Extensive studies are required to explore the antimicrobial nature of different parts of CS and to determine the phytoconstituents responsible for their antimicrobial activity. Scientists can also work on the preparation of nanoparticles with antimicrobial properties from different parts of CS.

### Table 2. Antimicrobial activity of different parts of CS against different microorganisms.

<table>
<thead>
<tr>
<th>Plant Part Used</th>
<th>Extract Used</th>
<th>Microorganisms</th>
<th>Key Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td>Methanolic extract</td>
<td>E. coli, B. subtilis, S. aureus, Streptococcus spp, A. niger</td>
<td>MIC—$125 \pm 10 \mu g/mL$, MIC—$512 \pm 43 \mu g/mL$, MIC—$110 \pm 28 \mu g/mL$, MIC—$165 \pm 20 \mu g/mL$, MIC—$256 \pm 30 \mu g/mL$</td>
<td>Sanwal and Choudhary [21]</td>
</tr>
<tr>
<td>Roots, leaves, and bark</td>
<td>95% Ethanol, methanol, and petroleum ether</td>
<td>E. coli DSM 1103, S. aureus ATCC 25923, S. aureus ATCC 25922, Proteus mirabilis, P. aeruginosa ATCC 35032 Escherichia coli</td>
<td>ZOI—2.33 ± 0.58–13.33 ± 1.53 mm, MIC—$312–5000 \mu g/mL$</td>
<td>Rubaka et al. [44]</td>
</tr>
<tr>
<td>Leaves</td>
<td>n-Hexane, ethyl acetate, and methanol</td>
<td>E. coli ATCC 25922, Proteus mirabilis, P. aeruginosa ATCC 35032 Escherichia coli</td>
<td>ZOI—15 mm at 0.5 mg/mL in ethyl acetate extract.</td>
<td>Feyissa and Melaku [16]</td>
</tr>
<tr>
<td>Fruit</td>
<td>Nanoemulsions</td>
<td>Staphylococcus aureus, Salmonella typhi, B. subtilis</td>
<td>MIC—$30–50 \mu g/mL$</td>
<td>Doshi et al. [50]</td>
</tr>
<tr>
<td>Root and Leaves</td>
<td>Petroleum ether, hexane, ethyl acetate, and chloroform</td>
<td>MRSA, E. coli, Proteus, P. fluorescens</td>
<td>ZOI—20–30 mm</td>
<td>Kumar et al. [17]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Methanol and its solvent fractions</td>
<td>S. aureus and S. pneumoniae, E. coli, K. pneumoniae</td>
<td>ZOI—7–13 mm</td>
<td>Tiruneh et al. [51]</td>
</tr>
</tbody>
</table>

ZOI: zone of inhibition; MIC: minimum inhibitory concentration.

### 3.4.6. Antidiabetic Activity

Plant-based drugs have always been attractive to humans for the treatment of diseases such as diabetes. Fatima et al. [13] investigated the antidiabetic effect of acetone extract of CS leaves on alloxan-induced diabetic rats. In overnight-fasted rats, alloxan
(150 mg/kg body weight) was injected intraperitoneally to induce diabetes mellitus. The oral administration of acetone extract of CS leaves in diabetic rats at doses of 200, 400, and 600 mg/kg was found to significantly decrease fasting blood glucose and urine sugar levels. The treatment also reduced cholesterol, triglyceride, low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) levels and increased high-density lipoprotein (HDL) levels significantly in the treated diabetic rats, thereby showing both anti-hyperglycemic and anti-hyperlipidemic activity. More research could be carried out to explore the antidiabetic potential of CS against various model systems. Moreover, studies could be conducted to investigate the mechanism of antidiabetic potential of CS and its phytocompounds.

3.4.7. Hepatoprotective Activity

The ethanolic extract of CS roots (100–400 mg/kg dose) also showed hepatoprotective activity against chloroform- and paracetamol-induced hepatotoxicity by decreasing the activities of bilirubin and lipid peroxidation, while significantly increasing the levels of uric acid, glutathione, superoxide dismutase, catalase, and protein in a dose-dependent manner [22]. Recently, Liu et al. [24] reported the hepatoprotective effects of (+)-isolariciresinol 3α-O-β-D-glucopyranoside and protocatechuic acid from root bark extract of CS using a H₂O₂-induced oxidative stress model in L02 cells. There were improvements in the viability of L02 cells in the H₂O₂ model group from 68.2% to 79.4% and 80.9% by (+)-isolariciresinol 3α-O-β-D-glucopyranoside and protocatechuic acid at concentrations of 5 µM, respectively, whereas the positive control (vitamin C) improved the cell viability from 65.2% to 82.7% at 5 µM. More research is required to validate the hepatoprotective effects of CS. Phytocompounds isolated from different parts of CS could be used to determine their hepatoprotective activity.

3.4.8. Anticancer Activity

The anticancer activity of CS has been reported using different cell lines. Sehar et al. [30] showed significant anticancer activity of CS root extract against HL-60 leukemia cell lines, with IC₅₀ = 34.58 µg/mL and GI₅₀ = 18.1 µg/mL. Later, cytotoxic effects of hexane, CE, and methanolic extracts of CS were reported on A375 melanoma cells using a sulforhodamine B staining assay, with IC₅₀ values of 40 µg/mL, 47 µg/mL, and >100 µg/mL, respectively. Cell-cycle analysis also validated the cytotoxic activity of hexane extract of CS through induction of cell-cycle arrest at the S phase, along with the induction of caspase-3/7 activities [31]. More research is required to validate the anticancer activity of different parts of CS using different types of cell lines. Researchers could also investigate the mechanistic aspects of the anticancer activity of CS phytocompounds.

3.4.9. Antioxidant Activity

Chloroform extract of CS stems (IC₅₀—47.03 µg), along with (-)-carissanol (IC₅₀—47.87µM) and (-)-carinol (IC₅₀—37.12µM), showed strong DPPH scavenging activity as compared to that of Trolox (IC₅₀—32.19µM) [47]. However, a study by Feyissa and Melaku [16] reported low antioxidant activity of EA extract of CS leaves (5% at 2.5 mg/mL). Moderate anti-DPPH activity was also reported by Wangteeraprasert et al. [27] for eight lignans isolated from CS stem extract. Afanyibo et al. [28] also reported strong radical scavenging of aqueous extract of CS roots, with an IC₅₀ value of 75.65 ± 5.02 µg/mL, as compared to that of hydroalcoholic extract (IC₅₀—96.10 ± 1.11 µg/mL). The antioxidant activity of crude CS root bark extract and its different fractions (i.e., PE, DCM, EA, and BUT) was also reported by Liu et al. [24] using a DPPH assay. The ethyl acetate fraction was found to have the highest DPPH scavenging activity (IC₅₀—31.8 ± 1.3 µg/mL), followed by the BUT (92.4 ± 8.6 µg/mL) and DCM (136.0 ± 7.6 µg/mL) fractions. Similarly, the EA fraction (14.9 ± 2.4 mmol Fe²⁺/g) also exhibited the best FRAP activity, followed by the BUT (4.1 ± 0.0 mmol Fe²⁺/g) and DCM (3.0 ± 0.7 mmol Fe²⁺/g) fractions. Protocatechuic acid from the root bark extract of CS showed strong DPPH activity, with an IC₅₀ value of 16.5 ± 1.2 µM, which was lower than that of the positive control (vitamin C, 25.5 ± 0.3 µM).
However, compounds such as (+)-isolariciresinol 3α-O-β-D-glucopyranoside, erythro-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol, threo-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol, and protocatechuic acid showed better FRAP activity as compared to that of vitamin C [24]. The antioxidant activity of neochlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, methyl 3,5-dicaffeoylquinic, methyl 4,5-dicaffeoylquinic, and 4-O-cafeoyl-3-O-syringoylquinic acid from the root bark of CS was reported by Liu et al. [23]. Recently, Nazareth et al. [51] also reported the strong antioxidant activity of ripe CS fruits based on a DPPH assay (IC_{50}—4.69 mg/mL). However, more studies are required to isolate the phytochemicals responsible for its antioxidant activity. In vivo studies also need to be conducted to determine the antioxidant activity of different parts of CS. The antioxidant activities reported in different parts of CS using different in vitro methods are listed in Table 3.

Table 3. Antioxidant activities showed by different extracts of CS using different in vitro assays.

<table>
<thead>
<tr>
<th>Part Used/Extracts</th>
<th>Used Methods</th>
<th>Key Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform extract of stems</td>
<td>DPPH</td>
<td>IC_{50}—47.03 µg/mL</td>
<td>Rao et al. [26]</td>
</tr>
<tr>
<td></td>
<td>DPPH</td>
<td>IC_{50}—1013 ± 2.00 µM AEAC/100 g dry wt.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRAP</td>
<td>IC_{50}—2118 ± 1.00 µM AEAC/g dry wt.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peroxidase (POX)</td>
<td>POX—0.001 ± 0.0003 Δ OD/min/g fwt</td>
<td>Nayak and Basak [54]</td>
</tr>
<tr>
<td></td>
<td>Catalase (CAT)</td>
<td>CAT—1.1119 ± 0.004 U/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Superoxide dismutase (SOD)</td>
<td>SOD—0.151 ± 0.001 Δ O.D/min/g tissue wt.</td>
<td></td>
</tr>
<tr>
<td>Fruit extract</td>
<td>DPPH</td>
<td>IC_{50}—75.65 ± 5.02 µg/mL (aqueous extract)</td>
<td>Afanyibo et al. [28]</td>
</tr>
<tr>
<td></td>
<td>DPPH</td>
<td>IC_{50}—96.10 ± 1.11 µg/mL (hydroalcoholic extract)</td>
<td></td>
</tr>
<tr>
<td>Hydroalcoholic and aqueous extract of root bark</td>
<td>DPPH</td>
<td>Ethyl acetate fractions showed strong DPPH and FRAP activity as compared to that of other fractions</td>
<td>Liu et al. [23]</td>
</tr>
<tr>
<td>Methanolic root bark extract and its sub-fractions</td>
<td>DPPH</td>
<td>IC_{50}—4.69 mg/mL</td>
<td>Nazareth et al. [55]</td>
</tr>
<tr>
<td>Ripe fruit extract</td>
<td>DPPH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DPPH: 1,1-Diphenyl-2-picrylhydrazyl; FRAP: ferric-reducing antioxidant power; POX: peroxidase; SOD: superoxide dismutase; CAT: catalase; IC_{50}: half-maximal inhibitory concentration.

3.4.10. Antiviral Activity

The antiviral activity of aqueous extracts of the root bark of CS against herpes simplex virus (HSV) was reported by Tolo et al. [56] based on in vitro and in vivo anti-HSV activity, using tests such as plaque inhibition assay, cell cytotoxicity assay, and virus yield reduction assay, as well as against BALB/C mice cutaneously infected with HSV. The root bark of CS significantly inhibited the formation of plaques in Vero E6 cells infected with 100 PFU of wild-type strains of HSV (7401H HSV-1 and Ito-1262 HSV-2) or resistant strains of HSV (TK−7401H HSV-1 and AP7 7401H HSV-1) by 100% at 50 µg/mL in vitro, with minimal cell cytotoxicity (CC_{50} = 480 µg/mL). When the extract was examined for in vivo efficacy in a murine model using BALB/C mice cutaneously infected with wild-type or resistant strains of HSV, the extract at an oral dose of 250 mg/kg significantly delayed the onset of HSV infections by over 50%. It also increased the mean survival time of treated infected mice by between 28 and 35% relative to the infected untreated mice (p < 0.05 versus control, according to Student’s t-test). The mortality rate for mice treated with the extract was also significantly reduced by between 70 and 90% as compared with the infected untreated mice, which exhibited 100% mortality. No acute toxicity was observed in mice at the oral therapeutic dose of 250 mg/kg. Further studies are required to investigate the antiviral activity of other parts of CS, using different model systems.

3.4.11. Other Pharmacological Properties

The different parts of CS are also reported to have antipyretic, wound-healing, vasorelaxant, anti-nociceptive, anti-venom, anti-herpetic, erythropoietic, antileishmanial, and antidepressant-like activities, as listed in Table 4.
Table 4. List of other pharmacological activities shown by CS plant as reported by various researchers.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Activity</th>
<th>Extract</th>
<th>Control</th>
<th>Model System Used</th>
<th>Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Antipyretic</td>
<td>Ethanolic root extract</td>
<td>Aspirin</td>
<td>Albino mice</td>
<td>Oral administration of 100 mg/kg, 200 mg/kg, and 400 mg/kg of CS resulted in reduction in body temperature by 0.15–2.55% as compared to aspirin (1.08–2.53%). CS root extract showed significant wound-healing activity, as evident from the rate of wound contraction and epithelization. Hydroxyproline expression and histological parameters were also well correlated with the observed healing pattern.</td>
<td>Hegde et al. [15]</td>
</tr>
<tr>
<td>2</td>
<td>Wound healing</td>
<td>Methanolic root extract</td>
<td>Silver sulfadiazine</td>
<td>Albino mice</td>
<td>All tested extracts caused concentration-dependent relaxation in pre-contracted aortic rings. Among all extracts, the dichloromethane-soluble extract from the leaves of <em>C. spinarum</em> (<em>EC_{50}—0.17 ± 0.01 mg mL^{-1}; E_{max}—85.72%</em>) was found to be highly active.</td>
<td>Sanwal and Chaudhary [21]</td>
</tr>
<tr>
<td>3</td>
<td>Vasorelaxant activity</td>
<td>Methanolic leaf extract</td>
<td>-</td>
<td>Wistar rats</td>
<td>High concentrations (100 mg/kg body weight) of the acetone leaf extracts of CS showed reduction in the writhing caused by formalin-induced pain. However, acetone leaf extract of CS at concentrations of 50 and 100 mg/kg of body weight showed similar reductions in acetic-acid-induced pain. The methanolic extract was found to inhibit acetylcholinesterase, phosphomonoesterase, phosphodiesterase, and S(^{\prime})-nucleotidase of viper venom, as well as the hyaluronidase, and phospholipase A2 of krait venom.</td>
<td>Fatiani et al. [25]</td>
</tr>
<tr>
<td>4</td>
<td>Anti-nociceptive activity</td>
<td>Acetone leaf extract</td>
<td>Diclofenac sodium</td>
<td>Swiss albino mice</td>
<td>Evomoside extracted from stems of CS was found to show more than 50% inhibition at 100 mg/mL against the Vero cell line, with no toxicity. In the inhibition method, the evomoside showed IC_{50} values of 120.2 and 168.3 mM against HSV-1 and HSV-2, respectively, and the IC_{50} value for acyclovir against HSV-1 was 2.8 mM.</td>
<td>Mworia et al. [57]; Mworia [58]</td>
</tr>
<tr>
<td>5</td>
<td>Anti-venom activity</td>
<td>Methanolic leaf extract</td>
<td>-</td>
<td>In vitro enzyme assays</td>
<td>Phospholipase A2 of <em>V. russelli</em> venom, hyaluronidase of <em>B. caeruleus</em> venom, and protease and L-amino acid oxidase enzymes in both venoms were not inhibited by the extracts. Evomoside extracted from stems of CS showed more than 50% inhibition at 100 mg/mL against the Vero cell line, with no toxicity. In the inactivation method, the evomoside showed IC_{50} values of 120.2 and 168.3 mM against HSV-1 and HSV-2, respectively, and the IC_{50} value for acyclovir against HSV-1 was 2.8 mM.</td>
<td>Janardhan et al. [59]</td>
</tr>
<tr>
<td>6</td>
<td>Anti-herpetic activity</td>
<td>Methanolic stem extract</td>
<td>Acyclovir</td>
<td>Plaque reduction assay</td>
<td><em>C. spinarum</em> at doses of 300 and 100 mg/kg was able to very significantly reverse anemia caused by phenylhydrazine after 45 days of treatment without anisocytosis.</td>
<td>Wangteeraprasert et al. [27]</td>
</tr>
<tr>
<td>7</td>
<td>Erythropoietic effect</td>
<td>Ethanolic root extract</td>
<td>Bioferon</td>
<td>Sprague Dawley rat</td>
<td>Hydroxyproline expression and histological parameters were also well correlated with the observed healing pattern. All tested extracts caused concentration-dependent relaxation in pre-contracted aortic rings. Among all extracts, the dichloromethane-soluble extract from the leaves of <em>C. spinarum</em> (<em>EC_{50}—0.17 ± 0.01 mg mL^{-1}; E_{max}—85.72%</em>) was found to be highly active.</td>
<td>Koffuor et al. [60]</td>
</tr>
</tbody>
</table>
Table 4. Cont.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Activity</th>
<th>Extract</th>
<th>Control</th>
<th>Model System Used</th>
<th>Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Antidepressant-like activity</td>
<td>Solvent fractions of the root bark</td>
<td>TW80</td>
<td>Rodents</td>
<td>Both the aqueous and ethyl acetate fractions significantly ( (p &lt; 0.001) ) decreased the duration of immobility. The locomotor test revealed that the activity was not due to non-specific psychostimulant effects. Serum corticosterone levels were reduced by both fractions, with the ethyl acetate fraction again being the most effective. Mechanistic studies showed the involvement of multiple neurotransmission systems, including the adrenergic, dopaminergic, and cholinergic systems, as well as the L-arginine-NO-cGMP pathway.</td>
<td>Ali, and Engidawork [61]</td>
</tr>
<tr>
<td>9</td>
<td>Antileishmanial activity</td>
<td>Polar and non-polar extracts of stems</td>
<td>Pentostam® and Amphotericin B®</td>
<td>Leishmania major</td>
<td>All of the polar and non-polar extracts of CS showed less activity against promastigotes as compared to that of Pentostam® and Amphotericin B® at all concentrations (50–200 ( \mu )g/mL). The activity of these extracts against the amastigote form of ( L. ) major was seen to be dose-dependent.</td>
<td>Njau et al. [62].</td>
</tr>
</tbody>
</table>

“-” indicates the absence of control in the study.
3.5. Biotechnological Applications of CS

3.5.1. Wine Production

Due to their astringent flavor and fruity aroma, the fruits of CS can be used for wine production. Mundaragi and Thangadurai [63] developed an optimized protocol for quality wine production using ripe CS fruits with 8.3% (v/v) ethanol content at a temperature of 25 °C, pH 3.5, and 10% (v/v) inoculum size. The physiochemical characteristics of CS fruit wine also revealed the presence of fair amounts of essential nutrients and significant antioxidant activity. Therefore, the utilization of CS fruits in the wine production industry can also result in significant increases in the economies of rural communities.

3.5.2. Anti-Quorum Sensing Activity

The anti-quorum sensing activity of ripe fruit extracts was higher at 1.8 mg/mL, with 78.65% inhibition of violacein production in Chromobacterium violaceum, as well as reducing swimming motility and biofilm formation in Pseudomonas aeruginosa and Yersinia enterocolitica (66.25% and 59.36%, respectively, at 1.2 mg/mL). This was attributed to the presence of syringic acid, resveratrol, and quercetin in ripe fruits [50]. Nazareth et al. [64] also reported the stability of anthocyanins by encapsulating concentrates of CS fruit juice with polyphenols in microemulsions (CSME) and nanoemulsions (CSNE). Increasing the amount of CS resulted in reduction in the particle size from 1154 to 70–300 nm, whereas the addition of Tween 80 reduced it optimally to 5–25 nm. The physiochemical characteristics of CSME and CSNE were found to have higher anti-quorum sensing activity than that of CSME. The degradation of anthocyanins in the control and ME/NE proceeded with zero- and first-order reaction rates, respectively, at 28 °C (half-life: 6, 25, and 40 days, respectively). CSNE was found to have higher anti-quorum sensing activity than that of CSME against C. violaceum (73.7%), and it inhibited biofilm formation by 70.1% and 64.4% in P. aeruginosa and Y. enterocolitica, respectively. Therefore, the fruits of CS can be exploited as a source of natural bioactive compounds with anti-quorum sensing activity to manage foodborne pathogens.

3.5.3. Natural Dye

The fruits of Carissa carandas—another member of the Carissa genus—have reportedly been used as natural dyes for fabric [65]. Like those of C. carandas, CS fruits are also colorful and can be used as natural colorants to dye fabrics. In future, extensive research could be conducted to explore the applications of CS fruit extracts in the dye or paint industries. Figure 5 shows various pharmacological applications and future areas of research for different parts of CS.

Figure 5. Pharmacological properties of CS reported in several sources, and future areas of research for CS.
3.6. Toxicity and Safety Aspects

Several studies have been conducted to evaluate the toxicity of different parts of CS. The safety of the ethanolic extract of CS roots at a dose of 0.5–1 g/kg body weight in rats was reported by Hegde and Joshi [22], and there was no mortality in the rats. The crude extracts of CS did not produce significant physical and behavioral changes at dose of up to 5000 mg/kg of the extracts, with no deaths. In subacute toxicity studies of the hydromethanolic and chloroform extracts, there were no significant changes (p > 0.05) in the hematological and physical parameters recorded in the treated groups when compared to the control groups [66]. The cytotoxicity of hydroalcoholic CS root extract on *Artemia salina*, along with the acute and subacute (28 days) oral toxicity on Wistar rats, was also investigated by Dossou-Yovo et al. [67]. The lethal concentration (LC50) of hydroalcoholic extract of CS roots was 0.9 mg/mL. The dose of 500–1000 mg/kg hydroalcoholic extract of CS roots for 28 days did not provoke death or toxicity signs. In terms of subacute toxicity, no signs of toxicity or mortality were observed during the experiment. Similar results were also reported for oral toxicity in Wistar rats with ethanolic CS root extract at a dose of 500–1000 mg/kg body weight [68]. Further studies could be performed to evaluate the oral/acute/subacute toxicity of CS leaves in different model systems.

4. Conclusions and Future Perspectives

The evergreen shrub CS has shown enormous medicinal and pharmacological activities. These pharmacological properties are due to the presence of a variety of phytochemicals, such as alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, phlobatannins, tannins, and terpenoids. Ethnopharmacological studies on CS also strengthen the argument for utilizing this plant for safe and effective treatments of several diseases. This review can provide researchers with a reference source for the botany, phytochemistry, ethnopharmacology, and toxicity of CS.

Owing to its safety and non-toxic nature, CS has proven applicable for the treatment of several disorders. However, extensive research with in vitro and in vivo models must be carried out to examine the therapeutic benefits of CS. Future studies could be conducted for the in vitro cultivation of this medicinal herb. The preparation of wine or juice from the fruits of CS can be promoted in rural areas to improve the villages’ economies. Scientists should explore the food preservative activity, antihypertensive activity, and wound-healing activity of CS. The fruits of CS could also be explored as natural colorants in the dye or paint industries.

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