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A comprehensive review on the ethnomedicinal uses, phytochemistry, pharmacological activities and therapeutic potential of *Casuarina equisetifolia* L.

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Abstract

Medicinal plants continue to play a major role in primary healthcare for billions of people around the world, especially in developing countries where modern medicines are unavailable or too expensive. In this worldwide tradition of phytomedicine, *Casuarina equisetifolia* L., the coastal she-oak plays a special role. It is a common coastal tree found throughout tropical and subtropical coastal regions of South Asia, Southeast Asia, Australia and the Pacific Islands. It has been used by indigenous communities for treating gastrointestinal diseases, inflammatory diseases, skin diseases, hepatic diseases and oral diseases. The bark, leaves, branchlets, root and seeds of the species contain many bioactive secondary metabolites, especially hydrolysable tannins such as the unique ellagitannins casuarinin and casuarictin, as well as flavonoids (quercetin, kaempferol, catechin), phenolic acids (gallic acid, ellagic acid), triterpenoids (lupeol, β -sitosterol), and saponins. Hydrolysable tannins and phenolic acids are mainly associated with antioxidant and antimicrobial activities, while flavonoids and triterpenoids contribute to anti-inflammatory, antidiabetic, hepatoprotective, wound healing and cytotoxic effects in various *in vitro* and *in vivo* studies. However, several critical research areas are missing such as clinical trials, unknown mechanistic pathways and inconstant standardization of bioactive extracts. This review brings together the available information on *C. equisetifolia* and critically examines its ethnomedicinal uses, phytochemistry and pharmacological activities to support future research on evidence-based therapeutic applications.

1. Introduction

The medicinal benefit of plants goes back to prehistoric times and is one of the oldest and longest practised forms of human activity. According to the World Health Organization, 26 out of 52 responding countries estimated that 60-99% of their populations use traditional medicine (WHO, 2025). This reliance reflects both economic necessity and the valuable, centuries old clinical observations of traditional healers. The value of the global herbal medicines market exceeded USD 130 billion in 2020 and the trend is anticipated to grow further due to increasing consumer demand for complementary and integrative medicine globally including developed nations (Grand View Research, 2021). Traditional medicine has led to identification of landmark drugs including morphine, quinine, aspirin, and paclitaxel. It is evident that natural products and their semi synthetic counterparts are central to drug discovery and have remained relevant since the last 40 years as they contribute to about 50% of all approved drugs (Newman and Cragg, 2020).

Coastal ecosystems are botanically diverse yet many of the coastal medicinal plants are relatively under explored. Salinity, high light

and low fertility in coastal soils can induce the development of secondary metabolites like polyphenols, tannins, and terpenoids. These compounds have important functions in plant defence against oxidative stress, UV radiation, herbivory and microbial attack (Isah, 2019). Tree species of coastal areas have been part of the ethnomedicinal use of various communities in South Asia, Southeast Asia and Pacific region for a long time but there are still many species not properly studied. *C. equisetifolia* is a unique, fast growing evergreen tree present on the coasts of South Asia, Southeast Asia, Australia and Pacific islands (family Casuarinaceae). It has several local names: Australian pine, coastal she-oak, beach she-oak and horsetail tree in English, Saru or Vilampatti in Tamil speaking parts of South India; Jangli jhar in Hindi and Agoho in the Philippines (Orwa *et al.*, 2009). *C. equisetifolia* is economically valuable for its hardwood timber, sand dune stabilization and ability to restore degraded areas through nitrogen fixing root bacteria (Parrotta, 1993).

Despite its massive traditional and ecological value, scientific research on *C. equisetifolia* remains fragmented across scattered ethnobotanical, phytochemical, and pharmacological studies. Unfortunately, there is no single critically evaluated and updated review which encompasses ethnomedicinal use, phytochemistry and experimental pharmacology of the species. The objectives of this review are : (i) summarise and critically discuss ethnomedicinal uses in different geographic and cultural traditions, (ii) systematically review the phytochemical constituents found in various parts of the plant, (iii) review the pharmacological effects reported in preclinical

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studies, (iv) discuss the mechanism underlying the reported pharmacological activities, (v) discuss the toxicity and safety data, and (vi) identify gaps for further investigation.

2. Taxonomy, botanical description and distribution

2.1 Taxonomic classification

Kingdom: Plantae; Division: Angiosperms; Class: Eudicots; Order: Fagales; Family: Casuarinaceae; Genus: *Casuarina*; Species: *C. equisetifolia*. The family Casuarinaceae comprises approximately 96 species across four genera: *Casuarina*, *Allocasuarina*, *Gymnostoma*, and *Ceuthostoma* of which *Casuarina* is the most widespread and pharmacologically studied (Johnson and Wilson, 1989).

2.2 Botanical description and distribution

C. equisetifolia is a 6-35 m tall, elegant, evergreen tree, with a straight trunk that is covered with coarse, deeply grooved, dark brown to greyish bark that flakes off in irregular plates on mature trees. The leaves are reduced to whorls of 6-8 scale like teeth surrounding jointed photosynthetic branchlets which closely resemble the aerial stems of *Equisetum*, the basis of the species epithet and the common name of horsetail tree. Flowers are unisexual, inconspicuous, male flowers are in terminal spikes, catkin like, female flowers in globose, woody, small pine cone type clusters. Fruits are ellipsoidal, cone shaped structures (10-24 mm) with numerous winged samaras that aid in wind dispersal (Johnson and Wilson, 1989).

The species occurs throughout coastal areas of Australia, Pacific Islands, Malay Peninsula, Philippines and Indian subcontinent. It has been planted extensively in India along the eastern and western coasts, especially in Tamil Nadu, Andhra Pradesh, Odisha, Kerala and Goa, both for timber and coastal afforestation projects (Parrotta, 1993). It has been introduced throughout the Caribbean, West Africa, East Africa, Hawaii and the southeastern United States, and is a known aggressive invader of disturbed coastal habitats in some of those areas (Orwa *et al.*, 2009).

3. Ethnomedicinal uses

3.1 Historical and cultural context

C. equisetifolia has been traditionally used for ethnomedicinal uses in the coastal areas of South and Southeast Asia, Australia and Pacific Islands. Various parts of the tree are traditionally used by indigenous people as medicine including bark, branchlets, root, seed and fruits. Traditional knowledge is the kind of empirical pharmacology accumulated over centuries which is still utilized in the course of contemporary drug discovery processes (Newman and Cragg, 2020; Tiwari and Talreja, 2023). In many coastal and island societies, *C. equisetifolia* became embedded in traditional medical systems as a versatile bush medicine tree, particularly within the wider Casuarinaceae family that supported indigenous livelihoods before the spread of Western medicine. Prior to European settlement in Australia, Aboriginal communities relied heavily on Casuarinaceae as traditional bush medicines, reflecting deep ecological knowledge and long-term empirical use (Tiwari and Talreja, 2023). Colonial records from northern Australia and the tropical Pacific similarly note *C. equisetifolia* bark as an astringent remedy in diarrhoea and dysentery, and describe the plant as part of the regional *materia medica* rather than a marginal resource. Over time, this local knowledge entered formal pharmacognostic and phytochemical investigations,

which now document multiple bioactive tannins, flavonoids and other constituents in bark, roots, needles and fruits that validate many of these long-standing uses and position *C. equisetifolia* as a candidate for developing modern phytotherapeutics (Tiwari and Talreja, 2023; K. and Gowrie, 2018).

3.2 Gastrointestinal applications

C. equisetifolia is especially linked to gastrointestinal diseases among its ethnomedicinal applications. Bark decoctions have been traditionally used in Sri Lanka, southern India and Philippines in the treatment of diarrhoea and dysentery (Orwa *et al.*, 2009). They are believed to be due to the high tannin content and astringent properties of their bark that may play a role in antimicrobial activity against enteric pathogens (Aroua *et al.*, 2025). In addition to its use against diarrhoea and dysentery, *C. equisetifolia* has been traditionally valued for its ability to alleviate digestive discomfort and support intestinal health. The bark is rich in tannin, flavonoids and other phenolics, which have known protective effects on the gastrointestinal tract. Tannins may complex with proteins of intestinal mucosa and thus help to maintain the integrity of the intestinal wall and prevent the loss of fluids and irritation of the intestinal mucosa, helping to control diarrhoeal conditions. In addition, the antimicrobial activity of these phytochemicals has the potential to inhibit gastrointestinal microorganisms that cause infections. It is also believed that the antioxidant properties of the phenolics can help reduce oxidative stress and inflammation in the digestive tract, leading to healing of digestive tract disorders. The traditional use of *C. equisetifolia* in various healthcare systems in Asia further reinforces its role as a natural medicine for gastrointestinal diseases and emphasizes the importance of conducting additional pharmacological studies to substantiate its therapeutic benefits (Orwa *et al.*, 2009).

3.3 Anti-inflammatory and analgesic applications

In some coastal regions of South India, bark paste was topically applied to reduce inflammation, muscle pain and joint swelling. The branchlet paste is also used in traditional medicine of some Southeast Asian countries as a remedy for headache. Based on these traditional uses some of the extracts of *C. equisetifolia* have shown to contain anti-inflammatory along with analgesic properties in experimental studies (Al-Snafi, 2015a). Bark extract showed anti-inflammatory effects across a range of concentrations (20-80 g/ml). Methanolic extract showed highest anti-inflammatory activity outperforming both the ethanol and water extract (Mamillapalli *et al.*, 2022). The anti-inflammatory potential of *C. equisetifolia* is largely attributed to its rich phytochemical composition, particularly phenolic compounds, flavonoids, tannins, and triterpenoids. These bioactive constituents are known to modulate inflammatory pathways by suppressing the production of pro-inflammatory mediators and reducing oxidative stress at the site of inflammation. In addition to its anti-inflammatory effects, the plant has demonstrated analgesic properties, supporting its traditional use in the management of pain-related conditions. The observed analgesic activity may be associated with the inhibition of inflammatory mediators that contribute to pain perception. Such pharmacological properties provide scientific support for the traditional application of bark and branchlet preparations in relieving muscle pain, joint inflammation, swelling, and headaches. The combined anti-inflammatory and analgesic activities of *C. equisetifolia* suggest its potential as a natural therapeutic agent for the management of inflammatory disorders and associated pain conditions (Al-Snafi, 2015a; Mamillapalli *et al.*, 2022).

3.4 Dermatological, wound healing and oral health applications

Across a wide range of tropical African, Asian and Southeast Asian cultures, leaf and bark extracts have been employed topically to cure variety of skin ailments including eczema, dermatitis, fungi and scabies. For wound healing, fresh bark extract is directly applied to wounds like lacerations, ulcers and burns to heal rapidly and prevent secondary infection. In South Asian communities, the roots and young branchlets are chewed to act as a natural toothbrush, the tannins released have astringent properties which are thought to help prevent bleeding gums and to be effective against toothaches and dental caries, with some pharmacological plausibility because of their proven antimicrobial properties in oral cavity pathogens such as *Streptococcus mutans* (K and Gowrie, 2018). A randomized clinical trial found 5% bark cream clinically comparable to benzoyl peroxide for acne vulgaris, supporting its traditional dermatologic use (Shafiq *et al.*, 2014). Additional pharmacological evidence further supports the traditional dermatological applications of *C. equisetifolia*. Bark and root extracts have demonstrated significant *in vitro* anti-inflammatory activity through protein denaturation inhibition and membrane stabilization assays, with effects comparable to those of diclofenac. These extracts also exhibit strong antioxidant properties, which may help alleviate skin inflammation and protect tissues from oxidative damage (K. and Gowrie, 2018). Furthermore, essential oil

obtained from the stem bark contains methyl salicylate and several terpenoid compounds that have exhibited anti-inflammatory and antinociceptive activities in experimental animal models, providing additional evidence for its potential use in the management of pain, swelling, and inflammatory skin conditions (Avoseh *et al.*, 2022).

3.5 Hepatoprotective, renal and respiratory applications

Traditionally, *C. equisetifolia* has been used in traditional and ayurvedic medicine to cure liver and urinary disorders. The plant extracts have also proved hepatoprotective in experimental studies in animal models (Pooja *et al.*, 2022). This hepatoprotective effect was confirmed with experimental studies in hepatotoxicity induced by paracetamol in rats, in which treatment with extracts of *C. equisetifolia* reversed the changes in liver enzymes (Rao *et al.*, 2018). The branchlets are used in Malaysia and also in some other Southeast Asian countries to cure respiratory diseases including cough, bronchitis and asthma (Orwa *et al.*, 2009). Regarding hepatoprotective and renal applications, diabetic rat studies show improved glucose, lipids, and liver enzyme profiles, suggesting metabolic and liver support, though direct kidney and respiratory validations are still limited (Rosalina *et al.*, 2021). The ethnomedicinal uses of *C. equisetifolia* reported in various traditional systems are summarized in Table 1.

Table 1: Documented ethnomedicinal uses of *C. equisetifolia*

Plant part	Traditional use	Preparation method	Region/community	References
Bark	Diarrhoea	Bark decoction administered orally	India	Rahman, 2012
Bark	Inflammation and digestive issues	Bark decoction administered orally	Indonesia	Handarini <i>et al.</i> , 2024
Leaves	Diabetes and hyperlipidemia	Leaf extracts administered orally	Nigeria	Muhammad <i>et al.</i> , 2022
Leaves	Diarrhoea, cough, ulcers, toothache, swelling and diabetes	Leaf decoctions and extracts	Middle Eastern and North Africa	Gumgumjee and Hajar, 2012
Leaves	Diarrhoea, dysentery, acne, sore throat, boils, diabetes and hypolipidemic	Leaf decoctions	Indonesia	Handarini <i>et al.</i> , 2024
Fruit	Inflammation, infections, ulcers, cough and diarrhoea	Fruit extracts administered traditionally	India	Mamillapalli <i>et al.</i> , 2020
Seeds	Diabetes, helminth infections, and spasmodic disorder	Seed powder and traditional preparations	India	Muthuraj <i>et al.</i> , 2020

4. Phytochemical constituents

4.1 Primary metabolites

The major metabolites profile of *C. equisetifolia* is similar to other woody angiosperms and contains carbohydrates, proteins and lipids as the major metabolites. Structural polysaccharides (cellulose and hemicelluloses) and soluble sugars (glucose, fructose, sucrose) are present in the bark and seeds. Palmitic, stearic, oleic and linoleic acids are some of the fatty acids found in lipid fractions of seeds. Fresh needles contain about 5.98% crude protein, 3.72% fat, 22.89% fiber, 54.81% carbohydrate, 2.34% ash and 10.27% moisture (Tiwari and Talreja, 2023). The primary metabolites do not contribute unambiguously to the documented pharmacological activities and are not considered to be the prime contributors to pharmacological activities (Kumar *et al.*, 2014).

4.2 Secondary metabolites: Phenolics and tannins

The bark of *C. equisetifolia* is especially remarkable for its very high tannin content, which explains its use as an astringent and which has led to its pharmacological applications. Hydrolysable tannins (ellagitannins) are the predominant ones. Monomeric phenolic compounds including gallic acid and ellagic acid are also found in significant quantities as a result of the tannins breaking down. These compounds are very efficient as potent radical scavengers because of their several hydroxyl groups which are equipped to donate hydrogen to the free radicals (Tiwari and Talreja, 2023). The methanolic bark extract contained a phenolic content of 71.2 mg gallic acid equivalents (GAE)/g extract and a tannin content of 77.59 mg tannic acid equivalents/g (Wapwera and Lori, 2021).

4.3 Flavonoids, terpenoids, saponins and alkaloids

Several flavonoids have been detected in *C. equisetifolia*, among which are aglycones such as quercetin and kaempferol, the most common ones reported (Kumar *et al.*, 2014). Quercetin is a flavonol that has

an unusually wide variety of biological activities and has been suggested as a major mediator of anti-inflammatory activity observed in part, because of its ability to inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. Bark and branchlet extracts contain a high amount of flavan-3-ols, such as catechin, which are known for their antioxidant and antimicrobial effects. The methanolic extracts

always give maximum total flavonoid content (Muthuraj *et al.*, 2020). Methanolic bark extract contained a total flavonoid content of 35.12 mg quercetin equivalents/g extract (Wapwera and Lori, 2021). The differences in total phenolic and total flavonoid content among various solvents extracts are illustrated in Figure 1, indicating differences in extraction efficiency.

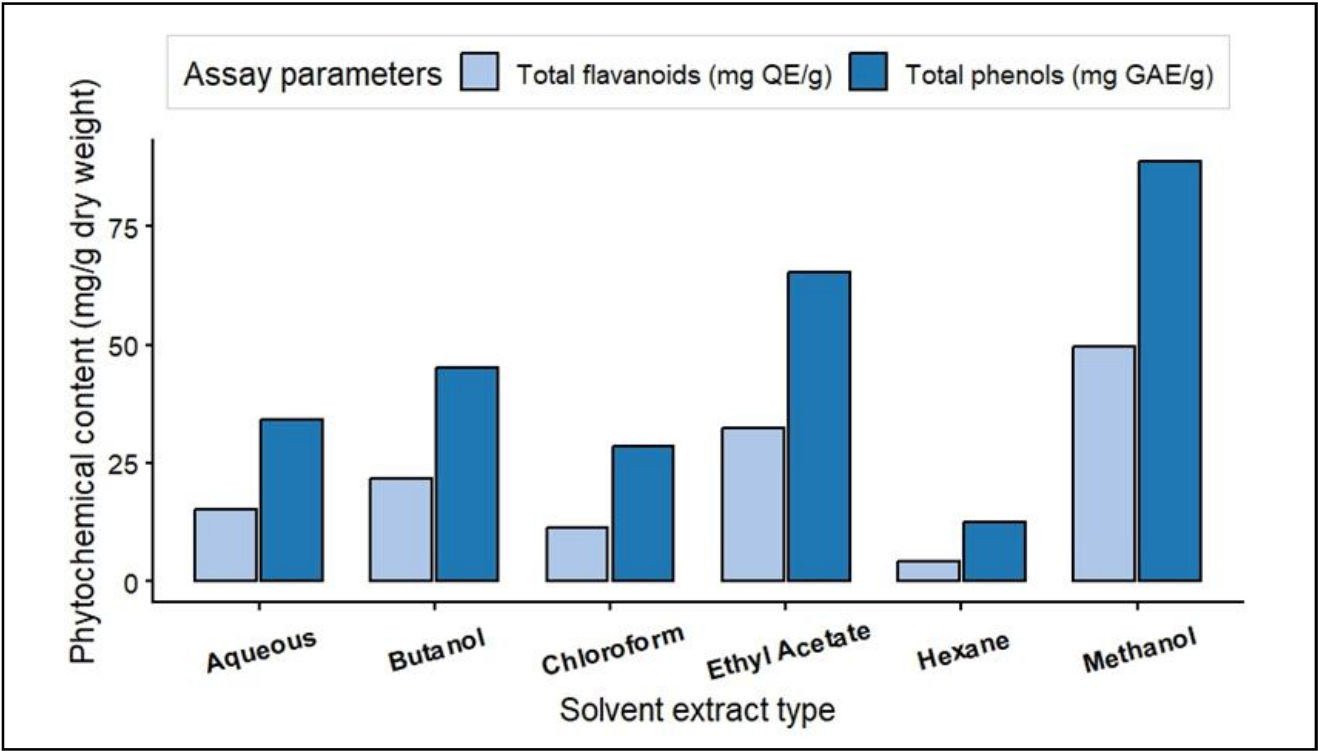


Figure 1: Comparative analysis of total phenols and total flavonoids in different solvent extracts of *C. equisetifolia* (Abdallah *et al.*, 2024).

Comparative analysis showed that methanolic and ethyl acetate extracts of *C. equisetifolia* yielded higher phenolic and flavonoid contents than other solvents, highlighting how solvent polarity drives

extraction efficiency. The quantitative composition of primary and secondary metabolites identified in fresh needles of *C. equisetifolia* is presented in Table 2.

Table 2: Primary and secondary metabolite composition of fresh needles of *C. equisetifolia*

Plant part	Compound	Quantity	References
Fresh needles	Ascorbic acid	61.00 ± 1.60 mg/100 g	Wapwera and Lori, 2021
Fresh needles	Crude protein	5.98 ± 0.20%	Wapwera and Lori, 2021
Fresh needles	Crude fat	3.72 ± 0.24%	Wapwera and Lori, 2021
Fresh needles	Crude fibre	22.89 ± 0.35%	Wapwera and Lori, 2021
Fresh needles	Ash	2.34 ± 0.07%	Wapwera and Lori, 2021
Fresh needles	Carbohydrate	54.81 ± 0.27%	Wapwera and Lori, 2021
Fresh needles	Moisture content	10.27 ± 0.48%	Wapwera and Lori, 2021
Fresh needles	Total phenolic content	58.44 ± 0.37 mg/g gallic acid eq.	Abdallah <i>et al.</i> , 2024
Fresh needles	Total flavonoids content	32.05 ± 0.30 mg/g quercetin eq.	Abdallah <i>et al.</i> , 2024
Fresh needles	Condensed tannins	50.3 ± 1.8 mg/g	Zhang <i>et al.</i> , 2009

Both bark and leaf fractions containing pentacyclic triterpenoid of lupane series namely lupeol have shown experimental anti-inflammatory and hepatoprotective activity. As a phytosterol closely

resembling cholesterol, beta sitosterol also has antidiabetic and anti-inflammatory actions. Strigolactones, contained in root extract, are signal molecules involved in mycorrhizal symbiosis but not used

extensively in the area of human diseases. The presence of triterpenoid saponins could be responsible for the antimicrobial and cytotoxic activity of the bark and root extracts; the presence of alkaloids in

trace amounts has not been systematically investigated yet (Muthuraj *et al.*, 2019). The major bioactive compounds reported from *C. equisetifolia* are summarized in Table 3.

Table 3: Major bioactive compounds identified in *C. equisetifolia*

Compound	Chemical class	Plant part	Biological activity	References
Gallic acid	Phenolic acid	Bark, leaves, fruit, seeds	Potent antioxidant, antimicrobial, cytotoxic, antidiabetic	Abdallah <i>et al.</i> , 2024
Ellagic acid	Polyphenol	Bark, fruit, seeds	Antioxidant, antimicrobial, cytotoxic, antidiabetic	Muthuraj <i>et al.</i> , 2020
Catechin	Flavonoid	Bark, fruit	Antioxidant, antimicrobial	Vaidya <i>et al.</i> , 2020
Quercetin	Flavonoid	Leaves, bark, seeds	Antioxidant, antimicrobial, antidiabetic, cytotoxic	Gumgumjee and Hajar, 2012
Lupeol	Triterpenoid	Leaf, fruit	Anti-inflammatory, antioxidant, antimicrobial, cytotoxic	Aher <i>et al.</i> , 2009
Kaempferol	Flavonoid	Leaves, seeds	Antioxidant, likely contributes to antidiabetic effects	Muthuraj <i>et al.</i> , 2020
Condensed tannins (procyanidins, epicatechin units)	Condensed tannins	Bark	Strong antioxidant, antimicrobial, gastro/nephro/hepatoprotective, antidiabetic	Aboulthana <i>et al.</i> , 2022
Salicylic acid	Phenolic acid	Leaves	Antimicrobial (identified as major phenolic)	Gumgumjee and Hajar, 2012
β -zingiberene, α -bisabolene, α -sesquiphellandrene, ar-curcumenone	Sesquiterpene	Stem bark essential oil	Anti-inflammatory, antinociceptive	Avoseh <i>et al.</i> , 2022

5. Pharmacological activities

5.1 Antioxidant activity

Oxidative stress, defined as the imbalance between the generation of reactive oxygen species (ROS) and the capacity of the endogenous antioxidant defence system, is widely recognized as an important pathophysiological process involved in numerous chronic diseases including cardiovascular diseases, diabetes mellitus, neurodegenerative disorders and cancers (Lobo *et al.*, 2010). The methanolic bark extract of *C. equisetifolia* exhibits potent radical scavenging activity with IC_{50} value of 4.7 μ g/ml, very close to that of standard antioxidant ascorbic acid (IC_{50} = 3.9 μ g/ml) (Aboulthana *et al.*, 2022). Total phenolic contents and antioxidant activity were positively related in *C. equisetifolia* and the most potent contributors to the radical scavenging activities were shown to be condensed tannins and others polyphenols. These phenolic compounds have potent redox properties, which allows them to be a source of hydrogen and electron, thus increasing their antioxidant activity. *C. equisetifolia* bark and root extracts also exhibited significant DPPH and FRAP radical scavenging activity. This confirms the polyphenols as the main antioxidant components for scavenging ROS and most importantly tannins (Zhang *et al.*, 2010).

5.2 Antimicrobial activity

Different extracts from needles, bark, roots, leaves and fruits have been tested *in vitro* and consistently show activity against a range of pathogenic bacteria and some fungi. Methanolic and other organic

extracts often show the strongest antibacterial effects, with notable inhibition zones against organisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and methicillin resistant *S. aureus* (MRSA) (Abdallah *et al.*, 2024; K. and Gowrie, 2018). In the detailed investigation of bark and root extracts of *C. equisetifolia* it has been observed that the plant contains active compounds such as alkaloids, flavonoids, phenols, tannins and terpenoids. The methanolic root extract was particularly rich in phenols (71.2 ± 0.51 mg/g) and flavonoids (35.12 ± 0.34 mg/g), indicating a strong phytochemical profile. In antibacterial testing, the extract showed notable activity against *E. coli* and *P. vulgaris*, with clear zones of inhibition measuring 23 ± 0.24 mm and 23 ± 0.32 mm, respectively. Along with this, the extracts also demonstrated good antioxidant potential and showed a dose dependent anti-inflammatory effect when compared with standard drugs, highlighting their possible therapeutic value (K. and Gowrie, 2018). In particular, the wide range antimicrobial activity of *C. equisetifolia* is of interest in light of the worldwide problem of antimicrobial resistance, because polyphenolics have multiple mechanisms of action, and are less likely to evolve single target resistance (Cushnie and Lamb, 2005).

5.3 Anti-inflammatory activity

Avoseh *et al.* (2022) showed the significant anti-inflammatory activity of stem bark essential oil of *C. equisetifolia* on Wistar rats. It inhibited inflammatory mediators and decreased carrageenan induced paw edema at 200 mg/kg, with similar activity to indomethacin (10 mg/

kg). The oil also showed good pain-relieving effects (60-63% inhibition in the neurogenic phase of formalin test) (Avoseh *et al.*, 2022). The aqueous and bark extracts of *C. equisetifolia* exhibited the potent anti-inflammatory activity mainly because of their rich number of flavonoids, phenols and tannins. The enzymes modulating and antioxidant effects of the polyphenolic compounds could mediate the inhibition of inflammatory pathway by reducing the inflammatory mediators. The anti-inflammatory properties are directly linked with the richness of the phytochemical in the *C. equisetifolia*. (Aboulthana *et al.*, 2022).

5.4 Cytotoxic and anticancer activity

Methanolic extract of *C. equisetifolia* showed significant cytotoxic activity by MTT assay on cancer cell lines, with IC values of 3.68 µg/ml (HepG-2) and 5.59 µg/ml (HCT-116), indicating dose dependent inhibition of cell viability under *in vitro* conditions (Abdallah *et al.*, 2024). Lupeol exhibits anticancer potential through regulation of different signal pathways involved in cancer development like PI3K/Akt, MAPK/ERK and Wnt/β-catenin which decrease proliferation and induce apoptosis (Chairez-Ramirez *et al.*, 2016).

Some experimental studies indicated that flavonoids and phenolics from *C. equisetifolia* show cytotoxic activities. That is to say, these substances may act *via* inhibiting proliferation of cancer cells (Tiwari and Talreja, 2023). However, these findings are mainly based on cell-line studies and further *in vivo* and clinical investigations are required to confirm therapeutic efficacy and safety.

5.5 Wound healing activity

Extracts from the bark of *C. equisetifolia* have shown to exhibit potent antibacterial, antioxidant and anti-inflammatory activities by virtue of the presence of flavonoids, phenols and tannins in it. These biological properties are essential in wound healing as they help in reducing microbial infection, controlling oxidative stress and minimizing inflammation, thereby supporting the natural tissue repair process. The wound healing activity is mechanistically explained by the synergistic activity of the antioxidant activity which prevents the oxidative damage to the proliferating cells at the wound margin, the antimicrobial activity which prevents the bacterial colonization at the wound and the procollagen synthetic activity of gallic acid which is documented in fibroblast culture system (K. and Gowrie, 2018).

Table 4: Summary of pharmacological activities of *C. equisetifolia*

Pharmacological activity	Extract used	Experimental model	Key findings	References
Antioxidant, antimicrobial, cytotoxic	Methanol, chloroform, ethyl acetate, n butanol, hexane, aqueous (needles)	<i>In vitro</i> antioxidant assays (DPPH, FRAP, ABTS)	Methanolic needle extract had highest phenolics/flavonoids, strongest antioxidant capacity and most potent cytotoxicity	Abdallah <i>et al.</i> , 2024
Antioxidant, cytotoxic, antidiabetic	Total methanolic bark extract and fractions: nano particles	<i>In vitro</i> antioxidant, scavenging assays, cytotoxicity, α-amylase inhibition; LD ₅₀ <i>in vivo</i>	Methanolic bark extract showed highest biological and cytotoxic activities; Au NP nano extract further enhanced antioxidant, cytotoxic and α-amylase inhibitory effects and was safer than native extract	Aboulthana <i>et al.</i> , 2022
Antibacterial, antioxidant, anti-inflammatory	Aqueous and organic bark extracts	<i>In vitro</i> agar well diffusion, DPPH and other antioxidant assays, protein denaturation anti-inflammatory assay	Methanolic bark extract rich in phenols, flavonoids, tannins, terpenoids; strong anti bacterial activity (up to 23 mm zones), good free radical scavenging and dose dependent anti inflammatory effect	K. and Gowrie, 2018
Anti inflammatory	Aqueous and ethanolic leaf and fruit extracts	<i>In vitro</i> HRBC membrane stabilization and protein denaturation	Ethanolic leaf and both aqueous/ethanolic fruit extracts had highest flavonoids; fruit extracts gave highest protection of HRBC	Mamillapalli <i>et al.</i> , 2020
Antidiabetic	Hexane, ethyl acetate, ethanol seed extracts	<i>In vitro</i> α-amylase inhibition, glucose uptake in 3T3 L1 cells	Ethanolic seed extract showed best antidiabetic activity <i>in vitro</i> , seeds contain quercetin, kaempferol, rutin, ellagic acid correlated with effect	Muthuraj <i>et al.</i> , 2020
Antibiofilm	Ethanol leaf extract	<i>In vitro</i> biofilm model using <i>Enterococcus faecalis</i>	Ethanol leaf extract (0.5-2%) significantly reduced <i>E. faecalis</i> biofilm formation in a concentration dependent manner	K. and Gowrie 2018
Antibacterial	Crude methanolic bark extract	<i>In vitro</i> disk diffusion, MIC /MBC, time kill assay, SEM	Bark extract produced larger inhibition zones than several antibiotics, low MIC /MBC, rapid killing of MRSA and others; SEM showed cell membrane rupture as killing mechanism	Avoseh <i>et al.</i> , 2022

5.6 Hypoglycaemic activity

The aerial parts and seeds of *C. equisetifolia* are traditionally used for the management of diabetes. The ethanolic bark extract of *C. equisetifolia* has been evaluated for its antidiabetic potential in streptozotocin induced diabetic male Wistar rats. Administration of the extract significantly reduced blood glucose levels after seven days of treatment, demonstrating notable hypoglycemic activity (Sriram, 2011). Furthermore, another study confirmed the antidiabetic and antihyperlipidemic effects of the ethanolic bark extract in streptozotocin-induced diabetic rats, indicating its therapeutic potential in diabetes management (Kantheti *et al.*, 2014).

5.7 Antihistamine activity

The methanolic extract of the wood of *C. equisetifolia* was tested for its antihistaminic activity in clonidine induced catalepsy model in mice. Catalepsy is an extrapyramidal effect that is linked to increased histamine release and dopaminergic neurotransmission. Clonidine induces histamine release from mast cells when given by the intraperitoneal route and produces catalepsy in experimental animals. The methanolic wood extract was effective in reducing the duration and severity of clonidine induced catalepsy, indicating that extract has antihistaminic activity. This action has been attributed to its mast cell stabilizing and histamine release blocking properties (Aher *et al.*, 2009).

5.8 Antidiabetic activity

Effect of ethanol extract of *C. equisetifolia* (400 mg/kg) on blood glucose level of diabetic rats induced by streptozotocin revealed a dose dependent activity with a fall in blood glucose level from 7th day to 21st day when compared with glibenclamide (Sriram, 2011). *In vitro* studies also demonstrated α -amylase inhibitory activity and enhanced glucose uptake, suggesting possible mechanisms underlying its antidiabetic effects. Similar study reported that phytosomes of *C. equisetifolia* extract showed enhanced antidiabetic and antihyperlipidemic activity compared to crude extract, due to improved absorption and bioavailability. The optimized formulation exhibited a reduced particle size (295 nm), high EE (82.43%) and superior glycaemic control in experimental models *in vivo* (Rani *et al.*, 2019). These findings indicate that *C. equisetifolia* possesses notable antidiabetic potential, which can be further enhanced through advanced formulation strategies that improve its bioavailability and therapeutic efficacy. The pharmacological activities of *C. equisetifolia* based on experimental studies are summarized in Table 4.

Although, *C. equisetifolia* has demonstrated promising antioxidant, antimicrobial, anti-inflammatory, antidiabetic and cytotoxic activities, most of the available evidence is derived from *in vitro* experiments and animal models. There is a lack of clinical studies to confirm the therapeutic efficacy and safety of plant in human beings. Thus, more in depth pharmacological, toxicological and well-designed clinical research is required to move its active constituents into clinical applications based on scientific evidence.

5.9 Nanoparticle synthesis from *C. equisetifolia*

Green nanotechnology involves utilizing plant extracts as natural mini-factories to decrease metal salts into nanoparticles that are more biocompatible and frequently medically active. *C. equisetifolia* is a polyphenol and tannin rich tree, which has been used to synthesize silver and gold nanoparticles and to prepare zinc oxide nanoparticles

with remarkable antimicrobial, antioxidant, and organ protective activity in lab and animal models. Silver nanoparticles (AgNPs) are synthesized by mixing the leaf or fruit extracts of *C. equisetifolia* with silver salts and phytochemicals present in the plant solutions act as the reducing and capping agents leading to the formation of mainly spherical shaped nanoparticles of 14-250 nm confirmed by UV-Vis, XRD, SEM/HRTEM and FTIR analysis (Moustafa *et al.*, 2020; Muthu and Rathika, 2016). Gold nanoparticles (AuNPs) are incorporated into methanolic bark extracts *via* green nanotechnology, exploiting bark metabolites as redox and stabilizing agents to create nano extracts (Aboulthana *et al.*, 2025). Zinc oxide nanoparticles (ZnONPs) can be synthesized from *Casuarina* leaf extract under UV A or UV C; UV C produces smaller (34-39 nm) oval particles with strong biological effects (Khan *et al.*, 2021).

C. equisetifolia derived AgNPs show strong antifungal and antibacterial activity, severely inhibiting *Candida albicans* and several bacteria *in vitro*, suggesting potential as alternative antimicrobial agents (Moustafa *et al.*, 2020). Bark AuNP nano extracts enhance antioxidant, antidiabetic and cytotoxic activities compared with native extracts *in vitro*, with improved safety in LD₅₀ testing (Aboulthana *et al.*, 2022). In rat models, *C. equisetifolia* bark AuNP nano extracts provide hepato and neuroprotection against organophosphate pesticides (chlorpyrifos and ethion), normalizing blood measures, improving oxidative stress and inflammatory markers, and reducing histopathological lesions in liver and brain, especially when given as pretreatment (Aboulthana *et al.*, 2023). *Casuarina* mediated ZnONPs display antibacterial activity and induce apoptosis in human liver cancer (HepG2) cells by increasing ROS, collapsing mitochondrial membrane potential, and activating caspase 3/3 7, while remaining biocompatible toward brine shrimp and human red blood cells (Khan *et al.*, 2021). Overall, *C. equisetifolia* extracts act as green factories to synthesize silver, gold, and zinc oxide nanoparticles with promising antimicrobial, antioxidant, antidiabetic, anticancer, and organ protective activities *in vitro* and in animal models. These studies highlight eco friendly synthesis, tunable particle size and morphology, and generally good biocompatibility, but translation to human medicine will require further toxicity, dosing, and clinical investigations.

6. Therapeutic mechanisms

Therapeutic activity of *C. equisetifolia* has been largely associated with its high content of bioactive phytochemicals present in the fruits, leaves, wood and bark which are extractable in methanolic solvent. Needle and bark extracts especially methanolic ones show strong antioxidant and cytotoxic actions, with high phenolic and flavonoid levels, potent free radical scavenging and growth inhibition of several cancer cell lines, suggesting potential anticancer leads under *in vitro* conditions (K. and Gowrie, 2018). Extracts from the bark, roots, leaf and fruit also demonstrated antimicrobial and anti-inflammatory activities, including inhibition against Gram-positive bacteria, Gram-negative bacteria and fungi in antimicrobial studies along with reduction of protein denaturation and membrane lysis in anti-inflammatory assays. These effects are believed to be associated with the presence of polyphenols, flavonoids and tannins, although the precise molecular mechanisms remain insufficiently characterized (Al-Snafi, 2015b). *In vivo*, studies further reported that leaf and fruit methanolic extracts exhibited antidiabetic, hypolipidemic, antiarthritic, nephroprotective and neuroprotective effects by

improving blood glucose, lipid profile, renal function markers, liver enzymes and oxidative stress parameters in diabetic, gentamicin or chlorpyrifos intoxicated and arthritis models (Muhammad *et al.*, 2022). Novel formulations such as phytosomes and gold nanoparticle nano extracts have also been reported to enhance antidiabetic,

antioxidant, cytotoxic and organ protective activities, while appearing safer than the corresponding crude methanolic bark extract in LD₅₀ testing (Rani *et al.*, 2019). An integrated representation of the morphology, phytochemicals, and pharmacological activities of *C. equisetifolia* is presented in Figure 2.

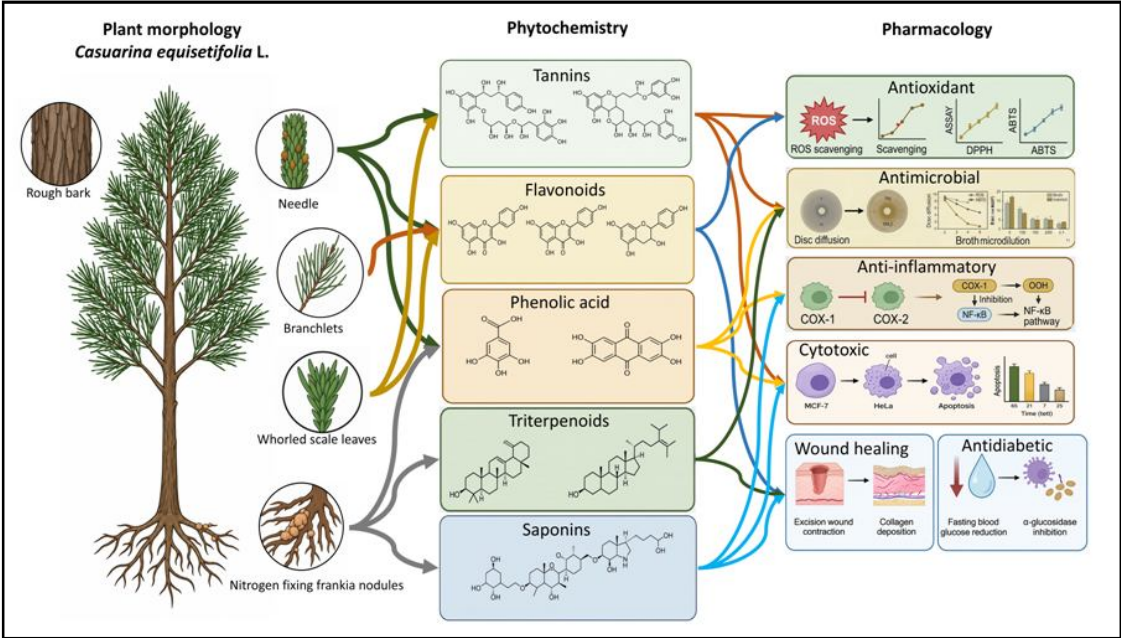


Figure 2: Schematic representation of the morphological features, phytochemical constituents and pharmacological activities of *C. equisetifolia* (Image generate by using the R software with version of 4.5.2.).

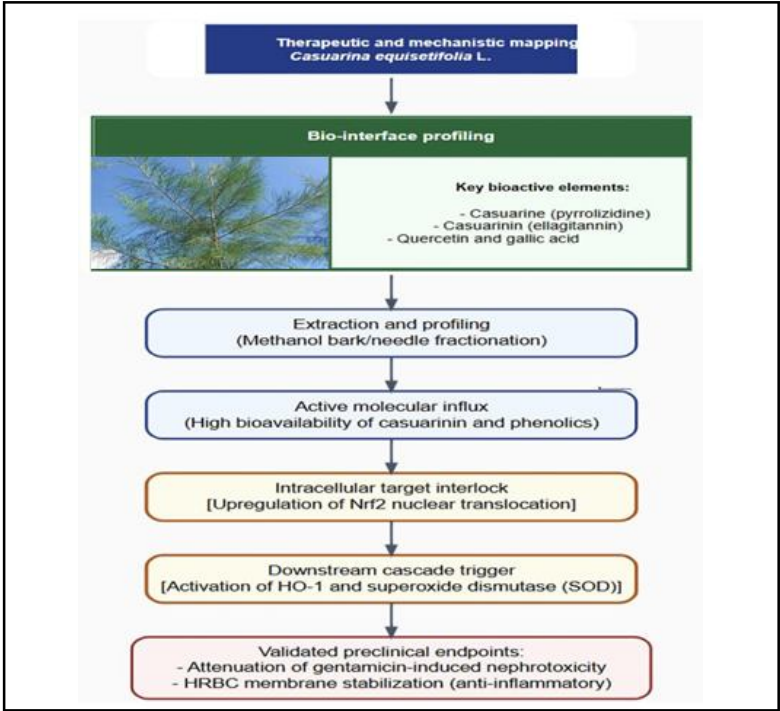


Figure 3: Mechanistic overview of extraction, bioactive compounds and pharmacological responses of *C. equisetifolia* (Prepared by us with the help of R software).

Reviews and pharmacognostic studies reinforce that these diverse activities (antimicrobial, antidiabetic, antioxidant, cytotoxic and hepatoprotective, spasmolytic, antinociceptive) align with the broad phytochemical profile of *C. equisetifolia* and justify its consideration as a multipurpose medicinal tree, though more systematic clinical and long term safety studies are still needed (Tiwari and Talreja, 2023). The mechanistic overview of extraction, bioactive compounds and pharmacological responses of *C. equisetifolia* is demonstrated in Figure 3.

7. Toxicity and safety evaluation

An OECD-423 acute oral toxicity study of ethanolic inflorescence extract in female Wistar rats (300 and 2000 mg/kg, single dose, 14 day observation) reported no mortality, no abnormal clinical signs, and no gross pathological changes; the LD₅₀ was >2000 mg/kg and the extract was classified as relatively low toxicity (Category 5), with a call for long term studies (Rao and Eswaraiyah, 2018). Bark methanolic extract and its gold nanoparticle nano extract also underwent LD₅₀ assessment, where the nano extract was described as safer than the native methanolic bark extract (Aboulthana *et al.*, 2022). Methanolic leaf extract at 300 mg/kg for 4 weeks protected against gentamicin induced renal dysfunction and oxidative stress in rats without causing any obvious hepatotoxicity as indicated by serum ALT and AST levels (El-Tantawy *et al.*, 2013). Bark and root extracts show strong antioxidant, antibacterial, anti-inflammatory and cytotoxic activities *in vitro*, but these studies do not include systemic *in vivo* toxicity evaluations (Muthuraj *et al.*, 2020). Similarly high phenolic and flavonoid needle extracted strong activity against cancer cell lines *in vitro*, showing a bioactivity but not whole animal safety margin (Abdallah *et al.*, 2024).

Current safety data are largely limited to single dose (acute) oral toxicity in rats and LD₅₀ type comparisons, with almost no subacute, subchronic or genotoxicity studies specifically on *C. equisetifolia*. Most pharmacological and nanoparticle enhancement studies emphasize efficacy, providing only indirect safety clues such as unchanged liver enzymes or improved histology under disease/toxin conditions (El-Tantawy *et al.*, 2013; Tiwari and Talreja, 2023). However, information regarding chronic toxicity, genotoxicity, reproductive toxicity and developmental toxicity of *C. equisetifolia* extracts remains limited. In addition, pharmacokinetic parameters such as absorption, bioavailability, metabolism and excretion of the major bioactive constituents have not been adequately characterized. Therefore, further long term toxicological and pharmacokinetic investigations are necessary to establish the safety profile of *C. equisetifolia* before clinical or therapeutic applications can be fully considered.

8. Future prospects and research gaps

The *C. equisetifolia* extract is found to contain the most diverse variety of secondary metabolites. Additionally, its antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hypolipidemic and antiarthritic activity has already been explored in predominantly, *in vitro* models and in rodents (Rahman, 2012). However, current evidence is still at an early preclinical stage, and substantial work is needed before its full therapeutic potential can be realized. Future studies should move beyond crude extracts and focus on bioassay guided isolation, structural elucidation and mechanism of action studies for key phenolics, flavonoids, tannins and terpenoids that underlie

observed activities such as cytotoxicity, anti inflammatory and antidiabetic effects (Vaidya *et al.*, 2020). Most pharmacological data come from *in vitro* assays or short term animal experiments; there is a major gap in subacute/chronic toxicity, pharmacokinetics, dose response and target organ safety needed to support rational dosing and formulation for humans (Muhammad *et al.*, 2022). While nano formulations and modified extracts (*e.g.*, Au NP bark nano extracts) enhance bioactivity and appear safer in LD₅₀ testing, their long-term safety, stability and *in vivo* efficacy remain underexplored (Aboulthana *et al.*, 2022). Ethnomedicinal claims for gastrointestinal, hepatic, respiratory and nervous system disorders are only partially supported by experimental data, highlighting the need for disease specific *in vivo* models and, ultimately well-designed clinical trials to validate traditional uses (Pawar *et al.*, 2021). Standardization is another critical gap: pharmacognostic and phytochemical profiles of stem bark, roots, leaves, fruits and seeds vary with solvent and location, so quality control markers and standardized extract specifications must be established to ensure reproducible pharmacological effects (Ramanathan *et al.*, 2012).

9. Conclusion

C. equisetifolia is an important coastal medicinal tree with diverse ethnomedicinal applications and significant pharmacological potential. The species contains considerable quantities of bioactive phytochemicals, including tannins, flavonoids, phenolic acid and terpenoids, which have been associated with antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, wound healing and cytotoxic properties. Many traditional applications of its parts have been substantiated by experimental research, particularly *in vitro* and animal studies. Despite these promising results, the therapeutic use of *C. equisetifolia* is restricted due to the lack of long-term toxicity studies, standardized extracts and clinical validation. The differences in phytochemical content of various plant parts, extraction techniques and geographical locations also underline the importance of quality control and standardization. Further studies should address isolation of active compound, studies with mechanism and pharmacokinetics, safety and clinical trials, properly designed to ascertain its utility and safety for human application. Overall, *C. equisetifolia* represents a promising source of bioactive compounds for future pharmacological and herbal research, although additional clinical evidence is required before definitive therapeutic applications can be established.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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