ABSTRACT

*Casuarina equisetifolia* contained carbohydrates, alkaloids, proteins, glycosides, saponins, phenolics, flavonoids, tannins, steroids, gum, reducing sugars and triterpenoids. It exerted many pharmacological activities including antimicrobial, antidiabetic, antioxidant, cytotoxic, hypolipidemic, gastroprotective, hepatoprotective and many other pharmacological effects. This paper will highlight the chemical constituents and pharmacological effects of *Casuarina equisetifolia*.

Key words: *Casuarina equisetifolia*, Chemical constituents, Pharmacological effects.

INTRODUCTION

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biocides and food additives. Medicinal plants are the Nature’s gift to human beings to help them pursue a disease-free healthy life. As a result of accumulated studies, *Casuarina equisetifolia* contained many active metabolites included carbohydrates, alkaloids, proteins, glycosides, saponins, phenolics, flavonoids, tannins, steroids, gum, reducing sugars and triterpenoids. It exerted many pharmacological activities including antimicrobial, antidiabetic, antioxidant, cytotoxic, hypolipidemic, gastroprotective, hepatoprotective and many other pharmacological effects. This paper is an attempt to highlight the chemical constituents and pharmacological effects of *Casuarina equisetifolia*.


Common Names

Amharic: (arzelibanos, shewshewe); Arabic: (casuarina); Bengali: (jau, jhau, belaiti jhao); Burmese: (pink-tinyu, tin-yu); Cantonese: (serve); Chinese: (mu ma huang,pu tong mu ma huang); Creole: (filao, pich pin); Creole Patois: (yar); Dutch: (Kazuarisboom); English: (Australian beefwood, beach she-oak, whistling pine, beefwood tree, common ru,swamp she oak, casuarina, she oak, wild pepper, sea pine, coast she-oak, horsetail casuarina, horsetail tree, ironwood, Australian pine); Fijian: (nokonoko); Filipino (agocho); French: (bois de fer, filao, pin d'Australia, fialo, pich pin); German (Keulenßaum, eisenholz,Strand- Kasuarine); Hindi: (vilayati saru,jangli jhao,jangli saru, savukku); Indonesian: (aru,tjemara laut,cemara laut,ai samara,eru); Japanese (mokumao, ogasawara-matsu); Khmer: (snga:w); Lao: (Sino-Tibetan) (‘son th la, pê:k namz, pêk nam, sôn th’ale); Malay: (ru,ru/ rhu laut,ru laut,aru); Sinhala: (kasa ghas); Spanish: (pino,pino d'Australia, Palo de buey); Swahili: (moinga,mvinje); Tamil: (chouk sabuku,savukku); Thai: (son-thale,ku); Tongan (toa); and Vietnamese: (caay phi lao,duong,filao,phi-lao) [1-4].

Classification

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Hamamelididae
Order: Casuarinales
Family: Casuarinaceae
Genus: Casuarina Rumph
Species: Casuarina equisetifolia.

Description
These trees are monoecious. The male and female flowers are light brown and inconspicuously tiny. The male flowers are at the tips of the leaf twigs, while the female flowers are on the branchlets below the leaf twigs. The female flowers are followed by rounded, 1/2 to 3/4 inch (1.3 to 1.9 cm) wide, hard, warty, brown, pinecone-like fruits. The seeds are winged samaras. The tiny, scale-like leaves are in whorls of 6 to 8 around the green, 9 to 15 inch (23 to 38 cm) long, 1/32 inch (1 mm) wide, pine needle-like leaf twigs. The leaf twigs are jointed. The dead, brown, fallen leaf twigs litter the ground under the trees like pine needles. The branches are slender and drooping, and the trees have a wispy appearance. The bark on older trees is rough, gray brown and flaking on the exterior and beefy red brown on the interior [2, 5].

Distribution
It is native to South-East Asia, Australia and Polynesia. It occurs naturally on subtropical and tropical coastlines from northern Australia throughout Malaysia, southern Myanmar, and the Kra Isthmus of Thailand, Melanesia and Polynesia. It is also cultivated as an ornamental for wind-breaks, or as a medicinal plant in some tropical countries in South pacific. It is common along the coast on beaches, rocky coasts, hill side and open forest in both wet and dry zones [5-7].

The wood of C. equisetifolia is very dense (basic specific gravity is 0.83), difficult to saw, and splits and warps when dried. Because the wood is strong, it is used as roundwood for fencing, pilings, beams, poles, and rafters and as split wood for fencing, pilings, and roofing shingles. As a high-quality fuelwood that burns with great heat (5000 Kcal per kg), it has been called the best firewood in the world. Used extensively in windbreaks, especially in China the trees are planted on coastal sand dunes to stabilize them. Although attractive in urban areas, C. equisetifolia was hazardous because it can snap or topple in hurricanes [1].

Traditional uses
It was used for the treatment of constipation, cough, diabetes, diarrhea, dysentery, gonorrhea, nervous disorders, acne, throat infections and stomach ulcer [8-10]. However, bark was used as an astringent and used in stomachache, diarrhea, dysentery and nervous disorders. Leaf: was used as antispasmodic in colic. Aerial parts: was used as hypoglycaemic [4,11]. Seeds were used as anthelmintic, antispasmodic and antidiabetic [12].

Parts used: Leaf, stem and fruit [13].

Physicochemical properties
The physicochemical properties of Casuarina equisetifolia stem bark (% of dry weight): loss on drying 2.3-0.42, total ash value 5.0-0.21, acid insoluble ash value 0.7-0.28, water soluble ash 1.6-0.35, sulphated ash 5.7-0.53, petroleum ether soluble extractive 1.4-0.16, chloroform soluble extractive 2.1-0.08, methanol soluble extractive 17.10 - 0.39 and water soluble extractive 11.0-0.48 [14].

Chemical constituents
Preliminary phytochemical screening of different parts of Casuarina equisetifolia revealed the presence of carbohydrates, alkaloids, proteins, glycosides, saponins, phenolics, flavonoids, tannins, steroids, gum, reducing sugars and triterpenoids [13, 15-24]. Seventy common triterpenoids, cholesterol, stigmasterol, campesterol cholesterol-5-en-3-beta-ol derivatives were isolated from the plant [24-25]. Seventy-six compounds comprising of monoterpenes hydrocarbons (29.3%), oxygenated monoterpenoids (16.2%), sesquiterpene hydrocarbons (2.7%), oxygenated derivatives (1.0%), aliphatic (40.6%) and non terpenoid (7.2%) compounds were observed in the leaf oils. The major compounds were pentadecanal (32.0%) and 1,8-cineole (13.1%). Significant quantities of α-phellandrene (7.0%), apiole (7.2%) and α- terpinene (6.9%) were present. The fruit oil was devoid of sesquiterpene hydrocarbon compounds [7,26-27]. The total phenol contents of Casuarina equisetifolia were 72.1 mg/g; it exhibited good reducing and antiradical powers [28-30]. The type and amount of flavonoids were differ according to the area from which the plant was taken, the high performance liquid chromatography analysis indicated the presence of 8 phenolic compounds in the leaves as major active constituent (gallic, protocatechuic, chlorogenic, syringic, p.hydroxy benzoic, p.coumaric, vanillic and salicylic acid). Results showed gallic, salicylic and protocatechuic in high concentration of 19.18, 11.57 and 6.84 μg/g, respectively. The concentration of other phenolic compounds ranged from 1.63 to 4.70 μg/g. The least concentration was chlorogenic with 1.63 μg/g [29]. However, El-Tantawy et al., determined the phenolics in the extract of Casuarina equisetifolia as gallic 21.41, protocatechuic 13.15, P-coumaric 0.73, chlorogenic 129.42, catechol 16.17, pyrogallol 1049.9, salicylic 3.14, chrysins 3.04 and benzoic 181.39 mg/100g [18]. While, Langui et al., mentioned that the methanolic extract of Casuarina equisetifolia leaves contained (mg/100 g): rutin 834.6, rosmarinic 384.6, quercitin 837.9, hesperitin 206.2, naringin 384.8, apigen 59.9 and kampferol 399.2 [18]. However, kaempferol-3-α-rhamnoside, quercetin-3-α-araboside, luteolin-3′, 4′-dimethoxy-7-β-rhamnoside, and kaempferol-3-β-rhamnoside, and protocatechuic in high concentration of 19.18, 11.57 and 6.84 μg/g, respectively. The concentration of other phenolic compounds ranged from 1.63 to 4.70 μg/g. The least concentration was chlorogenic with 1.63 μg/g [29]. However, El-Tantawy et al., determined the phenolics in the extract of Casuarina equisetifolia as gallic 21.41, protocatechuic 13.15, P-coumaric 0.73, chlorogenic 129.42, catechol 16.17, pyrogallol 1049.9, salicylic 3.14, chrysins 3.04 and benzoic 181.39 mg/100g [18]. While, Langui et al., mentioned that the methanolic extract of Casuarina equisetifolia leaves contained (mg/100 g): rutin 834.6, rosmarinic 384.6, quercitin 837.9, hesperitin 206.2, naringin 384.8, apigen 59.9 and kampferol 399.2 [18]. However, kaempferol-3-α-rhamnoside, quercetin-3-α-araboside, luteolin-3′, 4′-dimethoxy-7-β-rhamnoside, and kaempferol-3-β-rhamnoside, and protocatechuic in high concentration of 19.18, 11.57 and 6.84 μg/g, respectively. The concentration of other phenolic compounds ranged from 1.63 to 4.70 μg/g. The least concentration was chlorogenic with 1.63 μg/g [29]. However, El-Tantawy et al., determined the phenolics in the extract of Casuarina equisetifolia as gallic 21.41, protocatechuic 13.15, P-coumaric 0.73, chlorogenic 129.42, catechol 16.17, pyrogallol 1049.9, salicylic 3.14, chrysins 3.04 and benzoic 181.39 mg/100g [18]. While, Langui et al., mentioned that the methanolic extract of Casuarina equisetifolia leaves contained (mg/100 g): rutin 834.6, rosmarinic 384.6, quercitin 837.9, hesperitin 206.2, naringin 384.8, apigen 59.9 and kampferol 399.2 [18]. However, kaempferol-3-α-rhamnoside, quercetin-3-α-araboside, luteolin-3′, 4′-dimethoxy-7-β-rhamnoside,
kaempferol-3-β-dirhamnoside and quercetin-3-β-glucoside were isolated from *Casuarina equisetifolia* branchlet [31].

The structures of condensed tannins from the stem bark and fine root of *Casuarina equisetifolia* were identified using MALDI-TOF MS and HPLC analyses. The condensed tannins from stem bark and fine root consisted predominantly of procyanidin combined with prodelphinidin and propelargonidin, and epicatechin was the main extension unit. The condensed tannins had different polymer chain lengths, varying from trimers to tridecamer for stem bark and to pentadecamer for fine root [30]. Seasonal dynamics of total phenolics (TP), extractable condensed tannins (ECT), protein-bound condensed tannins (PBCT), fiber-bound condensed tannins (FBCT), total condensed tannins (TCT) and protein precipitation capacity (PPC) in young, mature and senescent branchlets of *Casuarina equisetifolia* were studied. In addition, nitrogen contents of branchlets at the different developmental stages were also determined. The contents of TP and ECT, and PPC in young branchlets were significantly higher than those in mature and senescent branchlets through the season. However, PBCT contents were significantly higher in senescent branchlets than those in young and mature branchlets; FBCT was fluctuated with season. Young branchlets had the highest N content, which decreased during branch maturity and senescence. The highest contents of TP and the lowest contents of TCT and N in young and mature branchlets were observed in summer. There was a significant negative correlation between TP and N contents. In contrast, TCT contents were positively correlated to N contents [32]. Cauasarine 1, (1R,2R,3R,6S,7S,7aR)-3-(hydroxymethyl) -1,2,6,7-tetrahydroxy pyrrolizidine was isolated from the bark of *Casuarina equisetifolia* L. (Casuarinaceae) [7].

**PHARMACOLOGICAL EFFECTS**

**Antimicrobial effects**

The crude methanolic extracts of bark, wood, leaf and fruits of *Casuarina equisetifolia* and chromatographically isolated compounds were studied for antibacterial and antifungal activity. The screenings of antibacterial and antifungal activities of isolated compounds were compared with ampicillin (10 units/disc) and ketokonazole (10 units/disc) respectively. The isolated compounds have shown activity against Gram negative bacteria and less activity against Gram positive bacteria. Among these, (gallic acid) and (lupeol) have shown good activity against Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria. Methanolic extracts of wood, bark and fruit has shown good activity (10.0, 12.0 and 10.0 mm respectively) against Gram positive microorganisms (*Staph. aureus*) while the extracts were without any effect against Gram negative microorganism. Fruit extract has resulted in good antifungal activity against *Candida albicans*. Lupeol, isolated from fruit, showed similar (8.0 mm) antifungal activity [24].

Antibacterial activity of *Casuarina equisetifolia* was tested by the disc diffusion method. Methanolic extracts of the leaves of *Casuarina equisetifolia* showed mild antibacterial activity against Gram positive (*Bacillus subtilis* and *Bacillus cereus*) and Gram negative (*Pseudomonas aeruginosa* and *E. coli*). The diameter of zone of inhibition (mm) for 250µg/disc of the methanolic extract was 8.02 ±0.23, 6.11 ±0.12, 7.23 ±0.27 and 7.14 ±0.33 against *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *E. coli* [16].

Aqueous extract of *Casuarina equisetifolia* was active against *S. epidermidis*, *B. subtilis*, *P. pseudoalcaligenes* and *S. typhimurium* (Zone of Inhibition 8-11mm), while, methanolic extract was active against *S. epidermidis*, *B. subtilis*, *P. pseudoalcaligenes*, *P. vulgaris* and *S. typhimurium* (Zone of Inhibition 12-18mm) [33].

The antibacterial effect of *Casuarina equisetifolia* was evaluated against *Bacillus cereus* ATCC11778, *Staphylococcus aureus* ATCC25923, *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* NCIM2719. The solvents used for the extraction of plant were water and methanol. The *in vitro* antibacterial activity was performed by agar disc diffusion and agar well diffusion method. Water extract of *Casuarina equisetifolia* showed antibacterial effect against *B. cereus* (13mm), *S. aureus* (11mm) and *K. pneumonia* (10mm), while methanolic extract was active against *B. cereus* (19mm), *S. aureus* (17mm), *E. aerogenes* (12mm), *E. coli* (12mm) and *K. pneumonia* (17mm) [13].

The antimicrobial activities of leaves extract was investigated against 7 medically important bacterial strains, *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Micrococcus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and 4 fungi. The antibacterial activity of aqueous and organic solvents was determined by agar well diffusion method. The most pronounced effect was shown by the methanol extract. The most susceptible bacteria were *S. aureus*, followed by *K. pneumoniae*, while the most resistant bacteria was *B. subtilis* followed by *Micrococcus*. The antifungal activity of aqueous and organic solvents was also determining. The most pronounced effect was shown by ethanolic extract. The most susceptible fungi were *Aspergillus flavus* while the most resistant fungi were *Candida albicans* isolates [29].

The anti-*Helicobacter pylori* and urease inhibition activities of extracts of *Casuarina equisetifolia* were investigated. The extracts exhibited lower activity than the standard antibiotics.

**Antidiabetic and antihyperlipidemic activity**

The antidiabetic activity of *Casuarina equisetifolia* leaves ethanolic extract (EECE) was evaluated against streptozotocin (STZ) induced experimental rats. Blood glucose levels were determined.
on 0, 7\textsuperscript{th}, 14\textsuperscript{th} and 21\textsuperscript{st} day after oral administration of ethanolic extracts of *Casuarina equisetifolia* (400mg/kg). An ethanolic extract of *C. equisetifolia* was found to reduce blood sugar in streptozotocin induced diabetic rats. Reduction in blood sugar could be seen from 7\textsuperscript{th} day after continuous administration of the extract. The effect of extracts of *Casuarina equisetifolia* on serum lipid, total cholesterol, triglycerides, low density and high density lipoprotein were also measured in the diabetic and non diabetic rats. There was significant reduction in total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats [6].

The effect of *Casuarina equisetifolia* bark incorporated into rat feed at 10-40% on the lipid profiles and blood sugar of albino rats was investigated. The parameters studied were triacylglycerol (TGL), total cholesterol (TC), total lipid (TL), phospholipids (PHOS), high-density lipoprotein (HDL) and random blood sugar (RBS). There was no significant change (P>0.05) in the TGL levels of all the rats, including the control, as they all range between 0.18-0.22(mg/dl). The effects on TC and TL were irregular as they did not display any dose dependence. The mean plasma PHOS levels did not change significantly (P>0.05) between the control and the rats fed on 10% feed (0.19± 0.00 vs 0.18± 0.00 mg/dl), but was significantly lowered (P<0.05) at 20-40% feed content. The mean HDL level rose, although insignificantly (P>0.05) with the percentage contents of the bark in the feeds; by implication, the low-density lipoprotein (LDL) was decreasing with the increase in the bark contents of the feeds. The RBS also decreased as the percentage bark contents of the feeds increased, indication that it could have anti-diabetic properties [35].

The effect of extracts of *Casuarina equisetifolia* bark on serum lipid profile, total cholesterol, triglycerides, low density, very low density and high density lipoprotein was evaluated in the diabetic and non diabetic rats. There was significant reduction in total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats [36].

### Antioxidant effect

The antioxidant activities were measured by two models: 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing/ antioxidant power (FRAP). The condensed tannins extracted from *C. equisetifolia* showed very good DPPH radical scavenging activity and ferric reducing/ antioxidant power [30].

Antioxidant activity of *Casuarina equisetifolia* was also tested using DPPH (2, 2-Diphenyl-1-picyrlhydrazyl) free radical scavenging assay. In comparison to ascorbic acid *Casuarina equisetifolia* showed strong antioxidant (DPPH free radical scavenging activity) activity where the IC50 = 25.71 µg/ml [16].

### Effects on smooth muscles

The bark extract reduced contractions in isolated ileum induced by spasmsogens like ACh, Histamine, KCl and BaCl and potentiated the effect of Nifedipine suggesting an antimuscarinic, antihistaminic and a calcium channel blocking action. The authors suggested that flavonoids and tannins present in the extract might be responsible for the observed antispasmodic effect [15].

The isolated goat tracheal chain was used to assess the antihistaminic activity of methanolic extracts of wood, bark, fruit and leaf of *Casuarina equisetifolia*. The extracts of wood and bark inhibited the histamine induced contraction of trachea (10-80 mcg/ml) in dose dependent pattern (P<0.05) while leaf and fruit extracts were without any effects. The successive chloroform extract demonstrated more activity (63.30 ± 10.33) as compare to petroleum ether (87.5 ± 13.24) and methanolic extract (166.66 ± 23.32) of wood (P<0.05). The chronic treatment of methanolic wood extract (100 mg/kg, ip) significantly reduced the clonidine induced catalepsy at 60 and 120 minutes (P<0.05) and mast cell degranulation (72.50±8.37) against standard, disodium cromoglycate, (85.19 ± 4.30) (P<0.001) [24].

### Gastroprotective effects

The anti-ulcer effect of the Ethanol extract of *Casuarina equisetifolia* (L.) extract was studied in albino rats. Ethanol extract at the doses of 200 and 400 mg/kg was administered orally to evaluate anti-ulcer activity in ethanol, indomethacin, and cold-restraint stress induced gastric ulcer models in Albino rats. Ethanol extract caused dose dependent inhibition in ethanol induced gastric lesions, ethanol extract showed 70.37 % protection at 400 mg/kg, and 52.7% protection at 200 mg/kg. In indomethacin induced gastric lesions, ethanol extract showed 68.3% protection at 400 mg/kg and 51.7% protection at 200 mg/kg. In clonidine induced gastric lesions, ethanol extract showed 75.02% protection at 400 mg/kg, and 52.7% protection at 200 mg/kg. The chronic treatment of ethanol extract showed 75.02% protection at 400 mg/kg, and 51.7% protection at 200 mg/kg. All the results were found statistically significant (p<0.05) [17].

The anti-*Helicobacter pylori* and urease inhibition activities of extracts of *Casuarina equisetifolia* were investigated. The extracts exhibited lower activity than the standard antibiotics used in this study [34].

### Anti-diarrhoeal activity

The anti-diarrhoeal effects of ethanolic (90%) extract of *Casuarina equisetifolia* Linn (EECE) was studied in rats. Anti diarrhoeal activity of 90% ethanol extract of *Casuarina equisetifolia* was investigated using castor oil-induced diarrhoea, enterpooling and small intestinal transit models in rats. The weight and volume of intestinal content induced by castor oil were studied by enterpooling method. Standard drug diphenoxylate (5 ml/kg, po) caused significant reductions in fecal output and
frequency of droppings whereas EECE at the doses of 200 and 400 mg/kg po significantly (P<0.001) reduced the castor-oil induced frequency and consistency of diarrhoea and enteropooling. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal divided by the total length of the small intestine. EECE at the doses of 200 and 400 mg/kg significantly inhibited (P<0.001) the castor oil induced charcoal meal transit. The EECE showed marked reduction in the number of diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as well as a modest reduction in intestinal transit [37].

Cytotoxic effect

Methanolic extracts of leaves of *Casuarina equisetifolia* showed moderate cytotoxic Activity in Brine Shrimp lethality bioassay test, where the LC$_{50}$ was 95.87 µg/ml [16].

Nephroprotective effects

The nephroprotective activity of methanolic extract of *Casuarina equisetifolia* leaves was studied in gentamicin-induced nephrotoxicity in Wistar rats. Subcutaneous injection of rats with gentamicin (80 mg/kg body weight/day) for six consecutive days induced marked acute renal toxicity, manifested by a significant increase in serum urea, creatinine and uric acid levels, along with a significant depletion of serum potassium level. Also oxidative stress was noticed in renal tissue as evidenced by a significant decrease in glutathione level, superoxide dismutase, glutathione-S-transferase activities, with a significant increase in malondialdehyde and nitric oxide levels when compared to control group. Administration of plant extract at a dose of 300 mg/kg once daily for 4 weeks restored normal renal functions and attenuated oxidative stress. *Casuarina equisetifolia* leaves extract ameliorates gentamicin-induced nephrotoxicity and oxidative damage by scavenging oxygen free radicals, decreasing lipid peroxidation and improving intracellular antioxidant defense [18].

Hepatoprotective effect

The methanol extracts of *Casuarina equisetifolia* were studied for hepatoprotective activity against liver damage induced in Swiss albino rats by carbon tetrachloride (CCl$_4$). It was found that the methanol extract of *C. equisetifolia* at a dose of 500 mg/kg body weight exhibited moderate protective effect by lowering the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol to a significant extent. The hepatoprotective activity was also supported by attenuation of the histopathological changes associated with CCl4 induced hepatotoxicity [37].

Toxicity

The study on acute toxicity of ethanolic extract of *Casuarina equisetifolia* showed that the extract was safe at the dose of 2000 mg/kg orally [17]. However, *Casuarina equisetifolia* pollen was recorded as an aeroallergen [38].

CONCLUSION

*Casuarina equisetifolia* is a plant with wide range of chemical constituents which exerted many pharmacological effects. There is a great promise for development of novel drugs from *Casuarina equisetifolia* to treat human diseases as a result of its effectiveness and safety.

REFERENCES