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Research Article

PHARMACOLOGICAL STUDIES: ANTIBACTERIAL, ANTIOXIDANT, AND ANTI-INFLAMMATORY EFFICACY OF *CASUARINA EQUISETIFOLIA* ROOT EXTRACTS

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ABSTRACT

Objective: The current study was aimed to investigate the potential phytoconstituents from *Casuarina equisetifolia* root extract. Qualitative, quantitative and gas chromatography–mass spectrometry (GC-MS) analysis of *C. equisetifolia* using various solvents of root extract was also carried out to characterize the presence of various bioactive compounds in the root. The research work was also targeted to reveal the antibacterial, antioxidant, and anti-inflammatory potential of the root extract of *C. equisetifolia*.

Methods: Root samples of *C. equisetifolia* were collected from Nimilenchery village, Pondicherry Union territory. The qualitative screening of the root extracts was carried out to check the presence of various phytoconstituents which was then followed by the quantitative analysis of phenols, flavonoids, and tannins. Further, the phytochemicals in the root extract were evaluated using GC-MS studies. *In vitro* antibacterial activity was performed by the agar well diffusion method using aqueous and organic solvent-based root extract against four different bacterial pathogens. *In vitro* antioxidant assay of different solvent extracts was elucidated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, hydrogen peroxide radical scavenging assay, and reducing power assay. Anti-inflammatory potential was also studied using protein denaturation of albumin.

Results: The qualitative phytochemical screening revealed the presence of various phytoconstituents which is of greater biological importance. Gallic acid equivalent (GAE) phenolic compound content (68.64±0.25 mg GAE/g of extract), quercetin equivalent flavonoid content (29.09±0.14 mg of QUE/g of extract), tannic acid equivalent (TAE) tannin content (51±0.42 mg TAE/g of extract), and terpenoid content (5.2%) were found to be significant in the methanol root extract of *C. equisetifolia* when compared with other solvents. GC-MS analysis revealed different peaks indicating the presence of different secondary metabolites. Prominent antibacterial activity was observed in methanol extract of root, with maximum zone of inhibition exhibited against *Proteus vulgaris* (23.45±0.28 mm). The methanol root extract was most effective with half maximal inhibitory concentration (IC_{50}) 52.74±0.65 µg/ml for DPPH and 64.94±0.24 µg/ml for hydrogen peroxide scavenging activity. Maximum absorbance was observed by 80 µg/ml (IC_{50} 51.79±0.26 µg/ml) of methanol root extract with respect to reducing power assay. *In vitro* anti-inflammatory activity had maximum inhibition of 84.6±0.26 with IC_{50} value of 33.6±0.23 µg/ml at 80 µg/ml.

Conclusion: From this study, it is revealed that the species of *C. equisetifolia* is a source of potential phytoconstituents exhibiting significantly various biological activities leading to the development of novel drug.

Keywords: *Casuarina equisetifolia,* Root, Phytochemicals, Antioxidant, Antibacterial, Anti-inflammatory, Gas chromatography–mass spectrometry.

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INTRODUCTION

Plants have been used as an efficient source in traditional medicine since ages. The potentiality of plants to act as therapeutic agents is based on its phytoconstituents such as phenols, flavonoids, tannins, alkaloids, proteins, amino acids, and steroids. Currently, about 75% of the population still depend on traditional medicine practices for the management of various diseases [1]. Better compatibility of plant-based medicines with the human body with very minor side effects has increased the interest to rediscover more plant-based chemical substances, which could serve as a better pharmacological active substance.

Casuarina equisetifolia belonging to the family Casuarinaceae is a rapid-growing species (3–4 years of crop rotation) found in regions of varied climatic conditions such as coastal dunes, hot humid tropics, mountain slopes, and semi-arid regions [2]. This species has greater ability to fix atmospheric nitrogen, with the help of its symbiotic association with *Frankia* - An Actinomycete [3]. *C. equisetifolia* has been documented as a suitable species for wasteland reclamation and development due to its adaptability to a wide range of habitat, salt tolerant [4], fast growth, drought resistant, and ability to stabilized sand dunes [5]. The plantation checks soil erosion and improves environmentally degraded soil [6]. Recently, there is also a great demand for *C. equisetifolia* in paper industries as wood is used for pulping, where root and bark are the waste materials.

In addition to this, various parts of this plant have been reported to have various biological activities such as antimicrobial [7], hepatoprotective [8], antiulceric [9], and antidiabetic due to its rich phytochemical efficacy. There is a growing interest in correlating the phytochemical constituents of medicinal plants with its pharmacological activity [10]. The tree species such as *C. equisetifolia* is still underexplored for their medicinal properties and pharmacological investigations [11]. This species is an established agroforestry tree crop in Tamil Nadu used as an intercropping tree species due to its disease-resistant (pest and pathogen) and nitrogen-fixing ability, which is mainly because of the presence of potential phytochemicals.

Thus, this study was aimed to analyze the qualitative and quantitative efficacy of phytochemicals including gas chromatography–mass spectrometry (GC-MS) analysis followed by the bioassays, namely, antibacterial, antioxidant, and anti-inflammatory potential of root extracts using various solvents.

METHODS

Collection of plant material

The root sample of *C. equisetifolia* was collected from Nimilenchery Village, Pondicherry Union territory (Latitude: 12.10'; Longitude 79.9'), Tamil Nadu. The plant sample was taxonomically identified and authenticated as *C. equisetifolia* Linn., Casuarinaceae, Ref No: BSI/SRC/5/23/2015/Tech/2012 by Botanical Survey of India, Coimbatore.

Preparation of root extract

Dried root sample of *C. equisetifolia* was cut into small pieces and surface sterilized. Extraction was carried out by adding distilled water and organic solvents (benzene, chloroform, methanol, and ethanol) in the ratio 1:10 w/v, for 48 h, by cold percolation method. The extract was filtered, concentrated, and dried and stored at 4°C until further use.

Qualitative phytochemical screening

Preliminary phytochemical screening of various root extracts (aqueous, benzene, chloroform, methanol, and ethanol) was screened for the presence of phytoconstituents. The test conducted was phenols, alkaloids, flavonoids, terpenoids, carbohydrates, saponins, proteins and amino acids, phlobatannins, glycosides, and tannins using standard procedures [12,13].

Quantitative analysis

Estimation of total phenol content

The content of total phenolic compound in aqueous, ethanol, and methanol extracts *of C. equisetifolia* root was determined by Folin–Ciocalteu reagent (FCR) [14]. Gallic acid was used as standard. To 1 ml of each extract (different concentrations for standard gallic acid solution), 1 ml of FCR was added in a test tube and the mixture was incubated for 5 min. 10 ml of 7% $Na_2 CO_3$ solution was added to the test solution and incubated in dark for 90 min at 23°C. The absorbance was read at 765 nm against the blank. The total phenolic content in the extracts was expressed in gallic acid equivalent (GAE) using the formula:

$C=(c/V)/m$
C=Total phenolic content, mg/g of plant extract in GAE
c=Concentration of gallic acid (mg/ml)
V=Volume of the extract (ml)
m=Weight of plant extract (g).

Estimation of total flavonoid content

The total flavonoid content was determined using aluminum chloride colorimetric method [15]. 0.5 ml of root sample was mixed with 2 ml of solvent and 0.3 ml of 5% sodium nitrite solution was added. To this mixture, 0.3 ml of 10% $AlCl_2$, 2 ml of 1 M sodium hydroxide, and 2.8 ml of distilled water were added and incubated at room temperature for 30 min. The absorbance was read at 510 nm using ultraviolet (UV)-visible spectrophotometer (UV 1650 Pc, Shimadzu). Total flavonoid concentration was expressed in terms of quercetin equivalent (QE) (mg of QAE/g of extract).

Estimation of tannins

The total tannin content in aqueous, ethanol, and methanol extracts of *C. equisetifolia* root was estimated by Folin–Denis method [16], with modifications. To 1 ml of plant extracts, 3 ml of water, 0.5 ml of Folin–Denis reagent, and 1.0 ml of 1 N sodium carbonate solution were added and diluted with water. The solution was mixed thoroughly and the absorbance was read at 515 nm. The calibration graph was plotted. Total tannin content was expressed as milligrams of tannic acid equivalent (TAE) per gram of dried sample.

Estimation of total terpenoids

Total terpenoid content was estimated [17] for ethanol, methanol, and aqueous extract of *C. equisetifolia* root. The crude extract was soaked in 20 ml of 95% ethanol for 24 h. The filtrate was extracted with petroleum ether (60°C–80°C). The residue of the extract obtained from the petroleum ether extract was dried and weighed to estimate the total terpenoids.

$$\text{Terpenoid content}\ (\%) = \frac{\text{Weight of terpenoid extract}\ (g)}{\text{Weight of sample}\ (g)} \times 100$$

GC-MS studies

The methanol root extract was subjected to GC-MS analysis to identify the bioactive compounds. This analysis was performed in Sophisticated Instrument Facility (SIF), Vellore Institute of Technology University, Vellore, Tamil Nadu. The Clarus 680 GC was used in the analysis that employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m×0.25 mm ID×250 μm df). The components were separated using helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. 1 μL of the root extract was injected into the instrument. The oven temperature was 60°C (2 min), followed by 300°C at the rate of 10°C/min and 300°C, where it was held for 6 min. The mass detector conditions were transfer line temperature 240°C, ion source temperature 240°C, and ionization mode electron impact at 70 eV, a scan time 0.2 s, and scan interval of 0.1 s for the fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Antibacterial activity

In vitro antibacterial activity assay of aqueous, ethanolic, and methanol root extract of *C. equisetifolia* was determined by agar well diffusion method, using Mueller–Hinton agar medium. Gentamycin (100 μg) was used as positive control and solvent was used as negative control for bacterial assay. Four different concentrations (25, 50, 75, and 100 μg) of various root extracts were tested against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* and *Proteus vulgaris* obtained from the Department of Microbiology, Ethiraj College for Women, Chennai. Zone of inhibition was measured after incubating the plates at 37°C for 24 h. Triplicates were maintained.

Antioxidant assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of various root extract of *C. equisetifolia* was tested using DPPH assay method [18]. 2 ml aliquot of DPPH solution was added to 0.5 ml of different concentrations (20, 40, 60, and 80 μg/ml) of various root extracts. The reaction mixture was incubated at room temperature for 30 min, and then, the absorbance was determined at 517 nm using UV-visible spectrophotometer (UV 1650 Pc, Shimadzu). The ascorbic acid was used as a standard. Half-maximal inhibitory concentration (IC_{50}) was calculated from the graph, by plotting percent inhibition against concentration. Experiments were performed in triplicates and average was calculated for each concentration.

$\text{Inhibition\%}=(OD_{blank}-OD_{test}/OD_{standard})\times 100$

Hydrogen peroxide scavenging assay

The ability of the extracts to scavenge hydrogen peroxide spectrometrically was determined according to the method [19]. 2 mM/l of hydrogen peroxide solution was prepared in phosphate buffer (pH=7.4). To different concentrations (20, 40, 60, and 80 μg/ml) of various extracts, 0.6 ml of hydrogen peroxide solution was added. Absorbance was measured at 230 nm against blank solution containing only phosphate buffer and was compared with the reference compound ascorbic acid. Experiments were performed in triplicates.

$\text{Hydrogen peroxide activity}=(Abs_{control}-Abs_{sample}/Abs_{(control)})\times 100$

Reducing power assay

The reducing power assay of the extracts was determined according to the standard method [20]. Different concentrations (20, 40, 60, and 80 μg/ml) of various root extracts of *C. equisetifolia* were mixed with 2.5 ml of 0.2 M phosphate buffer (pH=6.6) and 2.5 ml of 1%

potassium ferricyanide. The reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added to the reaction mixture and centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was collected and mixed with 0.5 ml of 0.1% $FeCl_3$. The absorbance was measured at 700 nm using UV-visible spectrophotometer (UV 1650 Pc, Shimadzu) and was compared with the reference compound ascorbic acid. Experiments were performed in triplicates.

In vitro anti-inflammatory activity

The anti-inflammatory activity of *C. equisetifolia* root extract using different solvents was studied by standard method of inhibition of albumin denaturation [21]. The reaction mixture consists of different concentrations (20, 40, 60, and 80 µg) of different solvent extracts and 1% of aqueous bovine albumin fraction, and pH was adjusted using 1 N HCl. The reaction mixture was incubated at 37°C for 10 min and then heated at 51°C for 20 min. The turbidity was measured at 660 nm using UV-visible spectrophotometer (UV 1650 Pc, Shimadzu) after cooling and was compared with the reference compound diclofenac sodium. Experiments were performed in triplicates.

Percent inhibition of protein denaturation was calculated using the formula:

$$\text{Percent inhibition}=(\text{Abs}_{\text{control}}-\text{Abs}_{\text{sample}}/\text{Abs}_{(\text{control})})\times 100$$

Statistical analysis

Results are expressed as mean±standard error. The experimental data were analyzed using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Phytochemical screening

Qualitative analysis of phytochemicals revealed the presence of phenols, flavonoids, terpenoids, carbohydrates, saponins, and tannins in aqueous, ethanol, and methanol root extract of *C. equisetifolia* (Table 1). Benzene and chloroform extract showed the presence of phenols, alkaloids, glycosides, and tannins. The presence of phlobatannins was observed only in ethanol and methanol root extract. The Observations from this study can be correlated with the polarity of extracted compounds in various solvents. Studies conducted by Gopichand *et al*., 2015 [22], showed the presence of alkaloids, flavonoids, phenols, terpenoids, and tannins in *C. equisetifolia* root extract.

Phytochemicals are naturally occurring substances produced by plants during primary and secondary metabolism. These phytochemicals play a key role in biological activities of the plant, plant growth, and defense against competitors, pathogens, or predators [23]. These plant secondary metabolites have the efficacy to stimulate the human immune system, act as an antioxidant [24], reduce inflammation and oxidative damages caused by the cell apoptosis, regulate intercellular signaling of hormones and genes, and slow down the growth rate of cancer cell [25] and also as potential antibacterial agents [26].

Thus, the phytochemical analysis revealed the presence of prominent phytochemicals, which could develop a symbiotic chemistry with human metabolism and help to treat various diseases. Since maximum solubility of phytochemicals was observed in aqueous, ethanol, and methanol root extracts in qualitative phytochemical analysis, further quantification and *in vitro* studies were carried out only with these three root extracts.

Quantitative phytochemical analysis

The total phenolic content was calculated using standard curve of gallic acid ($y=0.175x-0.118$, $R^2=0.995$) and expressed in GAE/g of extract. Methanol extract of root demonstrated higher total phenolic content (68.64±0.63 mg GAE/g of extract) which was followed by ethanol and aqueous extracts with 64.60±0.63 and 32.96±0.77 mg GAE/g of extract, respectively (Fig. 1).

The total flavonoid content was calculated using standard curve of quercetin ($y=0.013x-0.161$ $R^2=0.990$) and expressed in QE/g of extract. The total flavonoid content was high in methanol root extract (29.09±0.54 mg of QE/g of extract) followed by ethanol and aqueous extracts with 26.23±0.38 and 23.88±0.67 mg QE/g of extract, respectively (Fig. 1).

The total tannin content was calculated using standard curve of tannic acid ($y=0.003x-0.007$ $R^2=0.994$) and expressed in TAE/g of extract. Among the various solvents used, methanol extract revealed the highest tannin content of 51.6±0.82 mg TAE/g of extract followed by ethanol and aqueous extracts with 42.17±0.50 and 16.68±0.61 mg TAE/g of extract, respectively (Fig. 1).

The methanol root extracts showed maximum terpenoid content (5.20±0.52%) followed by ethanol extract (3.56±0.63%) and aqueous extract (2.58±0.77%).

Among the solvents used, there was a significant variation ($p<0.001$) in the phytochemicals quantified in which methanol extract was proved to be an effective solvent for biological assays.

Increase in the level of phenolic compounds enhances the quality of plants, serves as a tool for plant growth and reproduction, and is produced as a response to environmental factors such as light, pollution, and irradiation [27]. Epidemiological studies have proved the positive effect of phenols on human health since they decrease the coronary heart disease, control the platelet aggregations [28], have both *in vitro* and *in vivo* [29,30] antioxidant capacities, and also possess anticarcinogenic protection. Various biological activities of phenolic compounds include bile secretion, lowering of blood cholesterol and lipid levels, anti-inflammatory activity, and antibacterial activity against wide range of pathogens when taken in regular diet [31].

Flavonoid is a polyphenolic secondary metabolite [32]. Flavonoids give rich taste to plants that help in pollination and as a repellent to pest [33] and are also responsible for pigmentation of various parts of the plant and involved in nodule formation [34]. It is well known for their potential antioxidant property, which prevents the cell against the damage caused by free radicals by acting as scavengers of free radicals such as reactive oxygen species (ROS) and chelating metals [35].

Table 1: Phytochemical analysis of different extracts of root

S. No.	Phytochemical tests	Aqueous	Benzene	Chloroform	Ethanol	Methanol
1	Phenols	+	+	+	+	+
2	Alkaloids	+	+	+	+	+
3	Flavonoids	+	+	-	+	+
4	Terpenoids	+	-	-	+	+
5	Carbohydrates		-	-	+	+
6	Saponins	+	-	-	+	+
7	Protien and amino acids	+	-	-	-	-
8	Phlobatannins	-	-	-	+	+
9	Glycosides	-	+	+	+	+
10	Tannins	+	+	+	+	+

Flavonoids have been found to modulate the inflammation mediators such as Interleukin 6, which helps it to serve as an efficient anti-inflammatory agent [36].

Tannin is a polyphenolic complex, which serves as defensive secondary metabolite in plants, by protecting it from insects and herbivores, because of its bitter taste. The structure of tannic acid possesses hydrophobic core and hydrophilic shell which could be responsible for the antioxidant potential [37]. Antioxidant property of tannic acid is also attributed due to its ability to suppress the hydroxyl radical formation [38,39].

Terpenoids play a vital role by exerting metabolic control, mediating inter- and intraspecies interaction such as pollination and defense mechanism [40]. Terpenoids with provitamin A act as an important source for vision; it also influence the human immune system. Gap-junctional communication that influence the inflammatory mediators and prevents inflammation [41]. The potential of terpenoids to quench singlet oxygen, hydrogen transfer, and electron transfer proves it as a potent antioxidant agent [42].

GC-MS study

GC-MS chromatogram analysis of the root methanol extract of *C. equisetifolia* showed eight peaks indicating the presence of eight phytochemical constituents. The mass spectra were compared with the NIST library, and the eight phytocompounds were characterized and identified. The various phytochemicals characterized contribute to the medicinal activities of the plant. The eight compounds were identified as, trihydroxybenzoic acid, 2,4,10-trioxatricyclo, 1,3-dichloropentane, 2-butanone-4-hydroxy, D-galactonic acid- gamma lactone, butanoic acid - 3-cyano-3-hydroxy ethyl ester, 1-nitro 2-acetamido-1,2 – dideoxy-D-manitol, oxalic acid isohexyl pentyl ester (Fig. 2). Among the phytoconstituents, trihydroxybenzoic acid was reported to have antimicrobial, antioxidant, anti-inflammatory potential [43]. Oxalic acid and isohexyl pentyl ester are used as plasticizer [44]. Trihydroxybenzoic acid is a phenolic compound involved in the defense against certain pathogens causing human and animal diseases [45]. Their activity is a function of the lipophilic properties of the constituent, functional groups, and their solubility [46].

The presence of various bioactive compounds in *C. equisetifolia* justifies the use of root extracts for various ailments by traditional practitioners.

Antibacterial activity

Organic solvent root extracts exhibited more consistent antibacterial activity than aqueous root extract. Antibacterial activity increased with the increase in the concentration of the extract. The maximum zone of inhibition of 23.45±0.28 mm was exhibited by methanol root extract of *C. equisetifolia* against *P. vulgaris* at 100 µg concentration. The ethanolic and aqueous root extract showed maximum zone of inhibition against *P. vulgaris* (21.45±0.74 mm) and *B. subtilis* (16.55±0.48 mm), respectively, at 100 µg concentration (Fig. 3), whereas the study conducted by [47] in the methanol root extract of related *Casuarina* species (*Casuarina junghuhniana*) showed maximum zone of inhibition against *B. subtilis* (19±0.2 mm).

This activity could be due to the ability of the secondary metabolites to form a complex with extracellular proteins and with the cell wall of bacteria [48]. The inhibitory effect of methanol root extract, on the growth of bacteria, might be due to higher solubility of these potential secondary metabolites, which indicates the presence of broad spectrum of antibiotic compounds [43].

The organic solvent of the plant extract had more antibacterial potential than the aqueous extract. This observation can be correlated with the polarity of the solvent used for extraction, intrinsic bioactivity, and ability to diffuse in media used in assay [49].

The presence of alkaloids, phenols, carbohydrates, flavonoids, and tannins in the root extract may be responsible for the antibacterial efficacy of the root extracts [50].Phenolic compounds possess a C3 side chain which has a lower level of oxidation and containing no oxygen are often cited as the reason for the antimicrobial potential [51]. Terpenoids and flavonoids act as an effective antimicrobial agent due to its ability to disrupt the membrane of lipophilic compounds present in the microbial cell [52]. The antibacterial ability of the extract may also be due to the ability of tannin to hydrolyze the ester linkage between gallic acid and polyols [53]. Thus, tannin-rich plant parts have natural defense against microbial infection [54].

Antioxidant activity

DPPH radical scavenging activity

DPPH radical scavenging assay estimates the ability of the extract to

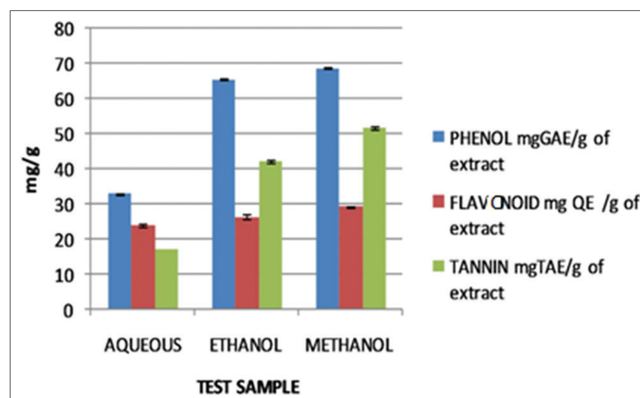

Fig. 1: Quantitative analysis (total phenol, total flavonoid, and total tannin)

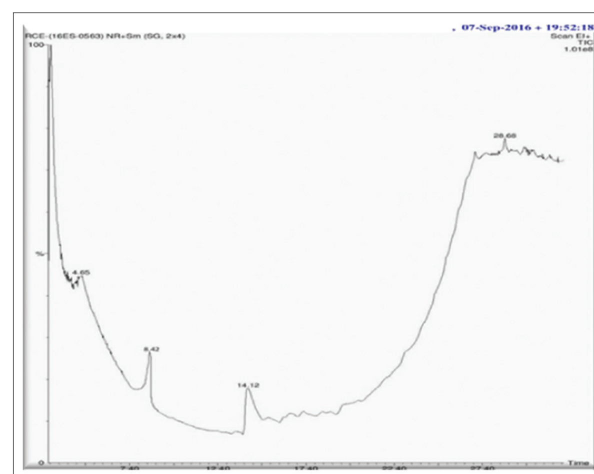

Fig. 2: Gas chromatography–mass spectrometry chromatogram of methanol extract of *Casuarina equisetifolia*

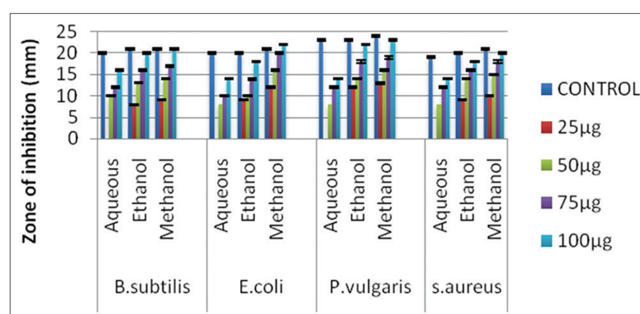

Fig. 3: Antibacterial activity of *Casuarina equisetifolia* root extracts against different bacterial pathogens

donate hydrogen or to scavenge the free radical. The results revealed that the methanol root extract of *C. equisetifolia* exhibited the highest scavenging activity with IC_{50} value followed by ethanolic and aqueous root extracts (Fig. 4). The methanol extract showed percent maximum inhibition at 80 µg concentration (86.82±0.65) followed by ethanol extract (85.46±0.55) and aqueous extracts (82.73±0.48), with IC_{50} value of 52.74±0.65, 54.86 ±0.86, and 57.83±0.72 µg/ml, respectively. The DPPH activity of standard ascorbic acid showed maximum percent inhibition of (96.34±0.82) at 80 µg concentration with IC_{50}value of 39.22±0.73 µg/ml. Lower IC_{50}value indicates greater antioxidant activity. Methanol extract of *C. equisetifolia* root showed significant ($p<0.001$) scavenging activity which may be attributed to the presence of greater amount of phenols and tannins.

Hydrogen peroxide scavenging assay

In the presence of metal ions and superoxide anion, hydrogen peroxide gets converted into hydroxyl radical and also produces singlet oxygen. Thus, it acts as a toxicant to the cell. Hydrogen peroxide can also degrade heme protein and release Fe ions, thus supporting the scavenging activity. The methanol extract showed percent maximum inhibition at 80 µg concentration (58.15±0.25) followed by ethanol extract (53.93±0.38) and aqueous extracts (50.47±0.56), with IC_{50} value of 64.94±0.24, 70.73±0.36, and 76.51±0.08 µg/ml, respectively. With regard to hydrogen peroxide scavenging activity, IC_{50} value indicates methanol root extract as an efficient scavenger. The IC_{50} value of standard ascorbic acid was 44.56±0.15 µg/ml with percent inhibition of 89.2±0.76 (Fig. 5).

Reducing power assay

The reducing power of the extract is marked by the reduction of Fe^{3+} complex (ferric cyanide) to ferrous that serves as an efficient indicator for potential antioxidant activity which is due to the presence of reductones. The reducing power of *C. equisetifolia* root extract expressed as the function of their concentration. There was an increase in reducing power with the increase in the concentration of aqueous, ethanol, and methanol root extract of *C. equisetifolia* (Fig. 6). Higher absorbance of the reaction mixture indicates more reducing potential. The methanol extract showed percent maximum inhibition at 80 µg concentration (77.46±0.63) followed by ethanol extract (72.30±0.37) and aqueous extract (65.25±0.65), respectively, with IC_{50} value of 51.79±0.26, 55.13±0.73, and 61.82±0.41 µg/ml, respectively. The reducing power of standard ascorbic acid showed maximum percent inhibition of 82.16±0.15 (IC_{50} value of 47.27±0.31 µg/ml) at 80 µg concentration.

A substance when present at lower concentration prevents oxidation of cellular substances like proteins, lipids, carbohydrates and DNA which contributes to the antioxidant property [55]. Plant polyphenols are diverse group of compounds, including tannins and flavonoids occurring naturally in plant products that acts as an effective antioxidant agent by quenching singlet and triplet oxygen, decomposing peroxidases and neutralizing free radicals [56].

The antioxidant property of the phenolic compounds may be related to its ability to inhibit lipoxygenase, chelate metal ions, and scavenge free radicals and thus controls ROS (O_2,H_2O_2, NO) [57]. The presence of tannin which has the ability to suppress hydroxyl radical formation [58] might also be responsible for the antioxidant action. Flavonoids effectively scavenges most of the oxidizing molecules, including singlet oxygen, and various free radicals [59] (Bravo., 1998)Similarly, terpenoids act as regulators of metabolism and plays a protective role as antioxidant.

Preliminary phytochemical analysis of root extract of various solvents confirmed the presence of phenol and phenolic compounds attributing to the potential antioxidant activity, of which methanol root extract of *C. equisetifolia* possesses efficient antioxidant activity. This result is in accordance with the study conducted in *C. equisetifolia* [24], where the root extracts exhibited maximum antioxidant-free radical scavenging activity. The bioactive compound present in the extract may be separated and purified which may also be employed as a drug in treating various human diseases in which free radicals are involved, such as cancer, cardiovascular diseases, and aging.

***In vitro* anti-inflammatory activity**

In anti-inflammatory assay of *C. equisetifolia* root extracts, the results revealed inhibition of thermally induced protein (albumin)

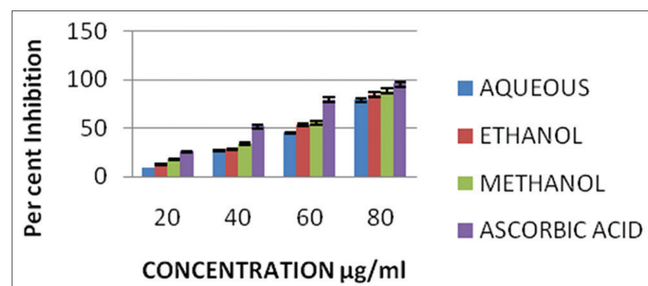

Fig. 4: 2,2-diphenyl-1-picrylhydrazyl-free radical scavenging activity of *Casuarina equisetifolia* root extracts

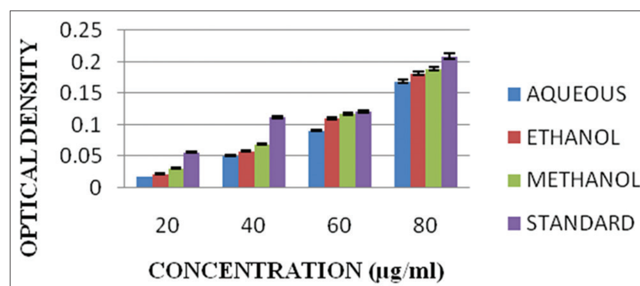

Fig. 6: Reducing power activity of *Casuarina equisetifolia* root extracts

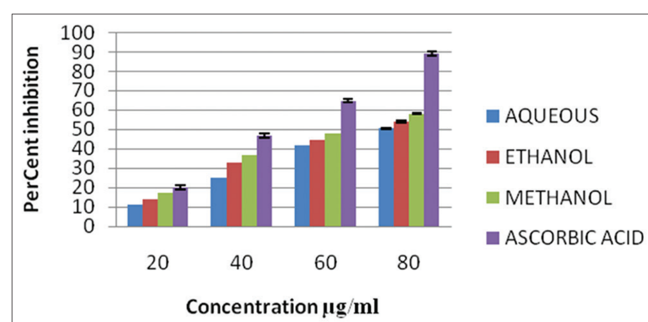

Fig. 5: Hydrogen peroxide scavenging activity of *Casuarina equisetifolia* root extracts

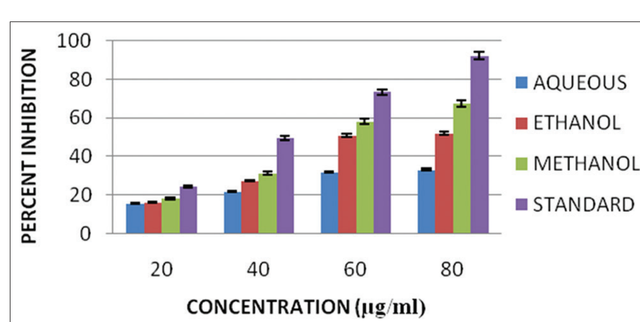

Fig. 7: Anti-inflammatory activity of *Casuarina equisetifolia* root extracts

denaturation in dose-dependent manner (Fig. 7). The methanol extract showed maximum percent inhibition of 84.6±0.23 when compared to ethanol extract of 76.3±0.31 and aqueous extract of 50.42±0.14 with IC_{50} value of 33.6±0.52, 52±0.47, and 65±0.31 μg/ml, respectively. The anti-inflammatory activity of standard diclofenac sodium showed maximum percent inhibition of 92.35±0.7 at 80 μg concentration with IC_{50} value of 40.80±0.47 μg/ml.

Inflammation is a biological defense mechanism, executed by the production of pro-inflammatory mediators. Overproduction of these mediators leads to chronic degenerative diseases such as, asthma, Alzheimer's disease, arthritis, and cancer [60]. Currently, inflammation is treated using nonsteroidal anti-inflammatory drugs - salicylate derivatives that produce many side effects such as renal failure, hypersensitive reactions, and gastrointestinal disorders [61]. Thus, secondary metabolites such as polyphenolic compounds obtained from plants could serve as an alternative in inflammation treatment, with minimal or null side effects. Anti-inflammatory activity of phenolic compounds may be due to its ability to inhibit the synthesis of pro-inflammatory mediators and ability to modify the eicosanoid synthesis and inhibit the activated immune cells through its inhibitory effects on nuclear factor-κB responsible for inflammation [62]. The tannins were found to antagonize the permeability-increasing effects of certain pro-inflammatory mediators and inhibit the migration of leukocytes (mainly lymphocytes - B-cells) to the inflammatory site, thus preventing inflammation [63]. The astringent property of tannins acts on the cell membrane, interfering in the inflammation of cell, and prevents inflammation [64]. Tannins also help to heal wound due to its antimicrobial property [65].

CONCLUSION

The above study clearly indicates that the polarity of solvents plays a vital role in extraction of phytoconstituents. The potential of solvent extract of *C. equisetifolia* root may be due to the presence of diverse phytoconstituents such as phenols, flavonoids, tannins, and terpenoids in crude extracts. In conclusion, this study has proved that the methanol root extract of *C. equisetifolia* in association with compounds of GC-MS analysis maybe an efficient therapeutic agent by being a potent antibacterial, antioxidant, and anti-inflammatory agent. Investigation is also being made to isolate, purify, and characterize the bioactive compound that is pharmacologically involved in these bioactivities. The isolated compound may serve as a powerful prototypes of antibacterial, antioxidant, and anti-inflammatory drugs with less or no side effects.

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AUTHOR'S CONTRIBUTION

Dr. S. Uma Gowrie: Design of the research and data analysis. Saranya: Execution of the research design and drafting of the article. All the authors contributed equally.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest in the publication.

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