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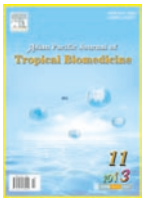

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In vitro analysis on bactericidal screening and antioxidant potentiality of leaf and root extracts of *Thottea siliquosa* (Lam.) Ding Hou. An ethnobotanical plant

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PEER REVIEW

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Comments

Normally researchers study the antimicrobial values only as MIC/MKC. Never into the phytochemicals. So the present results strongly support the bactericidal potentiality of the extract coupled with antioxidant nature So the outcome of the study can help the pharmaceutical people to find out the principle compound for further evaluation.
Details on Page 864

ABSTRACT

Objective: Natural products of plant origin are potential source of novel antimicrobial and antioxidative agents. *Thottea siliquosa* (Lam.) Ding Hou. (*T. siliquosa*). A medicinal herb used by local tribals for treating various ailments. The present study aims at the phytochemical screening, GC-MS analysis, *in vitro* antibacterial activity and antioxidant potentiality of root and leaf extracts of *T. siliquosa*.

Methods: Hot continuous Soxhlet extraction, GC-MS analysis, antibacterial analysis by disc diffusion, microdilution assay and antioxidant potentialities by hydroxyl radical and nitric oxide radical scavenging. The data was statistically analyzed.

Results: Phytochemical screening of the ethyl acetate and methanolic extract of leaf and root revealed the presence of phenols, alkaloids, tannins and saponin. The extract revealed a pool of phytochemicals by comparison with authentic standards from spectral library. Both the extracts has shown their broad spectrum of inhibition against the selected bacteria *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia* compared with standard antibiotic drug streptomycin. The extracts showed antioxidant activity by scavenging of free radicals such as hydroxyl and nitric oxide. The IC_{50} values of the ethyl acetate extracts leaf and root and standard in this assay were 167.5±0.67, 99.4±1.2, 192±2.5 μg/mL respectively. Similarly those methanolic extracts of leaf and root were 269.5±0.89 and 289.1±2.66 μg/mL respectively. Similarly, ethyl acetate and methanolic extracts also caused a moderate dose-dependent inhibition of nitric oxide with an IC_{50} range 65.5±1.55 to 148 ±3.09 μg/mL. The inhibitory activities were found to be dose dependent.

Conclusion: The present study provides evidence that ethyl acetate and methanol extract of leaf and root of *T. siliquosa* are potential source of natural antioxidants and bactericidal nature. It is essential that research should continue to isolate and purify the bio active components of this natural plant and use in drug discovery and development.

KEYWORDS

Antibacterial activity, Antioxidant potentiality, Phytochemicals, Soxhlet extraction, *Thottea siliquosa*.

1. Introduction

Plants and plant products have been used extensively to treat medical problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity[1]. It has been estimated that between 60-90% of

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population in developing countries use traditional and botanical medicines exclusively and consider them to be a normal part of primary healthcare[2]. Consumers are increasingly interested in complementary and alternative medicine, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in the use of alternative and complementary healthcare prompted scientists to investigate the various biological activities of medicinal plants. Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds (Cowan, 1999). These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities[3]. Similarly, studies have shown that many phytonutrients of fruits and vegetables might protect the human body against damage by ROS and thereby protecting against oxidative stress-related diseases, such as cancer, coronary heart disease, obesity, diabetes type 2, hypertension and cataract[4]. Antioxidant are compound that can delay or inhibit the lipid peroxidation or other free radicals by inhibiting the initiation or propagation of oxidative chain reactions and which can prevent or repair damage to the body cells caused by oxygen. They act by one or more of the following mechanisms: reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen[5]. Although there are synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used in processed foods, it has been reported that these compounds may have side effects. Therefore, there have been numerous researches on these bioresources to seek for potential natural and possibly economic and effective antioxidants to replace the synthetic ones.

Aristolochiaceae is represented by two genera, namely *Aristobchia* and *Thottea*. The later contains seven species, namely, *Thottea corymbosa, Thottea dependens, Thottea grandiflora, Thottea parviflora, Thottea sumalrana, Thottea lomentosa* and *Thottea tricornis*. *Thottea siliquosa* (Lam.) Ding Hou. (*T. siliquosa; Apama siliquosa* Lamk.or *Bragantia wallichii* R.Br. ex Wt. & Arn.), is an erect slender shrub of Aristolochiacea. Locally the plant in Kerala is referred as '*Alpam*' or '*Kuravankandamooli*', or '*Kuttila vayana*', growing in the evergreen and deciduous forests of peninsular India especially in the Western Ghat region as an under growth and it is used as herbal medicine for treating various ailments. We initially revealed the phytochemistry and cytotoxicity of *T. siliquosa* and now the study was extended to evaluate the antioxidant and antibacterial potentiality of this wonder herb[6].

2. Materials and methods

2.1. Plant material

T. siliquosa was collected from the Nedumangad taluk of Trivandrum district, Kerala, India. It was duly identified in herbarium of Tropical Botanical Garden and Research Institute, *Palode* and a voucher specimen was kept in the department for herbarium.

2.2. Soxhlet extraction

The fresh leaves and roots were washed with water, shade dried at room temperature and finely powdered. The leaf powder (100 g) and root powder (100 g) were successively extracted with petroleum ether, ethyl acetate, chloroform and methanol in a Soxhlet hot continuation extractor. Both leaf and root extracts were filtered, concentrated, lyophilized, the residue was weighed and stored at −20 °C.

2.3. GC- MS analysis

GC-MS analysis of the sample was carried out by using Agilant FC MS system and the identification of compounds is based on NIST Mass Spectral Library.

2.4. Antimicrobial screening

2.4.1. Micro organisms used

The screening of the antimicrobial activity of crude extracted from the leaf and root of *T. siliquosa* were carried out individually on active cultures of *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa) and Klebsiella pneumoniae (K. pneumoniae)*. All the strains were procured from the Microbial Type Culture and collection, Chandigarh, India.

2.4.2. Preparation of media

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/liter) Beef extract 2 g, casein acid hydrolysate 17.5 g, starch 1.5 g and agar 17 g; pH 7.4±0.2. About 38 g of MH agar was weighed and dissolved in 1000 mLof distilled water and adjusted to pH 7.4±0.2, sterilized by autoclaving at 121°C for 15 min at 15 psi pressure and was used for sensitivity tests[7, 8].

The four different concentrations of the leaf and root extracts were tested for antimicrobial activity using agar disc diffusion assay according to the method of Shilpa Satheesh and Murugan [3]. The strains of microorganisms obtained were inoculated in conical flask containing 100 mL of nutrient broth. These conical flasks were incubated at 37°C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia, Mumbai, India), poured on petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 μL of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Antimicrobial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10 μg/disc) is as standards[9].

Extracts were also evaluated to determine the Minimum Inhibitory Concentration (MIC) using the agar well diffusion technique. Serial dilutions of the test extracts were prepared in

DMSO to yield solutions of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 μg/mL. 20 μL aliquots of each dilution were introduced into wells in nutrient agar plates seeded with the standardized inoculums of bacteria. The test plates were incubated under the same condition as the screening stage. The lowest concentration of each extract showing a clear zone of inhibition and it was taken as the MIC.

2.5. Antoxidant potentiality

2.5.1. Hydroxyl radical scavenging

This was assayed as described by Hazra *et al*[10] with a slight modification. The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction). The reaction mixture contained, a final volume of 1 mL, 2-deoxy-2-ribose (2.8 mmol/L); KH_2PO_4-KOH buffer (20 mmol/L, pH 7.4); $FeCl_3$ (100 μM); EDTA (100 μM); H_2O_2 (1.0 mM); ascorbic acid (100 μM) and various concentrations (0-200 μg/mL) of the test sample or reference compound. After incubation for 1 h at 37°C, 0.5 mL of the reaction mixture was added to 1 mL 2.8% TCA, then 1 mL 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 min to develop the color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed six times. Mannitol, a classical OH. scavenger, was used as a positive control. Percentage inhibition was evaluated by comparing the test and blank solutions.

2.5.2. Nitric oxide radical scavenging

At physiological pH, nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions, which may be quantified by the Griess Illosvoy reaction[11]. The reaction mixture contained 10 mM SNP, phosphate buffered saline (pH 7.4) and various doses (0-70 μg/mL) of the test solution in a final volume of 3 mL. After incubation for 150 min at 25°C, 1 mL sulfanilamide (0.33% in 20% glacial acetic acid) was added to 0.5 mL of the incubated solution and allowed to stand for 5 min. Then 1 mL of napthylethylenediamine dihydrochloride (NED) (0.1% w/v) was added and the mixture was incubated for 30 min at 25°C. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED was measured spectrophotometrically at 540 nm against a blank sample. All tests were performed six times. Curcumin was used as a standard.

2.6. Statistical analysis

The mean values were expressed as mean±standard deviation (SD) and was analyzed using One way Anova (Turkeys studentized range) using the program SPSS 19.0 for windows. Differences were considered significant at $P<0.05$. The IC_{50} values were calculated by the formula $Y= 100\times A1/(X + A1)$, where $A1=IC_{50}$, Y=response (Y=100% when X=0), X=inhibitory concentration. The IC_{50} values were compared by paired tests.

3. Results

3.1. Phtochemical analysis

The petroleum ether, ethyl acetate, chloroform and methanol extracts of leaf and root of *T. siliquosa* having extractive value of 4.9, 7.9, 4.72 and 6.5 g respectively. Phytochemical screening showed the presence of alkaloids, saponins, steroids, terpenoids, flavonoids, glycosides and phenols as chemical constituents. The results are shown in Table 1.

Table 1
Preliminary phytochemical analysis in leaf and root of *T. siliquosa* using various solvents.

Chemical analysis		petroleum ether		ethyl acetate		chloroform		methanol	
		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Phenolics	$FeCl_3$ test	—	—	++	+++	—	—	+	+
	Lead acetate test	—	—	++	++	—	—	—	—
Flavonoids	NaOH test	—	+	+	+	—	—	+	+
	Shinoda test	—	—	—	+	—	—	+	—
	H_2SO_4 test	—	—	—	—	—	—	—	—
Saponins	Foam test	—	+	++	++	+	+	++	++
Alkaloids	Mayer's test	+	+	+++	+++	+	+	++	++
	Wagner's test	+	+	++	++	+	+	++	++
	Dragendorff's test	—	—	++	++	—	—	++	++
Tannins	Ferric chloride test	—	—	+	—	—	—	+	—
	Gelatin test	—	—	—	—	—	—	—	—

3.2. GC-MS analysis

In order to know the chemical constituents of these extracts, the root extract obtained successively with ethyl acetate was subjected to GC-MS analysis and the obtained result was compared with that of NIST Mass Spectral Library (Figure 1). This extract contains 3H-3a, 7-methanoazulene, 2, 4, 5, 6, 7, 8-hexahydro-1, 4, 9, 9-tetramethyl-, [3aR-(3a.α.,4.β.,7.α.)]-,santolina triene, ocimene, 1,2,4-triethyl benzene, 4-methyl-4-(2-methyl-2-propenyl)-tricyclo [3.3.0.0(2,8)] octan-3-one, 2-Isopropylidene-3-methylhexa-3, 5-dienal. Among these, 3H-3a, 7-methanoazulene, 2, 4, 5, 6, 7, 8-hexahydro-1, 4, 9, 9-tetramethyl-, [3aR-(3a. α.,4. β.,7.α.)]-, 2-isopropylide ne -3-methylhexa-3, 5-dienal are compared with authentic standards from spectral library (Table 2).

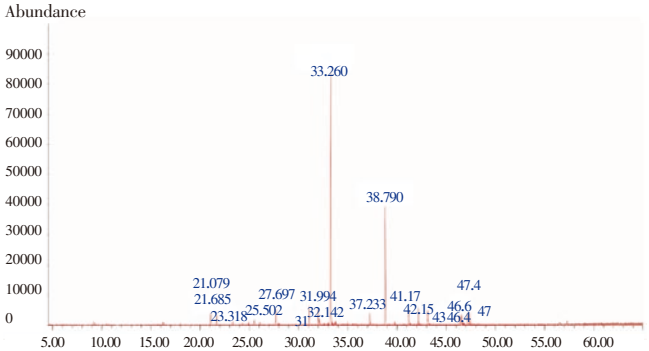

Figure 1. GC-MS analysis of ethyl acetate root extracts of *Thottea siliquosa* (Lam.) Ding Hou.

Table 2
Phytochemicals detected in the sample by GC-MS. Identification of compounds is based on NIST Mass Spectral Library comparison

RT(min)	Name of Compound	Match Quality (%) with NIST Library	% of Total Area
21.079	3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-, [3aR-(3a.alpha.,4.beta.,7.alpha.)]-	86	2.20
27.697	Santolina triene	53	2.16
31.072	Ocimene	47	2.37
31.994	1,2,4-triethyl benzene	35	2.07
33.260	4-methyl-4-(2-methyl-2-propenyl)-tricyclo[3.3.0.0(2,8)] octan-3-one	49	48.06
38.790	2-Isopropylidene-3-methylhexa-3,5-dienal	81	22.52

3.3. Antimicrobial assay

The antimicrobial effects of the tested ethyl acetate and methanolic extracts are presented in Table 3. Both the evaluated extracts demonstrated antimicrobial activity against the selected bacterial strains with inhibition zones greater than 0.3 cm (diameter of the well). The extract that exhibited high bactericidal activity (zone of inhibition>0.8 cm) was ethyl acetate followed by methanolic extract of the leaf and root of *T. siliquosa*.

Against all the bacterial strains tested, ethyl acetate extract against *Staphylococcus aureus* and *P. aeruginosa* was significant followed by *K. pneumoniae*. Methanolic extracts of leaf and root had the highest activity against *K. pneumoniae* with a zone of inhibition measuring 0.82±0.22 cm, followed by *E. coli*, a zone of inhibition of 0.75±0.03 cm. The antibacterial standard drug Streptomycin (10 μg/mL) had a zone of inhibition of 1±0.01 cm. Significant MIC value (25 μg/mL) was exhibited by ethyl acetate extracts against all the tested micro-organisms followed by the methanolic extracts of the leaf and roots (Table 3).

3.3. Antioxidant activity

3.3.1. Hydroxyl radical scavenging

This assay shows the abilities of the ethyl acetate and methanolic extract and standard mannitol to inhibit hydroxyl radical-mediated deoxy ribose degradation in Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture. The results are shown in Figure 2. The IC_{50} values of the ethyl acetate extracts (leaf and root and standard in this assay were 167.5±0.67, 99.4±1.2, 192±2.5 μg/mL respectively. Similarly those of methanolic extracts of leaf and root were 269.5±0.89 and 289.1±2.66 μg/mL respectively. The IC_{50} values of the extracts were comparable with that of the standard. At 200 μg/mL, the percentage inhibition values of *T. siliquosa* ethyl acetate extracts of leaf and root were 55 and 55.38 % and 52% for mannitol.

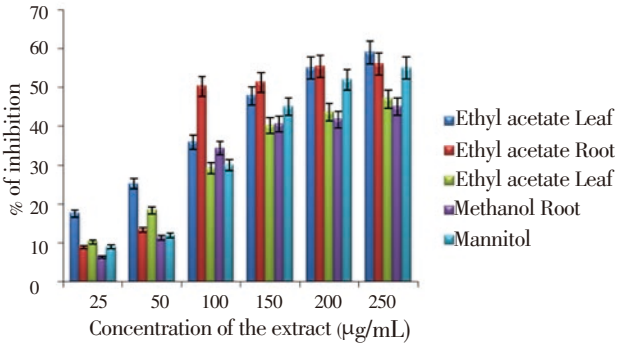

Figure 2. Hydroxyl radical scavenging activities of the *T. siliquosa* extract with the standard mannitol. The data represents the percentage inhibition of deoxyribose degradation. The results are mean±S.D. of six parallel measurements. Significant level at $P<0.001$.

3.3.2. Nitric oxide radical scavenging

T. siliquosa leaf and root ethyl acetate and methanolic extracts also caused a moderate dose-dependent inhibition of nitric oxide with an IC_{50} of 65.5±1.55, 80.9±4.32, 105.6±0.99, 148±3.09 μg/mL respectively (Figure 3). Similarly, the IC_{50} value of curcumin was 105±0.22 μg/mL. The IC_{50} value of the ethyl acetate extract was less than that of the standard.

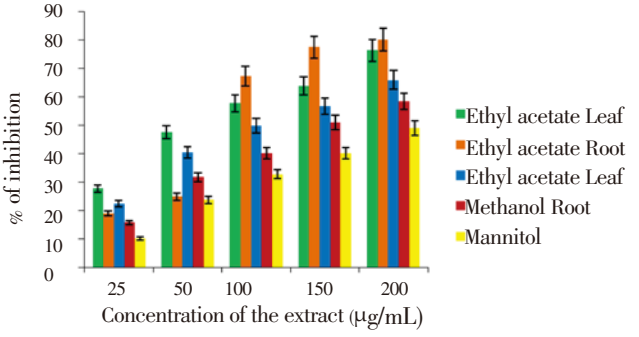

Figure 3. The nitric oxide radical scavenging activity of *T. siliquosa* extract with the standard curcumin. The data represents the percentage nitric oxide inhibition. Each value represents mean±S.D. (n=6). Significant level at $P<0.001$.

Table 3
Zone of inhibition (ZI in cm) and minimum inhibitory concentration (MIC in μg/mL) analysis were exhibited by leaf and root extracts of *T. siliquosa* with various pathogens.

Pathogens	Ethyl acetate				Methanol			
	Leaf		Root		Leaf		Root	
	ZI	MIC	ZI	MIC	ZI	MIC	ZI	MIC
S. aureus	0.88±0.04	25±0.01	0.76±0.02	50±0.02	0.66±0.011	50±0.06	0.67±0.013	50±0.08
P. aeruginosa	0.56±0.02	50±0.22	0.38±0.05	50±0.22	0.41±0.2	100±0.31	0.29±0.09	50±0.33
K. pneumoniae	0.48±0.01	100±0.05	0.35±0.1	100±0.32	0.82±0.22	25±0.08	0.59±0.07	50±0.43
E. coli	0.45±0.07	50±0.12	0.47±0.03	100±0.09	0.75±0.03	25±0.09	0.67±0.33	50±061

The values are mean of six independent replication±SD. Significant at $P<0.001$.

4. Discussion

Plants are an important source of biologically active substances; therefore they have been used for medicinal purposes since ancient times. The process of evaluating medicinal herbs is complex, and there is a need to carefully define a research strategy that addresses a solution to safe and efficacious herbal products. Notwithstanding the immense value of distilling the pharmacological activity of an herb into a chemical suitable for drug development, another approach is to develop a standardized herbal extract that yields consistent pharmacological activity[12].

Pathogenic microbes such as bacteria and fungi are becoming increasingly resistant to conventional antibiotics and resistance is emerging at alarming rate[13]. Thus the alarming increase in resistance to antibacterial has created desperate need for the search of new natural microbicidal agents.

Similarly, 'oxidative stress' that results from an imbalance between formation and neutralization of prooxidants initiates free radicals which cause decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to death[14]. These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy and degradation of essential fatty acids[11,15]. Antioxidant helps in treatment of above disorders. Synthetic drugs are potential against oxidative damage but they have adverse side effects. An alternative approach is to consume natural herbal antioxidants from food supplements and traditional medicines[16,17]. Currently, many herbal antioxidants have been isolated from different plant materials[18].

Hydroxyl radical is one of the major reactive oxygen species (ROS) which leads to biological dam-age via lipid peroxidation[19]. In the present study, they are produced by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH[11]. Ethyl acetate or methanolic extracts of *T. siliquosa* leaf and root were added to the reaction mixture; it removed the hydroxyl radicals from the sugar and prevented the reaction. The IC_{50} value and percentage of inhibition suggest that *T. siliquosa* extract is a better hydroxyl radical scavenger than the alternative standard mannitol.

Nitric oxide (NO) is generated by endothelial cells, macrophages, neurons and it is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity[20,21]. The toxicity of NO increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion (ONOO-)[12]. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The antioxidants inhibit nitrite formation by directly competing with oxygen in the reaction with nitric oxide. Diazotization takes place between nitrite and sulphanilamide, this diazotized product is coupled with naphthylene diamine to form chromophore, which is reduced by antioxidant when measured at 540 nm[11]. Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO[11] which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. The observed results indicate that ethyl acetate extracts of leaf and root exhibited better scavenging potential than the standard curcumin.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging[22].

The results indicate that extract of *T. siliquosa* contains significant amounts of 3H-3a, 7-Methanoazulene, 2, 4, 5, 6, 7, 8-hexahydro-1, 4, 9, 9-tetramethyl-,[3aR-(3a.α.,4.β.,7.α.)]-, Santolina triene, Ocimene, 1,2,4-triethyl benzene, 4- methyl-4-(2-methyl-2-propenyl)-tricyclo [3.3.0.0(2,8)] octan-3-one, 2-Isopropylidene- 3-methylhexa-3,5-dienal. Among these, 3H-3a, 7-Methanoazulene, 2,4,5,6,7,8- hexahydro-1, 4, 9, 9-tetramethyl-, [3aR-(3a. α.,4. β.,7.α.)]-, 2-Isopropylide ne -3-methylhexa-3, 5-dienal compounds. All these classes of compounds have good anti-oxidant potential and their effects on human nutrition and health are considerable[23].

The present results in *T. siliquosa* are in agreement with antioxidant and antimicrobial attributes of different solvent extracts from leaves, flowers and bark of *Delonix regia*[14]. Baskar *et al*[17]also have demonstrated *in vitro* antioxidant and antiproliferative potential of medicinal plants used in traditional Indian medicine to treat cancer. Meanwhile, the present results are not in agreement with the studies of Ben Hassine *et al*[17]where methanol extract demonstrate higher scavenging activities. However, Eucalyptus oleosa essential oils: from different plant parts (stems, leaves, flowers and fruits) shared a more or less similar pattern with the present study [15]. Merrine Raju and Ramesh made an extensive study of antioxidant properties of *T. siliquosa*[24]. The results reveal the correlation of antioxidant activities and the amount of total phenolic compounds of the extracts. In the present study the phytochemicals in *T. siliquosa* are different with significant hydroxyl radical scavenging and NO scavenging potential. Similarly, the antioxidant potentiality of the present study is comparable with *Plagiochila beddomei*, which is a liverwort[25].

Comparing the microorganism's inhibition, the ethyl acetate extract of *T. siliquosa* showed highest percentage of inhibition of *S. aureus* and this agrees with Arote *et al*.[26] who found that methanolic extract of *Pongamia pinnata* was effective against *S. aureus*. The factors responsible for this high susceptibility

of the bacteria to the extracts are not exactly known, but may be attributed to the presence of secondary plant metabolites or which is soluble in the tested solvents and to the structural differences in the cell envelope compositions of the *Gram* negative and the *Gram* positive bacteria[27]. The present study showed that *Gram* negative bacteria were more sensitive to the tested methanolic and ethyl acetate extracts as compared to the *Gram* positive bacteria. The highest sensitivity of *S. aureus* may be due to its cell wall structure and outer membrane. This also agrees with the findings of Ghulam Dastagir *et al*[28] who found that *S. aureus* was the most sensitive. The various levels of antimicrobial and antioxidant activity shown by leaf and root ethyl acetate and methanolic extracts may be due to the solvent extract containing different constituents. Ethyl acetate and methanolic extracts proved as the most effective solvents for extracting broad spectrum of bactericidal compounds from *T. siliquosa*.

5. Conclusion

In conclusion, the results obtained in this study provide a rationale for the use of *T. siliquosa* in traditional medicine as antiseptic. The leaf and root extracts exhibit significant antioxidant and free radical scavenging activities might be helpful in preventing the progress of various oxidative stresses. Further studies to establish the safety profiles of the bioactive extracts in an animal model and identification of their chemical constituents can be used as markers for standardization of antimicrobial herbal remedies are ongoing. Similarly, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

As a continuation of our previous preliminary work, "The *in vitro* cytotoxic activity of the leaf and root extract of *Thottea siliquosa* (Lam.) Ding Hou." using various solvents such as petroleum ether, ethyl acetate, chloroform and methanol was investigated by the trypan blue exclusion method. The level of cytotoxicity of the extracts were proportional to the concentration of the extract and significant percentage of cell death was observed with petroleum ether extract. These studies reveal the presence of cytotoxic compounds in the extracts of the leaf and roots, which can induce cell death.

Research frontiers

Phytochemistry of *Thottea tomentosa* revealed the presence of bioactive components of some pharmacological interests (or possibly toxic interests) especially aristolochic acids and aristolactam. In herbal medicine, the pounded leaves of *Thottea siliquosa* are applied to sore gums or tooth cavity for toothache. The plants are common along the hot spot of Westernghats. Also it is claimed that the shrub can be used even in drug form as an analgesic, antiasthmatic, antifertility and for treating impotence and snake-bite. Although the plant is widely used among the locals, to date, no study on the phytochemistry and antimicrobial activity has ever been reported.

Related Reports

Phytochemical analysis revealed phenanthrene derivatives and a sesquiterpene which posseses antifertility activity besides being a source of aristolochic acid, a tumour-inhibitory principle, rarely found in nature as it contains a NO_2 group. In China, *Aristolochia debitis* decoction known as 'qing mu xiang' has a direct constrictive action on blood vessels and shows an inhibitory action on the heart. Recently, it was found that aristolochic acids I and II were reported to cause interstitial nephritis. In this scenario, study shoule be done to explore pharmacoherb of *Thottea siliquosaan* in terms of antibacterial or other biological activities.

Innovations and breakthroughs

In this study, we investigated the antibacterial potentiality in selected pathogenic bacteria, antioxidant potentiality and GC-MS analysis of the phytochemicals in the root and leaf extract of the plant.

Applications

The results strongly suggest that the phytochemicals revealed by GC-MS are novel molecules as microbicidal and antioxidant nature. Purification of these compounds will leads to natural effective antibiotic or antioxidants in future.

Peer review

Normally researchers study the antimicrobial values only as MIC/MKC, but studies into the phytochemicals are still lack. So the present results strongly support the bactericidal potentiality of the extract coupled with antioxidant nature. So the outcome of the study can help people to find out the principle compound for further evaluation.

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