

Original Article

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF STEM BARK ESSENTIAL OIL
CONSTITUENTS OF *LITSEA GLUTINOSA* C. B. ROB.

ARUNODAYA H. S., KRISHNA V. *, SHASHIKUMAR R., GIRISH KUMAR K.

Department of PG Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Shankaraghatta 577451, Karnataka, India
Email: krishnabiotech2003@gmail.com

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ABSTRACT

Objective: To evaluate the chemical composition, antibacterial and antioxidant properties of stem bark essential oil of *Litsea glutinosa* C. B. Rob.

Methods: The essential oil isolated from stem bark of *L. glutinosa* and their chemical composition was analyzed by gas chromatography coupled with mass spectrometry detector. The *in vitro* antibacterial activity of the stem bark essential oil was investigated against eight human pathogenic bacterial clinical isolates using agar disc diffusion method and MIC value was determined by modified resazurin microtitre-plate assay. The antioxidant activity of essential oil was measured by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonate radical cation (ABTS) and β -carotene bleaching assay.

Results: GC-MS analysis of stem bark essential oil resulted in the identification of 37 compounds, off which 9,12-octadecadienoic acid (62.57%), hexadecanoic acid (12.68%), stigmast-5-en-3-ol (6.87%) and vitamin E (2.51%) were the main constituents representing 84.63% of the oil. The determination of *in vitro* antibacterial activity of stem bark essential oil resulted in significant inhibition zone (15.00 \pm 0.57 mm) and MIC value (0.15 \pm 0.15 \times 10⁻² mg/ml) against the pathogenic bacteria *Vibrio cholera* followed by *Pseudomonas aeruginosa* and *Salmonella typhi*. The results of DPPH radical scavenging (IC₅₀:4.540 \pm 0.06 μ g/ml), ABTS (IC₅₀:256.02 \pm 0.06 μ g/ml) and β -carotene bleaching assay (%I: 78.51 \pm 0.42 %) showed significant *in vitro* antioxidant property.

Conclusion: *L. glutinosa* stem bark essential oil showed potential antibacterial activity against the *Vibrio cholera*. The results of this investigation supported the ethnomedical claim of essential oil as a demulcent, antidiarrheal and antioxidant drug.

Keywords: *Litsea glutinosa*, Stem bark essential oil composition, GC-MS, Antibacterial and Antioxidant activity

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INTRODUCTION

Litsea glutinosa is an endemic and threatened aromatic medicinal tree which belongs to the Lauraceae family and found to be very sparsely distributed in the Western Ghats of Karnataka State, India. Leaves and stem bark essential oil possess allspice aroma. Traditional medicinal practitioners residing in the vicinity of Bhadra Wild Life Sanctuary are using *L. glutinosa* stem bark oil as the demulcent and mild astringent for diarrhea and dysentery. It is also reported for relieving pain, arousing sexual power, aches, sore eyes, skin infections, gouty joints, wounds and also for producing a soothing effect on the body [1-3]. The leaf extract has been evaluated for cardiovascular and anti-inflammatory activities [4]. The berries oil is used in the treatment of rheumatism and shampoo preparation [5].

Essential oils from aromatic and medicinal plants have been known from ancient times to possess biological activity [6]. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of foods from the toxic effects of oxidants, notably antibacterial [7], antifungal [8] and antioxidant properties [9].

Recently, aromatic medicinal plants and their essential oils have provoked interest in the isolation of novel biologically active compounds for the elimination of pathogenic microorganisms. Many pathogenic microbes evolved as multi-drug resistant (MDR) strains which acquire resistance against the known antibiotics [10]. Further, bacterial infections pose a greater threat to health, most notably in immune compromised subjects. Hence, it is essential to investigate the cheap and effective antimicrobial agents to combat microbial infections. The chemical composition and bioactivities of stem bark essential oil of *L. glutinosa* are unexplored. Therefore, an attempt was made to analyze the chemical composition of the stem bark essential oil and to evaluate the antibacterial and antioxidant activities to authenticate the traditional claim of the essential oil as the antidiarrheal drug.

MATERIALS AND METHODS

Chemicals and reagents

2,2-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) was obtained from Sigma Chemical Co. (St. Louis, MO, USA), butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), folin-ciocalteu reagent, dimethyl sulfoxide (DMSO) were purchased from Merck limited (Mumbai, India), resazurin, hi sensitivity broth, β -carotene, potassium persulfate, linoleic acid, ascorbic acid, nutrient agar (NA) media, sodium sulfate, gentamicin and ampicillin dish were procured from Himedia Pvt. Labs. (Mumbai, India). All other chemicals and solvents used were of analytical grade. Purified water from MilliQ water purification system (Millipore, Bedford, MA, USA) was used in all the experiments.

Plant material

The stem bark of *L. glutinosa* was collected from Bhadra Wild Life Sanctuary of the central Western Ghats, Karnataka, India during December 2013. The plant specimen was identified and authenticated by Tariq Husain, Head, Scientist, Biodiversity and Angiosperm Taxonomy, National Botanical Research Institute, Lucknow, India and the voucher specimen (No. 97294) was deposited.

Extraction of essential oil

A 250 g of air dried chopped and ground bark powder of *L. glutinosa* was subjected to hydrodistillation using a modified Clevenger-type apparatus [11]. Subsequently, obtained aromatic oil was dried over anhydrous sodium sulfate and preserved in sealed amber colored vial at 4 °C prior to analysis.

GC-MS analysis of the essential oil

The chemical composition of the stem bark essential oil was analyzed using Shimadzu GCMS-QP2010S instrument with GC-MS

solutions software and compounds were separated using Rtx-5, capillary column (0.25 mm, 0.25 μ m). Split ratio 1:25 injector temperature was 300 °C; the column temperature was maintained at 60 °C, followed by 10 min at 300 °C. About 1 μ l sample was injected into the column by the split mode. GC-MS was performed by an electron ionization system, with ionization of 70 eV. The helium was used as carrier gas at a flow rate of 1 ml/min; Mass scanning range was 40-500 m/z [12]. Interpretation of GC-MS chromatogram was conducted using the two databases of NIST05 and WILY7 mass spectral libraries which provide the best information about the identification of components. The name, molecular weight and structure of the components of stem bark essential oil were ascertained.

Antibacterial activity

Microbial strains

The antibacterial activity of the stem bark essential oil was individually tested against a set of seven bacterial human pathogenic clinical isolates obtained from Shivamogga Institute of Medical Sciences, Shivamogga, Karnataka. namely: *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholera*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptomyces pneumonia* and *Bacillus subtilis*. Bacterial isolates were cultured overnight at 37 °C in nutrient agar (NA) media.

Disk diffusion assay

Determination of antibacterial activity of stem bark essential oil was evaluated by agar disk diffusion method [13]. 100 μ l of the suspension containing 108 colony forming units CFU/ml of bacteria were spread on NA media, respectively. Sterile filter discs (6 mm in diameter) were placed onto the surface of the agar plates and 5 μ l of essential oil was pipetted onto the discs. Plates were kept at 4 °C for 1h and then incubated for 24 h at 37 °C. Gentamycin (10 μ g/dish) and ampicillin (10 μ g/dish) were used as a positive control for bacteria. While the discs without samples were used as a negative control. Antibacterial activity was assessed by measuring the diameter of the growth inhibition zone in millimeters (including disc diameter of 6 mm) for the test organisms compared to the control [14].

Resazurin microtitre-plate assay

The minimum inhibitory concentration (MIC) of the stem bark essential oil was evaluated by modified resazurin microtitre plate assay [15]. 50 μ l of test sample containing 250 μ g of essential oil [5 mg/ml (w/v)] solutions in 10% dimethyl sulfoxide (DMSO, v/v) and 50 μ g of standard antibiotic [1 mg/ml (w/v)] solutions in 10% DMSO was transferred into the first row of eight of 96 well plates. To all wells, 50 μ l of nutrient broth was added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 μ l of the test material in serially descending concentrations. 30 μ l of 3.3 times stronger hi sensitivity broth (3.3x) and 10 μ l of resazurin indicator solution (prepared by dissolving 27 mg resazurin in 4 ml of sterile distilled water) were added to each well. Finally, 10 μ l of bacterial suspension was added to the appropriate wells to achieve a concentration of approx 5×10^6 CFU/ml.

Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: column with all solutions with the exception of the test compound (as growth control), column with all solutions with the exception of the bacterial solution adding 10 μ l of nutrient broth instead (as sterility control) and a column with 10% DMSO (v/v) solution as a negative control. Gentamycin and ampicillin were used as positive controls. The plates were prepared in duplicate, incubated at 37 °C for 24 h and color change was assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which color change appeared was taken as the MIC value.

Determination of antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of *L. glutinosa* stem bark essential oil was assayed through TLC method [16]. 5 μ l of the essential oil (1:10 dilution in methanol) was applied on TLC plate and developed in ethyl acetate and methanol [1:1 (v/v)]. The plate was sprayed with 0.2% DPPH solution in methanol and left at room temperature for 30 min. Yellow spot formed due to bleaching of purple color of DPPH reagent indicates positive antioxidant activity of essential oil. Different concentrations of the essential oil (2 to 10 μ g/ml) and the standard BHA 2-10 μ g/ml were added to 0.004% methanolic solution of DPPH. After 30 min of incubation at room temperature (25 \pm 2 °C), the absorbance was read at 517 nm using a spectrophotometer. Radical scavenging activity was calculated by the evaluation of % of inhibition and IC₅₀ value [17].

ABTS radical scavenging activity

The radical scavenging capacity of the ABTS radical cation was determined as described by Re et al. [18]. The ABTS radical was generated by mixing equal volume (v/v) of 7 mmol ABTS and 2.6 mmol potassium persulfate and kept for incubation overnight at room temperature under dark condition. 150 μ l of essential oil and standard BHT at different concentrations were allowed to react with 2.85 μ l of ABTS mixture and were incubated at room temperature for 2 h in dark condition. The scavenging activity was determined by measuring the absorbance at 734 nm and the % of inhibition and IC₅₀ value was calculated [19].

β -carotene/linoleic acid assay

The antioxidant activity of stem bark essential oil was determined by measuring the efficacy of oil to inhibit the conjugated diene hydroperoxide formation arising from linoleic acid and β -carotene coupled oxidation in an emulsified aqueous system [20]. A stock solution of β -carotene and linoleic acid was prepared with 0.5 mg of β -carotene in 1 ml of chloroform, 25 μ l of linoleic acid and 200 μ l Tween 80. The chloroform was completely evaporated under vacuum in a Buchi-R3 rotary evaporator (Flawil, Switzerland) at 40 °C. 100 ml of oxygenated distilled water was then added to the residue and the resulting mixture was vigorously stirred to form a β -carotene-linoleic acid emulsion. The samples (2 g/l) were dissolved in DMSO and 350 μ l of each sample solution was added to 2.5 ml of the above mixture in test tubes. The test tubes were incubated in a water bath at 50 °C for 2 h, together with positive control and the same volume of DMSO used as blank. As the test samples added with β -carotene-linoleic acid emulsion, the zero time absorbance (A₀) was measured at 470 nm. Second absorbance (A_t) was measured after 2 h of incubation. BHA and ascorbic acid were used as positive controls.

Statistical analysis

The statistical analysis was performed by one-way ANOVA in Graphpad prism Version 5.01 Software. The results were expressed as mean \pm SEM.

RESULTS

Essential oil composition of stem bark

The amount of essential oil obtained from the stem bark of *L. glutinosa* was 2.3 ml/100 g dry weight, with characteristic aroma of allspice (*Pimenta dioica*). The GC-MS data revealed that *L. glutinosa* stem bark essential oil composed of 38 peaks as shown in the fig. 1.

Antibacterial activity of stem bark essential oil

The essential oil isolated from *L. glutinosa* stem bark exhibited varying antibacterial activity against the tested pathogenic microbial strains and are shown in table 2. The results of the disc diffusion assay followed by modified resazurin assay (fig 2A, B, C) indicated that the stem bark essential oil exhibited highest inhibitory activity against gram-negative bacteria *Vibrio cholera* with a significant inhibition zone of 15.00 \pm 0.57 mm and MIC value of 0.15 \pm 0.15 $\times 10^{-2}$ mg/ml.

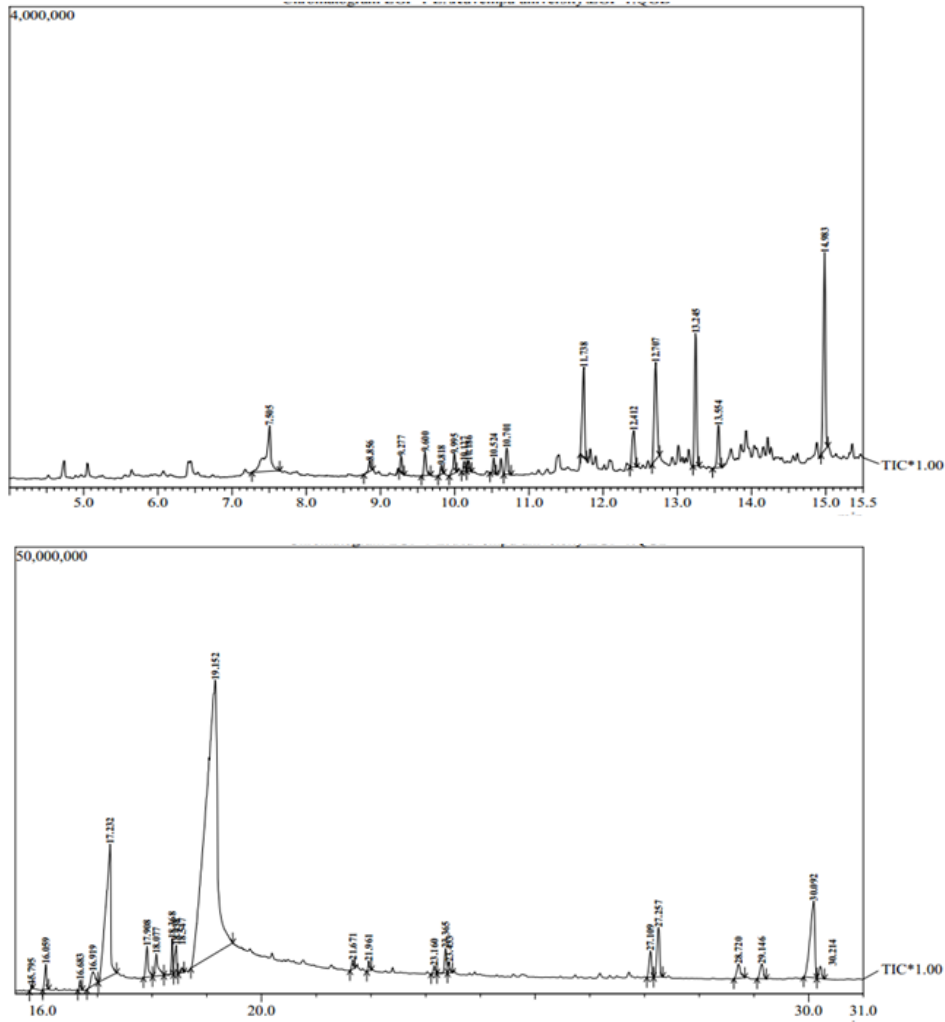


Fig. 1: It shows GC-MS chromatograms of *L. glutinosa* stem bark essential oil

Table 1: Showing chemical composition of *L. glutinosa* stem bark essential oil

S. No	R. Time	RI	Yield %	Compound
1	7.505	998	0.25	Octanoic acid (CAS) Caprylic acid
2	8.856	1078	0.02	Nonanoic acid
3	9.277	1102	0.03	2, 4-Dodecadienal, (E,E)
4	9.600	1123	0.06	2, 4 Decadienal
5	9.818	1136	0.02	1-Butene, 2, 3-dimethyl
6	9.995	1147	0.05	5-pentyl-2(5H)-furanone
7	10.127	1155	0.02	. alpha.-Cubebene
8	10.186	1159	0.02	Decanoic acid (CAS) Capric acid
9	10.524	1180	0.03	Copaene
10	10.701	1191	0.06	. beta.-elemene
11	11.738	1260	0.22	9-Oxononanoic acid
12	12.412	1304	0.11	1S, cis-calamenene
13	12.707	1325	0.28	Dodecanoic acid (CAS) Lauric acid
14	13.245	1363	0.28	(-)-Caryophyllene oxide
15	13.554	1385	0.10	Humulene oxide
16	14.983	1486	0.45	Tetradecanoic acid (CAS) Myristic acid
17	15.795	1543	0.11	Neophytadiene
18	16.059	1562	0.90	Pentadecanoic acid (CAS) Pentadecylic acid
19	16.683	1606	0.28	Hexadecanoic acid, methyl ester
20	16.919	1623	1.09	Octadec-9-enoic acid
21	17.232	1645	12.68	Hexadecanoic acid (CAS) Palmitic acid
22	17.908	1693	1.41	Oleic Acid
23	18.077	1705	1.30	Heptadecanoic acid (CAS) Margaric acid
24	18.368	1725	0.97	9, 12-Octadecadienoic acid (Z, Z)-, methyl ester
25	18.438	1731	1.13	9, 12, 15-Octadecatrienoic acid, methyl ester
26	18.547	1738	0.16	2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-

27	19.152	1781	62.57	9, 12-Octadecadienoic acid (Z,Z)-
28	21.671	1959	0.36	9-Octadecenoic acid, 1, 2, 3-propanetriyl ester
29	21.961	1979	0.29	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester
30	23.160	2064	0.31	Oleoyl chloride
31	23.365	2079!	0.96	Propyleneglycol monooleate
32	23.433	2083	0.38	Ethyl Linoleolate
33	27.109	2343	1.25	Unknown compound
34	27.257	2354	2.51	Vitamin E
35	28.720	2457	0.97	Ergost-5-en-3-ol, (3. beta.)-
36	29.146	2488	0.90	Stigmasta-5, 22-dien-3-ol, (3. beta., 22E)-(CAS) Stigmasterol
37	30.092	2554	6.87	Stigmast-5-en-3-ol, (3. beta., 24S)-(CAS) Clionasterol
38	30.214	2563	0.64	Cholestanol
	Total		100.00	

*RI: Relative Retention indices to C8-C29 n-alkanes on Rtx-5 capillary column, *Compounds listed in order of elution from Rtx-5 capillary column

Table 2: Antibacterial activity of the *L. glutinosa* essential oil

Microorganisms	Inhibition zone diameter (mm) and MIC (mgml ⁻¹)					
	Essential oil (5 µl/disc)		Gentamycin (10 µg/disc)		Ampicillin (10 µg/disc)	
	IZ	MIC	IZ	MIC	IZ	MIC
Gram-negative						
<i>E. coli</i>	11.00±0.57	6.25±0.10×10 ⁻²	29.67±0.33	1.56±0.10×10 ⁻³	16.33±0.88	8.33±0.20×10 ⁻³
<i>P. aeruginosa</i>	14.33±0.66	6.25±0.10×10 ⁻²	39.67±0.33	0.65±0.10×10 ⁻³	49.67±0.33	2.08±0.05×10 ⁻³
<i>S. typhi</i>	11.33±0.33	1.30±0.20×10 ⁻²	29.00±0.57	2.08±1.30×10 ⁻³	41.00±0.57	4.16±0.01×10 ⁻³
<i>V. cholera</i>	15.00±0.57	0.15±0.15×10 ⁻²	34.00±1.00	7.81±0.10×10 ⁻³	44.33±0.66	4.16±0.10×10 ⁻³
<i>K. pneumonia</i>	10.33±0.33	3.12±0.41×10 ⁻²	24.33±0.66	4.16±0.10×10 ⁻³	-----	25.0±0.10×10 ⁻³
Gram-positive						
<i>S. aureus</i>	09.66±0.33	12.5±0.01×10 ⁻²	24.67±0.33	4.16±0.10×10 ⁻³	12.67±0.66	5.20±0.10×10 ⁻³
<i>S. pneumonia</i>	10.00±0.57	3.12±0.01×10 ⁻²	23.33±0.88	4.16±0.10×10 ⁻³	08.33±0.33	16.6±0.10×10 ⁻³
<i>B. subtilis</i>	11.33±0.33	3.12±0.01×10 ⁻²	26.67±0.66	4.16±0.10×10 ⁻³	10.33±0.33	5.20±0.10×10 ⁻³

Values are mean±standard error (n=3) of three different samples, analyzed individually in triplicate, IZ, the diameter of inhibition zone (mm) including disc diameter of 6 mm, MIC, minimum inhibitory concentration (mg/ml).

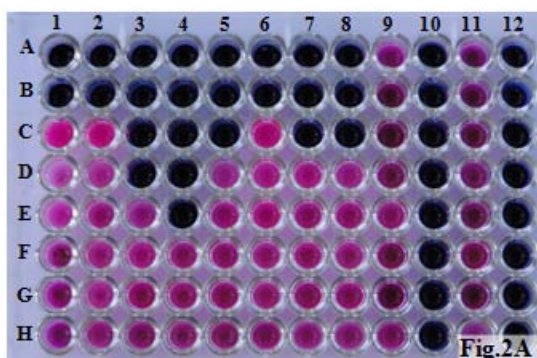


Fig. 2A: Modified resazurin assay results of stem bark essential oil in decreased concentrations of 50 µl to 0.39 µl

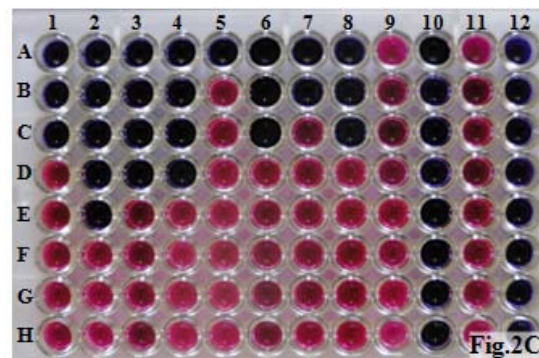


Fig. 2C: Modified resazurin assay results of ampicillin in decreased concentrations of 50 µl to 39 µg/ml

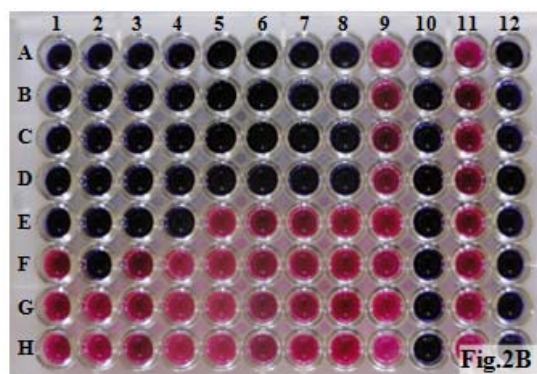


Fig. 2B: Modified resazurin assay results of gentamycin in decreased concentrations of 50 µl to 39 µg/ml

Antioxidant activity

Plants with radical scavenging property and antioxidant capacity are useful for medicinal applications and as a food additive. So, in the present study, the antioxidant capacity of *L. glutinosa* was evaluated using DPPH, ABTS free radical scavenging and β -carotene linoleic acid bleaching assay by comparing with the known antioxidant such as ascorbic acid, BHA and BHT respectively.

Discoloration of the purple color of the DPPH radical on TLC plates was assessed as a positive sign of antioxidant activity of oil. The stem bark essential oil showed DPPH and ABTS free radical scavenging activity in a dose-dependent manner and its IC₅₀ values of DPPH and ABTS assay is 4.540±0.06 µg/ml and 256.02±0.06 µg/ml respectively. In β -carotene bleaching assay, the inhibiting activity of oil was 78.51±0.42% which was higher than that of synthetic antioxidant ascorbic acid (63.33±0.71%) and less than BHA (93.42±0.40%) as shown in table 3.

Table 3: DPPH, ABTS radical scavenging and β -carotene bleaching assay

S. No	Activity	Concentration in $\mu\text{g/ml}$	% of Inhibition	IC ₅₀ $\mu\text{g/ml}$	Standard IC ₅₀ in $\mu\text{g/ml}$		
					BHA	BHT	Vit C
1	DPPH	2	5.70±0.52	4.540±0.06	19.13±0.05	----	----
		4	10.51±1.45				
		6	15.23±1.23				
		8	20.01±0.71				
		10	26.92±1.47				
2	ABTS	100	23.80±0.55	256.02±0.06	----	4.448±0.05----	
		200	46.66±1.65				
		300	70.15±2.48				
		400	81.90±1.10				
		500	89.52±1.10				
		600	98.09±0.55				
3	β -carotene	700	78.51±0.42	----	93.42±0.42	----	63.33±0.71

The results shown are averages of three independent experiments (n=3), values are mean±SEM.

DISCUSSION

Litsea glutinosa is an aromatic tree mainly growing in tropical and subtropical Asia [21] and many investigators explored the antimicrobial activity of the essential oil isolated from this species [22]. In the present investigation, major aromatic constituents were isolated and their biological activity was screened for antioxidant and antibacterial activity against the selected human pathogenic bacteria.

The GC-MS results of *L. glutinosa* stem bark isolate revealed the presence of 38 aromatic compounds. Among the major constituents identified, 9,12-octadecadienoic acid (*Z,Z*)-Linoleic acid (R/T 19.152) possesses anti-inflammatory, nematocidal, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, anti-histaminic, anti-acne, antiarthritic, antieczemic, antiandrogenic, 5-alpha reductase inhibitor and anti-coronary activity [23]. The hexadecanoic acid (R/T 17.235) can be a hypocholesterolemic, nematocidal, pesticide and lubricant activities and it was also reported as a more effective free radical scavenger than β -carotene [23, 24]. (3 β)-Stigmast-5-en-3-ol has shown an insulin-like effect, it stimulates glucose transport apart from its existing cholesterol-lowering efficacy. Therefore, it can play a beneficial role as an anti-diabetic agent [25]; it can also prevent diabetic neuropathy, a painful condition resulting from exposure of nerves to high glucose levels [26]. Vitamin E is known to possess anti-ageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, anti-dermatitic, anti-leukemic, anticancer, hepatoprotective, hypocholesterolemic, anti-ulcerogenic, vasodilator, antispasmodic, anti-bronchitic, and anticoronary activity [23]. Fruits, plants and vegetables are the main sources of antioxidant vitamins (vitamin E, vitamin C, the precursor of vitamin A i.e., β -carotene), which act as free radical scavengers, making these foods essential to human health.

Presently, much attention has been given to plant-derived essential oils, as they possess various pharmaceutical properties such as antibacterial, antioxidant, antiviral, anti-insecticidal, anti-mycotic, and anti-toxinogenic activities [27-30]. From the ancient times, many plant oils have been used as topical antiseptics and also it is used to improve food safety and quality [16]. The antimicrobial activities of fatty acids have been well known from many years [31, 32] and also these were bactericidal compounds against the pathogenic microorganisms including antibiotic-resistant *Staphylococcus aureus* [33, 34]. Linoleic, palmitic, linolenic, lauric, oleic, stearic myristic acids etc. Isolated from the stem bark of *L. glutinosa* were reported as the potential antibacterial drugs [35, 36]. GC-MS results of this study also confirmed the presence of a higher percentage of linoleic (9, 12-Octadecadienoic acid) and palmitic (Hexadecanoic acid) fatty acids in the stem bark.

The traditional claim indicated that the stem bark oil of *L. glutinosa* has been used to control diarrhea and dysentery which is caused by the gram-negative bacterial strain *Vibrio cholera*. In the present study also stem bark essential oil of *L. glutinosa* exhibited lowest MIC values than the tested gram-positive strains. Generally, gram-negative bacteria showed less sensitivity to plant extract when

compared to gram positive bacteria because of extra lipopolysaccharide and protein cell wall [37, 38]. Whereas, the zone of inhibition efficiency of stem bark essential oil was more (15.00±0.57 mm) against the *Vibrio cholera* culture. The results obtained in the present study are in agreement with the traditional medicinal claim as the potential anti-diarrheal drug. Vardar Unlu *et al.*, [39] Suggested that the simple relation involving cell structure and microbial sensitivity to essential oils is not yet well established and possible antagonistic or synergistic effects among the various active constituents of the oils should be taken into consideration. The previous investigator Parikh *et al.*, [40] also reported that decoction of stem bark was prescribed as the remedy for diarrhea and dysentery. So our results were in accordance with the earlier reports on the anti-diarrheal property of the stem bark essential oil.

The generation of reactive oxygen species (ROS) and free radicals beyond the antioxidant capacity of a biological system gives rise to oxidative stress during aerobic cellular metabolism and eventually associated with the pathogenesis of a variety of human diseases such as atherosclerosis, diabetes mellitus, hypertension, inflammation, cancer, neurodegenerative disorders, aging, ulcerative colitis, and cirrhosis [41-44]. Nowadays, there is growing interest in evaluating the availability of natural plant extracts as an alternative of synthetic antioxidants, such as BHT, BHA and ascorbic acid, which are commonly used in processed foods, it has been showed that these compounds have side effects in living organisms [45]. Hence, search for newer natural antioxidants, especially of plant origin, has ever since increased.

Reports also indicated that essential oil showed preservative action and they prevent lipid deterioration by the microorganism spoilage [46, 47]. Fatty acids can also attribute to antioxidant activity. In the case of the antioxidant assay, the DPPH, ABTS and β -carotene-linoleic acid test gave a significant result. The GC-MS analysis of the stem bark essential oil revealed the presence of palmitic acid in a higher percentage (12.68 %). Palmitic acid was reported to be a more effective free radical scavenger than β -carotene [48]. Stigmast-5-en-3-ol and vitamin c which are present in this oil may also contribute to the antioxidant activity which clearly indicates that essential oil possesses significant antioxidant activity, which is widely used as food preservatives in food technology [49, 50]. The higher content of fatty acids-linoleic acid, palmitic acid and Vitamin C in the stem bark essential oil could be responsible for the increased antibacterial and antioxidant activity.

CONCLUSION

In conclusion, the GC-MS analysis of *L. glutinosa* stem bark essential oil showed a higher concentration of 9, 12-octadecadienoic acid (62.57%) which exhibited promising anti-diarrheal activity against the human pathogenic bacterial strain. The DPPH radical scavenging, ABTS and β -carotene bleaching assay of essential oils also showed significant *in vitro* antioxidant property. The result of the present study further supports the ethnomedical claim of *L. glutinosa* stem bark oil as the demulcent and mild astringent for diarrhea and dysentery. The therapeutic action of the 9, 12-octadecadienoic acid

will be correlated with the effect of the reference standard dug in the future investigation.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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