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

Received: 20 Jun 2016 Revised and Accepted: 21 Oct 2016

© 2016 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)
DOI: http://dx.doi.org/10.22159/ijpps.2016v8i12.13577

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-ethylbenzthiazoline-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 was significant inhibition zone (15.00±0.57 mm) and MIC value (0.15±0.15×10^-2 mg/ml) against the pathogenic bacteria Vibrio cholerae followed by Pseudomonas aeruginosa and Salmonella typhi. The antioxidant activity of essential oil was measured by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), 2, 2-azinobis-3-ethylbenzthiazoline-6-sulphonate radical cation (ABTS) and β-carotene bleaching assay.

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

INTRODUCTION

Litsea glutinosa is an endemic and threatened aromatic medicinal tree which belongs to the Lauraceae family and found to be 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 Bhadrak 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-ethylbenzthiazoline)-6-sulfonic acid (ABTS) was obtained from Sigma Chemical Co. (St. Louis, MO, USA), butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), trichloroacetic acid (TCA), folin-cioalteu 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 sulfite, 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 Bhadrak Wild Life Sanctuary of the central Western Ghats, Karnataka, India during December 2013. The plant specimen was identified and authenticated by Tarig 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
Determination of antioxidant activity

**DPPH radical scavenging activity**

The antioxidant activity of *L. glutinosa* stem bark essential oil was assayed through TLR method [16]. 5 µl 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 the 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 µg/ml) and the standard BHA 2-10 µg/ml were added to 0.004% methanolic solution of DPPH. After 30 min of incubation at room temperature (25±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 persulphate and kept for incubation overnight at room temperature under dark condition. 150 µl of essential oil and standard BHT at different concentrations were allowed to react with 2.85 µl 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 µl of linoleic acid and 200 µl 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 µl 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 (A1) 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±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±0.57 mm and MIC value of 0.15±0.15×10⁻² 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.-Elmene                                                             |
| 11    | 11.738  | 1260 | 0.22    | 9-Oxoonanoic acid                                                         |
| 12    | 12.412  | 1304 | 0.11    | IS, cis-calamenene                                                        |
| 13    | 12.707  | 1325 | 0.28    | Dodecaneic 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    | Oliec 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-
Table 2: Antibacterial activity of the *L. glutinosa* essential oil

| Microorganisms | Inhibition zone diameter (mm) and MIC (mg/ml) |
|----------------|-----------------------------------------------|
|                | Essential oil (5 µl/disc) | Gentamycin (10 µg/disc) | Ampicillin (10 µg/disc) |
| Gram-negative  | IZ | MIC | IZ | MIC | IZ | MIC |
| *E. coli*      | 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 |
| *P. aeruginosa*| 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 |
| *S. typhi*     | 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 |
| *V. cholera*   | 15.00±0.57 | 0.15±0.15×10⁻² | 34.00±1.00 | 7.81±1.00×10⁻³ | 44.33±0.66 | 4.16±1.00×10⁻³ |
| *K. pneumonia* | 10.33±0.33 | 3.12±0.41×10⁻² | 24.33±0.66 | 4.16±1.00×10⁻³ | ------ | 25.00±1.00×10⁻³ |
| Gram-positive  | IZ | MIC | IZ | MIC | IZ | MIC |
| *S. aureus*    | 09.66±0.33 | 12.5±0.01×10⁻² | 24.67±0.33 | 4.16±1.00×10⁻³ | 12.67±0.66 | 5.20±1.00×10⁻³ |
| *S. pneumonia* | 10.00±0.57 | 3.12±0.01×10⁻² | 23.33±0.88 | 4.16±1.00×10⁻³ | 08.33±0.33 | 16.6±1.00×10⁻³ |
| *B. subtilis*  | 11.33±0.33 | 3.12±0.10×10⁻² | 26.67±0.66 | 4.16±1.00×10⁻³ | 10.33±0.33 | 5.20±1.00×10⁻³ |

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).

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 4.54±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 that of BHA (93.42±0.40%) as shown in table 3.
L. 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, nematicide, insectifuge, hypcholesterolemic, cancer preventive, hepatoprotective, anti-histaminic, anti-acute, antifibrinolytic, anticancer, anti-inflammatory, antidermatitic, anti-bacterial, antioxidative, antiviral, antimicrobial, antitoxicogenic, and antmycotic activities [23]. The hexadecanoic acid (R/T 17.235) can be a hypocholesterolemic, nematicide, pesticide and lubricant substances and was also reported as a more effective free radical scavenger than β-carotene [23, 24]. (3β)-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, anti-diabetic, anti-inflammation, anti-cancer, anti-dermatitis, anti- leukemic, antiscar, hepatoprotective, hypocholesterolemic, anti-ulcerogenic, vasodilator, antispasmodic, anti-bronchitic, and anticoagulant activity [23]. Fruits, plants, and vegetables are the main sources of antioxidant vitamins (vitamin A, 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, antinsecticidal, anti-myocytic, 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 analysis of L. glutinosa stem bark oil is reported to have 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 gram-negative bacteria strain Vibrio cholera. The present study also 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 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 antidiarrheal 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 antidiarrheal 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 lipids deterioration by the microorganism spoilage [46, 47]. Fatty acids can also contribute 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 antidiarrheal 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.

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

| S. No | Activity | Concentration in µg/ml | % of Inhibition | IC50 µg/ml | Standard IC50 in µg/ml |
|-------|----------|------------------------|-----------------|-----------|------------------------|
| 1     | DPPH     | 2                      | 5.70±0.52       | 4.54±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      | 25.60±0.06| 4.44±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| 63.3±0.71              |

The results shown are averages of three independent experiments (n=3), values are mean±SEM.
will be correlated with the effect of the reference standard dug in the future investigation.

ACKNOWLEDGEMENT
The authors wish to thank the UGC New Delhi [F No. 4-10/2010 (BSR)] for providing financial assistance and the Administrative authority Kuvempu University for providing facility for carrying out the investigation. We thank to the faculty of Shiwamogga Institute of Medical Sciences, Shiwamogga, Karnataka, India for providing human pathogenic clinical isolates.

CONFLICT OF INTERESTS
We declare that we have no conflict of interest.

REFERENCES
We declare that we have no conflict of interest.

1. Kirtikar K, Basu B. Indian medicinal plants. Allahabad; 1981. p. 2156–61.
2. Anonymous. The wealth of India, Material: L-M, CSIR, India; 1998. p. 229–32.
3. Rajendra K, Shakti upaday. Free radical scavenging activity screening of medicinal plants from Tripura, North east India. Indian J Nat Prod Resour 2009;9:17–22.
4. Kar A, Menon MK, Chauhan CS. Effect of essential oil of Litsea glutinosa (Lour.) C. B. Robinson on cardiovascular system and isolated tissues. Indian J Exp Biol 1976;2:61–2.
5. Chowdhury JU, Bhuyan NI, Nandi NC. Aromatic plants of Bangladesh; Essential oils of leaves and fruits of Litsea glutinosa (Lour.) C. B. Robinson. Bangladesh J Bot 2008;37:81–3.
6. Deans SG, Waterman PG. In: Volatile Oil Crops: Their Biology, Biochemistry and Production. ed. R.K. Ram, Waterman PG. Longman, London. 1993. p. 113.
7. Bachir RG, Benali M. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. Asian Pac J Trop Biomed 2012;2:739–42.
8. Mahilrajn S, Nandakumar J, Kailayalingam R, Manoharan NA, SriVijeyand S. Screening the antithungal activity of essential oils against decay fungi from palmryah leaf handcrafts. Biol Res 2014;47:35.
9. Preedy, V. Essential oils in food preservation, flavor and safety. Elsevier; 2016.
10. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;7:417–33.
11. European Council. European pharmacopoeia. 8th ed. Strasbourg: 2014.
12. Sahoo S, Singh S, Nayak S. Chemical composition, antioxidant and antimicrobial activity of essential oil and extract of Alpinia malaccensis roscoe (zingiberaceae). Int J Pharm Pharm Sci 2014;6:183–8.
13. Performance standards for antimicrobial disk susceptibility tests. Wayne PA: National Committee for Clinical Laboratory Standards; 1997.
14. Moorthy K, Aparna aravind, Punitha T, Vinodhini R, Suresh M. In vitro screening of antimicrobial activity of Wrightia tinctoria (Roxb.) R. Br. Indian J Clin Res 2012;5:54–8.
15. Sarker SD, Nahar L, Kumarasamy Y. Micromute plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 2007;42:321–4.
16. Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae). Food Chem 2005;90:333–40.
17. Archana D, Dixita M, Santhy KS. Antioxidant and anticlastogenic potential of piper Longum L. Int J Appl Pharm 2015;7:1–4.
18. Re R, Pellicani N, Protegente A, Pannala A, Yang M. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 1999;26:1231–7.
19. Raaman N. Antioxidant activities and phytochemical analysis of methanol extract of leaves of Hygrophila auriculata (Schumach) heine. Int J Curr Pharm Res 2015;7:100–5.
20. Ebrahimabadi AH, Ebrahimabadi EH, Djaliri-Bidgoli Z, Kashi FJ, Mazoochi A. Composition and antioxidant and antimicrobial activity of the essential oil and extracts of Stachys inflata Benth from Iran. Food Chem 2010;11:9452–8.
21. Wang YS, Liao Z, Li Y, Huang R, Zhang HR. A new megastigmagpane diglycoside from Litsea glutinosa (Lour.) C. B. Rob. J Braz Chem Soc 2011;22:234–9.
22. Saleh MA, Clark S, Woodard B, Deolu-Sobogun SA. Antioxidant and free radical scavenging activities of essential oils. Ethnicity Disease 2012;20:78–82.
23. Kumar P, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. Afr J Biochem Res 2010;4:191–5.
24. Sermalkandi M, Thangapandian V. GC-MS analysis of Cassia italic leaf methanol extract. Asian J Pharm Clin Res 2015;1:5–9.
25. Sujatha S, Anand S, Sangeetha KN, Shilpa K, Lakshmi J. Biological evaluation of (3β)-stigmast-5-en-3-ol as potent anti-diabetic agent in regulating glucose transport using in vitro model. Int J Diabetes Mellit 2012;2:101–9.
26. Pidl S, Raccach D, Gerbi A, Pieroni G, Vague P. At low doses, a gamma-linolenic acid- lipidic acid conjugate is more effective than docosahexaenoic acid-enriched phospholipids in preventing neuropathy in diabetic rats. J Nutr 2007;137:368–72.
27. Doughari JH, Ndalikudzi PA, Human IS, Benade S. Antioxidant, antimicrobial and antiproteorotic potentials of extracts of Curtisia dentata. Int J Ethnopharmacol 2012;141:1041–50.
28. Cannae S, Molicotti P, Ruggeri M, Cubeddu M, Sanguinetti M. Antimycotic activity of Myrtus communis L. towards Candida spp. from isolates. J Infect Dev Cities 2013;7:295–8.
29. Rottan H, Zulagarmn M, Antaraya Y, Tan EC, Rahman NA. Screening of antimicrobial activities in medicinal plants extracts against dengue virus using dengue NS2B-NS3 protease assay. Trop Biomed 2014;31:286–96.
30. Sukphan P, Sritulanak M, Bekoonsongkrip W, Lipipun V, Likhittawanvay K. Chemical constituents of Dendroblom venustum and their antimarial and anti-herpetic properties. Nat Prod Commun 2014;4:925–7.
31. Agaromorothy G, Chandrasekarakan M, Venkatesalu V, Hsu MJ. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-eye mango from India. Brazilian J Microbiol 2007;38:739–42.
32. Raynor L, Mitchell A, Walker R. Antifungal activity of four fatty acids against plant pathogenic fungi. Mycopathologia 2004;157:87–90.
33. Shiy SN, Rajpae VK, Kim HR, Kang SC. Antibacterial activity of eicosapentaenoic acid (EPA) against foodborne and food spoilage microorganisms. LWT-Food Sci Technol 2007;40:515–9.
34. Sado-Kamdem SL, Vannini L, Guerzoni ME. Effect of alpha-linolenic, capric and lauric acid on the fatty acid biosynthesis in Staphylococcus aureus. Int J Food Microbiol 2009;129:288–94.
35. McGaw LJ, Jager AK, van Staden J. Isolation of antibacterial fatty acids from Schotia brachypetala. Fitoterapia 2002;73:431–3.
36. Seidel V, Taylor PW. In vitro activity of extracts and constituents of Pelagonium against rapidly growing mycobacteria. Int J Antimicrob Agents 2004;23:613–9.
37. Adwan K, Abu-Hasan N. Gentamicin resistance in clinical strains of Enterobacteriaceae associated with reduced gentamicin uptake. Folia Microbiol (Praha) 1998;43:438–40.
38. Koor GJ, Arora DS. Antibacterial and phytochemical screening of Anethum graveleons, Foeniculum vulgare and Trachyspermum ammi. BMC Complement Altern Med 2009;9:30.
39. Vardar-Unlu G, Candan F, Sokmen A, Dalkerera D, Polissiou M. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of Thymus pectinatus Fisch, et Mey. Var. pectinatus (Lamiaceae). J Agric Food Chem 2003;51:63–7.
40. Parikh PH, Rangrez AY. Extraction and phytochemical evaluation of Litsea glutinosa bark methanolic extract. Int J Appl Pharm 2012;4:7–8.
41. Gutteridge, JMC, Halliwell B. Antioxidants in nutrition, health, disease. Oxford University Press, New York; 1994.
42. Maxwell SR. Prospects for the use of antioxidant therapies. Drugs 1995;49:345–61.
43. Sies H. Role of reactive oxygen species in biological processes. Klin Wochenschr 1991;69:965–8.
44. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Clarendon Press; 1989.
45. Ito N, Fukushima S, Hagiwara A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. J Natl Cancer Inst 1983;70:343–52.

46. Sacchetti G, Maietti S, Muzzoli M, Scaglioni M, Manfredini S. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem 2005;91:621–32.

47. Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa miller (Lamiaceae). Food Chem 2005;90:333–40.

48. Kim SY, Jeong SM, Park WP, Nam KC, Ahn DU, et al. Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. Food Chem 2006;97:472–9.

49. Nguefack J, Leth V, Amvam Zollo PH, Mathur SB. Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. Int J Food Microbiol 2004;94:329–34.

50. Prakash B, Singh P, Kedia A, Dubey NK. Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and in vivo efficacy in food system. Food Res Int 2012;49:201–8.

How to cite this article
• Arunodaya HS, Krishna V, Shashikumar R, Girish Kumar K. Antibacterial and antioxidant activities of stem bark essential oil constituents of Litsea glutinosa C. B. Rob. Int J Pharm Pharm Sci 2016;8(12):258-264.