Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2015; 7(1): 96-101

ISSN- 0975 1556

Research Article

# Chemical Profile, Antioxidant and Antimicrobial Activity of Essential Oils from *Boswellia ovalifoliolata* Bal. et. Henry

Prasanna Anjaneya Reddy Lebaka[1,5*], Venkata Ratnam K[2], Bhakshu Md Lepakshi[3], Narasimha Reddy Bhumi[1], Veeranjaneya Reddy Lebaka[4]

[1]*Deparment of Botany, Osmania University, Hyderabad, Telangana - 500 007, India*
[2]*Deparment of Plant Sciences, University of Hyderabad, Hyderabad, Telangana - 500 046, India*
[3]*Department of Botany, Govt. College for Men, Kadapa, Andhra Pradesh - 516 004, India*
[4]*Deparment of Microbiology, Yogi Vemana University, Kadapa, Andhra Pradesh - 516 003, India*
[5]*Lipidomics Centre, CSIR Central Food Technological Research Institute, Mysoe, Karnataka – 570020,India*

*Available Online: 1st January. 2015*

**ABSTRACT**
To study the phytochemical profile, antimicrobial and antioxidant activity of essential oils obtained from *Boswellia ovalifoliolata* (BO) leaf, stem bark and gum resin samples. Phytochemical profiles of the three essential oils were analyzed by GC-MS, antimicrobial activity was performed against two Gram positive, two Gram negative and two fungal species by using disc diffusion method. Minimum inhibitory concentrations were assayed by micro dilution method. Further, antioxidant capacity of three oil samples were determined by DPPH scavenging method. Gas chromatographic analysis of BO three essential oil (EO) samples revealed that 30 compounds were identified in leaf oil and 25 compouds in each were identified in stem bark and gum resin EOs. The results showed that 11 compounds i.e. α –amarphene, δ-cadinene, caryophyllene oxide, caryophyllene, β-farnesene, α-humulene, ledol, γ-murrolene, β-myrcene β-pinene, and (-)-zingiberene were common compounds in three oil samples. Three essential oils showed good antimicrobial activity against test pathogens. Among the tested pathogens, *Candida rugosa* found to be most sensitive organism to leaf, stem bark and gum EO samples, while Gram positive bacterium *Bacillus subtilis* was most resistant organism to the test oils. Further, the oil samples showed concentration dependent DPPH reducing capacity. The present study revealed that, the essential oils of BO exerts beneficial effects to inhibit human pathogenic microorganisms and as a source of natural antioxidant compounds.
**Key words**: *B. ovalifoliolata*; essential oils; phytochemical profile; antimicrobial activity; antioxidant activity

## INTRODUCTION

*Boswellia ovalifoliolata* Bal & Henry, is an endemic plant, restricted to Seshachalam and Nallamala hill range of Eastern Ghats, India. BO gum has been used as an alternative source of incense of *B. serrata* Roxb ex Colebr gum, by the tribal people inhabiting in and around the forest areas of Seshachalam and Nallamala forest region[1]. It has been used in traditional medicine to cure different ailments like, stomach ache, rheumatic pains, ulcers, amoebic dysentery and as immunostimulant by adivasi tribes of Seshachalam hills[2]. Thorough review of available literature on phytochemical studies, resulted two new heptanoids were isolated with antibacterial activity from BO stem bark[3]. Different parts of BO were reported for pharmacological properties such as leaf for antibacterial activity[4], silver nanoparticles synthesized from BO stem bark for antifungal and antibacterial activity[5, 6] and antiinflammatory activity in rat models[7] indicates its potentiality as medicine.

There are very few reports on essential oil composition and antioxidant and antimicrobial activity of the genus *Boswellia*[8-11]. The earlier scientific reports on pharmacological properties of *Boswellia* species indicated that, to the best of our knowledge no previous report was noticed on essential oil composition and pharmacological activity of different parts of *B. ovalifoliolata*.

## MATERIALS AND METHODS

*Collection of Plant Material*

*B. ovalifoliolata* leaf, stem bark and gum were collected from Tirumala hills. The voucher specimens were identified by Prof. R. R. Venkata Raju, S.K. University Anantapur, with the help of regional and local floras[12, 13] and deposited in Botanical Survey of India (BSI) at Deccan Circle, Hyderabad (Voucher number BSID 000829).

*Isolation of Essential Oil*

The collected plant material was cut into small pieces and subjected to hydro-distillation for 3-5 h in Clevenger type apparatus and isolated the essential oil[14]. The essential oils were dried over anhydrous sodium shulphate and stored at 4 ºC until used for chemical analysis and biological activities.

**Author for Correspondence*

*Gas Chromatography and GC-Mass Spectroscopic studies (GC and GC-MS Analysis)*

The essential oils were analyzed by using Shimadzu GC-MS2010 gas chromatograph and mass spectrometer equipped with QP 2010 and DB-5 column (30m, 0.25mm id, film thickness 0.25 µm). Helium was used as carrier gas at flow rate of 1.67 ml/min. The injection port was maintained at 250 ºC; the detector temperature was 220 ºC; oven temperature was maintained 100-240 ºC (10º/min). The split ratio was 1:30 and ionization energy was 70eV. The retention indices were calculated relative to $C_8$-$C_{20}$ n-alkenes. The constituents of the oil were identified by comparison of retention indices with those reported in the literature[15], by matching the mass spectral data with those stored in NIST 05s libraries.

Table 1. Essntial oil composition of three oils from *B. ovalifoliolata* leaf, stem bark and gum resin

| Compound name | Retention Index | BOLEO (%) | BOSEO (%) | BOGEO (%) |
|---|---|---|---|---|
| 2-Hexanal (E) | 814 | 0.64 | - | - |
| α-Pinene | 948 | 21.55 | - | 15.53 |
| Camphene | 943 | 1.42 | - | - |
| β-Myrcene | 958 | 4.55 | 17.46 | 13.82 |
| β-Pinene | 943 | 16.85 | 1.75 | 3.29 |
| 2-carene | 948 | 0.21 | - | - |
| Trans-β-Ocimene | 976 | 3.51 | - | - |
| D-Limonene | 1018 | 3.61 | - | 0.36 |
| α-Phyllandrene | 964 | 0.86 | - | - |
| Cis-β-Ocimene | 976 | 3.37 | - | - |
| γ-Terpinen | 902 | 0.42 | - | - |
| Geranyl nitrile | 1231 | 0.35 | - | - |
| p-Menth-1-en-4-ol acetate | 1327 | 2.91 | - | - |
| Nonanal | 1104 | 0.22 | - | - |
| 1-Terpinene-4-ol | 1137 | 2.03 | - | - |
| α-Terpineol | 1143 | 14.27 | 0.73 | - |
| Myrcenol | 1136 | 0.50 | - | 0.31 |
| Eucalyptol | 1059 | 1.86 | - | - |
| (-)-Zingiberene | 1451 | 2.13 | 0.61 | 0.90 |
| Caryophyllene | 1494 | 11.78 | 16.17 | 28.50 |
| β-Farnesene | 1440 | 0.18 | 1.80 | 1.55 |
| α-Humulene | 1579 | 0.77 | 1.69 | 3.2 |
| α-Farnesene | 1458 | 0.49 | - | - |
| γ-Murrolene | 1435 | 1.16 | 1.14 | 0.72 |
| α -Amarphene | 1440 | 0.43 | 10.32 | 7.73 |
| δ-Cadinene | 1469 | 1.42 | 2.01 | 2.97 |
| Caryophyllene oxide | 1507 | 1.02 | 1.46 | 0.37 |
| Guaiol | 1614 | 0.56 | - | - |
| Ledol | 1530 | 0.75 | 5.06 | 7.82 |
| N-Butylpyrrole | 975 | 0.18 | - | 0.61 |
| Cyclohexane | 1500 | - | 1.26 | - |
| Cyclofenchene | 729 | - | 8.99 | - |
| Copane | 1221 | - | 0.39 | - |
| α-Bergamotene | 1430 | - | 6.66 | - |
| β- Bisabolene | - | - | 0.97 | - |
| β-Sesquiphyllandrene | 1446 | - | 1.63 | - |
| α-Longipinene | 1403 | - | 0.54 | - |
| Cedr-8-ene | 1403 | - | 7.99 | - |
| α-Santalol | 1454 | - | 0.58 | - |
| γ-Eudesmol | 1626 | - | 0.89 | 0.59 |
| Hedycaryol | 1694 | - | 2.27 | - |
| Viridiflorol | 1530 | - | 3.39 | 6.84 |
| Farnesyl acetate | 1834 | - | 4.03 | - |
| Decalin | 1101 | - | - | 0.44 |
| Cineol | 1059 | - | - | 0.30 |
| Linalool | 1082 | - | - | 1.62 |

| | | | | |
|---|---|---|---|---|
| γ-Selinene | 1502 | - | - | 0.40 |
| Verbinone | 1119 | - | - | 0.32 |
| Azulene | 1490 | - | - | 0.52 |
| 1,4 Methano azulene | 1398 | - | - | 0.52 |
| Steviol | 2269 | - | - | 0.76 |

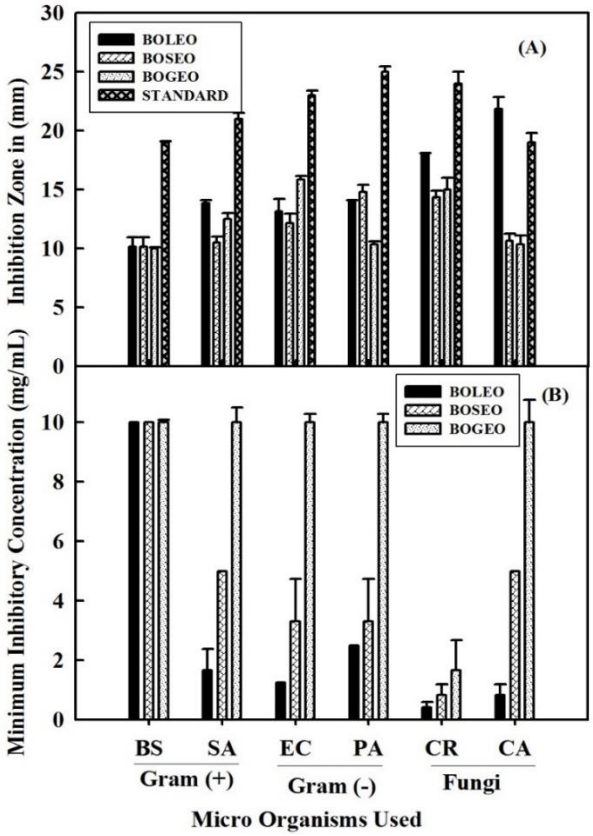


Figure 1: A. Antimicrobial activity of *B. ovalifoliolata* leaf, stem bark and gum resin essential oils, B. MIC of *B. ovalifoliolata* leaf, stem bark and gum resin essential oils against 4 bacteria and 2 fungi. BS: *Bacillus subtilis*, SA: *Staphylococcus aureus*, EC: *Escherichia coli*, PA: *Pseudomonas aeruginosa*, CR: *Candida rugosa* CA: *C. alibicans*.

*Antimicrobial studies*

*Test microorganisms:* The *in vitro* antimicrobial activity of the essential oil samples was studied against two Gram-positive bateria, *Bacillus subtilis* (BS - MTCC 1429), *Staphylococcus aureus* (SA - MTCC 737), two Gram-negative, *Escherichia coli* (EC - MTCC 1687), *Pseudomonas aeruginosa* (PA - MTCC 1688) and two fungal strains, *Candida albicans* (CA - MTCC 227), *Candida rugosa* (CR - NCIM 3462) by disc diffusion (Kirby-Bauer) method recommended by the NCCLS (2002). The continuous culture of test organisms were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh and National Collection of Industrial Microbiolgy (NCIM) NCL Pune, India. Test organisms (bacteria and fungi) were maintained on the nutrient agar (NA) and potato dextrose agar (PDA) respectively. The test organisms were sub cultured in to the fresh media prior to the testing antimicrobial studies.

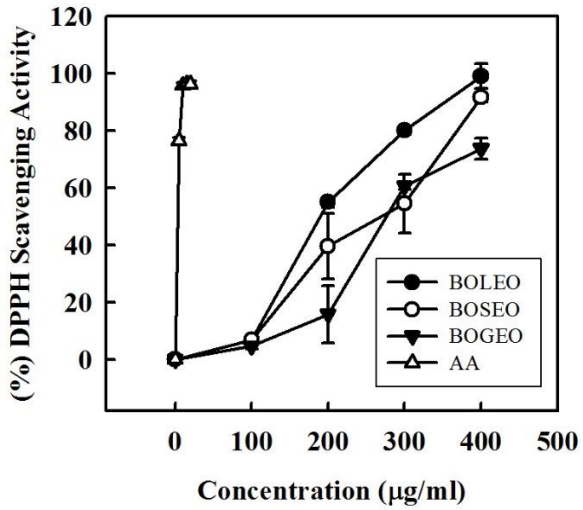


Figure 2: DPPH quenching activity of *B. ovalifoliolata*, oil samples (leaf, stem bark and gum resin). BOLEO: *B. ovalifoliolata* leaf essential oil, BOSEO: *B. ovalifoliolata* stem bark essential oil, BOGEO: *B. ovalifoliolata* gum resin essential oil, AA: Ascorbic acid.

*Antimicrobial assay:* The Nutrient agar (NA) and potato dextrose agar (PDA) medium (15%) was suspended in double distilled water (1000 mL) and heated to boiling until it dissolvs completely, the medium and Petri dishes were sterilized in a autoclave at pressure of 15 lb/inc$^2$ for 20 min.**.** The medium was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. After solidification of medium in plates, 0.5 ml of overnight culture of test organism was uniformly spread over the agar surface with a sterile L-shaped glass rod. Ten micro liters of the each oil sample of leaf, stem bark and gum resin were impregnated on to 6 mm sterile discs and placed on the pre seeded nutrient agar surface of the Petri dishes. The respective controls were maintained with DMSO (negative control) and standards (positive control) Penicillin, Streptomycin (30µg/disc) and Amphotericin B (30µg/disc) for bacterial and fungal species. The treated and the control Petri dishes were incubated at 35 ± 2 °C for 24 h for bacterial strains, while 28 ± 2 °C, 48 h for fungal strains. After

incubation, inhibition zones were measured and the diameter was calculated in millimeter using a metric scale. Three replications were maintained for each treatment.

*Determination of Minimum Inhibitory Concentration (MIC) of essential oils:* The minimum inhibitory concentration (MIC) was assayed by broth micro dilution method with slight modifications by using 96-well micro titer plate[16, 17]. A Micro plate with Mueller Hinton broth media (100µl) was added to 1-9 wells of 96 well micro titer plate. The test compound was dissolved in DMSO and concentration of 10 mg/ml of the test compound was added to the first well, which is serially diluted from 1 to 8 and the 9 well acts as control (concentrations ranged from 10 mg to 0.08 mg/ml). A fixed volume of 100µl overnight culture was added in all the wells and incubated at 37º C for 24 h for bacterial and 48 h for fungal species. The MICs were determined as the lowest concentration of oil inhibiting visible growth of each organism on the agar plate. After incubation period the micro plate was measured for turbidity with spectrophotometer (Tecan M200 infinite Micro Plate Reader) at 600 nm. Inhibition of bacterial growth in each well of the micro plate containing test oil was judged by comparison with growth in blank control plates. For fungal species MIC determined using the potato dextrose agar plates pre-treated with different concentrations of the essential oil and observed for visible colonies. The MICs were determined as the lowest concentration of oil showed inhibition of test organism[18]. Experiments were carried out in triplicate.

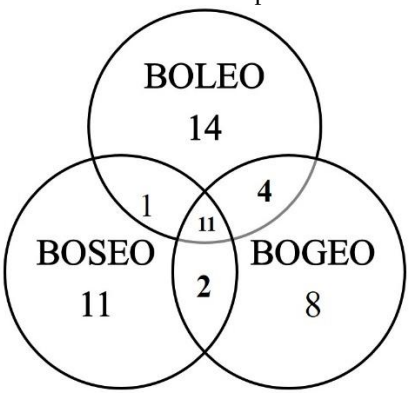


Figure 3: Common essntial oil compounds from *B. ovalifoliolata* leaf, stem bark and gum resin. BOLEO: *B. ovalifoliolata* leaf essential oil, BOSEO: *B. ovalifoliolata* stem bark essential oil, BOGEO: *B. ovalifoliolata* gum resin essential oil.

*Antioxidant activity*

*Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity:* The quenching effect of BO essential oil samples was assayed using the stable radical, 2,2-diphenylpicrylhydrazyl (DPPH), as a reagent[19, 20] by spectroscopic method. Twenty milligrams of oil samples were dissolved in 1 ml methanol. From this 5, 10, 15 and 20 µl were added to 1 ml of 0.004% methanol solution of DPPH. The discoloration of DPPH purple color in the presence of oil sample/standard indicated free radical scavenging activity. After 30 min incubation period at room temperature, the absorbance was measured against a blank at 517 nm. The percent (%) DPPH reducing capacity of BO oil samples was calculated by using the following formula:

$\% = [(A_c-A_s)/A_c] \times 100$

Where $A_c$ is the absorbance of the control reaction (containing all reagents except the test sample/standard), and $A_s$ is the absorbance of the test sample. Ascorbic acid was used as standard compound. The concentration of the oil sample required for 50% inhibition ($IC_{50}$) was calculated form the standard graph plotted of inhibition percentage against extract concentration. Tests were carried out in triplicate and mean values were tabulated along with standard error.

*Statistical Analysis:*

All Experiments were performed in triplicate, and mean value was calculated. The means were analyzed by two-way analysis of variance (ANOVA) used Window stat 8.5 advanced level statistical software. The results were expressed critical difference (CD) at 5% were considered as significant.

## RESULTS

*Chemical composition of B. ovalifoliolata leaf, stem bark and gum resin essential oils*

Fresh materials each 1 kg of leaves, stem bark and gum resin of *B. ovalifoliolata* were subjected to hydro distillation in Clevenger type of apparatus for 3-5 hours. Three samples yielded pale yellow color, greenish pale yellow and colorless oils with characteristic flavor. The yields of three essential oils (Eos) were calculated based on fresh weights of the plant samples *i.e*. BO leaf essential oil (BOLEO) – 0.17%, stem bark oil (BOSEO) – 0.2% and gum resin oil (BOGEO) – 5% respectively.

Gas chromatographic analysis of EO samples revealed the presence of 30 compounds in BOLEO and 25 compounds in each were identified in stem bark and gum resin EOs (Table 1). The major compounds found in leaf essential oil were α-pinene (21.55%), β-pinene (16.85%), α-terpineol (14.27%) and caryophyllene (11.78%), stem bark oil contain β-myrcene (17.46%), cyclofenchene (8.99%), caryophyllene (16.17%), α-amarphene (10.32%) and cedr-8ene (7.99%) and gum resin oil was dominated by caryophyllene (28.50%), α-pinene (15.53%), β-myrcene (13.82%) and ledol (7.82%).

*Antimicrobial screening*

The effect of three essential oils (BOLEO, BOSEO and BOGEO) were screened against two Gram positive, two Gram negative bacterial and two fungal strains by disc diffusion method. The results from the disc diffusion method revealed that BOLEO strongly inhibited CA and CR (21.83±1.04 mm and 18.0 ±1.00 mm), PA and CR were found to be highly sensitive to BOSEO (14.83±0.58 mm and 14.33 ±0.58 mm) whereas BOGEO strongly suppressed the

growth of EC and CR (15.83±0.29 mm and 15.00 ±1.00 mm) (Figure 1A).

Further, minimum inhibitory concentration (MIC) values of three EOs against the test pathogens were as follows, CR and CA expressed very less MIC values to BOLEO (0.43 and 0.83 mg/ml), whereas BOSEO and BOGEO both strongly inhibited growth of CR by expression of lowest MIC values (1.04 and 1.67 mg/ml) respectively (Figure 1B).

*Antioxidant activity*

Essential oils obtained from three samples of BO i.e. leaf, stem and gum resin were assayed using DPPH scavenging activity by spectrophotometric method. Three EOs as well as standard drug control, ascorbic acid exhibited dose dependent reducing activity on DPPH radical. Of these oils samples BOLEO showed strong inhibition (99%) on DPPH over other oil samples and ascorbic acid (Figure 2) indicates its significant antioxidant potentiality.

## DISCUSSION

The present investigation has been focused on evaluation of chemical constituents, antioxidant potential and antimicrobial activity of BO based on its popular usage in ethno medicine/folk medicine practiced by adivasi tribes. Gas chromatographic analysis of three essential oils (BOLEO, BOSEO and BOGEO) revealed that the oils contained a complex mixture of compounds mainly monoterpene hydrocarbons, oxygenated mono and sesquiterpenes. A total of 30 compounds were identified in BOLEO, whereas 25 compounds identified from BOSEO and BOGEO, constituting 100%, 99.79% and 99.38% respectively. BOLEO was predominant in monoterpene compounds (76.27%), while BOSEO and BOGEO dominated by sesquiterpene compounds by 57.08% and 54.14% respectively. Comparative study of three oil samples showed that 11 compounds i.e. α –amarphene, δ-cadinene, caryophyllene oxide, caryophyllene β-farnesene α-humulene, ledol, γ-murrolene, β-myrcene β-pinene, and (-)-zingiberene were common compounds in three oil samples. Each oil sample possess unique compounds i.e. fourteen present in BOLEO, 11 in BOSEO and 8 in BOGEO respectively. Four compounds i.e. α- pinene, D-limonene, myrcenol and N-butylpyrrol present in leaf-gum resin oils, α-terpineol present in leaf and stem bark oils and γ-eudesmol and viridiflorol were commonly found in stem bark and gum oils (Figure 3). Essential oils of *Boswellia* species were reported to possess antimicrobial, antioxidant and anticancer activities[9, 10, 11, 22] supports the present investigation. The potential antibacterial and antifungal activities of BO essential oils was found to be attributed to the presence of terpene compounds. Among the tested pathogens, *C. rugosa* found to be most sensitive organism to BOLEO, BOSEO and BOGEO samples, while Gram positive bacterium BS was most resistant organism to the test oils (Fig. 2 A and B). Of the tested oil samples, BOLEO showed strong inhibition on CR, CA, EC, SA and PA respectively. The presence of α and β-pinene as major compounds in BOLEO, may be attributed to its strong antifungal and antibacterial activity [22]. These two compounds are able to destroy cellular integrity of fungal and bacterial species [23]. β-myrcene and caryophyllene compounds found to be dominant in stem bark oil, may be responsible for its antibacterial and antifungal activities. Three compounds caryophyllene, α-pinene and β-myrcene were major compounds in gum resin oil, attributed for its biological activities[23, 27]. The hydrogen atom-or-electron donation ability of essential oils was measured from the bleaching of the purple colored methanol solution of DPPH. The results showed that all the tested oil samples have the strong reducing capacity to DPPH purple color in concentration dependent manner. Among the test samples BOLEO strongly reduced DPPH purple color than other oil samples ($IC_{50}$ value 200 µg/ml). The strong antioxidant capacity of BOLEO might be attributed to the presence of α and β-pinene as major compounds.

## CONCLUSION

The phytochemical analysis of BO three essential oil (EO) samples revealed that leaf oil sample have more (30) compounds than stem bark and gum resin oils. The results showed that 11 compounds i.e. α –amarphene, δ-cadinene, caryophyllene oxide, caryophyllene, β-farnesene, α-humulene, ledol, γ-murrolene, β-myrcene β-pinene, and (-)-zingiberene were common compounds in three oil samples. of the three tested oil samples, leaf oil showed strong antimicrobial activity against the test pathogens and strong DPPH quenching activity. Among the tested pathogens, *Candida rugosa* found to be most sensitive organism to leaf, stem bark and gum EO samples, while Gram positive bacterium *Bacillus subtilis* was most resistant organism to the test oils. The present investigation also provides strong support to the usage of BO as a medicament. The use of artificial mixture of potential components based on the phytochemical composition may provide control over the unprecedented dependence on *B. ovalifoliolata* an endangered and endemic plant which may provide the solution for its conservation. Moreover it serves as a good source for natural antioxidant and antibiotic compounds.

## ACKNOWLEDGMENTS

The author, LMB is thankful to (UGC- SERO, Hyderabad, F.No. MRP- 4851/14) for financial assistance.

## REFERENCES

1. Ahmedullah M., Nayar M. Endemic plants of the Indian region. Botanical Survey of India. Calcutta. 1986; 1:73.
2. Savitramma N, Sulochana C. Endemic medicinal plants from Tirumala hills, Andhara Pradesh, India. Fitoterepia 1998; 69: 253–254.
3. Reddy VLN, Ravinder K, Srinivasulu M, Venkateshwar GT, MallaReddy S, Srujankumar D, Prabhakar RT, Suryanarayana MU, Venkateswarlu Y. Two New MacrocyclicDiaryl Ether Heptanoids from *Boswellia*

*ovalifoliolata*. Chem Pharm Bull 2003; 51(9): 1081-1084.
4. Ratnam, VK, VenkataRaju, RR. In vitro antimicrobial activity of leaf extracts of *Boswellia ovalifoliolata*. Trop Med Plants 2008; 9(1): 27-9.
5. Savithramma N, Rao ML, Basha SKM. Antifungal efficacy of silver nanoparticles synthesized from the medicinal plants. Der Pharma Chemica 2011a; 3 (3): 364–72.
6. Savithramma N, Linga Rao M, Suvarnalatha Devi P. Evaluation of antibacterial efficacy of biologically synthesized silver Nanoparticles using stem barks of *Boswellia ovalifoliolata* Bal. and Henry and *Shorea tumbuggaia* Roxb. *J Biolog Scien* 2011b; 11(1): 39 – 45.
7. Sakunthala Devi PR, Adilaxmamma K, Srinivasa Rao G, Srilatha Ch, Alpha Raj M. Evaluation of anti-inflammatory activity of stem-bark of Boswelia ovalifoliata in rats. [Last accessed on 2010 Sept 28]; Inventi Rapid: *Ethanopharmac*. 2010; 1:ep159. Available from: http://www.inventi.in.
8. Yuxin Z, Zhangchi N, Cheng Lu, Siyu Z, Jianfen W, Baoqin L, Xuegong X, Yuanyan L. Triterpenoid resinous metabolites from the genus Boswellia: pharmacological activities and potentialspecies-identifying properties. Chemis Cent J 2013; 7:153.
9. Chen Y, Zhou C, Ge Z, Liu Y, Liu Y, Feng W, Li S, Chen G, Wei T. Composition and potential anticancer activities of essential oils obtained from myrrh and frankincense. Onco Let 2013; 6: 1140-46.
10. Hussain H, Ahmed AH, Ahmed AR, Javid H. Chemistry and Biology of Essential Oils of Genus *Boswellia.* Evidence-Based Complementary and Alternative Medicine 2013; ID 140509: 1-12.
11. Bekana D, Kebede T, Assefa M, Kassa H. Comparative Phytochemical Analyses of Resins of *Boswellia* Species (*B. papyrifera* (Del.) Hochst, *B. neglecta* S. Moore, and *B. rivae* Engl.) from Northwestern, Southern, and Southeastern Ethiopia. ISRN Analytical Chemistry 2014; ID 374678: 9.
12. Gamble JS. *Flora of Presidency of Madras*. Vol. I – III. Botanical Survey of India, Calcutta; 1915 – 1935.
13. Pullaiah T, Chennaiah E, Moulali A: *Flora of Andhra Pradesh (India)-I*. Scientific Publishers, Jodhpur; 1997.
14. Clevenger JF. Apparatus for determination of essential oil. J Am Pharm Assoc 1928; 17: 346-349.
15. Adams R.P, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing, Carol Stream, IL. 2001.
16. Villanova. National Committee for Clinical Laboratory Standards (NCCLS), Standard methods for dilution antimicrobial susceptibility tests for bacteria, which grows aerobically, 1982; 242.
17. NCCLS. Development of in vitro susceptibility testing and quality control parameters. Approved guideline, 2nd ed. NCCLS document M23-A2. NCCLS, Wayne, Pa. 2001.
18. Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Inter J Food Microbiol 2002; 74: 101–109.
19. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytotheraphy Res 2000; 14: 323–328.
20. Cuendet M, Hostettmann K, Potterat O. Iridoid glucosides with free radical-scavenging properties from *Fagraea blumei*. Helvetica Chimica Acta 1997; 80: 1144–1152.
21. MaheAlam, Hakimuddin KL. Samiullah K, Siddique M. A review on Phytochemical and Pharmacological studies of Kundur (*Boswellia serrata* Roxb ex Colebr.) -A Unani drug. J Appl Pharmac Scien 2012; 2 (3): 148-156.
22. da Silva ACR, Lopes PM, de Azevedo MMB, Costa DCM, Alviano CS, Daniela Sales ADS. Biological Activities of α-Pinene and β-Pinene Enantiomers. Molecules 2012; 17: 6305-6316.
23. Magwa LM, Gundidza M, Gweru N, Humphrey G. Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J Ethnopharm 2006; 103: 85–89.
24. Houghton PJ. Activity and constituents of Sage relevant to the potential treatment of symptoms of Alzheimer's disease. Herbal Gram 2004; 61: 38-54.
25. Knobloch K, Pauli A, Iberl B, Weigand H, Weis N. Antibacterial and antifungal properties of essential oil components. J Essen Oil Res 1989; 1: 119–128.
26. Cristani M, Arrigo M, Mandalari G, Castelli F, Sarpietro MG, Micieli D, Venuti V, Bisignano G, Saija A, Trombetta D. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. J Agric Food Chem 2007, 55: 6300–6308.
27. Leandro LM, Vargas FS, Barbosa PCS, Neves JKO, Silva JA, Veiga VF. Chemistry and biological activities of terpenoids from Copaiba (*Copaifera* spp.) oleoresins. Molecules 2012; 17: 3866-3889.