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Research Article

EVALUATION OF PHYTOCONSTITUENTS AND ANTIBACTERIAL POTENCY OF FRUITS OF *BOSWELLIA OVALIOFOLIOLATA*

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ABSTRACT
The aim of this study was to investigate the existence quantification of potent phytochemicals and *in vitro* antimicrobial activity of immature fruits of *Boswellia ovalofoliolata* aqueous fractions. Phytochemical analysis was performed to screen alkaloids, steroids, saponins, saponins, carbohydrates, flavonoids, polyphenols, tannins and glycosides. Quantification for Lipids, Phospholipis and Glycolipids were done using Thin Layer Chromatography. The aqueous extract of *Boswellia ovalifoliolata* of 25,50,75 and 100 mg/ml were tested using agar well-diffusion method against different bacterial pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and *E. coli* for their antimicrobial activity. Results revealed that aqueous fruit extract of *B. ovalfoliolata* was active against four bacteria and can be used as antimicrobial phytomedicine which can be therapeutically used against infections caused by tested microbes in our study.

***Key Words*:** *Boswellia ovalofoliolata, Phytochemical analysis, Antimicrobial activity, Tirumala Hills.*

INTRODUCTION
Phytochemicals are structurally distinct from microbially derived antibiotic natural products, it is likely that this chemical uniqueness will give rise to classes of antibacterials which have modes of action distinct from existing compounds. Plant-derived antibacterial also have potential topical materials rather than systemic drugs. There are considerable benefits to the topical route over the conventional systemic drug, in particular the speed to market and smaller amount of data needed to achieve this. phytochemicals display new mechanisms of action, or activity toward some of the newer targets. Scope of exploiting phytochemicals in dealing with drug-resistant bacteria has been reviewed by Gibbons (2008).
Boswellia ovalifoliolata N.P. Balakr. & A.N. Henry (Burseraceae) is a narrow endemic and endangered deciduous tree species. Its flowering, fruiting and seed dispersal events occur in a leafless state during the dry season. Leaf flushing occurs almost at the end of fruiting. In a few trees, leaf flushing is little bit early when fruits are still green and young. Natural fruit set rate is 9.3±4.63 (Range 2–24) at inflorescence level The average flower to fruit ratio is 3.7: 1. The fruit is initially light green simple septicidal trigonous capsule. The fruits dehisce along the septa to disseminate seeds into the air by the end of May (Raju, 2012). Chetty KM *et al*. (2013) reported that *B. ovalifoliolata* occur at the foothills of Seshachalam hill ranges of Eastern Ghats. Morphology of the entire plant has been studied by Kumar *et al* (2011). *Boswellia ovalifoliolata* acts as antihypercholesteromic agent (Satish Kumar *et al* 2014).In addition to the research done on *B. ovalifoliolata*, we are interested in quantifying the bioconstituents of immature fruits in order to screen for novel biomolecules.

MATERIALS AND METHODS
Plant material
Fresh immature fruits were collected from 2nd Ghat road, Tirumala Hills, Chittoor dt, Andhra Pradesh. The details of the plant were documented in the herbarium along with voucher number NBKR/BH/BR-211. and were authenticated by Taxonomist Dr. Madhava chetty, Department of Botany, Sri Venkateswara University, Tirupati.
Fresh material were washed in tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Figure 1: Tree Habitat of *Boswellia ovalifoliolata*

Figure 2: Immature fruits of *B. ovalifoliolata*

Preparation of Aqueous Extract

Freshly collected immature fruits were dried in shade and pulverized to a coarse powder and extracted with water using the soxhlet apparatus. The filtrate obtained was evaporated to dryness at 50-65°C in a rotary vacuum evaporator to obtain a molten mass. The obtained extracts were stored at 4^0C in refrigerator and were dissociated in dimethyl sulfoxide prior to use. Finally 200g of material was extracted in 500 ml of distilled water giving a concentration of 80 mg/0.1 ml. It was then autoclaved at 121^0C and 15 lbs pressure and stored at 4^0C. (Vaghasiya and Chanda, 2007)

Phytochemical analysis of collected plant extracts

The Phytochemical analysis was performed according to the methods described by Harborne (1998) with slight modifications Screening for the presence of the active ingredients in the aqueous fractions of *B. ovalifoliolata* were done for the presence of Steroids, terpinoids, tannins, flavonoids, alkaloids, saponins and glycosides.

Screening Of Lipid Compounds By Thin Layer Chromatography (TLC)

Thin layer plates were prepared by spreading a slurry of silica gel-G (50 g in 100 ml distilled water) to 0.5 mm thickness on thin glass plates with the help of spreader (Desaga Co., Heidelberg, Germany; Model No. 611). The plates were air dried and stored at room temperature. The plates were activated by heating them at 110°C for 30 minutes in a hot air oven, just before use. About 1 g equivalent of lipid extract was spotted on TLC plates with the help of micropipette. The spot areas were dried immediately with the help of a hair drier. Then the plates were run in unidimensional ascending chromatography by using flat rectangular TLC glass chambers. The chambers were saturated with the developing solvents one day before the plates developed. Solvent systems are Chloroform: Methanol : Acetic acid : Water (170:25:25:3 v/v/v/v) and Acetone : Benzene : Water (91:30:8 v/v/v)

The plates were placed in the chambers and made airtight. The developed plates were removed and then dried at room temperature and exposed to iodine vapours to visualize all the lipid compounds.For the detection of various lipids separated on the TLC they were sprayed with the following chromogenic spray reagents with the help of an automizer. The lipids were identified by comparison of R_f values, colours and with those of the authentic samples by co-chromatography (Devi, 2014)

The glycolipids and phospholipids were identified by using specific colour developing reagents as method followed by Skipski *et al.,* (1965).

Antimicrobial Studies

Antimicrobial assay were done according to agar well diffusion method (Murray *et al.,* 1995 later modified by Olurinola, 1996)

Microorganisms

The standard microorganisms *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis and E. coli* were obtained from the Department of Microbiology, Sri Venkateswara University, Tirupati. The bacterial strains were maintained regularly sub cultured on same nutrient agar medium and stored slants at

4^0C.Media preparation and Bacterial inoculums were prepare according to the method followed by Devi SL(2013).

Antibacterial activity of the plant extract

The prepared culture plates were inoculated with different selected strains of bacteria (*Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, E. coli)* (each plate with single selected bacterial strain grown as lawn) using streak plate method followed by Devi SL (2013). The extracts with different concentrations (25, 50, 75 and 100 mg/ml)were loaded into the wells. The plates were then kept in refrigerator for 15min. to allow diffusion of the extracts into the media. The plates were incubated 24 hours at 37±2^{o}C. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were scored after the inhibition zone formation around the well in three different fixed directions in all three replicates and the average values were tabulated.

Test against standard controls

Commercially available antibiotic Gentamycin (1.0 mg/ml) were used as standard control for the entire test microorganism. The sensitivity patterns were recorded and the readings were interpreted according to the critical diameter given by National Committee for Clinical Laboratory Standards (NCCLS, 1997).

RESULTS

Phytochemical evaluation

Results showed were tabulated in Table 1 and 2 for phytochemical analysis.

Table 1: Phytochemical Analysis

phytochemical test	*Boswellia ovalifoliolata*
Saponins	++
Alkaloids	+
Terpenoids and steroids	+++
Lignins	++
Tannins	+
Indoles	-
Anthroquinones	-
Proteins	+
Carbohydrates	++
Anthocyanidins	+
Phenolic compounds	+++
Flavonoids	++

Table 2: Preliminary Quantification of Lipids

Lipid Compound	***Boswellia ovalifoliolata***
Phosphatidic acid	-
Phosphatidyl serine	-
Phosphatidyl inositol	++
Phosphatidyl choline	+
Phosphatidyl ethanolamine	-
Digalactosyl diglyceride	+++
Phosphatidyl glycerol	+
Unidentified galactolipid	+
Sulphoquinovosyl diglyceride	++
Diphosphatidyl glycerol	+
Monogalactosyl diglyceride	+
Steryl glycoside	+
Unidentified lipid (U_1)	+++

+++ = Present in high quantity; *++ = Present in appreciable quantity*; *+ = present in low quantity.*

Lipids detected are ***a***. phosphatidic acid, c. phosphatidyl inositol, d. phosphatidyl choline, f. digalactosyl diglyceride, g. phosphatidly glycerol, h. unidentified galactolipid, i. sulpho-quinovosyl diglyceride, j. diphosphatidyl glycerol,k. monogalactosyl diglyceride, l. steryl glycoside, m. unidentified lipid (U_1).

Phospholipids detected are c. phosphatidyl inositol, d. phosphatidyl choline, g. phosphatidyl glycerol, j. diphosphatedyl glycerol.

Glycolipids detected are f. digalactosyl diglyceride, i. sulphoquinovosyl diglyceride, k. monogalactosyl diglyceride.

Figure 2 (2.1,2.2,2.3) : Thin Layer Chromatography

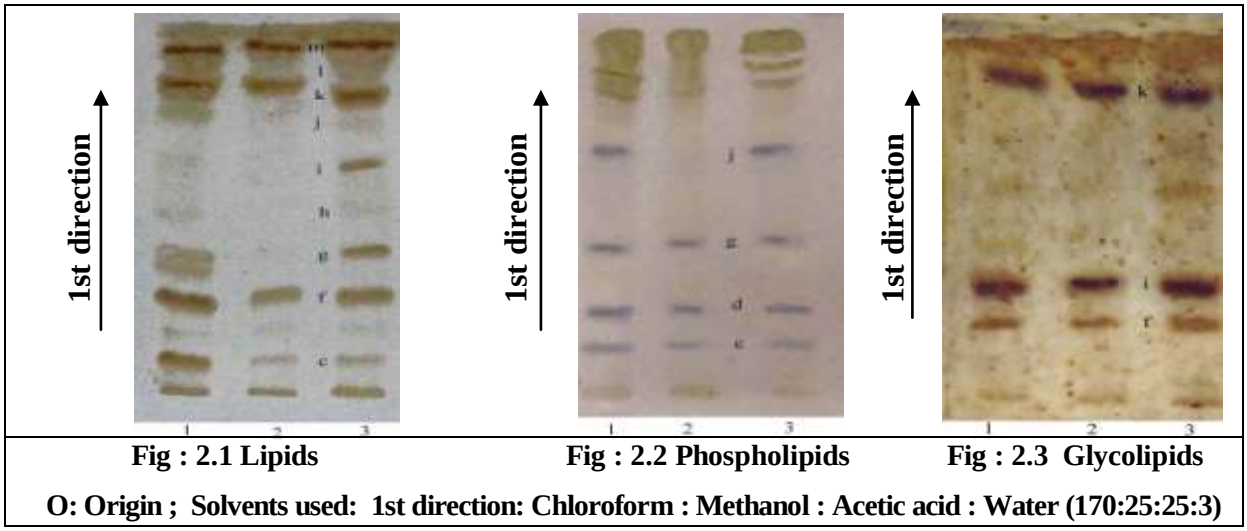


Fig : 2.1 Lipids **Fig : 2.2 Phospholipids** **Fig : 2.3 Glycolipids**

O: Origin ; Solvents used: 1st direction: Chloroform : Methanol : Acetic acid : Water (170:25:25:3)

Amino Acids detected are Arginine, Asparagine, α-Alanine, β-Alanine, Cysteine, Cystine, Glutamic acid, Glutamine, Glycine, Histidine, Leucine, Lysine, Proline, Valine. Anthocyanidin compounds detected are Peonidin, Malvindin, Petunidin, Delphinidin. Flavonoids Compounds detected are Rutin, Quercetin, Kaempferol, Luteolin, Apigenin ,Orientin, Vitexin

Figure 3 (3.1,3.2,3.3,3.4,3.5,3.6): *Antimicrobial Evaluation*

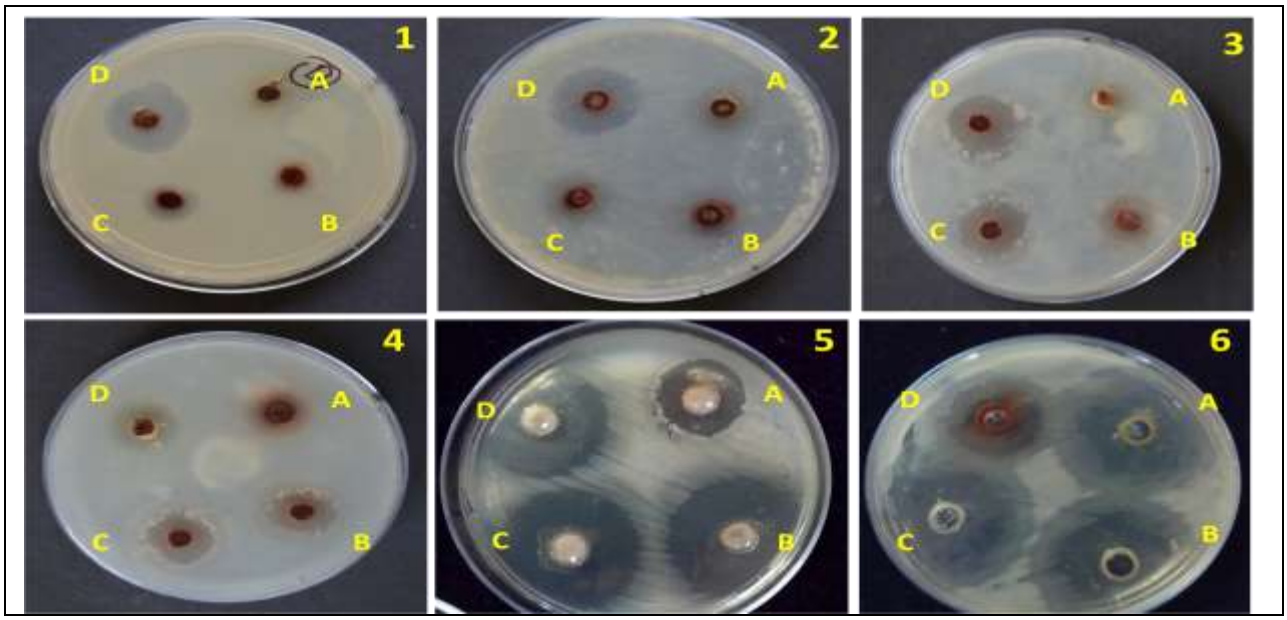
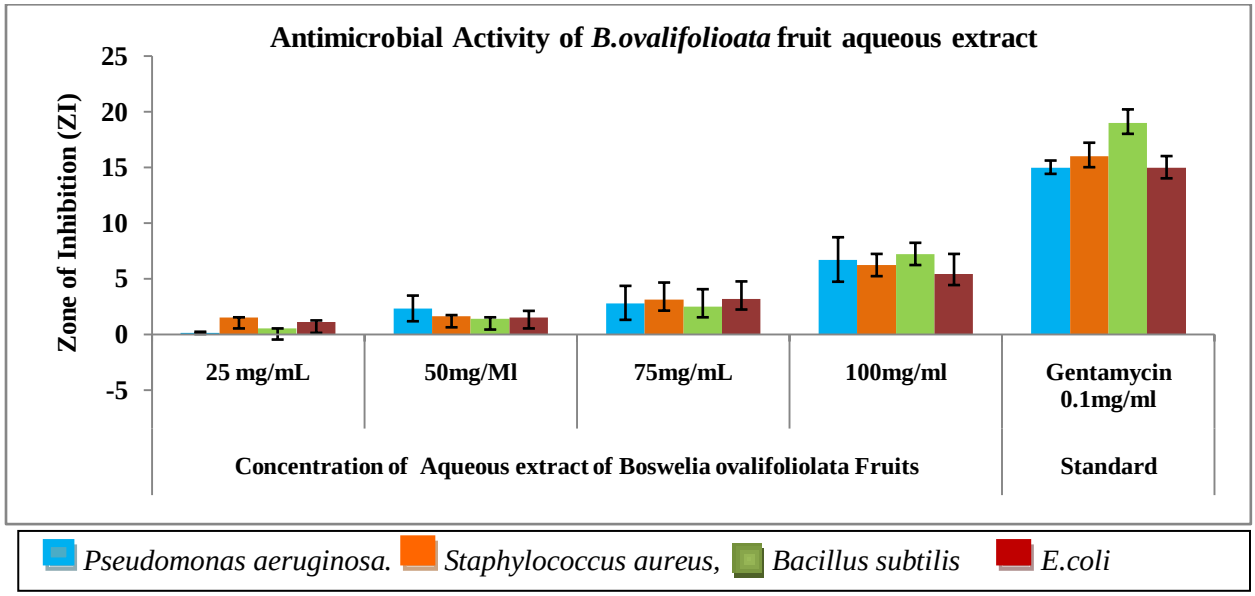


Figure 3.1-3.4 : Plates (1-4) **1.** *Pseudomonas aeruginosa 2. Staphylococcus aureus 3. Bacillus subtilis 4. E.coli* . Plate 5 – Standard Antibotic (Gentamycin 0.1mg/ml); Plate 6- Antibiotic + Extract 75mg/mL on *Bacillus subtilis*; A :25 mg/ml , B: 50 mg/ml c: 75 mg/ml D: 100 mg/ml.

The results of antimicrobial activity of aqueous extracts of *Boswellia ovalifoliolata* are shown in Figure 4 & 5. This is a first report of in vitro antimicrobial activity of aqueous extracts of *Boswellia ovalifoliolata*. The maximum zone of inhibition i.e. 7mm by *Pseudomonas aeruginosa* and *Bacillus subtilis*, whereas 6mm of inhibition by *Staphylococcus aureus and E.coli.*

Figure 4: Graphical representation Antimicrobial Activity of *B. ovalifolioata* fruit aqueous extract



DISCUSSION

Plant antimicrobials also offer potentially new classes of agents to deal with the threat of biowarfare (Gibbons, 2008). Attention to the discovery of novel plant antimicrobials must be paid in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles (Cowan, 1999).Tests to determine the concentration of solvent above which toxicity occurs should always be carried out before the experiment proper, and controls with potential solvent toxicity in mind should be incorporated into the experiment (Wadhwani *et al*., 2009; Houghton and Raman, 1998). Pathogen resistance to synthetic drugs and antibiotics already in use makes search for plants with antimicrobial activity more important, as they can substitute for synthetic antibiotics and drugs (Kothari *et al*., 2010).

The results revealed that the extracts showed moderate to high antimicrobial activity against all the tested microbial strains. The antimicrobial activity was evaluated from the zone of inhibition. With increase in concentration of the extracts from 25 to 100 mg/ml, all extract were showed good antimicrobial activity. In this diffusion assay, only at 100mg/ml plant extract showed maximum zone of inhibition (6 -7 mm). against the tested strains. The present study concludes that the plant species with numerous more effective phytochemical compounds like saponins, steroids, alkaloids, indoles, lignins, terpenoids, tannins, anthroquinones, proteins, carbohydrates and several types of anthocyanidins, phenolic compounds, flavonoids, amino acids and lipid compounds having multiple medicinal applications, used in wide range of formulations, higher therapeutic, commercial and economic values indicating that the Tirumala hills is a green gold treasure house of high valued medicinal plants.

CONCLUSION

In this study evaluation of phytoconstituents and antibacterial potency of fruits of *Boswellia ovaliofoliolata* has been done. We conclude with the positive result in the antibacterial efficacy at 75 mg/ml as minimal concentration to act against *Pseudomonas aeruginosa,, Staphylococcus aureus, Bacillus subtilis* and *E. coli* in crude form. Since *Boswellia ovaliofoliolata* is an endemic plant and an alarming status in the population ,there is an urgent need to conserve *in vitro* and *in vivo.*

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