INTRODUCTION

Santalum album Linn. (family: Santalaceae) is an evergreen small tree, a partial root parasite, attaining a height of 1 to 2.4 meters with slender drooping as well as erect branching [1]. Historical review reveals that sandalwood has been referred to in Indian mythology, folklore and ancient scriptures. In India, S. album L. is found all over the country, with over 90% of the area in Karnataka and Tamil Nadu [2, 3]. Medicinally S. album is useful in biliousness, fever and thirst. Extremely, a paste of S. album is used at scorpion bites, inflamed skin, and skin eruption. It is commonly used in cosmetic and hair oil. Sandalwood oil relieves itching, pruritus, inflammation of the skin. It is most effective in relieving dehydrated skin so that it makes great for anti-ageing skin care. The sandalwood oil is apopular remedy in gonorrhea, chronic bronchitis, cystitis, glut, urethral haemorrhage and scabies. Salubrium L. is bitter, cooling, sedative diuretic, expectorant, stimulant and has astringent actions. It is disinfectant to mucous membrane in genitor-urinary and bronchial tracts. Good for memory and act as blood purifier [2, 3]. The essential oil has antibacterial, antifungal action and used in dysuria, urethral discharges and gallbladder diseases. Sandalwood is bacteriostatic against gram-positive bacteria and used as a urinary antiseptic in chronic cystitis and sexually transmitted diseases [4].

The heartwood contains essential oil, dark resin and tannic acid [2]. The essential oil contains mixture of sesquiterpene alcohols especially α-trans-bergamotol, cis-α-santalol, cis-β-santalol, epι-cis-β-santalol with a small amount of trans-β-santalol and cis-lanceol [5]. Other chemical constituent’s present in the heartwood of S. album L. includes hydrocarbons α-santene and β-santene, the alcohols santenol, teresantalol, the aldehydes nortricylocasantal and isovaleraldehyde, the ketones santene, santalene, santalicol with santalic acids [6]. Other hydrocarbons such as α-santene, β-santene, α-bergamotene and epi-β-santalene are also present in the oil as well as α-curcumene, β-curcumene, γ-curcumene, β-bisabolene and α-bisabolol are also reported in heartwood [6]. New antitumor sesquiterpenoid from S. album L. also reported in methanolic extract of heartwood [7]. From Indian origin heartwood of S. album, some new bisabolane and santalene type of sesquiterpenoids along with (+)-α-nuciferol, (+)-citronellol and geraniol were isolated [8]. More oil constituents including santalone, 3-dien-1-yl methyl ketone, 4-methylcyclohexa-1, (6)-5-[2, 3-dimethyl-3-nortricycleyl]-pent-3-en-2-one and 5, 6-dimethyl-5-norbornene-2-ohwere identified [9]. Indian sandalwood oil also confirmed two new sesquiterpene aldehydes as Cyclosantalal and epicyclosantalal [10]. The heartwood oil of S. album L. Contains bisabolensals A to E and α-trans-bisabostenol [11].

The seed oil of S. albumis dark red viscid fixed oil containing santallic acid (or Ximenynic acid) and stearic acid (9-octadecenoic acid) [12]. The seed oil from young and mature trees contains santalic acid, saturated fatty acid, nitrogen, protein, K₂O, CaO, MgO, Fe₂O₃, P₂O₅. A calcium-dependent protein kinase is expressed in sandalwood seeds under developmental regulation, and it is localized with spherical storage organelles in the endosperm [14]. Aim of the present study was to perform phytochemical evaluation and antimicrobial screening of petroleum ether and ethanol extracts of Santalum album seeds to find out the most effective extract.

MATERIALS AND METHODS

Chemicals and reagents

All reagents and chemicals were of analytical grade. Silica gel GF254, was purchased from Merck Life Science Private Limited, Mumbai, India. Dimethyl sulfoxide and other reagents were purchased from Sigma Chemical Co. (USA). Santalbic acid marker was obtained from the Sam Labs Ltd., Bangalore. All media were used of Hi media Pvt Ltd.
Weighed 9.8 mg of marker santalbic acid and dissolved in n-hexane by HPTLC method. Santalbic acid marker was obtained from the biomarkers. Quantitative estimation of santalbic acid was carried out by HPTLC. Determination of santalbic acid content in petroleum ether extract of S. album seeds by preparative thin layer chromatography (TLC).  
To speed up the separation and to make them online for continuous recording varrious modification of preparative TLC have been developed. In general microgramamount of mixture are separated in analytical TLC. In preparative TLC thicker layer of sorbent are employed. Separated band of thecompound are scraped from the plate and subjected to solvent extraction with modern spectrometric method for structure determination.  
Preparative TLC plates were prepared using aslurry of silica gel GF254 by pouring technique. The plates were activated at 110°C for 1 hr. The sample was prepared in petroleum ether and applied as a band on the plate. Sample overloading was avoided to reduce tailing effect. Then the plates were dried in air and developed in the pre-saturated developing chamber using the solvent system n-hexane/diethyl ether/acetic acid (85:15:5). The substances separated as distinct bands. The spots were scraped and dissolve in petroleum ether. Prepared the dilution of the solution for UV spectrophotometer analysis to measure absorbance.  
Column chromatography of petroleum ether extract of S. album seeds  
Column chromatography is an important useful technique used for the separation and purification of both solids and liquids. Based on TLC profile of extract, the petroleum ether extract of S. album was further fractionated by silica gel column chromatography.  
The stationary phase Silica (Merck, 60-120 mesh) bed was prepared in mobile phase in the glass column (size 45 x 4.5 cm), the bed (final geometry 35 cm x 4.5 cm) was allowed to settle with gentle tapping after each addition, in order to ensure the uniform packing. Mobile phase-hexane/diethyl ether/acetic acid (85:15:5) was run through the column after loading of the sample to elute different fractions. A linear solvent flow rate was maintained as 42 drops/min and 50 ml of each fraction were collected. The collected fractions were subjected to TLC and were concentrated to dryness at normal temperature. The collected fractions were also subjected to ultraviolet spectrophotometer analysis. Selected fractions were characterized by FTIR and NMR spectral analysis.  
Determination of santalbic acid content in Salvia by high-performance thin layer chromatography (HPTLC) method  
HPTLC is a versatile technique for qualitative and quantitative analysis of herbal extracts. This technique includes fingerprint profiles of extract and estimation of different markers and biomarkers. Quantitative estimation of santalbic acid was carried out by HPTLC method. Santalbic acid marker was obtained from the Sansi Labs Ltd., Bangalore, as a gift sample.  
Weighed 9.8 mg of marker santalbic acid and dissolved in n-hexane (1.96 mg/ml). Marker solution was stored in an amber colored flask. The sample solution was prepared as 74.46 mg/ml of petroleum ether extract 5 ml of n-hexane. The sample solution was stored in an amber colored flask. Precoated TLC plates of Silica gel G 60 F254 (E. Merck), 10 x 20 cm in size on the aluminium support of 200 μm thickness were used as stationary phase. A mixture of n-hexane: diethyl ether: acetic acid (85:15:5) was used as mobile phase. A marker and sample solutions was applied as band length 10 mm from lower edge of the plate using 100 μl syringe on CAMAG LINOMAT5 automatic sample applicator on precoated TLC plates. Plates were developed in previously saturated, 20x20 cm twin trough (CAMAG) chamber. Plates were then air dried for 10 min and scanned by using CAMAG TLC scanner 3 in Reflection/Fluorescence Mode with WINCATS software. The temperature should be 25±2°C and relative humidity 40%. The profile obtained was documented at 254 nm, 366 nm through the visible camera (CAMAG Visuinalzer: 150503).

In vitro screening for antimicrobial activity  
Test organisms and other materials  
Selected test organisms were gram-positive bacterium Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 6538), gram-negative Escherichia coli (ATCC 10538), Pseudomonas aeruginosa (ATCC 27853), and fungi Candida albicans (ATCC 10231) obtained from the Department of Microbiology, R. C. Patel Art Science and Commerce College, Shirpur Maharashtra, India. All test organisms were cultured aseptically at 37 °C in nutrient agar medium for overnight. Fungi were cultured at 30 °C in potato dextrose agar medium for 5-7 d. All media were used of Himedia Pvt Ltd. Nutrient agarmedia for bacteria and potato dextrose agar were selected for fungi. The sterilization of media and all glassware were done by autoclaving at temp 121°C and pressure 15 Psig for 15 min.  
Disc diffusion method  
Filter paper disks were prepared by cutting paper disks of about 6 mm in diameter from whatman filter paper (No. 41). The paper disks were flattened by spreading on a clean smooth surface of the petri dish and pressed by rolling a bottle repeatedly and sterilized in an autoclave [16].  
The stock solution was prepared in dimethyl sulfoxide (DMSO) at a concentration 5000 μg/ml. Accurately weighed 2 g of the each extract and dissolved in 400 ml of DMSO solution. The solutions were stored in the refrigerator at 4°C. Stock cultures were maintained at 4°C on slopes of nutrient agar in test tubes. Active cultures for experiments were prepared by selecting at least three to five isolated colonies from the same morphological type from the stock cultures [17]. The colonies is transferred with a loop into a test tube having 4 to 5 ml of a nutrient agar media for bacteria and incubated without agitation for 24 h at 37 °C. The experiment was performed under strict aseptic conditions. Potato dextrose agar medium was used for fungi and incubated without agitation for 3-5 d at 30°C.  
Application of discs to inoculated agar plates  
Sterile 6 mm disc filter paper disc were impregnated with 100 μl of the plant extracts. Discs were transferred on the agar with sterilized forceps. Each disc must be pressed down to ensure complete contact with the agar surface. They distributed evenly so that they are no closer than 24 mm from centre to centre. The plates are placed in freezing condition for 5 min and were incubated at 37±0.1 °C for 24 hr, while yeast plates were incubated at 28±0.1 °C for 48 h in the incubator. After incubation, each plate is examined. The diameters of the zones of complete inhibition are measured, including the diameter of the disc with the help of aruler. The diameter of these zones was measured in millimeters.  
Determination of minimum inhibitory concentration (MIC)  
MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). Broth dilution technique was utilized in which a stock solution of test extract was prepared with the highest concentration of 5 mg/ml in sterile water and makes serially dilutions concentration ranging from 0.5 mg/ml to 5 mg/ml using nutrient broth. These were inoculated with 0.2 ml suspension of test organisms. The test tubes were observed for turbidity after 18 h of incubation at 37°C. The least concentration
which does not show turbidity was observed and noted as the minimum inhibitory concentration (MIC) value [18, 19].

**Determination of minimum bacterial concentration (MBC)**

MBC is defined as the lowest concentration at which no bacterial growth is observed (bactericidal concentration). MBC was determined by subculturing of MIC tubes in antimicrobial-free agar plates. The content of the test tubes was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37°C for 18 h. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC.

**Statistical analysis**

All experiments were performed in a triplicate manner in antimicrobial test analysis. The data were expressed as mean values±SD using In Stat software and tested with analysis of variance followed by the multiple comparison tests of Tukey-Kramer with P<0.01 were considered significant.

**RESULTS**

**Phytochemical screening and preparative TLC**

Preliminary screening of petroleum ether extract (yield was 14.66% w/w) was revealed the presence of steroids, terpenoids, and fatty acids. The presence of glycosides and phenolic compounds was confirmed in the ethanolic extract (yield 9.03%). Based on the type of chemical constituents, the different solvents were tried to find out best separation in thin layer chromatography. The best separation was found insolvent system, n-hexane/diethyl ether/acetic acid (85:15:5). The plates were observed under ultraviolet light and bands recorded with respect to retardation factor (Rf) = 0.7 (fraction A), 0.6 (fraction B), and 0.4 (fraction C) were scraped off. UV absorbance of fraction A, B and C having Rf value was 0.7, 0.6, 0.4 respectively. UV spectrum of petroleum ether extract and standard santalbic acid showed absorption at λ max 202.0 nm and 209.8 nm.

**Column chromatography of petroleum ether extract of S. album seeds**

Column chromatography of petroleum ether extract produces different fractions. After TLC of different fractions was confirmed those fractions from 9 to 11 have a single spot with similar Rf 0.27 that was identical to the Rf value of santalbic acid. Further isolated fraction 9 to 11 were combined and characterized by IR and NMR spectral analysis. IR spectrum showed (fig. 1) characteristic broad peak at 3430.83 cm⁻¹ ruled out the possibility of presence of an acid. IR spectrum sharp peaks characteristic at 893 cm⁻¹ (-C=C-disubstituted olefinic), 1298 cm⁻¹ (-C=O), 1760 cm⁻¹ (Acetic carbonyl-C=O), 3019 cm⁻¹ (-C=CH-), 2959 cm⁻¹ (Alkane-C-H), 1008.62 cm⁻¹, 2361 cm⁻¹ (-C≡C-), 2328 cm⁻¹, 1610 cm⁻¹ (-C=C-Aliphatic), 1423 cm⁻¹, 1126 cm⁻¹ (-C=O Hetro atom).

![FTIR spectrum of isolated santalbic acid](image1)

**Fig.1: FTIR spectrum of isolated santalbic acid**

![H-NMR spectrum of isolated santalbic acid](image2)

**Fig.2: H-NMR spectrum of isolated santalbic acid**
Proton NMR spectrum showed unsaturation at 6.039 ppm and peak at 5.426 ppm due to ethylenic and methylene proton atom respectively. These are comparable with reported data of synthetic methyl ester of santalbic acid.[18] While other peak and signals at δ value 0.94 (T, 3H), 1.24 (Q, 28H), 1.44 (M, 4H), 2.0 (Q, 5H), 2.26 (Q, 6H), 4.0 (T, 1H), 4.4 (T, 1H), 5.5(T, 1H), 6.0 (M, 1H) was also seen in proton NMR (fig. 2). The result obtained from 13C NMR spectrum showed strong peak due ethylenic carbon atom at 143.33 ppm and 109.768 ppm. While due to E-enyne system it shows a peak at 32.968 ppm and 19.297 ppm (Methylene carbon atom) which are comparable with reported data of synthetic methyl ester of santalbic acid. Also the other peak and signals at δ value 110 (C-9, 10 S), 130 (C-7, 8 D), 173 (C-18, S), 29 (C-1 Q), 30 (C-2 T), 31.8 (C-5, 13, 12 T), 34 (C-11, 12, 4, 16, 6, 17 T), 22.8 (15, 14, 3 T) was seen (fig. 3).

HPTLC analysis of petroleum ether extract of Santalum album

HPTLC chromatogram of crude petroleum ether extract of S. album Linn. seed at 235 nm (Sample 10 µl) shows a spot with Rf value 0.21 (100.00)(table 1, fig. 4). HPTLC chromatogram of marker Santalbic acid at 235 nm (Sample 10 µl) shows Rf value at 0.22 (100.00). From above Rf values of extract and marker Santalbic acid, specificity of one marker component with Rf 0.21 was Santalbic acid in S. album by comparing with marker Santalbic acid[20]. HPTLC chromatogram for fraction of petroleum ether extract of S. album Linn. at 235 nm (Sample 10 µl) shows Rf value at 0.21 (100.00) while HPTLC chromatogram of marker Santalbic acid at 235 nm (Sample 10 µl) shows Rf value at 0.22 (100.00). From above Rf values of S. album Linn. seed and marker Santalbic acid, specificity of one marker component with Rf 0.21 was 4.7% w/w Santalbic acid in S. album Linn. seeds by comparing with marker Santalbic acid (fig. 5).

In vitro antimicrobial activity

Ethnobotanical importance of S. album has been investigated and studied by various authors. They reported antimicrobial activity of essential oil obtained from heartwood, yet was no work on antimicrobial activity of fixed oil obtained from seeds of S. album L. The data obtained from disc diffusion method are used for comparative study of two extracts of S. album L. seed such as petroleum ether extract and alcoholic extract was shown in table 2 (fig. 6 and 7).

Table 1: HPTLC profile of Santalum album extract, fraction and standard santalbic acid

| Track | Peaks | Start Rf | Start height (AU) | Max Rf | Max height (AU) | Max % | End Rf | End height (AU) | Area (AU) | Area % |
|-------|-------|----------|------------------|--------|----------------|-------|--------|-----------------|-----------|--------|
| 1     | 1     | 0.21     | 0.0              | 0.25   | 302.0          | 100   | 0.27   | 2.3             | 7277.0    | 100    |
| 2     | 1     | 0.21     | 5.4              | 0.24   | 314.6          | 100   | 0.27   | 0.4             | 6414.5    | 100    |
| 3     | 1     | 0.22     | 0.1              | 0.25   | 654.2          | 100   | 0.29   | 0.4             | 111077.3  | 100    |
Fig. 5: HPTLC chromatogram of (A) Petroleum ether extract of *Santalum album* seeds (B) Fraction of petroleum ether extract of *Santalum album* seeds (S) Standard santalbic acid at 235 nm

Table 2: Antimicrobial activity of plant extracts against test microbial

| Test organisms | Strains   | Conc. (mg/ml) | mean±SD of diameter for zone of inhibition (mm) Petroleum ether extract | Alcoholic extract | Standard gentamicin (30µg/ml) |
|----------------|-----------|---------------|---------------------------------------------------------------------|-------------------|-------------------------------|
| Gram positive  | B. subtilis | 5.0           | 13.66±2.08*                                                          | 9.01±0.35         | 14.25±0.89                    |
| Gram positive  | S. aureus | 5.0           | 11.33±0.57*                                                          | -                 | 12.57±1.04                    |
| Gram negative  | P. aeruginosa | 5.0       | 6.4±0.30                                                             | -                 | 10.43±0.76                    |
| Gram negative  | E. coli   | 5.0           | 7.3±0.26                                                             | 6.2±1.00          | 9.61±0.52                     |
| Fungi          | C. albicans | 5.0          | 13.66±0.5*                                                           | -                 | 15.94±1.60                    |

n=3, value represented as mean±SD. *P < 0.01 were considered significant; (- = absent)

Petroleum ether extract of seed of *S. album* was more active against *B. subtilis* and fungus *C. albicans* (zone of inhibition 13.66±2.08 mm, 13.66±0.5 mm respectively). While the alcoholic extract was not sufficient active against all five microbes. Petroleum ether extract of
seed of *S. album* was also active against gram-positive bacteria *S. aureus* (zone of inhibition 11.33±0.57 mm) while it is less effective against gram-negative bacteria *P. aeruginosa* and *E. coli* (zone of inhibition 6.4±0.30 mm and 7.3±0.26 mm, respectively). Observations found that the MIC and MBC values of the petroleum ether extract for *S. aureus* was same as 156.25µg/ml.

In general most bactericidal antimicrobials, the MIC and MBC are approximately same or near as in present study it has typically been observed (table 3 and 4). It was seen that the MIC value for *B. subtilis*, *P. aeruginosa*, *E. coli* and *C. albicans* was 78.125 µg/ml, 19.331 µg/ml, 4.882 µg/ml and 9.765 µg/ml respectively while MBC was 39.062 µg/ml, 31.25 µg/ml, 31.25 µg/ml and 9.765 µg/ml, respectively.

### Table 3: Minimum inhibitory concentration (MIC) values for bacterial strain against petroleum ether extract of *S. album* seeds

| Concentration (µg/ml) of petroleum ether extract | *B. subtilis* | *P. aeruginosa* | *S. aureus* | *E. coli* | *C. albicans* |
|-----------------------------------------------|--------------|----------------|-------------|-----------|--------------|
| 5000                                          | +            | +              | +           | +         | +            |
| 2500                                          | +            | +              | +           | +         | +            |
| 1250                                          | +            | +              | +           | +         | +            |
| 625                                           | +            | +              | β           | +         | +            |
| 312.5                                         | +            | +              | -           | +         | +            |
| 156.25                                        | +            | +              | β           | -         | +            |
| 78.125                                        | B            | +              | -           | +         | +            |
| 39.062                                        | -            | +              | -           | -         | β            |
| 19.331                                        | -            | β              | -           | -         | -            |
| 9.765                                         | -            | -              | -           | -         | -            |
| 4.882                                         | -            | -              | -           | -         | -            |
| 2.441                                         | -            | -              | -           | -         | -            |
| 1.2205                                        | -            | -              | -           | -         | -            |
| 0.610                                         | -            | -              | -           | -         | -            |
| 0.305                                         | -            | -              | -           | -         | -            |

-Resistance (growth of bacteria/fungi or turbidity); +:Concentrations show no turbidity (inhibition of bacterial growth); β: Least concentration showing no turbidity (MIC)
ethanol extracts. Petroleum ether extract of seed of *Santalum album* Linn showed prominent antibacterial activity than activity against all five microorganisms. From zone of inhibition,红外分析, petroleum ether extracts exhibited antimicrobial activity against all five microorganisms. Presence of Santalbic acid in petroleum ether extract was confirmed by supporting results of UV, IR, and NMR analysis. Petroleum ether extracts exhibited antimicrobial activity against all five microorganisms. From zone of inhibition, petroleum ether extract showed prominent antibacterial activity than ethanolic extracts. Petroleum ether extract of seed of *S. album* was more active against *B. subtilis* and fungus *C. albicans*. Petroleum ether extract of *S. album* seed was also active against gram-positive bacteria *S. aureus* while it is less effective against gram-negative bacteria *P. aeruginosa* and *E. coli*. The MIC and MBC values of petroleum ether extract for *S. Album* was observed similarly as 156.25 μg/ml. It is worth mentioning that in the present study petroleum ether extract exhibited significant antimicrobial activity towards gram-positive bacterial strains studied and comparable to the standard antibiotic drug. These results may justify the use of *S. album* in traditional medicines for treating skin diseases and urinary infectious disease. Thus the present study could have clinical importance also.

**Antimicrobial activity of sandalwood oil against bacterial and fungal pathogens shown with MIC values in the limit of 50-1000 ppm [21]. In addition, it is well recognized that cytoplasmic membrane coagulation, electron flux and imbalance of active transport, the breakdown of protons motive force are major actions that involve in antimicrobial property of phytochemicals. Santalbic acid is a major constituent of the *S. album* seeds. In some standardized bioassays it was found effective against gram-positive bacteria and several pathogenic fungi whereas the unapplied oil was observed inactive [22].**

In the present study, terpenoid-rich sandalwood oil is a strong bacteriostatic agent, as recommended according to MIC values (up to 500 μg/ml) based classification of plants [23]. According to the previous report, ethanolic extracts of *S. album* seeds does not have antibacterial properties [24], study indicating that ethanol does not extract out such principles, whereas petroleum ether from seeds shows antimicrobial potential in the present study. Phenolics and terpenoids are major categories of a compound already reported for the antibacterial property. *S. album* seeds oil constituents and their synthetic analogues are reported as strong antimicrobial and antibiotic agents [25, 26]. In the previous research work, *S. album* essential oil found effective significantly against *E. coli* and *Bacillus mycoides* [27]. It also showed significant inhibitory effect most of the bacterial strains except *Pseudomonas aeruginosa* [28].

In the chromatographic study, the presence of santalbic acid as an active constituent was confirmed and may be responsible for the antimicrobial activity of sandalwood. Santalbic acid is a very nonpolar constituent present in *S. Album* seeds have been found effective against some pathogenic fungi and Gram-positive bacteria [22]. In another statement, essential oils that consist of various ranges of terpenoid compounds are also known for their strong _in vitro_ antifungal properties [29].

**CONCLUSION**

Terpenoids are also a wide group of a compound that can alter the cell morphology by manipulating the osmotic pressure of the cell, resulting in disrupting the cytoplasmic membrane and causing the release of cell constituents. Although further research work is required to identify responsible constituents in the extract, the petroleum ether extract showed the lowest MIC values, suggesting that nonpolar constituents are most active antimicrobial compounds. From above results, it was concluded that petroleum ether extract has antimicrobial activity against both gram positive, gram negative and fungal strain selected in the present study. Our findings also indicate that santalbic acid from petroleum ether extract of *S. album* seeds with possible high potency could serve as chemotherapeutic agents.

**ACKNOWLEDGEMENT**

The authors are thankful to the BSI (Botanical Survey of India), Pune, Maharashtra, India, for providing the necessary facilities to identify the plant material. Author also grateful to Department of Microbiology, R. C. Patel Art Science and Commerce College, Shirpur, Maharashtra, India, to provide microbial stains.

**AUTHORS CONTRIBUTION**

The experimental work was done by Vaishali Patil under the supervision of Dr. Gautam P Vadnere. Md. Rageeb Usman participated in manuscript preparation and revised in final version by Santram Lodhi.

**CONFLICT OF INTERESTS**

Authors do not have any conflict of interest.

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**Table 4: Minimum bacterial concentration (MBC) values for bacterial strain against petroleum ether extract of seed of Santalum album Linn**

| Concentration of petroleum ether extract(μg/ml) | *B. subtilis* | *P. aeruginosa* | *S. aureus* | *E. coli* | *C. albicans* |
|-----------------------------------------------|--------------|----------------|-------------|-----------|--------------|
| 5000                                         | +            | +              | +           | +         | +            |
| 2500                                         | +            | +              | +           | +         | +            |
| 1250                                         | +            | +              | +           | +         | +            |
| 625                                          | +            | +              | +           | +         | +            |
| 312.5                                        | +            | +              | β           | +         | +            |
| 156.25                                       | +            | +              | β           | +         | +            |
| 78.125                                       | +            | β              | -           | -         | -            |
| 39.062                                       | B            | +              | -           | -         | +            |
| 19.331                                       | -            | +              | -           | -         | +            |
| 9.765                                        | -            | +              | -           | -         | β            |
| 4.882                                        | -            | β              | -           | -         | -            |
| 2.441                                        | -            | -              | -           | -         | -            |
| 1.2205                                       | -            | -              | -           | -         | -            |
| 0.610                                        | -            | -              | -           | -         | -            |
| 0.305                                        | -            | -              | -           | -         | -            |

-Resistance (growth of bacteria/fungi or turbidity); +Concentrations show no turbidity (inhibition of bacterial growth); β: Least concentration showing no turbidity (MIC)
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