IJPSR (2016), Vol. 7, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 February, 2016; received in revised form, 28 March, 2016; accepted, 15 May, 2016; published 01 July, 2016

SANTALUM ALBUM: CLINICAL ASPECTS FOR TREATMENT OF *CANDIDA* INFECTIONS

Parveen Surain, Neeraj Kumar*, Romika Dhiman and Vikas Meashi

Department of Microbiology, Kurukshetra University, Kurukshetra, Harayana, India

Keywords:

Anticandidal activity, *S.album* bark, Candida sp.

Correspondence to Author:
Dr. Neeraj kumar

Department of Microbiology, Kurukshetra University, Kurukshetra, Harayana-136119, India

Email: neerajkuk26@gmail.com

ABSTRACT: Background: In present study, we aim to assess the anticandidal activity of crude extracts and screening of bioactive compounds and their identification by NMR, IR and GC-MS. **Results:** A total of 192 isolates were isolated from patients having oral and vaginal candidiasis and a total of 134 isolates showed positive results with Gram staining. The selected isolates APKU-2, APKU-3 and APKU-4 were identified as *C.albicans*, *C.lusitaniae* and *C.krusei* by studying its morphological, biochemical, and molecular characteristics. Chemical characterization of the extracted compounds was done by H1NMR, Fourier transform infrared spectroscopy (FTIR), Gas chromatography–mass spectrometry (GC-MS) analysis to determine the structure, melting point, and molecular mass of the purified compounds. **Conclusions:** The crude extracts and purified compounds from the sandalwood holds potential as medications for various antifungal therapeutics. However, further research on their potential incorporation into different preparations, safety and cost- effectiveness remains to be done.

INTRODUCTION: *Candida* is a diploid, dimorphic yeast producing three morphologic forms: yeast cells, pseudohyphae and true hyphae. It is an opportunistic pathogen causing various types of candidiasis which are on the increase around the globe. Candidiasis (or moniliasis) is an opportunistic fungal infection of humans caused by various species of *Candida*, especially *Candida albicans*. Superficial candidiasis of the oral cavity called thrush, seen as white patches, are common complications of AIDS, diabetes or prolonged antibiotic therapy [1, 2]. *Candida* can invade the lungs, kidneys, and heart or to be carrier in blood, where it causes a severe toxic reaction.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(7).2813-29

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (7).2813-29

Of the 17 pathogenic species, *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* are often involved in 90% of invasive infections. *C. kefyr*, *C. guilliermondii*, *C. lusitaniae*, *C. stellatoidea*, and *C. dubliniensis* are the other major pathogens [3, 4]. Majority of the clinically used antifungals suffer from various drawbacks in terms of toxicity, drug-drug interactions, lack of fungicidal efficacy, high cost and emergence of resistant strains resulting from frequent usage [5].

The phenomenon of drug resistance has raised interest in substances of natural origin as a therapeutic alternative [6, 7]. Majority of the clinically used antifungals suffer from various drawbacks in terms of toxicity, drug-drug interactions, lack of fungicidal efficacy, high cost and emergence of resistant strains resulting from frequent usage. The phenomenon of drug resistance has raised interest in substances of natural origin as a therapeutic alternative [7]. The known success of traditional

medicine has guided the search for new chemotherapeutic alternatives to eliminate the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotics. Medicinal plants and corresponding preparations have been used for a wide range of purposes and for many centuries people have been trying to treat diseases as well as alleviate symptoms by using different plant extracts and formulations [8]. Medicinal plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids and phenols, which have been used worldwide in traditional medicine to treat several diseases and infection. Among the plants investigated to date, one showing enormous potential is the pepper family, The *Santalaceae, S.album*, commonly known as sandalwood, Safed chandan, A small evergreen glabrous tree with slender drooping branches the sapwood white and odorless.

It generally occurs at altitudes of 2000-3000 feet. The tree attains the height of 60-65 feet and is actually an obligate hemi parasite plant on various hosts—"*Cassia siamea, Pongamia glabra* and *Lantana acuminata*" is a native of the highlands of southern India mainly Coorg, Chennai and Mysore? The chief constituents of the oil is santalol (90% or more) a mixture of two primary sesquiterpene alcohols, C15H24O viz, α-santalol (bp-166- 1670C) and β-santalol (b.p-177-1780C) in which α– form predominates. More than hundred constituents of sandalwood oil in categories of tannins, terpenes, resins and waxes have been reported [9, 10, 11, 12 13].

METHODS:

Isolation of oral and vaginal candidiasis pathogens:

To diagnose fungal infection, debris/exudates samples were collected from the vaginal and oral sites, aseptically with the help of sterile cotton swabs and maintained at 4°C and brought to the Microbiology laboratory and processed to have immediately the exact nature of fungal flora involved. A detailed history was taken with particular reference to age, sex, presence of predisposing factors, onset and duration of complaints, initial appearance and progression of the lesions, treatment taken, marital status, exposure to sexually transmitted diseases and HIV status in relevant cases also noted. A detailed history regarding similar incidents in the family members were enquired into.

The samples both from vaginal and oral sites were processed for direct isolation and examination of the *Candida* spp. on malt yeast extract agar (MEA) media. For direct microscopic examination, a small portion of the specimen was mounted in 10% KOH and Careful microscopic examination will reveal oval-shaped blastospores, pseudo hyphae and occasionally chlamydiospores. The size and shape of the yeast observed may also suggest the presence of non-*albicans* yeast. The budding cells of *C. krusei* for example are noticeably larger and more elongate than those of *C. albicans*. For selective isolation of a *Candida* spp. the samples were inoculated on four different media: Malt yeast extract agar, Candidchrom agar, Biggy agar, Cornmeal with tween 80 agar. For isolation, sample swab was rolled and inoculated over the surface of agar plates of all the four media and incubated at 25^0 C, 37^0 C and 40^0 C for 24-48 hrs. The plates were examined for the presence of growth and sporulating structures.

Microscopic and cultural characterization of isolates:

The purified isolates were identified on the basis of cultural characteristics (colour, exudates, texture of colony) by consulting various books, and available monographs. The identification of yeasts was done on the basis of morphological characteristics in lacto phenol cotton blue wet-mount microscopy (presence of budding). Direct examination of samples with KOH until enough dissolution of keratinized cells is achieved to microscopic visualization of the fungal cells can be performed. Gentle pressure applied to coverslip placed on sample, mixed with KOH solution, and should flatten the sample completely in order to allow good microscopic resolution. Different isolates were characterized with respect to growth on four different media i. e. MEA, Candidchrom agar, Biggy agar (containing fluorogenic or chromogenic substrates) and Corn meal agar for production of chlamydospores, Germ tube test, Carbohydrate assimilation and fermentation and Esterase production (lipolytic activity).

Molecular characterization:
The molecular characterization of isolates has been done by Xcelris Labs Ltd. Ahmedabad, India.

Identification of a microbial culture using D1/D2 region of LSU based molecular technique:
DNA was isolated from the culture provided by the scientist. Its quality was evaluated on 1. 2% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of D1/D2 region of LSU (Large subunit 28S rDNA) gene was amplified by PCR from the above isolated plasmid DNA. A single discrete PCR amplicon band of 650 bp was observed when resolved on Agarose Gel (Gel Image-1). The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with DF and DR primers using BDT v3. 1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 596 bp of D2 region of 28S rDNA gene was generated from forward and reverse sequence data using aligner software. The D1/D2 region of LSU (Large subunit 28S rDNA) gene sequence was used to carry out BLAST with the database of NCBI gene bank database. Based on maximum identity score first ten sequences were selected the phylogenetic tree was constructed using MEGA 4. The purity of genomic DNA using agarose gel electrophoresis indicated the good quality and integrity of DNA.

Anticandidal activity of *Santalum album* plant extract:
The bark of *S. album* were collected from Ch. Devi lal park, Khizrabad. The taxonomic identity of this plant was confirmed by Dr. B. D. Vashishta, Kurukshetra University, Kurukshetra.

Extraction:
The bark samples were carefully washed under running tap water followed by sterile distilled water and air dried at 35-40°C for 4-5 days, homogenized to a fine powder using a sterilized mixer grinder and stored in air tight bottles. Six different solvents ethanol, methanol, acetone, petroleum ether, dichloromethane and sterile water were used for extraction. Ten grams of sample was separately soaked in conical flasks each containing 100ml of solvents [14]. Each preparation was filtered through a sterilized Whatman No. 1 filter paper and finally concentrated to dryness under vacuum at 40°C using a Rota evaporator. The dried extracts, thus, obtained were sterilized by overnight UV-irradiation, checked for sterility on agar plates and stored at 4°C in labelled sterile bottles until further use [15,16].

Purification and identification of active metabolites:
The purification and identification of the active compounds were done from the acetonic extracts of *Santalum album* (bark) showed best anticandidal activity against all the tested *Candida* pathogens. The purification of bioactive compounds was made by using thin layer chromatography (TLC- TLC Silica gel 60 F254 plates (Cat. No.1. 05554. 0007) of Merck, Germany were used for performing TLC. The TLC was performed at room temperature using properly equilibrated chromatography glass chamber). Column chromatography and Gas chromatography and Mass spectrometry (GC-MS). All the purified fractions collected were evaluated for their anticandidal efficacy against all the test organisms.

Structural elucidation of isolated metabolites:
Identification of compounds usually involves a combination of different techniques such as nuclear magnetic resonance (NMR) spectroscopy (The ^{1}H NMR spectra of pure, dried isolated compounds (5-10 mg) were done at the Department of Chemistry, Kurukshetra University, Kururkshetra using Bruker Advance DRX 300 FT-NMR in deuterated solvents, either dimethylsulphoxide (DMSO-d_6) or chloroform ($CDCl_3$) using tetramethylsilane (TMS) as internal standard). Infrared spectrometry (IR- IR analyses of three purified compounds was performed at Department of Chemistry, Kurukshetra University, Kururkshetra using ABB MB 3000 DTGS FT-IR Spectrophotometer using the KBr pellet technique. It has a resolution of 1 cm^{-1} and can scan range of 450-4000 cm^{-1}).

Gas and mass spectrometry (GC-MS- GC-MS of the compounds was got done through Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University (JNU), New Delhi, India. For molecular analysis of purified polymer a coupled GCMS was performed using a GCMS-QP

2010 Plus model with capillary Column- Rtx-5 MS (30 mX 0. 25 mm i. d. X 0. 25 micrometer film thickness). The samples were injected (1 μL) in the split mode and the injection temperature was 270°C and column oven temperature was 100°C).

Test microorganisms:
The anticandidal activity was evaluated against different *Candida* spp. i.e. against three isolates (*C. albicans* strain-2 (KC139704) and *C. krusei* (KC616318) and one standard species (*C. albicans* strain-1 (MTCC No. 4748) as a control by agar well diffusion method. These were sub cultured on malt yeast agar (MEA) and incubated aerobically at 37°C.

Screening for anticandidal activity:
Anticandidal activity of six solvent extracts (acetone, methanol, ethanol, petroleum ether, dichloromethane and aqueous) of the leaves was determined by the agar well diffusion method. In this method, pure isolate of each yeast was sub cultured on the MEA plates at 37°C for 24h. Minimum of inoculum of the isolates were transferred into normal saline (0. 85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 106 cells/ml (standardized by 0. 5McFarland standard) and used as the inoculum for performing agar well diffusion assay. 100µl of inoculum of each test organism was spread onto the MEA plates.

The inoculated agar plates were allowed to dry, wells of 8mm were made with a sterile borer and the lower portion of each well was sealed with a little molten MEA medium. 100µl of it was propelled directly into each well (in triplicates) of the inoculated agar plates for each test organism.

The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24h. Sterile DMSO served as the negative control and ketoconazole as the positive control. The anticandidal activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8mm [15]. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.

Determination of minimum inhibitory concentration (MIC):
MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24h. The MIC of all the extracts was determined following the modified agar well diffusion method of. A twofold serial dilution of each extract was prepared by first reconstituting the powder in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 50mg/ml to 0. 39mg/ml.

A 100µl volume of each dilution was introduced into wells (triplicate) of the MEA plates already seeded with 100µl of standardized inoculum (106cells/ml) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 h and observed for the inhibition zones. The lowest concentration of the test extract showing a clear zone of inhibition (>8mm), considered as the MIC, was recorded for each test organism [16].

RESULTS AND DISCUSSION:
Isolation of oral and vaginal candidiasis pathogens:
A total of 134 samples from vaginal (108) and oral (26) patients were found to be positive, of which 73. 4% from vaginal and 57. 7% from oral were found to be positive for the presence of yeasts during examination.

Microscopic, cultural, biochemical and molecular characterization of selected isolates:
The selected isolate was classified up to genus level using the morphological and biochemical characteristics (**Tables 1, 2** and **3**). For further characterization, almost complete 28S rDNA gene sequences were determined. The obtained sequences were aligned and compared with the yeast sequences available in the Gene Bank database. The phylogenetic analysis (**Fig.1, 2** and **3**) was done using MEGA 4 software by neighbor-joining tree and distance matrix-based nucleotide sequence homology which revealed that isolates APKU-2, 3 and 4 were *C.albicans*, *C.lusitaniae* and *C.krusei*.

TABLE 1: COLONIAL FEATURES OF *CANDIDA* SPP. ON FOUR DIFFERENT MEDIA.

S. No.	Standard/Isolate	Species/Source	Colony characteristics			
			Candidchrom agar	Biggy agar	On corn meal tween 80 agar	Malt yeast extract agar
1	Standard	*C. albicans*/blood	Creamish	Brownish	Creamish	Whitish with a sheen
2	Isolate	APKU-2/vaginal	Bluish	Dark brown	Creamish	Creamish
3	Isolate	APKU-4/oral	Light white greenish	Brown black	Creamish	Creamish

TABLE 2: MORPHOLOGICAL FEATURES OF CANDIDA SPP.

Species	Clamydospores	Germ tube	Pseudohyphae	Size of yeast (µm)
Ca	+	+	+	4-6×9-13
APKU-2	+	+	+	5-6×6-9
APKU-4	-	-	-	3-5×6-10

TABLE 3: BIOCHEMICAL FEATURES OF *CANDIDA* SPP.

Tests	Ca	APKU-2	APKU-4
Catalase	+ve	+ve	+ve
Urease	-ve	-ve	+ve
Carbohydrate assimilation			
Cellobiose	+ve	+ve	+ve
Trehalose	+ve	+ve	-ve
Galactose	+ve	+ve	-ve
Melibiose	+ve	+ve	-ve
Arabinose	-ve	-ve	+ve
Carbohydrate fermentation			
Glucose	+ve	+ve	+ve
Trehalose	+ve	+ve	-ve
Galactose	+ve	+ve	-ve
Mannitol	+ve	+ve	-ve
Lactose	-ve	-ve	-ve
Cycloheximide resistance			
Susceptible/Resistance	+ve	+ve	-ve

Molecular characterization of *Candida* spp.:

The purity of genomic DNA using agarose gel electrophoresis indicated the good quality and integrity of DNA. PCR amplification of 28S rDNA gene with DF and DR primers for all the *Candida* spp. viz. APKU-2, APKU-3 and APKU-4 produced an amplification product of approximately 609 bp, 560 bp and 605 bp.

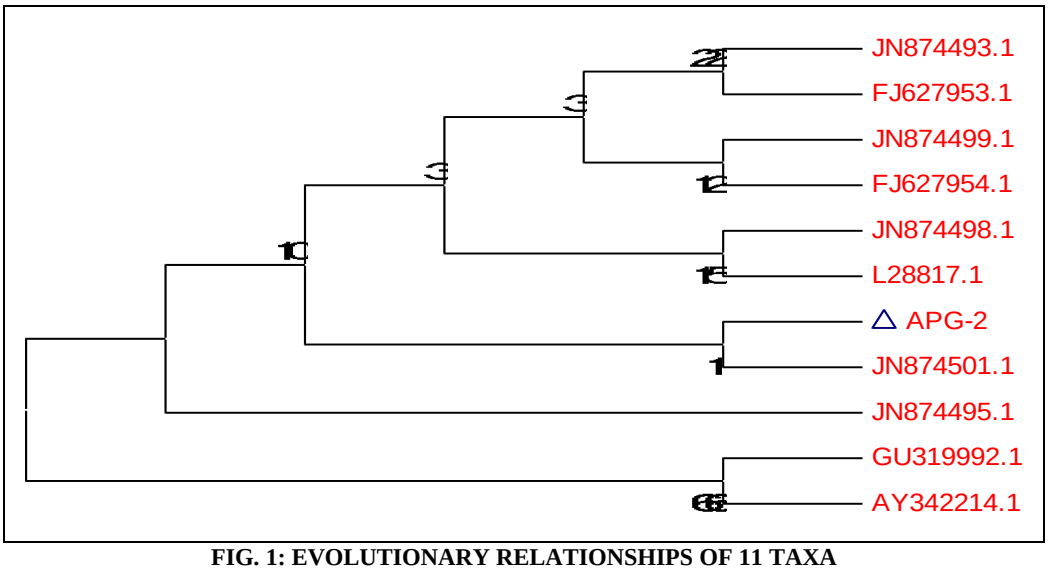


FIG. 1: EVOLUTIONARY RELATIONSHIPS OF 11 TAXA

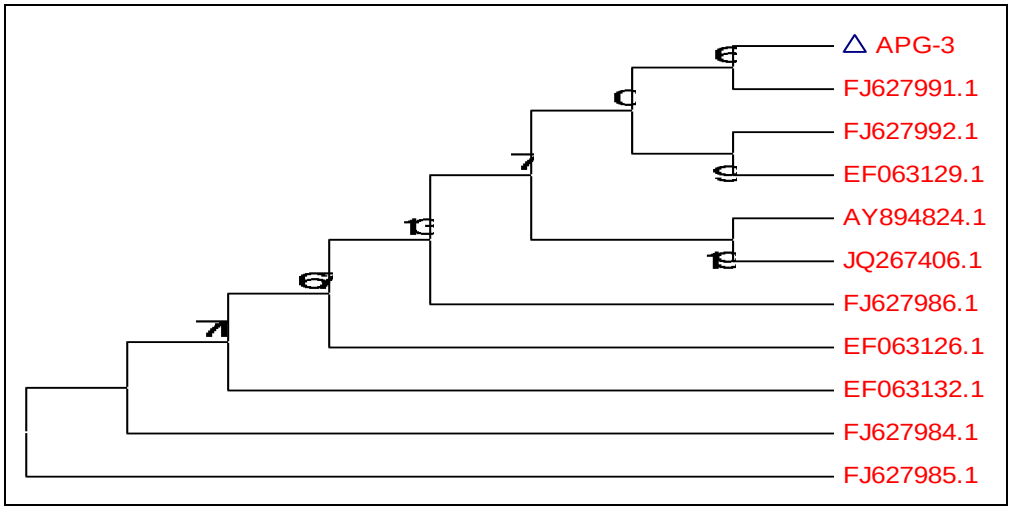


FIG.2: EVOLUTIONARY RELATIONSHIPS OF 11 TAXA

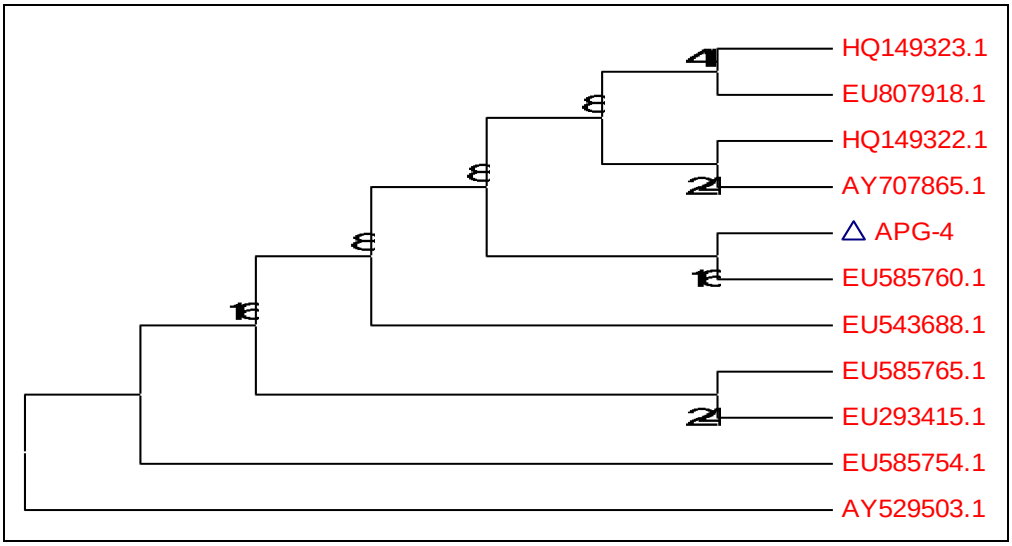


FIG. 3: EVOLUTIONARY RELATIONSHIPS OF 11 TAXA

TABLE 4: SIMILARITY PATTERN OF DIFFERENT ISOLATES WITH THE GENE BANK DATABASE SEQUENCES.

Isolate	Similarity pattern	Identity percentage	Gene bank submitted name	Accession number
APKU-2 (vaginal)	*Candida albicans* strain ATCC MYA-4780	100%	*Candida albicans*	KC139704
APKU-3 (vaginal)	*Clavispora lusitaniae* strain EXOC7	100%	*Candida lusitaniae*	KC616317
APKU-4 (oral)	*Issatchenkia orientalis* strain QD2.1	100%	*Candida krusei*	KC616318

Anticandidal activity of *Santalum album*- bark

The organic extracts of *S. album* bark extracts showed inhibition of all the tested species. Acetonic extract with highest zone of inhibition against all the species. *C. albicans* strain-1 was most susceptible with maximum zone of inhibition 34. 6mm followed by *C. albicans* strain-2 (30. 6mm), *C. krusei* (22. 6mm). Aqueous extract showed activity against *C. albicans* strain-2.

TABLE 5: ANTICANDIDAL ACTIVITY OF PLANT EXTRACT OF *SANTALUM ALBUM* BARK.

Solvent extract (mg/ml)	Diameter of zone of inhibition (mm)		
	Ca-1	Ca-2	Ck
Ethanol	25. 6±1. 52	28. 6±1. 15	18. 6±0. 57
Methanol	25. 6±1. 52	30. 6±1. 52	20. 3±0. 57
Acetone	34. 6±1. 52	30. 6±1. 52	22. 6±1. 15
Petroleum ether	19. 3±0. 57	20. 6±0. 57	12. 0±0. 57
Dichloromethane	28. 6±1. 15	26. 6±1. 15	19. 3±1. 52

Aqueous	15. 0±0. 57	14. 0±0. 57	-
	Antifungal drugs (%w/v)		
Ketoconazole (2%)	40. 6 [a] ±0. 57†	30. 3±0. 57	22. 3±1. 52
Neem ras (9%)	13. 6±0. 57	12. 3±0. 57	-

Ca, *C. albicans* strain-1; Ca, *C. albicans* strain-2; Ck, *C. krusei*, - No activity; [a] Values, including diameter of the well (8mm), are means of three replicates, ± † Standard deviation. *****Anticandidal activity was determined by agar well diffusion method on MEA medium.

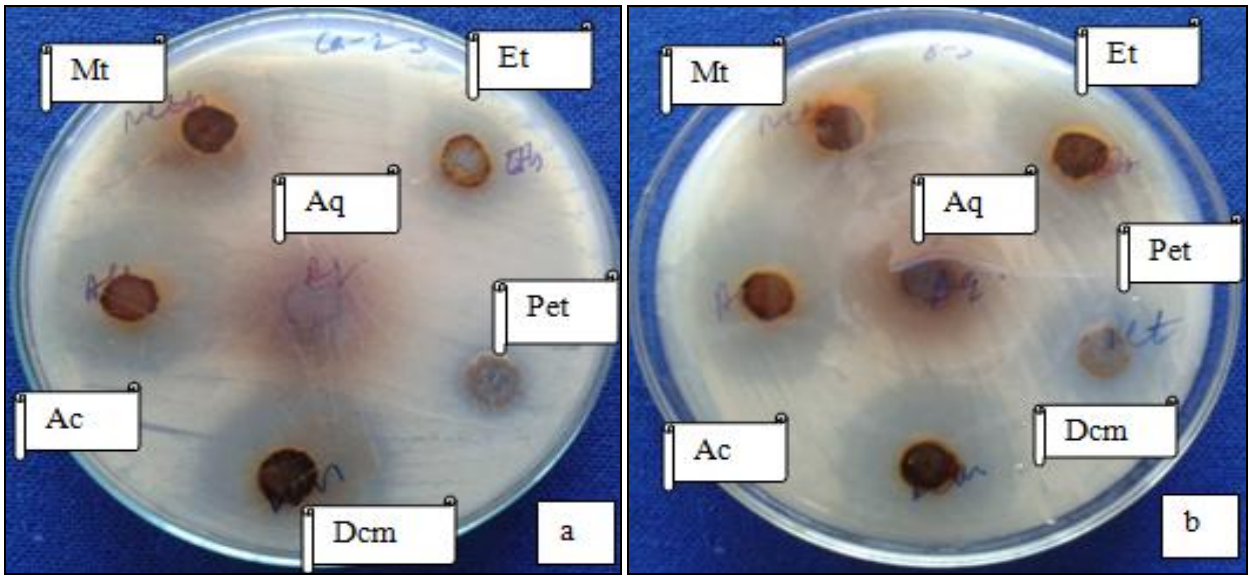


FIG. 4: ZONES OF INHIBITION PRODUCED BY THE ORGANIC AND AQUEOUS BARK EXTRACTS OF *S. ALBUM* AGAINST (a) *C. ALBICANS* STRAIN-1 (MTCC No. 4748), (b) *C. ALBICANS* STRAIN-2, Mt-METHANOL; Et-ETHANOL; Ac-ACETONE; PET- PETROLEUM ETHER; DCM- DICHLOROMETHANE; Aq-AQUEOUS.

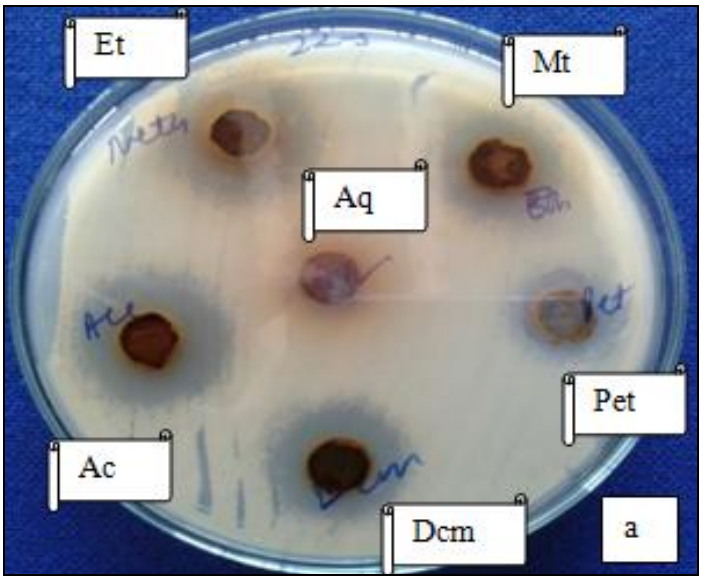


FIG. 5: ZONES OF INHIBITION PRODUCED BY THE ORGANIC AND AQUEOUS BARK EXTRACTS OF *S. ALBUM* AGAINST (a) *C. KRUSEI* ; MT-METHANOL; Et-ETHANOL; Ac-ACETONE; Pet- PETROLEUM ETHER; DCM- DICHLOROMETHANe; Aq-AQUEOUS

MIC of *Santalum album* -bark

Excellent bioactivity has been found in all the organic extracts of *S. album* bark, the acetonic extract showing MIC values against all the species followed by dichloromethane, methanolic, ethanolic and petroleum ether extracts. The MIC of 0. 39mg/ml was found against *C. albicans* strain-1 followed by 1. 56mg/ml against *C. glabrata* and *C. albicans* strain-2.

TABLE 6: MIC OF *SANTALUM ALBUM* BARK EXTRACTS.

Solvent extract	Ca-1	Ca-2	Ck
Ethanol	6. 25	3. 12	25
Methanol	6. 25	1. 56	25
Acetone	0. 39	1. 56	12. 5
Petroleum ether	25	25	50
Dichloromethane	3. 12	6. 25	25
Aqueous	50	50	nt

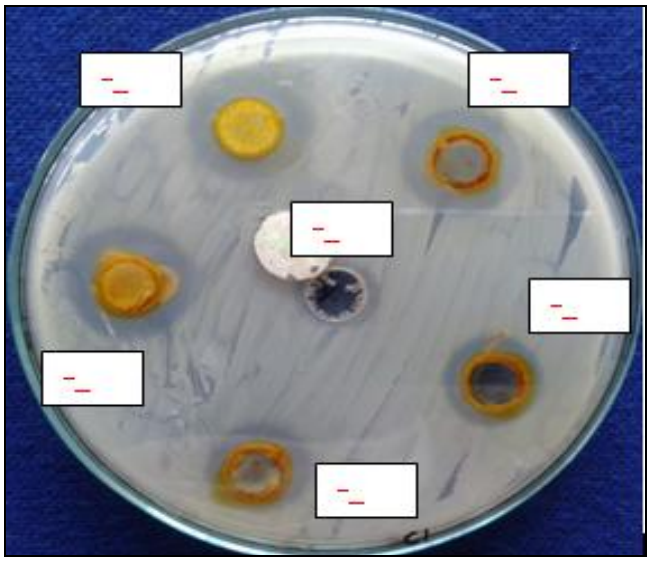


FIG. 6: PLATES SHOWING MINIMUM INHIBITORY CONCENTRATION (MIC) OF *S. ALBUM* BARK AGAINST *C. ALBICANS* STRAIN-1 (MTCC No. 4748) (1-50mg/ml; 2-25mg/ml; 3-6. 25mg/ml; 4-3. 12mg/ml; 5-1. 56mg/ml; 6-0. 39mg/ml).

Purification and identification of active compounds from *Santalum album:*

A total of 92 sub fractions, in 100ml each were collected from the acetonic extract of *Santalum album* by column chromatography. A gradient of benzene: acetone ranging between 100:0 and 0:100 were used to elute the compound. All the 92 sub fractions were analyzed by TLC. The fractions showing similar profile of Rf values were pooled and dried in Rota evaporator. Pooling of same fractions resulted in total of 6 sub fractions. The 6 sub fractions collected from column chromatography were evaluated for their anticandidal activity at 4.0 mg/ml by agar well diffusion method.

GC-MS analysis of acetone extract of sandalwood bark:

GC-MS analysis helps in elucidating the structure of components. The key compounds of concern were identified based on their retention peak. A total of 107 compounds were identified from this plant with different retention time and area of percentage.

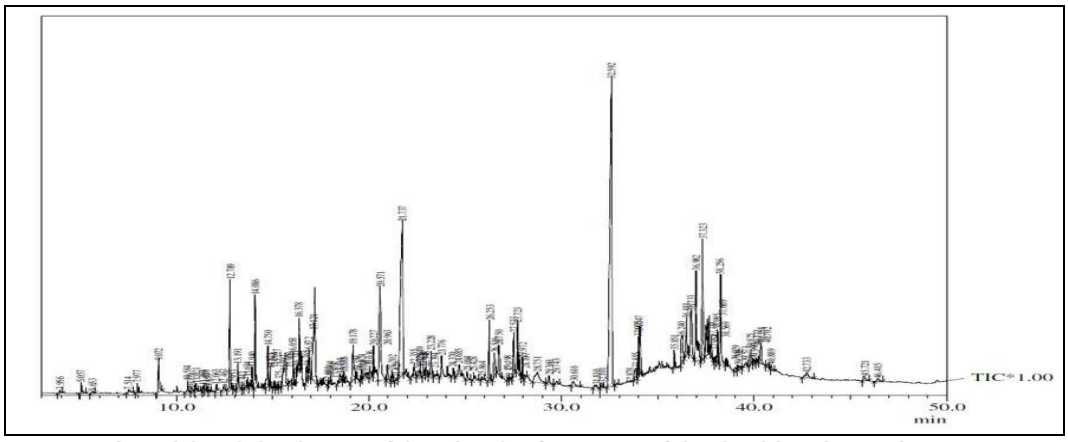


FIG. 7: GC-MS OF CRUDE ACETONIC BARK EXTRACT OF *SANTALUM ALBUM*.

Structural elucidation of pure compounds:

Identification of compound GP-3 and GP-2:

The two compounds, GP-3 from *Santalum album* were purified and were subjected to structural elucidation by NMR, IR and GC-MS spectroscopy. The compound GP-2, isolated from acetonic extract of *S. album* bark, was crystallized as green colored oil from the silica gel column after elution with benzene-acetone.

The compound GP-3, isolated from acetonic extract of *S. album* bark, was crystallized as colourless oil from the silica gel column after elution with benzene- acetone.

IR Spectroscopy:

IR (KBr) cm^{-1}: 3348 (O-H stretch), 1427-1597 (C-C). It was indicated from the spectrum that there was one functional groups present in the compound. The strong absorption bands at 3348 and 1427-1597 cm^{-1} correspond to characteristic O-H and C-C stretch. The bands pointed towards the presence of aliphatic region. Thus, IR spectrum supported the results obtained from chemical analysis of GP-2 (**Fig. 8-a).**

IR (KBr) cm^{-1}: 1674 (C=C) stretch, 1481-1582 (C-C). It was indicated from the spectrum that there was one functional groups present in the compound. The strong absorption bands at 1674 and 1481-1582 cm^{-1} correspond to characteristic C=C and C-C stretch. The bands pointed towards the presence of aromaticity. Thus, IR spectrum supported the results obtained from chemical analysis of GP-3 (**Fig.9-a**).

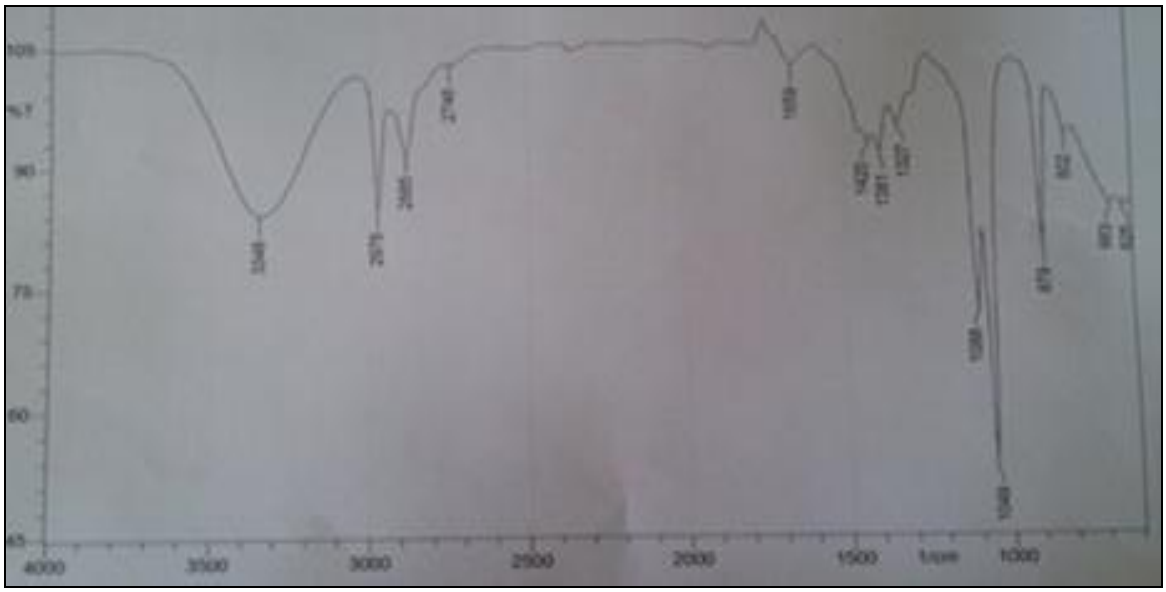
NMR Spectroscopy:

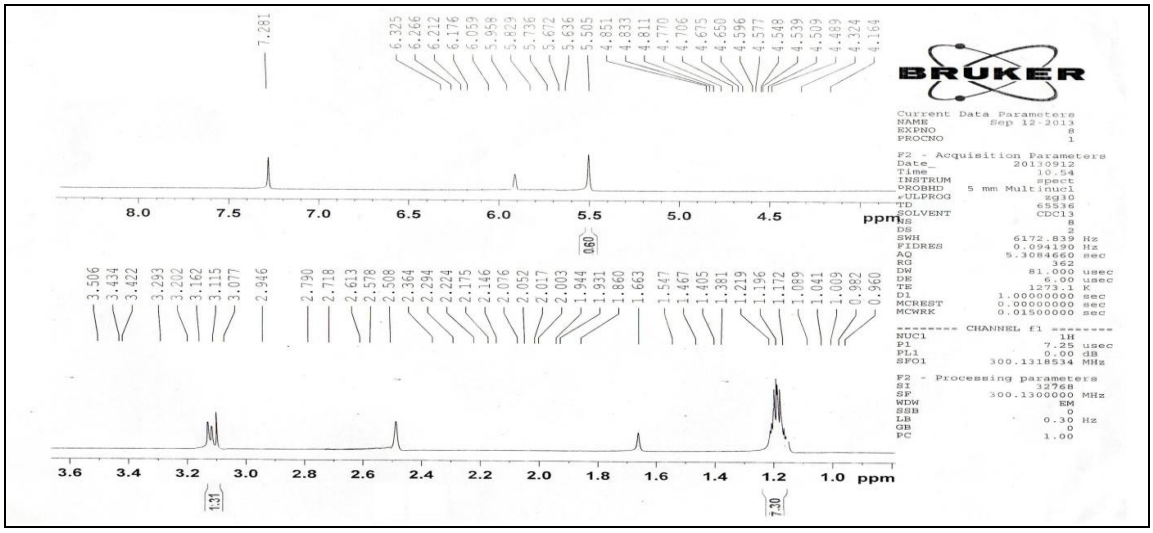
^{1}H NMR (300MHz, $CDCl_3$): **δ** 1. 172-1. 219 (m, 15H), 3. 077-3. 202 (m, 3H), 5. 505 (s,O-H). The ^{1}H NMR spectrum showed a multiplet at **δ** 1. 172-1. 219 due to fifteen protons. A multiplet at **δ** 3. 077-3. 202 due to three protons. A singlet appearing at **δ** 5. 505 due to O-H was ascribed for hydroxyl group (**Fig. 8-b**).

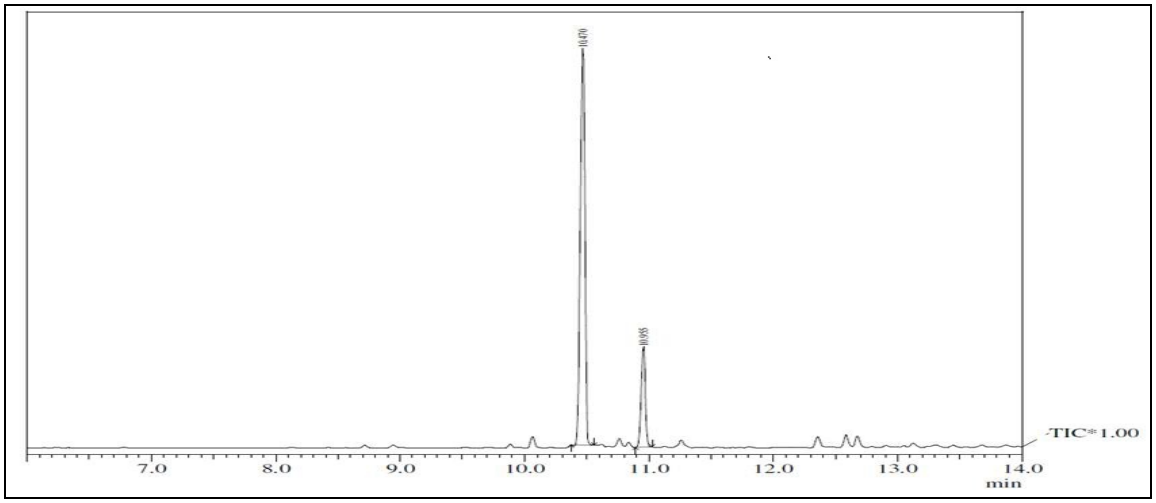
^{1}H NMR (300MHz, $CDCl_3$): δ 8. 347-8. 329 (d,1H,J=Hz), 8. 224-8. 251 (d, 1H, J=8. 1Hz), 7. 633-7. 607 (d,1H,J=8. 1Hz),7. 827-7. 809 (d,1H,J=7. 2Hz), 5. 141 (s, 1H), 2. 564 (s, 3H). The ^{1}H NMR spectrum showed doublet at 8. 347, 8. 224, 7. 633 and 7. 827due to one proton each which was assigned for aromatic protons. A singlet appearing at 5. 141 and 2. 564 due to one and three protons (**Fig. 9-b**).

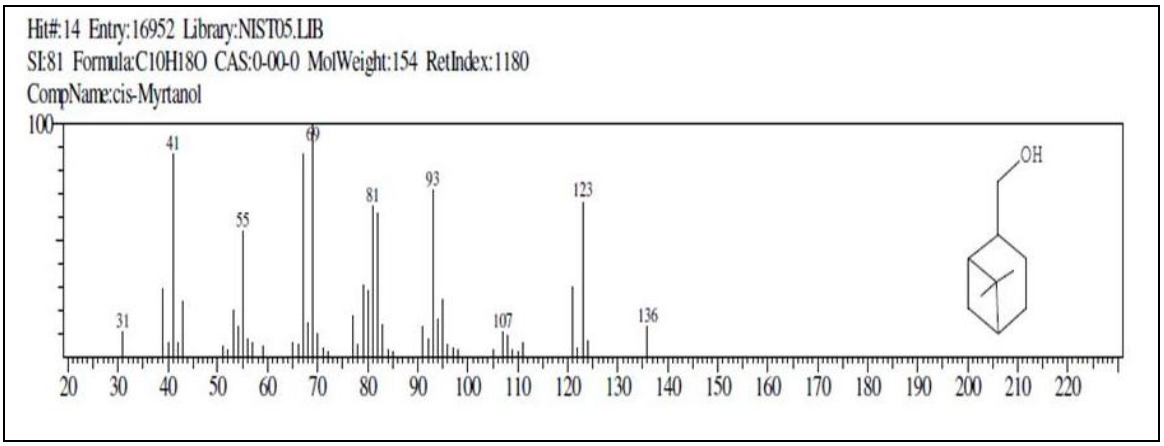
Based on IR, NMR and GC-MS spectrometry, the compound GP-2 from *S. album* bark was identified as Cis-myrtanol (**Fig. 8-c**).

Based on IR, NMR and GC-MS spectroscopy, the compound GP-3 from *S. album* bark was identified as Curcumen (**Fig. 9-c**).



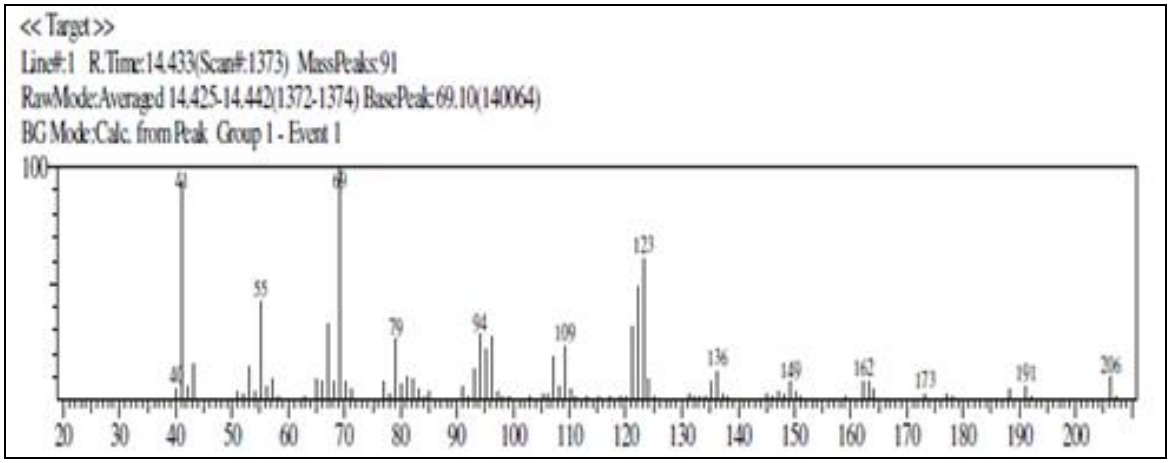


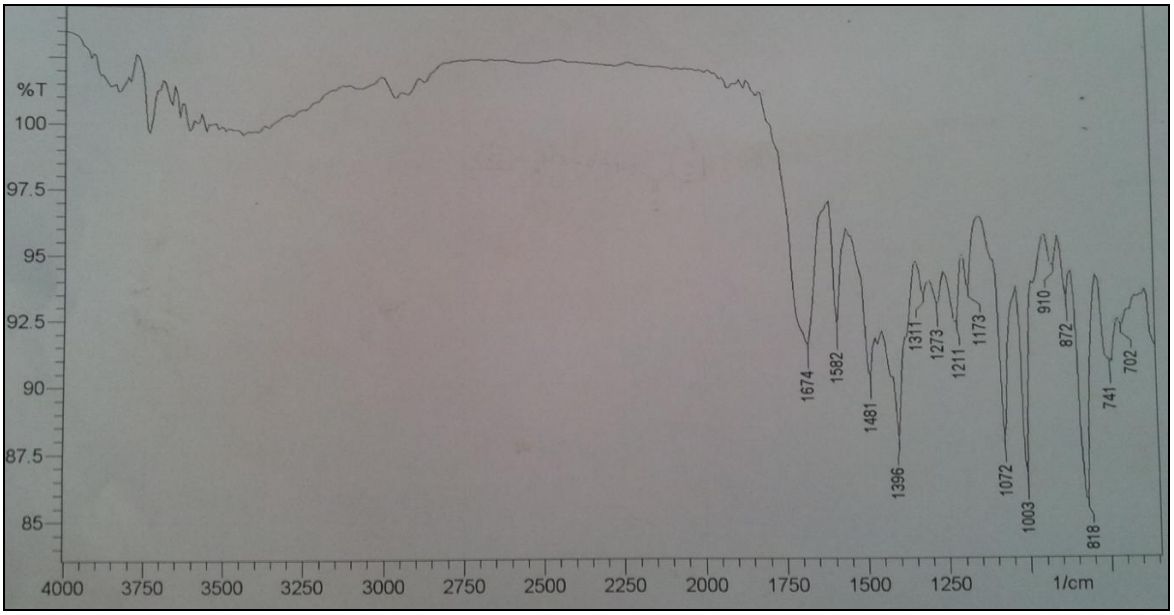


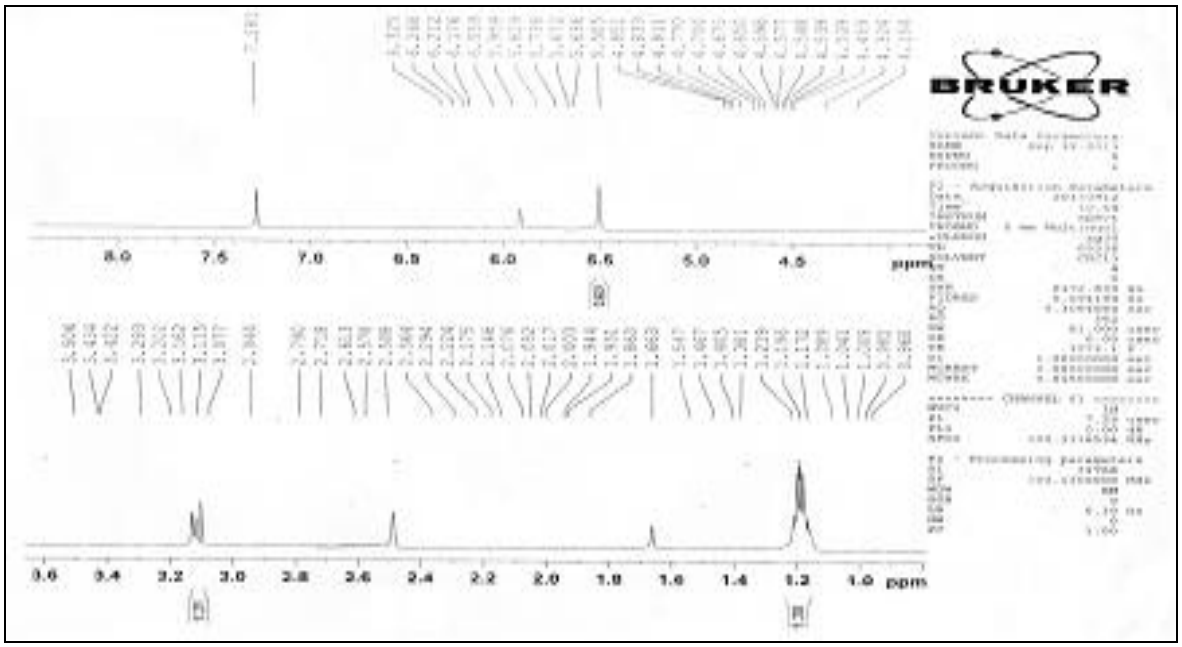


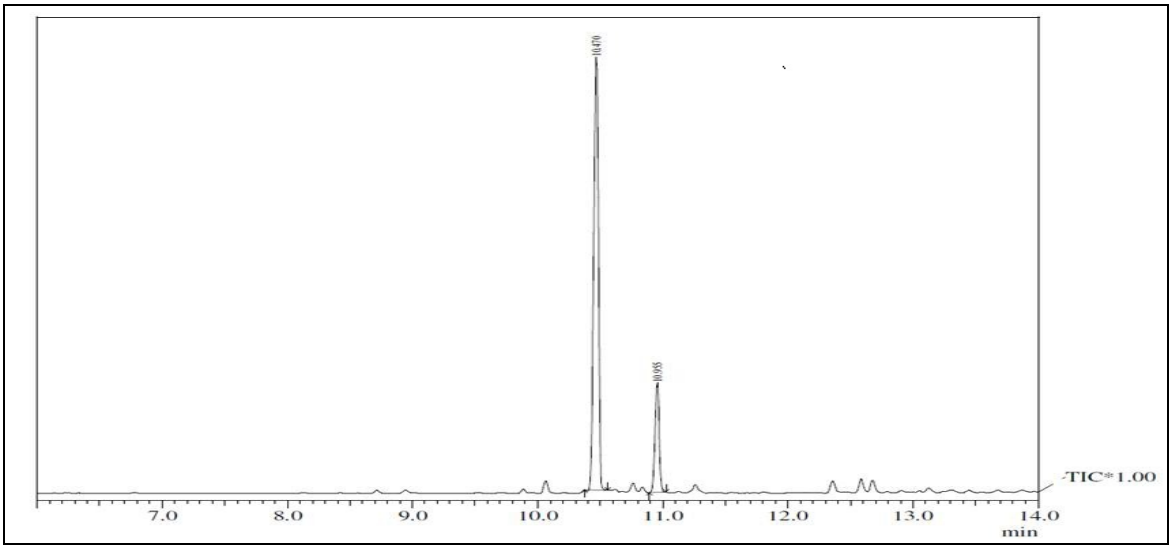
Peak Report TIC

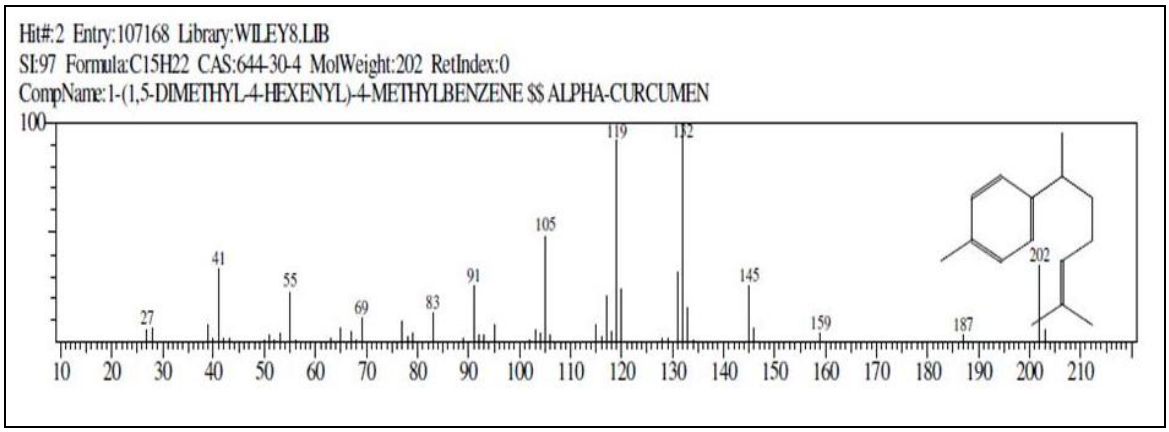
Peak#	R.Time	Area	Area%	Name
1	14.433	3584115	100.00	cis-Myrtanol
		3584115	100.00	


<< Target >>
Line#:1 R.Time:14.433(Scan#:1373) MassPeaks:91
RawMode:Averaged 14.425-14.442(1372-1374) BasePeak:69.10(140064)
BG Mode:Calc. from Peak Group 1 - Event 1
100
41
69
55
123
79
94
109
136
149
162
173
191
206
20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200


%T
100
97.5
95
92.5
90
87.5
85
1674
1582
1481
1396
1311
1273
1211
1173
1072
1003
910
872
818
741
702
4000 3750 3500 3250 3000 2750 2500 2250 2000 1750 1500 1250 1/cm

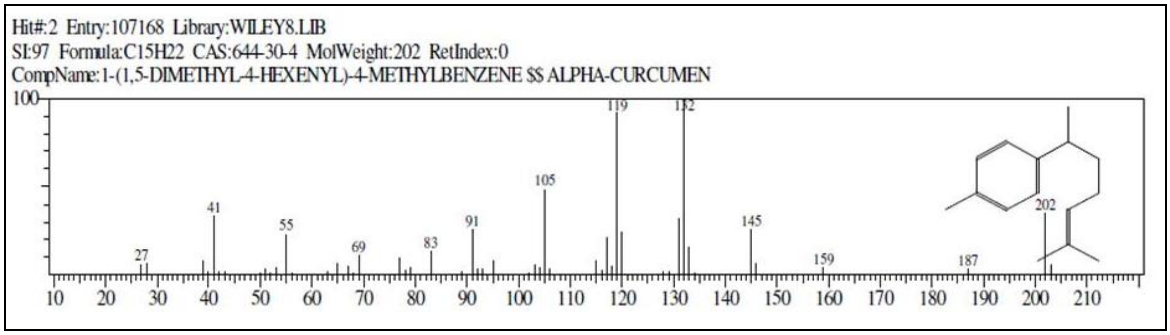

BRUKER
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 ppm
3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 ppm





Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	10.470	16138704	81.25	Curcumene
2	10.955	3725433	18.75	Beta-Bisabolene
		19864137	100.00	



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	10.470	16138704	81.25	Curcumene
2	10.955	3725433	18.75	Beta-Bisabolene
		19864137	100.00	

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.956	1149271	0.09	Benzoic acid <hexahydro->
2	5.057	3470563	0.28	2-PENTANOL, 2,3-DIMETHYL-
3	5.653	1160707	0.09	
4	7.514	1896495	0.15	BENZOIC ACID, 2-[[[4-[(ACETYLAMINO)SULFONYL]PI
5	7.977	2396522	0.19	Terpinyl acetate <alpha->
6	9.072	11420656	0.93	Vanillin
7	10.584	3163633	0.26	LONGIPINENE <ALPHA-> DB5-1267
8	10.771	1951382	0.16	2-AZIDO-1,2-DIPHENYLETHYL ACETATE
9	11.021	2712164	0.22	1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetran
10	11.394	1696201	0.14	Sesquisabinene
11	11.558	1435626	0.12	HIMACHALENE <GAMMA-DEHYDRO-AR-> DB5-1705
12	11.659	879672	0.07	BENZALDEHYDE, 3-METHOXY-4-[(TRIMETHYLSILYL
13	12.063	1509147	0.12	ROSE PHENONE
14	12.463	1967672	0.16	OXIDO HIMACHALENE DB5-1819
15	12.789	32998742	2.68	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER
16	12.953	1325975	0.11	5-METHYL-2-(1-METHYL-1-PHENYL-ETHYL)-CYCLOH
17	13.191	8110004	0.66	HIMACHALENE OXIDE <BETA-> DB5-1906
18	13.442	511511	0.04	Epicubenol
19	13.694	4252528	0.35	Cadin-4-en-10-ol
20	13.940	8464843	0.69	Cadin-4-en-10-ol
21	14.086	28003786	2.27	Tumerone <ar->
22	14.750	14316737	1.16	EPI-ALPHA-PATSCHULENE
23	14.871	4638658	0.38	1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]dodec-8(9)-ene
24	15.064	1729922	0.14	BENZENE, (3-NITROPROPYL)-
25	15.227	1021231	0.08	DRIMENOL DB5-2239
26	15.325	710686	0.06	Hexadecane, 2,6,10,14-tetramethyl-
27	15.592	19498464	1.58	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
28	15.908	2897470	0.24	Benzyl benzoate
29	16.058	8653921	0.70	Atlantone <trans-, alpha->
30	16.241	2362396	0.19	Fluoranthene, hexadecahydro-
31	16.378	13998245	1.14	2,6,10-TRIMETHYLUNDECAN-(5E)-2,5,9-TRIEN-4-ONE
32	16.765	5456042	0.44	2,6,10-TRIMETHYLUNDECAN-(5E)-2,5,9-TRIEN-4-ONE
33	16.872	11040041	0.90	Bicyclo[3.2.0]hept-2-en-6-one, 5-methoxy-7-phenyl-, (Z,exo+e
34	17.121	52361970	4.25	Manool
35	17.474	2940356	0.24	1H-Naphtho[2,1-b]pyran-8(4aH)-one, 3-ethenyldecahydro-3,4
36	17.880	468769	0.04	HEXADECANE, 2,6,10,14-TETRAMETHYL-
37	18.004	2042044	0.17	Heptadecane <n->
38	18.494	4815999	0.39	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-me
39	18.688	3149937	0.26	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-me
40	19.178	18876834	1.53	9-OCTADECENOIC ACID (Z)-
41	19.359	2600641	0.21	Manool
42	19.624	2530991	0.21	Heptadecane, 2,6,10,15-tetramethyl-
43	19.791	3552197	0.29	CATALPONOL <EPI-> DB5-2712

Peak#	R.Time	Area	Area%	Name
44	20.036	4222116	0.34	4,4'-BIS(TRIMETHYLSILYL)-1,1'-BIPHENYL #
45	20.227	8318381	0.68	1,4-Naphthalenedione, 2-hydroxy-3-(3-methyl-2-butenyl)-
46	20.571	53271366	4.32	[1,1'-Biphenyl]-2-ol, 5-(1,1-dimethylethyl)-
47	20.963	7540443	0.61	2H-Benz[e]indol-2-one, 1,3,3a,4,5,9b-hexahydro-
48	21.202	3616524	0.29	TRICOSANE
49	21.425	1192679	0.10	CINNOLINE, 4-METHYL-, 1-OXIDE
50	21.737	129353589	10.50	9,10-Anthracenedione, 2-methyl-
51	21.928	3219294	0.26	
52	22.355	7051499	0.57	Indane-1,3-dione, 2-(3-hydroxyphenyl)-
53	22.649	5976426	0.49	1-CHLOROOCTADECANE
54	22.826	2792124	0.23	Dec-5-ene-3,7-diyne, 2,9-dimethyl-
55	22.948	3568196	0.29	9,10-Anthracenedione, 2-(1,1-dimethylethyl)-
56	23.228	9514010	0.77	1-Hydroxy-4-methylanthraquinone
57	23.394	1166843	0.09	Trimethyl-[1-(2-methyl-propenyl)-1,2,3,4-tetrahydronaphthale
58	23.776	10749502	0.87	1-Formylanthraquinone
59	24.370	3896510	0.32	1-[1-(2,6-DIMETHYLPHENYL)-1H-TETRAAZOL-5-YL]-N
60	24.685	7411045	0.60	Bicyclo[2.2.1]heptane, 2-(dicyanomethylene)-1,7,7-trimethyl-
61	25.094	3317092	0.27	2-BUTANONE, 3-METHYL-1-(TRIMETHYLSILYL)-
62	25.428	4305279	0.35	Heneicosane
63	25.864	1218152	0.10	2-Methyl-cis-7,8-epoxynonadecane
64	26.253	15724049	1.28	Benzonitrile, m-phenethyl-
65	26.516	10961157	0.89	2-(Hydroxymethyl)anthraquinone
66	26.750	11221682	0.91	1,3,5-TRIOXA-2,4,6,8-TETRASILACYCLOOCTANE, 2,2,4
67	27.198	674124	0.05	TETRADECANAL
68	27.325	591267	0.05	2-BUTENOIC ACID, ETHYL ESTER, (E)-
69	27.523	16830359	1.37	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
70	27.723	21220145	1.72	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
71	27.972	5915037	0.48	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
72	28.107	1590126	0.13	
73	28.751	16207255	1.32	3-ISOPROPYL-7-METHYL-3-OCTENE-1,5-DIYNE
74	29.360	4329216	0.35	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
75	29.743	1842174	0.15	
76	30.616	3561899	0.29	9,10-Anthracenediol, diacetate
77	31.810	2876178	0.23	Squalene
78	32.101	2872985	0.23	Decanedioic acid, bis(2-ethylhexyl) ester
79	32.592	234017430	18.99	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexameth
80	32.878	902449	0.07	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene
81	33.855	6200960	0.50	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexar
82	33.975	7466904	0.61	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15
83	34.047	18252074	1.48	2,6,10,14,18-PENTAMETHYL-2,6,10,14,18-ICOSAPENTA
84	35.851	4219027	0.34	Neoisolongifolene-8-ol
85	36.240	17308971	1.40	Ergost-5-en-3-ol, (3.beta.)-
86	36.481	12099284	0.98	STIGMASTA-5,22-DIEN-3-OL
87	36.733	26762145	2.17	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-

Peak#	R.Time	Area	Area%	Name
88	36.982	42187375	3.42	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-
89	37.323	46092413	3.74	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-
90	37.669	29453911	2.39	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-
91	37.788	6235614	0.51	Thunbergol
92	37.954	2277563	0.18	7,22-Ergostadienone
93	38.095	9549559	0.78	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.b
94	38.256	34903087	2.83	<NO NAME>
95	38.569	2099352	0.17	Manool
96	39.029	1705234	0.14	DI-(T-BUTYL)-HYDROXYPHENYL T-BUTYL-HYDROX
97	39.208	2480799	0.20	Friedelan-3-one
98	39.573	3656044	0.30	(5,16-DIMETHOXYDINAPHTHO[2,3-A:2',3'-G]AZULENE
99	39.825	6897749	0.56	RT:39.825
100	39.990	1666555	0.14	Betulin
101	40.172	1356072	0.11	Stigmastane-3,6-dione, (5.alpha.)-
102	40.358	10417323	0.85	2H-NAPHTHO[1,2-B]PYRAN, BIMOL. DERIV.
103	40.702	2210374	0.18	
104	40.889	2125613	0.17	TRICYCLO[20.8.0.0E7,16]TRIACONTAN, 1(22),7(16)-DIE
105	42.733	5373317	0.44	Benzene, 1,1'-[1-(2,2-dimethyl-3-butenyl)-1,3-propanediyl]bis
106	45.721	3533686	0.29	HEPTACYCLO[28.2.2.2(14,17).1(4,8).1(7,11).1(20,24).1(2
107	46.415	2288344	0.19	MORUSIN-DIMETHYL ETHER
		1231998602	100.00	

Anticandidal activity of *S. album* bark purified compounds:

The results of anticandidal properties of *S. album* subfractions- cis-myrtanol, curcumen and the negative control (DMSO) are presented in Table. 7. A persual of the data reveals that of the two pure subfractions, cis-myrtanol showed good activity against all the tested species, the zone of inhibition ranging between 20.6mm and 23.6mm, with highest zone of inhibition 23.3mm against *C. albicans* strain-1 (MTCC No. 4748) followed by *C. albicans* strain-2 (22.6mm) and *C. krusei* (20.6mm). In case of curcumen, the zones of inhibition ranged between 15.6mm and 20.6mm with maximum against *C. albicans* strain-2 (20. 6mm) followed by *C. albicans* strain-1 (19. 3mm) and *C. krusei* (15. 6mm).

TABLE 7: ANTICANDIDAL ACTIVITY OF CIS-MYRTANOL AND CURCUMEN AGAINST *CANDIDA* SPP.

Purified compounds (mg/ml)	Ca-1	Ca-2	Ck
Cis-Myrtanol	23. 6[a]±0. 57†	22. 6±0. 57	20. 6±0. 57
Curcumen	19. 3±1. 52	20. 6±0. 57	15. 6±1. 52
DMSO	-	-	

Ca, *C. albicans* strain-1 (MTCC No. 4748); Ca-,*C. albicans* strain-2; Ck, *C. krusei*, No activity; [a] Values, including diameter of the well (8mm), are means of three replicates. ± † Standard deviation.*Anticandidal activity was determined by agar well diffusion method on MEA medium.

Evaluation of synergistic/antagonistic anticandidal activity of pure compounds among themselves and with commercially available antifungal drugs:

The synergistic anticandidal potential of the two pure compounds isolated from *S. album* bark, determined by agar well diffusion method, are presented in **Table 8**. A persual of the data reveals that the combination of two pure compounds (cis-myrtanol + curcumen, 1:1) showed antagonistic effect against all the tested *Candida* spp. thus reducing the diameter of inhibition of microbial growth. The two compounds in combination produced zones in the range of 17. 3mm and 12. 3mm, maximum against *C. krusei* (17. 3mm) which were lesser than their individual zones of inhibition.

TABLE 8: SYNERGISTIC ACTIVITY OF PURE COMPOUNDS (INDIVIDUALLY AND IN COMBINATION) ISOLATED FROM *S. ALBUM* BARK.

Purified compounds (mg/ml)	Ca-1	Ca-2	Ck
Cis-Myrtanol	23. 6[a]±0. 57†	22. 6±0. 57	20. 6±0. 57
Curcumen	19. 3±1. 52	20. 6±0. 57	15. 6±1. 52
Cis-Myrtanol + Curcumen	16. 6±0. 57	-	17. 3±0. 57
DMSO	-	-	-

The two compounds when tested in combination of 1:1 with the best active active (Neem ras) in order to determine their synergistic/antagonistic effects on *Candida* spp. through agar well diffusion method, revealed that antifungal neem ras showed antagonistic effect reducing the diameter of inhibition of *Candida* growth.

TABLE 9: EFFECT OF PURE COMPOUNDS ON THE ANTICANDIDAL ACTIVITY OF NEEM RAS ON *CANDIDA* SPP.

Purified compounds (mg/ml)	Ca-1	Ca-2	Ck
NR+Cis-myrtanol	17. 3	14. 0	-
NR+ Curcumen	13. 0	15. 6	15. 3
NR	16. 6	17. 6	-
DMSO	-	-	-

CONCLUSION: In this study, the crude extract of bark of *Santalum album* by GC-MS study results in the presence of 107 compounds. *Santalum album* was found to be the most potent plant possessing anticandidal activity against all the species of *Candida* in all the six solvents. *C. albicans* (4748) was most susceptible with maximum zone of inhibition 34. 6mm followed by *C. glabrata* (32. 6mm), *C. tropicalis* (14. 3mm). *C. albicans* (30. 6mm), *C. krusei* (22. 6mm) and *C. lusitaniae* (20. 3mm) were different isolates showing activity. On the basis of results obtained in the GC-MS analysis, the column chromatography of the plant part was done. The column sub fractions lead to the isolation of two and one active pure sub fractions each, GP-2 and GP-3 from *S. album* bark.

The purified compounds isolated from the acetonic extract of *S. album* bark namely, GP-2 was crystallized as green coloured oil from the silica gel column after elution with benzene-acetone. Based on spectra obtained through IR, NMR and GC-MS spectrometry, the compound GP-2 was identified as cis-myrtanol, C10H18O. Another purified compound GP-3, was crystallized as colourless oil from the silica gel column after elution with benzene- acetone. Based on spectra obtained through IR, NMR and GC-MS spectrometry, the compound GP-3 was identified as curcumen, C15H22. Among the two pure subfractions, cis-myrtanol showed good activity against all the tested species, the zone of inhibition ranging between 15.0mm and 24.3mm, with highest zone of inhibition 24.3mm against *C. tropicalis* (Fig) followed by *C. albicans* (4748) (23.6mm), *C. albicans* (22.6mm), *C. krusei* (20.6mm), *C. glabrata* (20.3mm) and *C. lusitaniae* (15. 0mm). In case of curcumen, the zones of inhibition ranged between 15.6mm and 24.6mm with maximum against *C. tropicalis* (24.6mm) followed by *C. albicans* (20.6mm), *C. albicans* (4748) and *C. lusitaniae* (19.3mm) and *C. krusei* (15.6mm).

Furthermore, the integration of herbal medicine into modern medical practices must take into account the interrelated issues of quality, safety, and efficacy. Thus, after studying various plants for their anticandidal activity, the crude extracts and purified compounds from the plant can be incorporated into medications for various antifungal therapy. However, further studies for their incorporation into different preparations, safety and cost- effectiveness has to be conducted.

COMPETING INTERESTS: The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS: We are grateful to Dr. B.D. Vashishta, Chairman, Department of Botany, Kurukshetra University, Kurukshetra, for helping with the identification of the plant samples. The authors are very thankful to the Department of Chemistry, Kurukshetra University, Kurukshetra, for providing the necessary facilities for NMR, FTIR analysis of the compounds and also thankful to AIRF (Advanced instrumentation research

facility), JNU (Jawaharlal National University) for providing facilities for GC-MS.

CONFLICT OF INTEREST: With the submission of this manuscript I would like to undertake that all authors of this research paper have directly participated in the planning, execution, or analysis of this study. All authors of this paper have read and approved the final version submitted. The contents of this manuscript have not been copyrighted or published previously. The contents of this manuscript are not now under consideration for publication elsewhere. The contents of this manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the Journal is under consideration. There are no directly related manuscripts or abstracts, published or unpublished, by any authors of this paper. My Institute's representative is fully aware of this submission.

REFERENCES:

1. Imamura Y, Izumikawa K and Kohno S: Candidiasis 2008; 12: 2341-2344.
2. Pfaller MA and Diekema DJ**:** Epidemiology of invasive candidiasis: a persistent public health problem. Clincal Microbiol Rev 2007; 20: 133-163.
3. Achkar JM and Fries BC: *Candida* Infections of the Genitourinary Tract. Clin Micro Reviews 2010; 253–273.
4. Fenn JP: Update of medically important yeasts and a practical approach to their identification. Labmedicine 2007; 38 (3): 178-183.
5. Kirk PM, Cannon PF, Minter DW and Stalpers JA: Ainsworth and Bisby's Dictionary of the Fungi. Wallingford: CABI, Tenth Edition 2008.
6. Boklari FM: Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia. Mycopathologia 2009; 7(1): 51-57.
7. Al-Juraifani, AA: Antimicrobial activity of some medicinal plants used in Saudi Arabia. Canadian journal of pure & applied sciences 2011; 5(2): 1509-1512.
8. Aneja KR and Mehrotra RS: Fungal diversity and biotechnology. New age International (P) Ltd. New Delhi, Third Edition 2011.
9. Doran JC, Thomson L, Brophy JJ, Goldsack B, Bulai P, Faka'osi T and Mokoia T: Variation in heartwood oil composition of young sandalwood trees in the south Pacific (*Santalumyasi*, *S. album* and F1 hybrids in Fiji, and *S. yasi* in Tonga and Niue). Sandalwood Research Newsletter 2005; 20: 3-7.
10. Brand JE, Fox JED, Pronk G and Cornwell C: Comparison of oil concentration and oil quality from *Santalumspicatum*, *S. album* plantations, 8-25 years old, with those from mature *S. spicatum* natural stands. Australian Forestry 2007; 70(4): 235-241.
11. Baldovini N, Delasalle C and Joulain D: Phytochemistry of the heartwood from fragrant *Santalum* species: a review. Flavour and Fragrance Journal 2011; 26: 7−26.
12. Jones CG, Moniodis J, Zulak KG, Scaffidi A, Plummer JA, Ghisalberti EL, Barbour EL and Bohlmann J: Sandalwood fragrance biosynthesis involves Sesquiterpene synthases of both the Terpene Synthase (TPS)-an and TPS-b subfamilies, including santalene synthases. The Journal of Biological Chemistry 2011; 286 (20): 17445-17454.
13. Jones CG, Keeling CI, Ghisalberti EL, Barbour EL, Plummer JA and Bohlmann J: Isolation of cDNAs and functional characterisation of two multiproduct terpene synthase enzymes from sandalwood, *Santalum album* L. Archives of Biochemistry and Biophysics 2008; 477(1): 121−30.
14. Aneja KR, Joshi R, Sharma C and Aneja A: Antimicrobial efficacy of fruit extracts of two *Piper* species against selected bacterial and oral fungal pathogens. Braz. J. Oral. Sci 2010; 9(4): 421-426.
15. Aneja KR, Joshi R and Sharma C: Potency of *Barleriaprionitis* L. bark extarcts against oral diseases causing strains of bacteria and fungi of clinical origin. New York Sci J 2010b; 3(11): 5-12.
16. Ahmad N, Fazal H, Abbasi BH, Farooq S Ali, M and Khan, MA: Biological role of *Piper nigrum L*. (Black pepper): A review, Asian Pacific Journal of Tropical Biomedicine 2012; S1945-S1953.

How to cite this article:
Surain P, Kumar N, Dhiman R and Measht V: *Santalum Album*: Clinical Aspects for Treatment of *Candida* Infections. Int J Pharm Sci Res 2016; 7(7): 2813-29.doi: 10.13040/IJPSR.0975-8232.7(7).2813-29.