Sandalwood, an Indian medicinal plant attenuates the microbial growth and influence up/down regulation of the metabolites

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Running title: Metabolite up/down regulation in *E. coli*

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Abstract

The methanolic extract of sandalwood (SwME) was prepared by soxhlet apparatus and the antibacterial assay was performed. Further, the metabolite profiling of SwME and lysates of *E. coli* and *E. coli* grown in the presence of SwME was generated. SwME showed maximum inhibition against *E. coli* (MTCC 443) i.e. 82.71%, and minimal against *B. subtilis* (MTCC 736) i.e. 26.82%. The metabolome profiles of *E. coli* and SwME were generated using gas chromatography-mass spectrometry (GC-MS) technique. Comparative studies were done to understand to what extent metabolite modifications differ between SwME, *E. coli* lysate and the *E. coli* strain grown in presence of extract. Result revealed 23 peaks with major compounds present in *E. coli* were 9-Octadecenoic Acid (Z)-, Methyl Ester (26.85%), Hexadecanoic Acid, methyl ester (20.5%) and Hexadecanoic acid, trimethylsilyl ester (15.79%). When *E. coli* was grown in the presence of SwME, GC-MS analysis showed 25 peaks with major compounds such as 9-Octadecenoic Acid, Methyl Ester (21.97%), Hexadecanoic Acid, Methyl Ester (17.03%), and Hexadecanoic Acid, Trimethylsilyl Ester (14.96%). Correlating the metabolic profiles with the changes occurring is essential to progression their comprehension and in the development of new approaches to identify the metabolomics regulation in *E. coli* in response to SwME.

Keywords

Antibacterial activity; Metabolite profiling; GC-MS analysis; Quenching; *Santalum album*; Chromatography

1. Introduction

With the advancement in human livings effected by technology and such innovations, they say our disease causing organism have also developed themselves. This is currently the most focused aspect of humans, the new or improved ways of defense and cure from the life threatening disease. The present chemical based pharmaceutical is looking new hopes in traditional or natural origin medicines which would be efficiently effective against disease causing microorganism which are now said to immune themselves from the chemical based medicine available in the market (Baker et. al. 2007, Beghyn et. al. 2008 and Brinskin 2000). Nature has all the solutions to mankind problems; such is the natural or ayurvedic medicine derived from the plants, animals or marine. Therefore, several plants have been selected based on their use in traditional systems of medicine (Ji et. al. 2009). Among the thousands of such therapeutic plants, Sandalwood is one of the well-known plants from the Indian origin that has been used in cosmetics and therapeutics and is used by Ayurvedic medicinal practitioners in India for almost 3000 years. It is known for its multi-therapeutic properties and is being used since a long time in this part of world as a cosmetics and therapeutic agent. The Indian plant, *Santalum album* may serve as a dietary antioxidant, with several modes of action to protect the body organs against oxidative damage and age-related cognitive decline. Aqueous extract of *Santalum album* showed good activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* and the aqueous extract showed maximum activity against *S. aureus* (Shamsi et. al. 2014).

Plants are known to possess the various complex phytochemical compounds that may be used in the prevention and treatment of several human diseases (Hall et. al. 2002). Identification of phytochemicals or bioactive compounds and their characterization are required to explore the medicinal value of the plant which is an ongoing challenge in this era. This process needs the several extraction methods for the metabolites extraction and the advanced techniques that can identify, detect and characterize these bioactive compounds (Wolfender et. al. 2015). Metabolomics is a field where various analytical strategies and platform in application of natural extracts develop the chemical profiles. The information obtained from this approach might be useful in the determination of various biological aspects of plants such as defense, growth, development, productivity, responses to external stresses etc. (Desai and Alexander 2013, Misra et. al. 2014). Gas chromatography (GC) coupled with mass spectrometry (MS) is the analytical platform and strategy that can be employed for the analysis of metabolites found in the plant extracts

qualitatively and quantitatively (Wong et. al. 2015). GC-MS analysis can identify up to approximately 100 metabolites in the simple plant extracts and can generate a metabolite profile (Benina et. al. 2013, Obata et. al. 2013, Sauter et. al. 1991, Lee et. al. 2014). The effective and better identification of the obtained metabolites can be easily done by comparing the results with the database stored in the large mass spectral library based on GC-MS electron impact (EI) (Musharraf et. al. 2013). The sample preparation for the extraction of metabolites also plays a key role in this analytical platform of metabolomics and is known to be a major factor that affects the profiling of metabolite (Ying et. al. 2009). Metabolites extraction can be done in various organic solvents such as ethanol, water, methanol, and hexane and can be carried out by using various conventional extraction methods followed by lyophilization and then monitoring and generating the profiles of extracted metabolite using several analytical platforms (Lapornik et. al. 2009, Ahmad and Shah 2015). Various factors such as type of solvent, extraction method, temperature etc. affect the metabolic content of crude extracts of plants (Kindt et. al. 2009). For the study of above mentioned properties of *Santalum album*, antibacterial assay and GC-MS analysis was performed to know the metabolite composition of *Santalum album* methanolic extract, *E. coli* (the most susceptible bacterial strain) lysate and the *E. coli* grown in presence of *Santalum album* methanolic extract.

2. Materials and methodology

2.1. Plant material/ Chemicals

The medicinal plant sandalwood (*Santalum album*) was purchased from Local Ayurvedic Clinic, Rampur, Uttar Pradesh, India. The chemicals of analytical grade were purchased from Merck and HI media.

2.2. Test microorganism

The identified bacterial strains *Staphylococcus aureus* (MTCC902), *Escherichia coli* (MTCC443), *Bacillus subtilis* (MTCC736), and *Pseudomonas aeruginosa* (MTCC2453) were obtained from NCCS, Pune, India. Stock cultures of all the tested bacterial strains were maintained on nutrient agar slants at 4°C.

2.3. Preparation of methanolic crude extract of sandalwood

The sandalwood was ground finely in a grinder mixer and then 10 g of powdered sandalwood was placed in a soxhlet apparatus. Extraction was carried out with 250 ml of methanol for 36-48 h. The temperature was maintained below the boiling point of the solvent. Further, the extract was filtered through a 0.45 µm filter. The filtrate was then concentrated by Rotary Evaporator and it served as the methanol extract of sandalwood (SwME) for the further exploration and activities. The extract was stored in a refrigerator at 4°C for further use.

2.4. Determination of antibacterial potential of sandalwood methanolic extract (SwME)

SwME was evaluated for its antibacterial potential against several bacterial strains as the method described by Shamsi et al. 2014 with some modifications. A single colony from agar plates of each bacterial strains was inoculated in autoclaved Luria broth (LB) media for bacterial growth. The cultures were incubated at 37°C for 12-24 h. Next day, the LB broth was added to the bacterial cultures to dilute it up to 1×10^4 colony-forming units (CFU). Approximately 50 µl of 5mg/ml SwME (250µg SwME) was mixed with diluted bacterial cultures in 96-well plate. The final volume in each well was maintained up to 300 µl using LB media. Ampicillin (Stock 10 mg/ml) was used as positive control and 25 µl of it (250 µg ampicillin) was applied in each well containing LB media and bacterial broth culture. The results were compared with negative controls which contained the LB media and bacterial culture. Blanks contained LB media only and the LB media with SwME. 96-well plate was further incubated at 37°C for 14-16 hrs. After incubation, absorption measurements were determined using ELISA microplate reader at 600 nm. The percentage mean growth inhibition (MGI %) was calculated by $\% \text{ MGI} = [(A_c - A_t)/A_c] \times 100$.

Where, A_c and A_t are the absorbance of negative control and SwME treated strains respectively.

The EC_{50} value (concentration causing 50 % reduction in the bacterial growth) was calculated using Microsoft office Excel 2007, based on the readings.

2.5. The GC-MS analysis

2.5.1. Homogenization and extraction

Homogenization and extraction were the preliminary step to quench biomass for the release of metabolites. Homogenization is carried out by a grinder. The solvent methanol was degassed and cooled before the extraction procedure.

2.5.2. Biomass Quenching

SwME, and the lysates of *E. coli* and *E. coli* grown in the presence of SwME were taken and the biomass was centrifuged for 20-30 min to pellet down the biomass at 5000 rpm at 4°C. Then, the supernatant was discarded and an approximately 1 ml aliquot was preserved for the determination of leakage of internal metabolites. Further, the pellets and supernatants were frozen in liquid nitrogen followed by storing them at -80°C and then the further analysis was done.

2.5.3. Extraction of metabolites

The biomass in the form of pellets were mixed with 500 µl of 100% chilled methanol (-48°C), followed by freezing in liquid nitrogen and then allowed to thaw on dry ice. The process of freezing followed by thawing was performed several times which helped in the permeability of the cell membrane, which resulted in the metabolite leakage from the cells. The samples were then centrifuged at 10000 g for 5-10 min at 4°C. Then, the supernatant was obtained and preserved on dry ice. Approx. 500 µl aliquot of 100% methanol (-48°C) was further mixed with the pellet and then stored on dry ice to perform further analysis.

2.5.4. Mass spectrometric analysis

GC-MS analysis of lysate of *E. coli*, SwME and *E. coli* grown in presence of SwME was performed on a GC-MS equipment. The dimensions of GC-MS column (non-polar column) used were about 30 m × 0.25 mm with a film thickness of 0.25 mm based on capillary standard. The flow rate was maintained up to 0.5 ml/min and approximately 1 µl of the concentrated sample was utilized. The methanolic samples were run fully at a range of 50-650 m/z and the inlet temperature was maintained up to 250°C. The samples were run for approx. 90 min and GC-MS was examined by using electron impact ionization at 70eV. The obtained spectrums of the samples were compared with the database of spectrum of known compounds which are stored in the GC-MS NIST library to evaluate the total ion count for compound identification and robust quantification. Further, the peak areas, retention time etc. were measured and data processing was performed by Turbo-Mass software. The raw GC-MS chromatograms were transferred to a server station by recording two scans per second in full scan mode.

2.5.5. Data acquisition by GC-MS

Peak identifications were carried out by matching retention indices and mass spectral similarity against a user-defined metabolite library. Metabolite peaks were quantified by area of target ion traces. The obtained number of metabolites was counted per chromatogram.

2.5.6. Identification of components

Identification of the obtained compounds were done by comparing their GC-MS mass spectrum by using the database of National Institute Standard and Technology (NIST) with software Turbomas 5.2 which stores approximately 62,000 patterns which was based on their molecular mass, molecular structure, calculated fragments. The database then generated a table which included the name, molecular weight and structure of the tested components by comparing to the database. The % relative of each compound was calculated by comparing the average peak area in the chromatogram to the total areas. The detection of compounds helped in the determination of the current commercial, industrial and traditional use of crude extracts as an herbal medicine. This method also helped in the determination of the best extraction methods for these components. The identified compound now further can be explored for their putative biological or therapeutic relevance. GC-MS software that was able to perform multi-target analysis also included quantitation by area and height of the peak in the chromatogram.

2.6. Statistical analysis

The experiments were performed in triplicates (n=3). Graph Pad Prism Software was used to prepare Graphs and to calculate the statistical parameters such as mean, standard error of mean (SEM) etc. The data obtained were expressed as 'mean±SEM'.

3. Results

3.1. Determination of antibacterial activity of SwME

Antibacterial potential of the SwME was evaluated by assessing the percentage mean growth inhibition in the presence of SwME and the results were compared to the proper positive control. The results exhibited fair antibacterial property

188 *in-vitro* against all the tested strains i.e. the bacterial growth was diminished in the presence of SwME as shown in Fig.
189 1. Results showed that SwME had strongest inhibitory activity against *E. coli* (MTCC 443) i.e. 82.71% and minimal
190 inhibition against *B. subtilis* (MTCC 736) i.e. 26.82% at approx. 0.833 mg/ml concentration of SwME. Results were
191 quite comparable with synthetic antibacterial drug ampicillin which showed almost complete inhibition of bacterial
192 pathogen at the same concentration of antibiotic.

193 Generally, *B. subtilis* was the only strain which was less susceptible to the antibacterial effect of the SwME and
194 ampicillin as compared to the other strains. SwME exhibited different degree of growth percentage inhibition among
195 tested bacterial strains which were presented in the form of EC₅₀ values (Table 1).

196 3.2. GC-MS analysis

197 Identification and quantification up to 100 metabolites can be performed using an important and critical technique i.e.
198 GC-MS based metabolite profiling. These metabolites may incorporate sugars, hydrocarbons, phenolic compounds,
199 carboxylic acids, sugar alcohols, alcohol acids, esters, amino acids, and polyamines etc. and provide a far reaching
200 scope of the primary metabolism central pathways. The present study emphasizes on use of the GC-MS analysis for
201 identification and quantification of the metabolites or compounds present in the methanolic extract of sandalwood, *E.*
202 *coli* and the *E. coli* grown in presence of SwME. The GC-MS analysis spectra of SwME, *E. coli* and *E. coli* +SwME
203 showed and identified the presence of several phytocompounds that could contribute to the ethno-medicinal quality
204 and the pharmacological nature of the plant. Phytocompounds were identified and confirmed by comparing their
205 retention time, peak area, molecular formula etc. to the database of NIST library.

206

207 3.2.1. Identification of compounds in SwME

208 GC-MS chromatogram of the SwME (Fig. 2) showed 43 peaks indicating the presence of forty one compounds. The
209 chemical compounds identified in the methanolic extract of the sandalwood extract are presented in Table 2. Based on
210 abundance, the most prevailing compounds present in the methanolic extract were Piperidine, 1-[5-(1,3-Benzodioxol-
211 5-yl)-1-oxo-2,4-Pentadienyl] (15.08%), 2-Penten-1-ol, 5-(2,3 Dimethyltricyclo[2.2.1.0(2,6)]Hept-3-yl)-2Methyl
212 (14.44%) and 2-Penten-1-ol, 2-Methyl-5-(2-Methyl-3-Methylene-2-Norbornyl) (8.83%). Acetonyl Decyl Ether
213 (0.09%), Pregnane, Silane Derivative (0.1%) and 1, 2-Benzenedicarboxylic Acid, Diisooctyl Ester (0.2%) were present

in low amount. The result of GC-MS analyses unveiled that the SwME is primarily composed of oxygenated hydrocarbons and prevalently phenolic hydrocarbons. The phytochemical compounds revealed through GC-MS analysis are those phytochemicals which are responsible for various pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant activities etc.

3.2.2. Identification of compounds in *E. coli* lysate

Further, the lysate of *E. coli* revealed the presence of 23 peaks in the GC-MS chromatogram which indicates the presence of 20 compounds. The major compounds obtained were 9-Octadecenoic Acid (Z)-, Methyl Ester (26.85%), Hexadecanoic Acid, methyl ester (20.5%) and Hexadecanoic acid, trimethylsilyl ester (15.79%) and the 1-Hexyl-1-Nitrocyclohexane (0.23%), Pentadecane (0.32%) and Heptadecanoic Acid, Methyl Ester (0.35%) were found in rare amount (Fig. 3, Table 3).

3.2.3. Identification of compounds in *E. coli* grown in the presence of SwME

While, when *E. coli* was grown in the presence of SwME to find the impact of bioactive compounds of the SWME extract on the metabolites present in the *E. coli*, the methanolic lysate was explored to find the up or down-regulated metabolites using GC-MS technique. The results obtained showed 25 peaks indicating the presence of twenty one compounds. This time, some different compounds were observed which was neither present in SwME nor in *E. coli*. The results obtained here suggested that, sandalwood up regulated few metabolites in *E. coli* which may be present in the negligible amount and hence this up regulation might be responsible for the bacterial death. The different metabolites observed were 3-Ethyl-8-Methyl-2-Oxatetracyclo [4.4.0.0(1, 4).0(6, 8)] Decane, Benzene, Ethoxy, Trans-9-Octadecenoic Acid, Trimethylsilyl Ester, Oleic Acid, Trimethylsilyl Ester, Octadecanoic Acid, Trimethylsilyl Ester, 1-Hexyl-2-Nitrocyclohexane, Tetracosanoic Acid, Methyl Ester, 9-Octadecenoic Acid, 2-[(Trimethylsilyl)Oxy]-1-[[[(Trimethylsilyl)Oxy]Methyl]Ethyl Ester, Silane, [(10-Chlorodecyl)Oxy]Trimethyl, 9,12-Octadecadienoic Acid (z,z)-, 2-[(Trimethylsilyl)oxy]-1-[[[(Trimethylsilyl)Oxy]Methyl]Ethyl Ester etc. The major compounds were 9-Octadecenoic Acid, Methyl Ester (21.97%), Hexadecanoic Acid, Methyl Ester (17.03%), and Hexadecanoic Acid, Trimethylsilyl Ester (14.96%) and Tetradecanoic Acid, 12-Methyl-, Methyl Ester (0.35%), 1-Hexyl-2-Nitrocyclohexane (0.36%), 9,12-Octadecadienoic Acid (z,z)-, 2-[(Trimethylsilyl)Oxy]-1-[[[(Trimethylsilyl)Oxy]Methyl]Mthyl Ester (0.38%) were found in minimal percentage. GC-MS analysis showed up/down regulation of various compound. Some compounds

were turned out to be rare and some compounds get up regulated and showed high peak in the chromatogram (Fig. 4, Table 4).

There are some phytocompounds such as Pentadecane (0.52%), 9-Octadecenoic Acid (Z)- Methyl Ester (26.85%), Docosanoic Acid, Methyl Ester (2.16%), Tetradecanoic Acid, 12-Methyl-, Methyl Est (0.68%) found in *E.coli* lysate. These compounds get downregulated with peak area 0.5%, 21.97%, 1.68% and 0.35% respectively when grown in presence of SwME.

The present investigation is only a preliminary characterization of the presence of some particular properties in sandalwood extract. Further investigation and in-depth knowledge is required to identify the biochemical and phytochemical functions of the herbs. The metabolites which were identified here by GC-MS analysis revealed that they possess many biochemical and pharmacological potential and can be based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA.

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4. Discussion

Medicinal plants are considered new as new assets for producing agents that go about as alternatives to antibiotics against antibiotic-resistant bacteria. It is very important for an antimicrobial agent to be eco-friendly and biologically safe. This study revealed that sandalwood could be a promising antibacterial agent as it showed inhibition against various bacterial strains i.e. the maximum and minimum inhibition of SwME was found to be against *E. coli* and *B. subtilis* respectively. Also the compounds which get up/down regulated might be responsible for this activity. Shamsi et al. in their study investigated that the aqueous extract of *S. album* showed effective inhibitory activity against *S. aureus* (MTCC 902) (Shamsi et. al. 2014). The results from antibacterial activity of SwME revealed that *E. coli* was the most sensitive strain with highest % inhibition towards methanolic extract. (Stenz et. al. 2008) in their study showed when 9-octadecenoic acid was added after primary adhesion was developed, it stimulated biofilm formation and hence bacterial growth. Through our study, it is confirmed that when *E. coli* was grown in presence of SwME, 9-octadecenoic acid was down-regulated which confirms the result of the above study.

The presence of different compounds in SwME through GC-MS analysis showed sandalwood to be the promising therapeutic agents as the compounds present in methanolic extract possess antimicrobial, antioxidant, antiulcer, anti-

268 asthmatic activity etc. Hexadecanoic Acid present in methanolic extract of sandalwood possess Anti-inflammatory,
 269 Antioxidant, hypocholesterolemia nematocide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase
 270 inhibitor, potent mosquito larvicide (Aparna et. al. 2012, Kumar et. al. 2010, Rahuman et. al. 2000, Miustapha et. al.
 271 2016), 9-12 Octadecenoic Acid (Z)-, Methyl Ester shows anti-cancer and antimicrobial activity (Yu et. al. 2005,
 272 Rahman et. al. 2014). Piperidine has characteristic antiasthmatic activity (Matsushita et. al. 1998). The literature survey
 273 also confirmed the presence of 9 Octadecenoic Acid as a plant metabolite in the hexane soluble extract of the *Croton*
 274 *tiglium* seeds (Saputera et. al. 2006). Santha et al. in their study revealed the presence of α -santalol as chemopreventive
 275 agent appears to be very promising in skin cancer control (Sreevidya and Chandradhar 2015). The present study also
 276 confirms the anti-cancerous activity of *S. album* through the presence of 9 Octadecenoic Acid and Sentlyl acetate in
 277 the methanolic extract.

278 The results pertaining to GC-MS analysis of the methanolic extract of *S. album* lead to the identification of various
 279 compounds which have manifold therapeutic benefits. The identified metabolites were known to possess various
 280 bioactivities such as antifungal, anti-inflammatory, skin disorders, antibacterial etc. The extract showed the presence
 281 of a variety of fatty acids, anti-inflammatory compounds such as hexadecanoic acid, fragrance and flavoring agents
 282 such as tetradecanoic acid are found in the plant extract (Burdock and Carabin 2007).

283 This study explored the therapeutic significance of the sandalwood which has great effect on bacteria causing the
 284 metabolite regulation and can be proposed as an herb of psychopharmacological importance. Various chemical
 285 compounds which are identified in methanolic extract of sandalwood, *E. coli* lysate and *E. coli* grown in methanolic
 286 extract of sandalwood help in the determination of the possible synergistic benefits of the plant and their potential as
 287 antimicrobial agent. This also opens up the ways to investigate it further by isolating the single bioactive compounds
 288 leading to the further investigation of specific biological and pharmacological potentials (Sermakkani and
 289 Thangapandian 2012).

290 5. Conclusions

291 This study evaluated the antibacterial potential of SwME and the profiling of bioactive compounds generated by GC-
 292 MS analysis in SwME extract and in the lysate of *E. coli*. The results concluded that SwME was strong antimicrobial
 293 agent showing fair antibacterial activity against all the bacterial strains. GC-MS chromatogram also showed various

294 compounds in SwME with maximum peak intensity which could contribute to the medicinal value of the sandalwood.
 295 This study was performed to analyze the effect of sandalwood methanolic extract on the bacterial metabolites when
 296 grown in presence of extract. The result showed the presence of compound in the extract such as hexadecanoic acid
 297 and 9 Octadecenoic acids which exerts the antimicrobial activity. Antibacterial activity of methanolic extract was
 298 found to be impressive against *E. coli* proved that sandalwood has great potential for development of therapeutic
 299 agents against microbial ailments and it can also be used as alternative or antibiotic. However, isolation and
 300 purification of single bioactive compound and their biological activity will be certainly giving productive outcomes
 301 and will open a new area of investigation of individual components and their pharmacological potency. From these
 302 results, it could be inferred that "*Santalum album*" contains diverse bio-active compounds. Evaluation of
 303 pharmacological activity is under progress. Along these lines, it is suggested as a plant of phyto-pharmaceutical
 304 importance.

305

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311 The authors report no declarations of interest. The work is not submitted elsewhere for publication. It has not been
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Table and figure captions

Table 1: Determination of EC₅₀ values of SwME and ampicillin and represented in mg/ml.

Table 2: Biologically active chemical compounds of methanol extract from sandalwood, showing retention time, molecular formula and peak area (%).

Table 3: GC-MS analysis revealed the presence of compounds in the methanolic lysate of *E. coli* showing retention time, molecular formula and peak area (%).

Table 4: GC-MS analysis revealed the presence of compounds in the methanolic lysate of *E.coli* grown in presence of sandalwood methanolic extract showing retention time, molecular formula and peak area (%).

Figure 1: Bar diagrammatic representations of *in vitro* antibacterial activity of sandalwood methanolic extract. The bars represent the percentage mean growth inhibition by methanolic extract of sandalwood when tested against 4 bacterial strains.

Figure 2: A typical chromatogram of the bioactive compounds present in methanol crude extract of sandalwood plant showing different peaks.

Figure 3: A typical chromatogram of the metabolites found in the methanolic lysate of *E.coli*.

Figure 4: A typical chromatogram of the metabolites present the methanolic lysate of *E. coli* grown in presence of sandalwood methanolic extract.

Figure 1

Figure 2

Figure 3

Figure 4

Table 1

Bacterial strains	EC ₅₀ (mg/ml)
<i>E. coli</i> (MTCC 443)	0.50
<i>S. aureus</i> (MTCC 902)	0.95
<i>P. aeruginosa</i> (MTCC 245)	0.57
<i>B. subtilis</i> (MTCC 736)	1.54

R. Time	Area%	Name	Molecular formula
9.320	0.09	ACETONYL DECYL ETHER	C13H26O2
12.477	0.21	5-EXO-(PHENYLSULFONYL)-5-ENDO-METHYLBICYCLO[2.2.2]OCT-2-ENE	C15H18O2S
12.997	0.3	1-PROPYNE, 3-ETHOXY	C5H8O
13.107	14.44	2-PENTEN-1-OL, 5-(2,3 DIMETHYLTRICYCLO[2.2.1.0(2,6)]HEPT-3-YL)-2METHYL	C15H24O
13.277	1.58	2-PENTEN-1-OL, 5-(2,6-DIMETHYLBICYCLO[3.1.1]HEPT-2-EN-6-YL)-2-METHYL-, [1S [1.ALPHA.,5.ALPHA.,6.ALPHA.(Z)]]-	C15H24O
13.457	0.79	(-)-E.-BETA.-SANTALYL ACETATE	C17H26O2
13.62	8.83	2-PENTEN-1-OL, 2-METHYL-5-(2-METHYL-3-METHYLENE-2-NORBORNYL)	C15H24O
14.207	2.62	1,3-CYCLOHEXADIENE, 1-METHYL-4-(1-METHYLETHYL)	C10H16
14.757	0.49	5-(2-METHYLCYCLOPENT-1-ENYL)-2,2-DIMETHYLPENT-4-ENOIC ACID	C13H20O2
15.157	0.1	PREGNANE, SILANE DERIVATIVE	C27H50O2Si2
15.903	2.63	MYRTENE ACID BROMIDE	C10H13BrO
16.543	3.96	HEPTADECANOIC ACID, METHYL ESTER	C18H36O2
16.997	2.41	NAPHTHALENE, DECAHYDRO-2,2-DIMETHYL	C12H22
17.440	0.39	4-VINYL-IMIDAZOLE	C5H6N2
17.690	2.09	(4R,8R)-8,9-EPOXY-P--MENTH-1-ENE	C10H16O
18.207	0.33	2-HYDROXY-3-PYRAZIN-2-YL-ACRYLIC ACID	C7H6N2O3
18.450	0.44	2-HYDROXY-3-PYRAZIN-2-YL-ACRYLIC ACID	C7H6N2O3
19.123	5.35	HEXADECANOIC ACID, TRIMETHYLSILYL ESTER	C19H40O2Si
19.293	0.68	BICYCLO[2.2.1]HEPT-2-ENE, 2,3-DIMETHYL-	C9H14

20.007	6.99	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	C19H36O2
20.09	0.83	EICOSENOIC ACID, METHYL ESTER	C21H40O2
20.343	0.71	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER	C16H32O2
20.717	0.36	7-OCTEN-1-YN-4-OL, 7-METHYL	C9H14O
21.43	1.12	TRIMETHYLSILYL (9E)-9-OCTADECENOATE	C21H42O2Si
21.653	0.63	METHYL (3A.ALPHA. 7A.BETA.)-5-OXO-HEXAHYDROINDANE-3A-OXOACETATE	C12H16O4
22.33	1.25	CYCLOHEXANOL, 1-(1-CYCLOHEXEN-1-YL)	C12H20O
22.640	0.44	1-Penten-3-one, 4,4-dimethyl-1-phenyl-	C13H16O
23.84	1.55	METHYL DIHYDROMALVALATE	C19H36O2
24.013	0.45	HEPTADECANOIC ACID, METHYL ESTER	C18H36O2
24.23	0.2	1,2-BENZENEDICARBOXYLIC ACID, DIISOCTYL ESTER	C24H38O4
24.313	0.55	SILANE, [(1-ETHYLDECYL)OXY]TRIMETHYL	C15H34OSi
25.19	2.63	BIS[3,4-METHYLENEDIOXYBENZYL]SULFONE	C16H14O6S
25.74	0.55	DOCOSANOIC ACID, METHYL ESTER	C23H46O2
25.883	1.21	GLUCITOL, 4,6-O-HEPTYLIDENE-	C13H26O6
26.113	1.37	OXIRANEMETHANOL, 3-(1-PENTADECYNYL)-, (2R-TRANS)	C18H32O2
26.27	0.99	17-Octadecynoic acid	C18H32O2
27.667	0.53	RETINOIC ACID, 9,10-EPOXY-9,10-DIHYDRO-, (9R,10S)	C20H28O3
28.417	15.08	PIPERIDINE, 1-[5-(1,3-BENZODIOXOL-5-YL)-1-OXO-2,4-PENTADIENYL]	C17H19NO3
28.947	3.63	14-METHYL-8-HEXADECYN-1-OL	C17H32O

32.923	1.28	1H-INDAZOLE, 4,5,6,7-TETRAHYDRO	C7H10N2
33.922	1.28	1H-INDAZOLE, 4,5,6,7-TETRAHYDRO	C7H10N2
38.290	5.1	2-PENTEN-1-OL, 5-(2,3-DIMETHYLTRICYCLO[2.2.1.0(2,6)]HEPT-3-YL)-2-METHYL	C15H24O
39.79	3.53	BICYCLO[2.2.1]HEPTANE, 2-METHYL-3-METHYLENE-2-(4-METHYL-3-PENTENYL)-, (1S-EXO)	C15H24

Table 2

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R. Time	Area%	Name of the compound	Molecular Formula
11.951	0.52	Pentadecane	C ₁₅ H ₃₂
14.398	0.32	Pentadecane	C ₁₅ H ₃₂
15.754	2.83	1-Hexadecanol	C ₁₆ H ₃₄ O
16.555	20.5	Hexadecanoic Acid, Methyl Ester	C ₁₇ H ₃₄ O ₂
18.493	0.51	9-Octadecenoic Acid, Methyl Ester	C ₂₁ H ₃₈ O ₄
19.135	15.79	Hexadecanoic acid, trimethylsilyl ester	C ₁₉ H ₄₀ O ₂ Si
19.787	9.83	1-Octadecanol	C ₁₈ H ₃₈ O
20.022	26.85	9-Octadecenoic Acid (Z)-, Methyl Ester	C ₁₉ H ₃₆ O ₂
20.097	3.4	9-Octadecenoic Acid (Z)-, Methyl Ester	C ₁₉ H ₃₆ O ₂
20.352	2.16	Docosanoic Acid, Methyl Ester	C ₂₃ H ₄₆ O ₂
20.552	0.72	Hexadecanoamide	C ₁₆ H ₃₃ NO
20.725	2.06	1-Dimethyl(Chloromethyl)Silyloxyoctadecane	C ₂₁ H ₄₅ ClO ₂ Si
21.436	5.43	Trimethylsilyl (9e)-9-Octadecenoate	C ₂₁ H ₄₂ O ₂ Si
21.59	0.49	Hexadecanoamide	C ₁₆ H ₃₃ NO
21.689	0.43	Tetradecanoic Acid, Trimethylsilyl Ester	C ₁₇ H ₃₆ O ₂ Si
22.26	0.23	1-Hexyl-1-Nitrocyclohexane	C ₂₁ H ₂₃ NO ₂
22.478	0.68	Tetradecanoic Acid, 12-Methyl-, Methyl Est	C ₁₆ H ₃₂ O ₂
23.658	0.57	1h-Indole-3-Ethanamine	C ₁₀ H ₁₂ N ₂
23.848	2.57	Cyclopropanecarboxylic Acid, 2-Hexyl-, Meth	C ₁₈ H ₃₄ O ₂
24.024	0.35	Heptadecanoic Acid, Methyl Ester	C ₁₈ H ₃₆ O ₂
24.324	1.43	Palmitic Acid, 2-(Trimethylsiloxy)Ethyl Ester	C ₂₁ H ₄₄ O ₃ Si
25.105	0.64	Naphthalene, 2-(2-Nitro-2-Propenyl)-	C ₁₃ H ₁₁ NO ₂
25.899	0.4	1-Decanol, 9-[(Trimethylsilyl)Oxy]-	C ₁₅ H ₂₉ F ₃ O ₃ Si

Table 3

R. Time	Area %	Compound name	Molecular formula
11.957	0.5	PENTADECANE	C15H32
13.107	10.93	2-PENTEN-1-OL, 5-(2,3-DIMETHYLTRICYCLO[2.2.1.0(2,6)]HEPT-3-YL)-2-METHYL	C15H24O
13.277	1.01	2-PENTEN-1-OL, 5-(2,6-DIMETHYLBICYCLO[3.1.1]HEPT-2-EN-6-YL)-2-METHYL-, [1S-[1.ALPHA.,5.ALPHA.,6.ALPHA.(Z)]]	C15H24O
13.457	0.61	E.-BETA.-SANTALYL ACETATE	C17H26O2
13.623	5.95	2-PENTEN-1-OL, 2-METHYL-5-(2-METHYL-3-METHYLENE-2-NORBORNYL)	C15H24O
13.76	0.59	(-)-E.-BETA.-SANTALYL ACETATE	C17H26O2
14.217	0.63	1,3-CYCLOHEXADIENE, 1-METHYL-4-(1-METHYLETHYL)	C10H16
14.293	0.43	(-)-E.-BETA.-SANTALYL ACETATE	C17H26O2
15.915	1.20	3-ETHYL-8-METHYL-2-(2,3,4,5-TETRAMETHYLTRICYCLO[4.1.0.0(1,4).0(6,8)]DECANE	C12H18O
16.57	17.03	Hexadecanoic acid, methyl ester	C17H34O2
17.463	0.5	BENZENE, ETHOXY	C8H10O
19.147	14.96	Hexadecanoic acid, trimethylsilyl ester	C19H40O2Si
20.033	21.97	9-OCTADECENOIC ACID, METHYL ESTER	C19H36O2
20.110	2.8	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	C19H36O2
20.360	1.68	DOCOSANOIC ACID, METHYL ESTER	C23H46O2
21.443	11.88	trans-9-Octadecenoic acid, trimethylsilyl ester	C21H42O2Si
21.51	1.03	OLEIC ACID, TRIMETHYLSILYL ESTER	C21H42O2Si
21.697	1.11	OCTADECANOIC ACID, TRIMETHYLSILYL ESTER	C21H44O2Si
22.263	0.36	1-Hexyl-2-nitrocyclohexane	C12H23NO2
22.48	0.35	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER	C16H32O2
23.853	2.07	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	C19H36O2
24.033	0.67	TETRACOSANOIC ACID, METHYL ESTER	C25H50O2
24.327	0.6	9-Octadecenoic acid, 2-[(trimethylsilyl)oxy]-1-[[trimethylsilyl]oxy]methyl]ethyl ester	C27H56O4Si2
25.900	0.71	Silane, [(10-chlorodecyl)oxy]trimethyl	C13H29ClOSi
26.103	0.38	9,12-OCTADECADIENOIC ACID (Z,Z)-, 2-[(TRIMETHYLSILYL)OXY]-1-[[TRIMETHYLSILYL)OXY]METHYL]ETHYL ESTER	C27H54O4Si2

Table 4