**PROSPECTING OF PHENYLPROPANOIDS AND
TERPENOIDS IN EAST INDIAN SANDALWOOD
(SANTALUM ALBUM L.)**

Biswapriya Biswas Misra

**PROSPECTING OF PHENYLPROPANOIDS AND
TERPENOIDS IN EAST INDIAN SANDALWOOD
(SANTALUM ALBUM L.)**

*Thesis submitted to
Indian Institute of Technology, Kharagpur
for the award of the degree*

of

Doctor of Philosophy

by

Biswapriya Biswavas Misra

under the guidance of

Prof. Satyahari Dey

**DEPARTMENT OF BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY, KHARAGPUR
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PREFACE

The East Indian Sandalwood (*Santalum album* L.) is a highly valuable and precious woody tropical tree, much admired for its fragrant heartwood, scented oil for its aesthetic uses and pharmacological applications.

Pioneering efforts of Indian Institute of Science (IISc), Bangalore and Bhabha Atomic Research Centre, Mumbai allowed early inroads into sandalwood tree biotechnology through several phases, i.e., (1) development of biotechnological means of micropropagation through tissue cultural practices, (2) improvisation in conventional silvicultural methods and timber quality improvement, (3) revelation of underlying biochemical processes associated with growth and development and (4) work on accumulation patterns, quantitative studies and chemical properties of sandalwood oil. The laboratory of Prof. Satyahari Dey, at Indian Institute of Technology Kharagpur had significant contributions since, 1990; in large scale production of *in vitro* tissues through bioreactors as a means for rapid mass propagation and commercialization through institute and industry collaborative Technology Development Mission (TDM) project sponsored by Govt. of India and the Tata Tea Ltd. The next phase of sandalwood research in this laboratory went one step ahead through the 'Bioprospecting of Novel Genes and Molecules from Sandalwood' in the form of a substantial grant from Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India.

This thesis is based upon studies conducted during September, 2004 to December, 2009 at the Plant Biotechnology Laboratory, Department of Biotechnology, IIT Kharagpur under the supervision of Prof. Dey and written to address the potential of this lesser explored tropical forest tree, as a rich source of phytaceuticals and genetic information to provide future guidance towards the development of value-added products, thus 'bioprospecting'. The framework within which this research was supported has certainly left its mark on large parts of this thesis, where the focus was to uncover the underlying metabolite pools and diversities using modern technological solutions, and addressing the intricacies of the biochemistry, metabolite pools, proteins and genes by implementation and thorough evaluation of both newly adopted and existing techniques for this work. It provides fairly new information that has many facets that remain to be explored both, by people in academia, related to terpenoid biology, forest biotechnology and pharmacology, as well as people in industry involved with development of alternative medicine and pharmaceuticals. Though the sandalwood fragrance related research have been dominating the scientific literature in the past, this work would take it a step further to reveal the potential of *in vitro* grown tissues as alternative sources for production of sandalwood oil fragrance constituents. Chemosynthesis of sandal-fragrance-mimic molecules, increased adulteration of commercially available oil and upsurge in marketability of taxonomically and chemically unrelated plant essential oils as 'sandalwood

oil' put a serious question mark towards identification and authentication of true oils. *In vitro* biology of sandalwood is extended a step forward to pave way to uncover the hidden commercialization potentials through this work. This thesis will seek to help familiarize people with the bioactive molecules involved in regards to its ethnopharmacological properties and shed light on some of the genetic basis that produces such fragrance. The work included as Appendix is found in the attached CD with the thesis as well as at the personal webpage of author, <http://sites.google.com/site/bbmisranow/>.

This investigation covered basic biochemistry addressing some questions unique to this typical plant, to sometimes reaching the state-of-the-art hyphenated techniques to gather the complicated information embedded within the metabolome, instrumentation to fingerprint the developmental stages while at times unraveled the genetic machinery involved and finally explored the biologically active molecules to get insights into their potential activity for use as commercial therapeutic plant- products.

Within the available time frame and instrumental resources, this first time attempt on metabolite profiling/ bioprospecting resulted in new information on metabolites biosynthesized in cultured cells and tissues in sandalwood. The author realized that further exploration will reveal huge scope of producing *in vitro*, the aromatherapeutic phytomedicinals. It would have been the author's satisfaction had the biological activities been conducted with isolated compounds. But he is hopeful that his laboratory will soon achieve this success of scaling up, so that individual compounds will be available for this exercise. The thesis reports the first ever detection of santalols from cultured cells and tissues and the IPR protection is ensured.

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IIT Kharagpur

January, 2010

(Biswapriya Biswavas Misra)

LIST OF ABBREVIATIONS

2, 4-D	2, 4-Dichlorophenoxyacetic Acid
2, 5-DHB	2, 5-DiHydroxy Benzoic acid
2D	Two Dimensional
3D	Three Dimensional
Aa	Amino Acid
ABTS	2, 2' Azino Bis (3-ethylbenzo) Thiazoline-6-Sulfonic acid
ACT	Actin
AD	Alzheimer's Disease
ALP	Alkaline Phosphatase
ANOVA	Analysis Of Variance
AOAC	Association of Official Analytical Chemists
APase	Acid Phosphatase
ATCC	American Type Culture Collection
ATP	Adenosine TriPhosphate
BA	6-BenzylAminopurine
BHT	Butylated Hydroxyl Toluene
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
BSA	Bovine Serum Albumin
BW	Body Weight
CA	Callus
CA	Cluster Analysis
CAD	Coniferyl Alcohol Dehydrogenase
CAS	Chemical Abstracts Service
CAT	Catalase
CDD	Conserved Domain Database
cDNA	Complementary DeoxyriboNucleic Acid
CFU	Colony Forming Unit
ChE	Cholinesterase
CLSM	Confocal Laser Scanning Microscopy
CNS	Central Nervous System
CoA	Coenzyme A
CTAB	Cetyl Trimethyl Ammonium Bromide
CV	Color Value
CVD	CardioVascular Disease
D2	Deuterium
Da	Dalton (= 1 amu)
DAB	3, 3-DiAminoBenzidine tetra hydrochloride
DABC	Drug Administration Bureau of China
DAD	Diode Array Detector
DAPI	4', 6-DiAmidino-2-PhenylIndole
ddH ₂ O	double distilled water
DMAPP	DiMethyl Allyl Pyrophosphate
DMEM	Dulbecco's Modified Eagle Medium

DMSO	Dimethyl SulfOxide
DNA	DeoxyriboNucleic Acid
dNTP	DeoxyNucleotide TriPhosphate
DPPH	1, 1-DiPhenyl-2-PicrylHydrazyl
DTNB	5', 5-DiThio-bis- (2-NitroBenzoate)
DTT	DiThioThreitol
DW	Dry Weight
DXP	1-Deoxy-D-Xylulose 5-Phosphate
DXS	1-Deoxy-D-Xylulose 5-Phosphate Synthase
<i>E. coli</i>	<i>Escherichia coli</i>
EA	Enzyme Activity
EAS	Epi-Aristolochene Synthase
EB	Elution Buffer
EBV	Epstein-Barr Virus
EC	Enzyme Commission
Ecm	Extracellular Matrix
EDC	1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide
EDTA	Ethylenediaminetetracetic Acid
ELISA	Enzyme Linked Immuno Assay
EMEA	European Medicines Agency
ESI-MS	Electron Spray Ionization-Mass Spectroscopy
EST	Expressed Sequence Tags
EtOH	Ethyl Alcohol
<i>E</i> -value	Expected value
FARM	First Aspartate-Rich Motif
FBS	Fetal Calf Serum
FCR	Folin Ciocalteau's Reagent
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FITC	Fluorescein IsoThioCyanate
FPP	Farnesyl Pyrophosphate (Diphosphate)
FPPS	Farnesyl Pyrophosphate Synthase
FRAP	Ferric Reducing Assay Power/ Ferric Reducing Ability of Plasma
FTIR	Fourier Transform Infra Red Spectroscopy
FW	Fresh Weight
GA3	Gibberellic Acid
GAP	GlycerAldehyde-3-Phosphate
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detection
GC-MS	Gas Chromatography-Mass Spectrometry
GelDoc	Gel Documentation
GFC	Gel Filtration Chromatography
GGPP	Geranyl Geranyl Pyrophosphate
GLC	Gas Liquid Chromatography
GPP	Geranyl Pyrophosphate
HBSS	Hanks Balanced Salt Solution
HEPES	4-(2-HydroxyEthyl)-1-PiperazineEthaneSulfonic acid

HMGCoAR	3-Hydroxy-3-Methyl-Glutaryl-CoA Reductase
HPLC	High Pressure Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
HPV	Human Papilloma Virus
HRP	Horse Radish Peroxidase
HSV	Herpes Simplex Viruses
HW	Heartwood
IAA	Indole-3-Acetic Acid
IBA	Indole-3-Butyric Acid
IC ₅₀	50 % Inhibition Concentration
IgG	Immunoglobulin G
IPP	Isopentyl Pyrophosphate
Kbp	Kilo basepair
kDa	kiloDalton
KI	Kovat's Index
LB	Luria-Bertani
LC-MS	Liquid Chromatography-Mass Spectroscopy
LD ₅₀	50 % Lethal Dose
LRI	Linear Retention Index
m/ z	mass to charge ratio
MALDI-ToF	Matrix-Assisted Laser Desorption Ionization-Time of Flight
MDA	MalonDiAldehyde
MEP	2-C-Methyl-Erythritol 4-Phosphate
MES	2-(N-Morpholino) EthaneSulfonic acid
MGYP	Malt extract-Glucose-Yeast extract-Peptide
MHB	Mueller Hilton Broth
MIC	Minimal Inhibitory Concentration
mM	milli Molar
MMP	Matrix Metallo-Proteinase
MOPSO	3-(N-MOrPholino)- 2-hydroxypropaneSulfOnic acid
mRNA	messenger RiboNucleic Acid
MS	Murashige and Skoog Medium
MTBE	Methyl-Tertiary-Butyl Ether
MTCC	Microbial Type Culture Collection
MTPS	Monoterpene Synthase
MTT	3, [4, 5- dimethylthiazol, 2yl], 2, 5, diphenyl tetrazolium bromide
MTPS	MonoTerPene Synthase
MVA	Mevalonic Acid
MW	Molecular Weight
MWCO	Molecular Weight Cut Off
NADB	NADP Binding
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center for Biotechnology Information
NCIM	National Collection of Industrial Microorganisms
NIDDM	Non-Insulin-Dependent Diabetes Mellitus/ Type 2 Diabetes
NIST	National Institute of Standards and Technology
nkat	nano katal

NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
Nr	non-redundant
nt	nucleotide
OD	Optical Density
OECD	Organisation for Economic Co-operation and Development
ON	Overnight
ORD	Optical Rotatory Dispersion Spectroscopy
ORF	Open Reading Frame
OT	Old Tree
PAL	L-Phenylalanine Ammonia Lyase
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PD	Parkinson's disease
PDA	Potato Dextrose Agar
PDB	Protein Data Bank
PEG	Polyethylene Glycol
pH	potenz Hydrogen
PHYRE	Protein Homology/analogY Recognition Engine
PIPES	L-piperazine-N-N-bis (2-ethane sulfonic) acid
PMSF	PhenylMethylSulfonyl Fluoride
POD	Peroxidase
PPO	PolyPhenol Oxidase
PROSITE	Protein Sites and Patterns
PT	Prenyltransferase
PUFA	PolyUnsaturated Fatty Acid
PVPP	PolyVinyl Poly Pyrrolidone
RBC	Red Blood Cell
Rf	Relative Front
RLCCC	Rotary Locular Counter Current Chromatography
RNase	RiboNuclease
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RP-HPLC	Reverse Phase-High Pressure Liquid Chromatography
RPMI	Roswell Park Memorial Institute
rRNA	ribosomal RiboNucleic Acid
RTP	Room Temperature (25 ± 3 ° C) and Pressure
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
<i>Sa</i>	<i>Santalum album</i>
SARM	Second Aspartate-Rich Motif
SCOP	Structural Classification of Proteins
SD	Seedling
SD	Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis
SE	Somatic Embryo
SWO	SandalWood Oil
SOD	Superoxide Dismutase

sq. km	Square Kilometers
sqRT-PCR	semi quantitative Reverse Transcription-Polymerase Chain Reaction
STPS	SesquiTerPene Synthase
TAC	Total Antioxidant Capacity
TAE	Tris-Acetate-EDTA
TAIR	The Arabidopsis Information Resource
TBRs	ThioBarbituric acid Reactive Substance
TCA	Trichloro Acetic Acid
TEMED	N, N, N', N'- Tetramethylene ethylene diamine
TFA	TriFluoroacetic Acid
TLC	Thin Layer Chromatography
T _m	melting Temperature of DNA
TM	Trade Mark
TPP	Thiamine diphosphate
TPS	Terpene Synthase
Tris	Tris(hydroxymethyl)aminomethane
TTC	2, 3, 5-TriphenylTetrazolium Chloride
USFDA	United States Food and Drug Administration
UTPS	Unusual TerPene Synthase
UV	Ultra Violet
VLDL	Very-Low-Density Lipoprotein
WHO	World Health Organization
WPM	Woody Plant Medium (Lloyd and McCown, 1980)
wrt	with respect to
YT	Young Tree
β-ME	β-MercaptoEthanol

LIST OF SYMBOLS

Amino acid	1-Letter symbol	3- Letter symbol
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamic acid	E	Glu
Glutamine	Q	Gln
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

LIST OF TABLES

Table 1.1 Major achievements in research and development work in sandalwood.	28
Table 1.2 Biological activities associated with sandalwood oil and molecules thereof.	29
Table 2.1 Chromatographic and spectral characteristics of phenolics detected in different sandalwood tissues fractions by RP- HPLC- DAD.	42
Table 2.2 LC- MS/ MS- based profiling of sandalwood phenylpropanoids.	44
Table 3.1 HPTLC- based metabolite profiling of sandalwood tissue sesquiterpenoid constituents.	74
Table 3.2 GC- based metabolite profiling of sandalwood tissue sesquiterpenoid constituents.	75
Table 3.3 GC- ITQ- MS analysis results obtained for volatile constituents from sandalwood tissues.	77
Table 3.4 Total terpenoid content from sandalwood tissues.	78
Table 3.5 Cross-reactivity of the polyclonal antibody.	88
Table 4.1 Physico- chemical and phytochemical analyses of extracts.	115
Table 4.2 Biological activities of sandalwood extracts.	121
Table 4.3 Investigated biochemical parameters in different mice groups for <i>in vivo</i> antioxidant activity of α - santalol and sandalwood oil.	122
Table 4.4 Investigated biochemical parameters in different mice groups for <i>in vivo</i> anti-hyperglycemic activity of α - santalol and sandalwood oil.	123
Table 4.5 Correlations between phytoconstituents and biological activities.	124
Table 5.1 GenBank genetic resource relevant to sandalwood research till date.	136
Table 5.2 The oligonucleotide (primer) sequences used for cloning the genes of interest.	138
Table 5.3 Table showing condition PCR conditions used for amplification of sesquiterpene synthase genes (STPS 1- 3).	139
Table 5.4 Primers used in sq- RT- PCR reactions for amplification of 6 genes.	144
Table 5.5 Comparative bioinformatic analyses of PDB templates and obtained sandalwood clones.	154
Table 5.6 Important features of generated models by homology- based modeling and respective PDB templates.	155
Table 5.7 Comparison of overall quality factors between generated models by homology- based modeling and respective PDB templates.	155
Table 5.8 Relative tissue level expression profiling of sandalwood genes by sqRT- PCR.	156

LIST OF FIGURES

Figure 1.1 Distribution of East Indian sandalwood in Indian subcontinent.	2
Figure 1.2 General phenylpropanoid biosynthetic pathway leading to a wide range of phenylpropanoids and vascularization.	12
Figure 1.3 General terpenoid biosynthetic pathways operative in plants, with cross talk of intermediates. Sandalwood sesquiterpenoid biosynthetic pathway is highlighted.	16
Figure 1.4 Detailed MEP and MVA biosynthetic pathway, showing formation of various terpenoids and several pathway inhibitors.	18
Figure 1.5 Domain architecture of plant terpene synthases.	20
Figure 2.1 Schematic overview of the proposed investigation on profiling of phenylpropanoids.	32
Figure 2.2 Phenylpropanoid enzyme activities in sandalwood.	39
Figure 2.3 Levels of PP metabolites in sandalwood tissues.	40
Figure 2.4 Reverse phase high performance liquid chromatograms (RP- HPLC) for the combined phenolic fractions of five tissues.	41
Figure 2.5 Occurrence of PPs in five sandalwood tissues.	43
Figure 2.6 Histochemical studies on PP accumulation, lignification and vascularization events in various growth/developmental stages.	46
Figure 2.7 Overall trends in biochemical, histochemical and enzymatic events in sandalwood <i>in vitro</i> and <i>in vivo</i> development.	47
Figure 3.1 Schematic representation of various approaches adopted in the investigation.	55
Figure 3.2 A. DXP synthase and B. HMGC _o A reductase activities in sandalwood developmental stages.	70
Figure 3.3 Representative sesquiterpene synthase activity from callus.	71
Figure 3.4 Compositional analysis of sandalwood oil by HPTLC and GC analysis.	72
Figure 3.5 A. Typical representative TLC chromatogram for sandalwood oil constituents. B. GC- MS/ MS chromatogram for sandalwood oil, showing the two of the most abundant constituents, i.e., α - santalol and β - santalol. C. GC- MS/ MS spectra for (Z)- α - santalol. D. GC- MS/ MS spectra for (Z)- β - santalol.	72
Figure 3.6 HPTLC- based standard curve for α - santalol.	73
Figure 3.7 Metabolite profiling of sandalwood tissues by HPTLC.	73
Figure 3.8 GC- based sesquiterpenoid profiling of sandalwood tissue sesquiterpenoid constituents.	75
Figure 3.9 Profiling of sandalwood volatile constituents by GC- MS/ MS in 5 developmental stages.	76
Figure 3.10 A. Chemical structures and log P values of the farnesylated metabolites. B. The absorption spectrum of farnesene, farnesol and FPP on HPTLC plates. C. Representative HPTLC chromatograms. D. Quantification of farnesylated metabolites (C- 15, FPP, farnesene and farnesol) as detected by HPTLC.	78
Figure 3.11 Pigment quantification in sandalwood tissues, e.g., chlorophyll a content, chlorophyll b content, total chlorophyll, total carotenoids and total xanthophylls.	79

Figure 3.12 A. Scheme showing purification of sesquiterpenoid from somatic embryo and the approaches followed. B. HPTLC- profile of combined organic somatic embryo extract.	79
Figure 3.13 ¹³ C- NMR spectra for (Z)- β- santalol in d ₆ - DMSO.	80
Figure 3.14 MALDI- ToF mass spectrum for (Z)- β- santalol. In the inset same spectrum (unzoomed) to show the single peak.	80
Figure 3.15 The ESI- MS mass spectra for (Z)- β- santalol and adducts formed thereof.	81
Figure 3.16 The FTIR spectra for (Z)- β- santalol.	81
Figure 3.17 The GC- MS/ MS spectra for (Z)- β- santalol.	82
Figure 3.18 The LC- APCI- MS (MS ²) spectra for (Z)- β- santalol.	82
Figure 3.19 Preparative scale purification of α- santalol from sandalwood oil. A. RLCCC- aided separation of Z- α- santalol and Z- β- santalol separation, B. silica- gel column chromatography, C. Sephadex LH20 TM - aided separation and lastly, D. Preparative HPTLC- based purification of α- santalol. E. Purification events followed to obtain α- santalol from sandalwood oil.	83
Figure 3.20 A. UV- Vis spectrophotometric spectral scan for antigen, conjugate and carrier proteins. B. Spectrofluorimetric analysis for BSA and BSA- α- santalol conjugate. C. SDS PAGE (15 %) analysis of carrier protein with and without conjugate.	85
Figure 3.21 Analysis of antigen for confirmation of conjugation by MALDI- ToF analysis.	86
Figure 3.22 Antibody production during the immunization period.	86
Figure 3.23 ELISA checkerboard results for determination of antibody titre against antigen.	87
Figure 3.24 A. Correlation between ELISA and HPTLC analysis methods for free α- santalols. B. Standard curve generated against α- santalol, by TLC- immuno- dot blot, using polyclonal antibodies against α- santalol- BSA conjugate with anti - rabbit IgG goat- FITC labeled secondary antibody to aid in detection in UV light. C. Immuno dot blot using a nitrocellulose strip for detection of α- santalol using rabbit polyclonal antibody against α- santalol- BSA conjugate. D. Dipstick assay developed with toothpicks, E. Immuno TLC dot blot for detection of α- santalol using the rabbit polyclonal antibody against α- santalol- BSA conjugate.	87
Figure 3.25 Affinity purification of polyclonal anti- α- santalol- BSA conjugate antibody using 4 CL- B TM matrix.	88
Figure 3.26 Confocal and bright field microscopy images for alpha- santalol and sesquiterpenoid localization in samples.	89
Figure 3.27 A. Sesquiterpene synthase (STPS) protein purification scheme followed. B. Gel filtration elution profile of pooled anion exchange elutes (250- 500 mM KCl) run in a Superdex- 200 GF column. C. 12 % SDS- PAGE analysis of anion exchanger (Source Q 15) elutes. D. 12 % SDS- PAGE analysis of gel filtration (Superdex 200) elutes. E. GC- chromatogram showing GC- based activity profiling of partially purified STPS, displaying production of santalols, santalenes and bergamotol from FPP.	91
Figure 3.28 Overview of terpenoids in various developmental stages in sandalwood.	92

Figure 4.1 Schematic overview of the proposed investigation of biological activities.	102
Figure 4.2 A. HPTLC- based fingerprint of sandalwood extracts. B. Cluster analysis (CA) of the metabolite compositions and biological activities for the 5 extracts of sandalwood.	124
Figure 4.3 Visual interpretation of the range of biological activities conducted with sandalwood extracts, sandalwood oil and positive controls.	125
Figure 5.1 Schematic representation of the proposed investigation.	136
Figure 5.2 Amplicons separated on 1.5 % agarose gels, obtained by PCR- based methods.	145
Figure 5.3 Restriction digestion analysis of TA- clones.	145
Figure 5.4 SaACT3 displaying the actin features, i.e., the profilin binding sites, the gelsolin binding sites and the ATP- binding sites.	146
Figure 5.5 Assignment of the translated SaCAD clone (3- 87 aa length) to the NADB- Rossmann superfamily of proteins.	147
Figure 5.6 Assignment of the translated SaDXS1 clone to the TPP and transketolase- pyrophosphatase super family.	147
Figure 5.7 The SaSTPS3 is an isoprenoid biosynthetic superfamily gene showing a substrate binding pocket, a Mg^{2+} divalent metal ion binding site as well as the aspartate- rich regions.	149
Figure 5.8 Homology- based modeling of SaACT3, SaCAD and SaDXS2.	152
Figure 5.9 Homology- based modeling of SaFPPS, SaMTPS and SaSTPS3.	153
Figure 5.10 Semi quantitative RT- PCR measured expression levels of 6 genes in sandalwood tissues.	156
Box 1. Analytical methods for plant metabolite profiling and chromatographic fingerprinting.	9
Box 2. Assay schemes and methods for antioxidants.	24

LIST OF APPENDIX

Appendix A.1 Drug- likeliness and physico- chemical and biological properties of α - santalol.	I
Appendix A.2 CAD genes characterized till date.	II
Appendix A.3 DXS genes characterized till date.	II
Appendix A.4 FPPS genes characterized till date.	III
Appendix A.5 Monoterpene synthases characterized till date.	III
Appendix A.6 Sesquiterpene synthases characterized till date.	IV
Appendix B.1 Definitions of materials under study.	V
Appendix B.2 RP- HPLC- based acquisition of DAD spectra of up to 15 phenylpropanoids.	VI
Appendix B.3 Representative LC- MS/ MS spectra in negative and positive modes, showing the presence of phenolics.	VI
Appendix B.4 LC profile of the sandalwood tissue extracts.	VII
Appendix C.1 Mice and rabbit immunization schemes.	VIII
Appendix C.2 Identification of sesquiterpene synthase assay products from various tissues.	VIII
Appendix C.3 Kovat's indices for santalanes and known sesquiterpenoids from sandalwood for polar and non- polar GC columns.	VIII
Appendix C.4 Sesquiterpenoid constituents in sandalwood oil as revealed by HPTLC and GC analysis.	IX
Appendix C.5 Schemes, mechanisms and approaches followed for antibody development against Z- α - santalol.	IX
Appendix C.6 Schemes shown for antibody generation in mice and rabbits by two methods.	X
Appendix D.1 Methodology for phytochemical analyses of sandalwood extracts.	XI
Appendix D.2 Correlations between groups of metabolites quantified. Group 1 and 2 represent the two groups of metabolites quantified and plotted against each other to obtain the correlation.	XII
Appendix D.3 Spectral characteristics of phytoconstituents in sandalwood extracts.	XII
Appendix D.4 Percentage of each characterized metabolite groups in the tissue extracts.	XIII
Appendix D.5 MIC values ($\mu\text{g}/\text{ml}$) of sandalwood oil against 14 bacterial strains (both Gram +ve and Gram -ve) determined by the broth dilution method.	XIV
Appendix D.6 Antibacterial activity of tissue extracts based on disc diffusion method.	XIV
Appendix D.7 Agar spot detection method for antibacterial activity of the tissue extracts and sandalwood oil.	XV
Appendix D.8 Antifungal activity of sandalwood oil against some plant pathogenic fungi and other laboratory strains.	XV
Appendix D.9 TLC- bioautography of five extracts and sandalwood oil, against the 13 bacterial strains.	XVI
Appendix D.10 The TLC- bioautographic screening for tyrosinase inhibitors from sandalwood extractives and sandalwood oil.	XVII
Appendix D.11 The TLC- bioautographic screening for acetylcholinesterase and butyrylcholinesterase inhibitors from sandalwood extractives and sandalwood oil.	XVII
Appendix D.12 Allelopathic effects of sandalwood extracts and sandalwood oil against 10 plant species during seed germination.	XVII
Appendix D.13 Bacterial growth inhibition assays by germinating sandalwood seeds, against 8 various bacteria, with zones of inhibitions.	XVII
Appendix E.1 Multiple sequence alignment of homologous plant ACTs.	XVIII
Appendix E.2 Multi- alignment of nucleotide sequences homologous plant CADs.	XIX
Appendix E.3 Multi- alignment of nucleotide sequences of homologous plant DXSs.	XX
Appendix E.4 Multi- alignment of nucleotide sequences of homologous plant FPPSs.	XXI
Appendix E.5 Multi- alignment of nucleotide sequences of homologous plant UTPSs.	XXII
Appendix E.6 Multi- alignment of nucleotide sequences of homologous plant STPSs.	XXIII
Appendix E.7 Sequence of the sandalwood actin genomic clone SaACT1.	XXIV
Appendix E.8 Blast results obtained for actin genomic clone, SaACT1.	XXIV

Appendix E.9 Sequence of the sandalwood actin genomic clone SaACT2.	XXV
Appendix E.10 Blast results obtained for actin genomic clone, SaACT2.	XXV
Appendix E.11 Sequence of the sandalwood actin genomic clone SaACT3.	XXV
Appendix E.12 Blast results obtained for actin genomic clone, SaACT3.	XXVI
Appendix E.13 Comparison of the three different actin clones (SaACT1, SaACT2, SaACT3) obtained from sandalwood tissues.	XXVI
Appendix E.14 Sequence of the sandalwood CAD genomic clone SaCAD.	XXVI
Appendix E.15 Blast results obtained for the CAD genomic clone, SaCAD.	XXVI
Appendix E.16 Sequence of the sandalwood DXS genomic clone SaDXS1.	XXVII
Appendix E.17 Blast results obtained for the first DXP synthase genomic clone, SaDXS1.	XXVII
Appendix E.18 Sequence of the sandalwood DXS genomic clone SaDXS2.	XXVIII
Appendix E.19 Blast results obtained for the second DXP synthase genomic clone, SaDXS2.	XXVIII
Appendix E.20 Comparison of the two deoxy- xylulose phosphate synthase clones (SaDXS1 and SaDXS2) from sandalwood.	XXVIII
Appendix E.21 Sequence of the sandalwood FPPS genomic clone SaFPPS.	XXVIII
Appendix E.22 Blast results obtained for the farnesyl pyrophosphate synthase genomic clone, SaFPPS.	XXVIII XXIX
Appendix E.23 Sequence comparisons among sandalwood SaFPPS with other plant FPPS homologues.	XXIX
Appendix E.24 Sequence of the sandalwood monoterpene synthase genomic clone SaMTPS.	XXIX
Appendix E.25 Blast results obtained for the monoterpene synthase genomic clone, SaMTPS.	XXX
Appendix E.26 Sequence of the sandalwood STPS genomic clone SaSTPS1.	XXX
Appendix E.27 Blast results obtained for the first sesquiterpene synthase genomic clone, SaSTPS1.	XXX
Appendix E.28 Sequence of the sandalwood STPS genomic clone SaSTPS2.	XXX
Appendix E.29 Blast results obtained for the second sesquiterpene synthase genomic clone, SaSTPS2.	XXXI
Appendix E.30 Sequence of the sandalwood STPS genomic clone SaSTPS3.	
Appendix E.31 Blast results obtained for the third sesquiterpene synthase genomic clone, SaSTPS3.	XXXI
Appendix E.32 Comparison of the three SaSTPS clones obtained from sandalwood among other plant sesquiterpene synthase sequences aligned from GenBank sequences.	XXXII
Appendix E.33 Multiple sequence alignment of homologous plant ACTs.	XXXIII
Appendix E.34 Multiple sequence alignment of homologous plant CADs.	XXXIV
Appendix E.35 Multiple sequence alignment of homologous plant DXSs.	XXXV
Appendix E.36 Multiple sequence alignment of homologous plant FPPSs.	XXXVI
Appendix E.37 Multiple sequence alignment of homologous plant MTPSs.	XXXVI
Appendix E.38 Multiple sequence alignment of homologous plant STPSs.	XXXVII
Appendix E.39 Phylogenetic analysis of plant ACTs.	XXXVIII
Appendix E.40 Phylogenetic analysis of plant CADs.	XXXIX
Appendix E.41 Phylogenetic analysis of plant DXSs.	XL
Appendix E.42 Phylogenetic analysis of plant FPPSs.	XLI
Appendix E.43 Phylogenetic analysis of plant MTPSs.	XLII
Appendix E.44 Phylogenetic analysis of plant STPSs.	XLIII
Appendix E.45 Vector map of pTZ57R/ T (MBI, Fermentas) used for TA- cloning.	XLIII
Appendix F.1 Composition of the MS (Murashige and Skoog, 1962) medium used in the investigation to raise <i>in vitro</i> developmental stages.	XLIV
Appendix F.2 Composition of the WPM (Lloyd and McCown, 1980) used in the investigation to raise <i>in vitro</i> developmental stages.	XLIV
Appendix F.3 Flowchart showing the Harborne protocol (Harborne, 1998).	XLV
Appendix F.4 Proposed sandalwood sesquiterpenoid biosynthetic pathway schemes (modified from Sell, 2003).	XLVI

GLOSSARY

Antioxidants- Protect the body against free radicals by neutralizing, reducing or scavenging the oxidants before they can damage cells, tissues, DNA, lipids and proteins (Sanchez-Moreno, 2002).

Bioprospecting- The search for and examination of biological resources (plants, animals, microorganisms) that have features that might be of value for commercial development; is the search for useful biological materials [i.e, non human biological materials (NHBMs) in common pool resources (CPRs)] (Polski, 2005); the purposeful evaluation of wild biological material in search of valuable new products, has always been a central activity in human development; involves the application of advanced technologies to develop new pharmaceuticals, agrochemicals, cosmetics, flavorings, fragrances, industrial enzymes, and other products from biodiversity (Artuso, 2002).

Chromatographic Fingerprinting- Method to show chemical information of medicines or herbals with chromatograms, spectrograms and such graphical means of chemical analytical techniques allowing visual inspection of chromatographic fingerprint as identification.

Condensed Tannins/ Pro (antho) cyanidins/ Oligomeric Proanthocyanidins- Polymers of 2 to 50 (or more) flavonoids, such as catechins that are joined by carbon-carbon bonds, which are not susceptible to being cleaved by hydrolysis.

Enzymatic antioxidant defense-superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc.

Flavan-3-ols/ Flavanols- A class of flavonoids having a 2- phenyl-3, 4-dihydro- 2H-chromen-3-ol skeleton, and are building blocks of proanthocyanidins. Examples: catechins.

Free Radicals- Chemical species that contain one or more unpaired electrons, are capable of independent existence, are highly unstable and reactive thus cause damage to other molecules by extracting electrons from them in order to attain stability (Sanchez-Moreno, 2002).

Metabolic Fingerprinting- Rapid and global analysis for classification of samples on the basis of provenance of either their biological relevance or origin (Fiehn, 2001).

Metabolite / Metabolic Profiling- Analysis focused on a group of metabolites, for example, a class of compounds such as carbohydrates, amino acids or those associated with a specific pathway (Fiehn, 2001).

Metabolite Target Analysis- Analysis restricted to metabolites of, for example, a particular enzyme system that would be directly affected by abiotic or biotic perturbation (Fiehn, 2001).

Metabolome- Defined as the complete complement of all small molecule (< 1500 Da) metabolites found in a specific cell, organ or organism.

Metabolomics- Comprehensive qualitative and quantitative analysis of all metabolites simultaneously under a given set of conditions (Fiehn, 2001).

Metabonomics- Quantitative measurement of the dynamic multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification (Nicholson et al., 2002).

Non-enzymatic Antioxidant Defense-ascorbic acid (vitamin C), α -tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, etc.

Polyphenols- A group of chemical substances found in plants, characterized by the presence of more than one phenol unit or building block per molecule. Polyphenols are generally divided into hydrolyzable tannins (gallic acid esters of glucose and other sugars) and phenylpropanoids, such as lignins, flavonoids, and condensed tannins

Quinones- Compounds having a fully conjugated cyclic dione structure derived from aromatic compounds by conversion of an even number of $-\text{CH}=\text{}$ groups into $-\text{C}(=\text{O})-$ groups with any necessary rearrangement of double bonds. Example: benzoquinone, phylloquinone etc.

Reactive Nitrogen Species (RNS)- nitric oxide (NO), peroxyxynitrite.

Reactive Oxygen Species (ROS)- superoxide anion, peroxy, hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\text{OH}^\cdot$).

Tannins- Astringent, bitter plant polyphenols that precipitate proteins and are divided into hydrolyzable tannins [i.e., gallic acid] and condensed tannins/ proanthocyanidins [i.e., flavones].

TERMINOLOGY

Callus (CA): *In vitro* grown highly proliferating cell line (IITKGP/ 91), grown on solid MS/ WPM media, supplemented with 2, 4- D (1 mg/ l), 14 days old.

Somatic Embryo (SE): *In vitro* grown embryos from somatic cells, grown on solid MS/ WPM media, supplemented with IAA (0.5 mg/ l) and BAP (0.5 mg/ l), 2 weeks old.

Seedling (SD): *In vitro* grown somatic seedlings, grown on solid MS/ WPM media supplemented with IAA (1 mg/ l), IBA (1 mg/ l) and GA (0.5 mg/ l), 6 weeks old.

Young Tree (YT): Field grown tree growing in the backyard of BT, IIT Kgp, non- oil producing tree, mostly vegetative twigs sampled, 7 years old.

Old Tree (OT): Field grown tree growing in the backyard of BT, IIT Kgp, oil producing tree, mostly vegetative twigs sampled, 14 years old.

ABSTRACT

The East Indian Sandalwood, *Santalum album* L., a tropical woody tree, deposits sesquiterpenoid rich essential oil in the heartwood. In view of its century-old traditional uses in perfumery and healthcare this investigation in prospecting of phenylpropanoid and terpenoids from *in vitro* tissues, in comparison to the *in vivo* trees, was undertaken to throw light on the phytochemistry of the healthcare molecules, explore *in vitro* tissues as alternate avenues for the supply and profiling of metabolites as a pre-requisite towards future sesquiterpenoid biosynthetic studies in this plant.

Important phenylpropanoid enzymes i.e., phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and lignin peroxidases (POX) and their products, i.e., phenolic, hydroxycinnamic and benzoic acid, flavonoid, anthocyanin and lignin contents showed concomitant increase with lignification/ vascularization as visualized with histochemical analyses. HPLC analysis revealed identities and quantities of 20 phenolics, whereas LC-MS analysis identified ~52 phenylpropanoids through out development.

Differential accumulation of terpenoid biosynthetic pathway enzymes, i.e., DXP synthase, HMGC_oA reductase and sesquiterpene synthase and metabolites i.e., sesquiterpenoid hydrocarbons and alcohols, photosynthetic pigments and farnesylated intermediates were evident from GC and HPTLC analyses. A putative sesquiterpene synthase (~60 kDa) was partially purified from leaves. GC-MS/ MS studies revealed the presence of ~47 volatile constituents in sandalwood. The fragrant sandalwood oil constituent, β -santalol was isolated from somatic embryos and characterized by ¹³C-NMR, ESI-MS, LC-APCI-MS, MALDI-ToF, GC-MS/ MS and FTIR analyses whereas the bioactive constituent α -santalol, bulk-purified from sandalwood oil by HPTLC, RLCCC and column chromatography.

Tissue extracts rich in terpenoids and phenylpropanoids revealed antimicrobial, antioxidant, α -amylase inhibition, anti-tyrosinase and neuroprotective potentials *in vitro*. Sandalwood oil and α -santalol showed strong *in vivo* antioxidant and anti-hyperglycemic potentials.

Homology-based cloning using degenerate primers, yielded partial clones of a housekeeping gene (actin), a phenylpropanoid gene (CAD) and 4 terpenoid biosynthetic pathway genes (DXP synthase, FPP synthase, monoterpene synthase and sesquiterpene synthase), that showed homology and phylogenetic relationships with other plant genes. Homology-based modeling revealed their protein structure-function relationships, i.e., catalytic sites, domains and motifs. Structures were validated by Ramachandran plots. Transcriptional profiling by sqRT-PCR revealed higher expression levels of five transcripts in callus and young tree.

Keywords: Sandalwood, *Santalum album* L., prospecting, phenylpropanoid, sesquiterpenoid, santalol, terpene synthase, bioactivity.

CONTENTS

	Pages
Title Page	i
Certificate of Approval	iii
Certificate by Supervisor	v
Declaration	vii
Preface	ix
Acknowledgement	xi
List of Abbreviations	xiii
List of Tables	xix
List of Figures	xxi
List of Appendix	xxv
Glossary	xxvii
Abstract	xxix
 Chapter 1 Introduction, Review of the Literature and Objectives of the Present Investigation	 1
1.1 Introduction	1
1.2 Review of literature	2
1.2.1 Taxonomy	2
1.2.2 Diseases and pests	3
1.2.3 Sandalwood essential oil	3
1.2.4 Santalenes as major constituents	3
1.2.5 Chemo- synthesis of santalenes	4
1.2.6 Synthetic analogues of santalanes	4
1.2.7 Sandalwood odor chemistry	5
1.2.8 Organoleptics and physicochemical properties	5
1.2.9 Santalol biosynthetic pathway	5
1.2.10 <i>In vitro</i> micropropagation and plant development	7
1.2.11 Secondary metabolism in plants	7
1.2.12 Plant cell cultures as sources of natural products	7
1.2.13 Approaches to characterize secondary metabolome	8
1.2.14 Phenylpropanoid biosynthetic pathway	10
1.2.14.1 Phenylalanine ammonia lyase (PAL)	10
1.2.14.2 Cinnamyl alcohol dehydrogenase (CAD)	10
1.2.14.3 Peroxidase (POX)	11
1.2.14.4 Polyphenol oxidase (PPO)	11
1.2.14.5 Phenolics	11
1.2.14.6 Lignin biosynthesis and lignification	13
1.2.14.7 Flavonoids and anthocyanins	13
1.2.14.8 Phenylpropanoids in heartwood	14
1.2.15 Terpenoids	15
1.2.15.1 Importance of terpenoids	15
1.2.15.2 Terpenoid biosynthetic pathway	15
1.2.15.3 Terpenoid production <i>in vitro</i>	16
1.2.15.4 Chlorophylls and carotenoids	17
1.2.15.5 1- deoxy- D- xylulose phosphate synthase (DXS)	18
1.2.15.6 3- Hydroxy- 3- methylglutaryl- coenzyme A reductase (HMGCoAR)	19
1.2.15.7 Farnesyl diphosphate synthase (FPPS)	19
1.2.15.8 Terpene synthase (TPS)	19
1.2.15.9 Monoterpene synthase (MTPS)	20

1.2.15.10 Sesquiterpene synthases (STPS)	21
1.2.16 Biological activities of natural products	22
1.2.16.1 Essential oils as bioactives	22
1.2.16.2 Phytoconstituents as antimicrobials	22
1.2.16.3 Phytoconstituents as antioxidants	22
1.2.16.3.1 Phenylpropanoids as antioxidants	23
1.2.16.4 Phytoconstituents as tyrosinase inhibitors	24
1.2.16.5 Phytoconstituents as antidiabetic principles	25
1.2.16.5.1 Diabetes mellitus	25
1.2.16.5.2 Hypoglycemic potential of plants	26
1.2.16.6 Cholinesterase inhibitors as neuroprotective agents	26
1.2.16.7 Phytoconstituents as cytotoxic agents	27
1.2.16.8 Anticancer drugs from plants	27
1.2.16.9 Phytoconstituents as MMP inhibitors	27
1.2.17 Research and development work in sandalwood	28
1.3 Objectives of the investigation	29
Chapter 2 Profiling of Phenylpropanoids	31
2.1 Introduction	31
2.2 Materials and methods	32
2.2.1 Preparation of crude enzyme extracts	32
2.2.2 Enzyme assays	32
2.2.2.1 Phenylalanine ammonia- lyase (PAL, EC 4.3.1.5) assay	32
2.2.2.2 Cinnamyl alcohol dehydrogenase (CAD, EC 1.1.1.195) assay	32
2.2.2.3 Peroxidase (POX, EC 1.11.1.7) assays	33
2.2.3 Polyphenol oxidase (PPO) (EC 1.14.18.1) enzyme preparation and assay	33
2.2.4 Profiling of phenylpropanoids	34
2.2.4.1 Isolation of free and wall- bound phenolics	34
2.2.4.2 Folin- Ciocalteu assay for phenolics	34
2.2.4.3 High Pressure Liquid Chromatography (HPLC) analysis of phenolics	34
2.2.4.4 Liquid Chromatography Mass Spectroscopy (LC- MS) analyses of phenylpropanoids	35
2.2.4.5 Anthocyanin quantification	36
2.2.4.6 Flavonoid quantification	36
2.2.4.7 Concentrations of total UV- absorbing compounds	36
2.2.4.8 Quantification of lignin	36
2.2.4.9 Quantification of shikimic acid	37
2.2.5 Microscopy and histochemistry	37
2.2.5.1 Fluorescent microscopy studies	37
2.2.5.2 Wesner reaction for lignification	37
2.2.5.3 Maule reaction for lignification	38
2.2.5.4 Double staining for visualization of soft and hard tissues	38
2.2.5.5 Polychromatic staining by Toluidine Blue O	38
2.2.6 Data analysis	38
2.3 Results	38
2.3.1 Phenylpropanoid enzymes	38
2.3.2 Phenylpropanoid metabolites	39
2.3.3 RP- HPLC and LC- MS/ MS based profiling of phenylpropanoids	41
2.3.4 Histochemical evidences for differentiation and maturation events	45
2.4 Discussion	47
Chapter 3 Profiling of Terpenoids	55
3.1 Introduction	55

3.2 Materials and methods	56
3.2.1 Extraction of enzymes involved in terpenoid biosynthesis	56
3.2.2 3- Hydroxy- 3- methylglutaryl coenzyme A reductase, (HMGR, EC 1.1.1.34) assay	56
3.2.3 1- deoxy- D- xylulose 5- phosphate synthase (DXS, EC 2.2.1.7) assay	56
3.2.4 Sesquiterpene synthase (STPS, EC 4.2.3.41 ?) assay	56
3.2.4.1 Extraction of sesquiterpene synthase enriched crude fractions	56
3.2.4.2 Sesquiterpene synthase (STPS) assay	57
3.2.5 Profiling of sesquiterpenoids	57
3.2.5.1 Extraction of sesquiterpenoids from tissues	57
3.2.5.2 Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) analysis of tissue extracts and sandalwood oil	58
3.2.5.3 Gas chromatography (GC) analyses of sandalwood oil and tissues	58
3.2.5.4 Quantification of total terpenoids in tissues	59
3.2.6 Quantification of sesquiterpenoid biosynthesis intermediates	59
3.2.6.1 Extraction of farnesylated compounds	59
3.2.6.2 Preparation of standard farnesylated compounds	59
3.2.6.2.1 Extraction of acid phosphatase (APase) rich fractions from potato	59
3.2.6.2.2 Acid phosphatase assay	60
3.2.6.2.3 Enzymatic hydrolysis of prenylpyrophosphates	60
3.2.6.3 HPTLC- based analyses for detection and quantification	60
3.2.7 Quantification of chlorophylls, carotenoids and xanthophylls	60
3.2.8 Purification and characterization of β- santalol from somatic embryos	60
3.2.8.1 Extraction and purification of β- santalol	60
3.2.8.2 Nuclear Magnetic Resonance (NMR) analysis	60
3.2.8.3 Matrix Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry (MALDI- ToF- MS) analysis	61
3.2.8.4 Electron Spray Ionization Mass Spectroscopy (ESI- MS) analysis	61
3.2.8.5 Fourier Transform Infrared Spectroscopy (FTIR) analysis	61
3.2.8.6 Gas Chromatography Mass Spectroscopy (GS- MS/ MS) analysis	61
3.2.8.7 Liquid Chromatography- Atmospheric Pressure Chemical Ionization- Mass Spectroscopy (LC- APCI- MS/ MS) analysis	62
3.2.9 Purification of α- santalol from sandalwood oil	62
3.2.9.1 Rotatory locular counter current chromatography (RLCCC)	62
3.2.9.2 Column chromatography	62
3.2.10 Immunodetection of α- santalol	63
3.2.10.1 Polyclonal antibodies against α- santalol	63
3.2.10.2 Antigen (α- santalol- BSA conjugate) preparation	63
3.2.10.2.1 Method 1	63
3.2.10.2.2 Method 2	63
3.2.10.3 Confirmation of conjugation of BSA- α- santalol	63
3.2.10.3.1 UV- visible spectrophotometry	63
3.2.10.3.2 Spectrofluorimetry	64
3.2.10.3.3 SDS PAGE analysis	64
3.2.10.3.4 MALDI- ToF- MS analysis	64
3.2.10.4 Raising antibodies in mice and rabbit	64
3.2.10.4.1 Immunization of rabbits	64
3.2.10.4.2 Immunization of mice	65
3.2.10.4.3 Antisera titer determination by indirect ELISA	65
3.2.10.5 Standard curve generation for α- santalol using antibody	66
3.2.10.6 Immunodetection of α- santalol on solid surface	66
3.2.10.7 Immuno TLC- Dot Blot assay (DAB- based detection system)	66
3.2.10.8 Immuno Dot Blot assay (FITC- based detection system)	67

3.2.10.9 Dip- stick ELISA assay (nitrocellulose membrane)	67
3.2.10.10 Dip- stick ELISA (wooden toothpicks)	67
3.2.10.11 Polyclonal antibody (IgG) purification by affinity chromatography	68
3.2.10.12 Immunohistolocalization of α - santalol	68
3.2.10.12.1 Localization of α - santalol by confocal laser scanning microscopy (CLSM)	68
3.2.10.12.2 Histochemical localization of santalols	69
3.2.11 Purification of sesquiterpene synthase from leaf	69
3.2.11.1 Extraction of crude proteins	69
3.2.11.2 Anion exchange chromatography	69
3.2.11.3 Gel filtration chromatography	69
3.3 Results	70
3.3 Terpenoid biosynthesis enzymes	70
3.3.1 DXS and HMGRCoAR enzyme activities	70
3.3.1.2 Sesquiterpene synthase assay and GC- based identification of products	70
3.3.2 Profiling of sesquiterpenoids	71
3.3.2.1 Sesquiterpenoid composition of the sandalwood oil	71
3.3.2.2 Sesquiterpenoid composition of sandalwood (HPTLC- based)	73
3.3.2.3 Sesquiterpenoid composition of the sandalwood tissues (GC- based)	74
3.3.2.4 GC- MS/ MS- based profiling of sandalwood volatile constituents	75
3.3.2.5 Farnesylated compounds in sandalwood (HPTLC- based)	76
3.3.2.6 Terpenoid content	78
3.3.2.7 Photosynthetic pigments in sandalwood	78
3.3.3 Purification of β - santalol from somatic embryo	79
3.3.3.1 Confirmation of (Z)- β - santalol by ^{13}C - NMR, MALDI- ToF- MS, FTIR, GC- MS/ MS and LC- APCI- MS/ MS analyses	80
3.3.4 Isolation of (Z)- α - santalol	83
3.3.4.1 Preparative- scale purification of (Z)- α - santalol by column chromatography, RLCCC and HPTLC	83
3.3.5 Immunodetection of Z- α - santalol	84
3.3.5.1 Immunogen design	84
3.3.5.2 Confirmation of conjugation of α - santalol to BSA	84
3.3.5.2.1 UV- vis spectrophotometry	84
3.3.5.2.2 Spectrofluorimetry	84
3.3.5.2.3 SDS- PAGE analysis	85
3.3.5.2.4 MALDI- ToF- MS analysis	85
3.3.5.3 Production of polyclonal antibodies	86
3.3.5.4 Optimization of antibody and α - santalol- BSA conjugate concentrations	86
3.3.6 Immunolocalization of (Z)- α - santalol in sandalwood developmental stages by confocal laser scanning microscopy (CLSM)	88
3.3.7 Partial purification of sesquiterpene synthase (SaSTPS) protein from sandalwood leaves	90
3.4 Discussion	92
Chapter 4 Screening of Biological Activities of Extracts	
Rich in Phenylpropanoids and Terpenoids	101
4.1 Introduction	101
4.2 Materials and Methods	103
4.2.1 Microorganisms	103
4.2.1.1 Bacterial strains	103
4.2.1.2 Fungal strains	103
4.2.2 Physical characterization of the tissues and extracts	103
4.2.2.1 Dry weight, moisture and ash content	103
4.2.2.2 Preparation of extracts	103

4.2.2.3 pH of the extracts	104
4.2.2.4 Determination of plant extract yield	104
4.2.3 Phytochemical profiling of extracts	104
4.2.3.1 High performance thin layer chromatography (HPTLC) analysis	104
4.2.4 Biological activity screening of the extract	104
4.2.4.1 Minimum inhibitory concentration (MIC) determination	104
4.2.4.2 Disc- diffusion method for antibacterial activity	105
4.2.4.3 Agar spot method for antibacterial activity	105
4.2.4.4 TLC bioautography for antibacterial screening	105
4.2.4.5 Quantitative evaluation of antimicrobial activity	105
4.2.4.6 Antifungal activity by disc diffusion method	105
4.2.4.7 Lipid peroxidation inhibition in mice liver and brain microsomes (TBA method)	106
4.2.4.8 Reducing power determination	106
4.2.4.9 Hydroxyl radical scavenging activity (deoxyribose method)	106
4.2.4.10 Metal (ferrous) ion chelating activity	106
4.2.4.11 Ferric reducing antioxidant power (FRAP) assay	106
4.2.4.12 Total antioxidant capacity (TAC)	106
4.2.4.13 β - carotene linoleate bleaching activity	106
4.2.4.14 Nitric oxide free radical scavenging activity	106
4.2.4.15 ABTS' free radical scavenging activity	106
4.2.4.16 DPPH free radical scavenging activity	107
4.2.4.17 DPPH dot blot (qualitative) assay	107
4.2.4.18 Tyrosinase inhibition (spectrophotometric)	107
4.2.4.19 Tyrosinase inhibition (bioautography)	107
4.2.4.20 Alpha- amylase (EC 3.2.1.1) inhibition assay (spectrophotometric)	107
4.2.4.21 Alpha- amylase inhibition assay (disc- diffusion)	108
4.2.4.22 Glucose diffusion inhibition assay	108
4.2.4.23 Cholinesterase (ChE) inhibition assay (TLC bioautographic)	108
4.2.4.24 Cholinesterase (ChE) inhibition assay (spectrophotometric)	108
4.2.4.25 MMP inhibition assay	108
4.2.4.26 Cytotoxicity screening in fibroblast cell lines	109
4.2.4.27 Cytostatic potential in carcinoma and melanoma cell lines	109
4.2.4.28 <i>In vivo</i> anti- hyperglycemic assay in alloxan- induced diabetic mice model	109
4.2.5.28.1 Experimental animals	109
4.2.5.28.2 Administration of compounds to normal mice	109
4.2.5.28.3 Preparation of diabetic mice	110
4.2.5.28.4 Estimation of blood glucose level	110
4.2.5.28.5 Estimation of liver glycogen content	110
4.2.5.28.6 Estimation of serum bilirubin level determination	110
4.2.5.29 <i>In vivo</i> antioxidant assay	110
4.2.5.29.1 Preparation of oxidative stress induced mice	111
4.2.5.29.2 SGPT/ ALT (Alanine Aminotransferase- Serum Glutamate Pyruvate Transminase- E.C.2.6.1.2) assay	111
4.2.5.29.3 SGOT/ AST (Aspartate Aminotransferase- Serum Glutamate Oxaloacetate Transminase- E.C.2.6.1.1) assay	111
4.2.5.29.4 Serum alkaline phosphatase (ALP) assay	111
4.2.5.29.5 Serum bilirubin level determination	111
4.2.5.29.6 Superoxide dismutase activity	111
4.2.5.29.7 Catalase activity	111
4.2.5.29.8 Free sulfhydryl groups in serum as reduced glutathione (GSH)	112
4.2.5.29.9 Quantification of protein carbonyl content	112
4.2.5.29.10 NO assay (for nitrite determination)	112

4.2.5.29.11 Serum antioxidant activity (DPPH) based	112
4.2.5.30 Splenocyte (spleen lymphocyte) and macrophages (activated monocytes) isolation	112
4.2.5.31 Splenocyte proliferation assay	112
4.2.5.32 Nitrite determination (NO assay) in macrophages	113
4.2.5.33 Cluster analysis (CA)	113
4.2.5.34 Statistical analysis	113
4.3 Results	113
4.3.1 Physico- chemical characterization of tissues and extracts	113
4.3.2 Phytochemical analysis of extracts	114
4.3.2.1 Terpenoid metabolites	114
4.3.2.2 Phenylpropanoid metabolites	114
4.3.3 Biological activities of sandalwood extracts and sandalwood oil	116
4.3.3.1 Antimicrobial (antibacterial and antifungal) activities	116
4.3.3.2 Antioxidant, antityrosinase, antidiabetic and neuroprotective potentials	117
4.3.3.3 Cytotoxicity in fibroblasts and cancer cell lines, NO production in macrophages and MMP inhibition <i>in vitro</i>	119
4.3.3.4 <i>In vivo</i> antioxidant potential of sandalwood oil and α - santalol	119
4.3.3.5 <i>In vivo</i> hypoglycemic potential of sandalwood oil and α - santalol	120
4.4 Discussion	126
Chapter 5 PCR- based Cloning and Evaluation of Phenylpropanoid and Terpenoid Biosynthetic Pathway Genes	135
5.1 Introduction	135
5.2 Materials and methods	137
5.2.1 Bacterial growth media	137
5.2.2 Antibiotics	137
5.2.3 Bacterial strains	137
5.2.4 Molecular cloning techniques	137
5.2.4.1 Quantification of nucleic acids	137
5.2.4.2 Synthesis of oligonucleotides (primers)	137
5.2.4.3 Nucleic acid isolation	138
5.2.4.4 Polymerase chain reaction (PCR) amplification	139
5.2.4.5 Reverse transcriptase- polymerase chain reaction (RT- PCR) and complimentary DNA (cDNA) preparation	139
5.2.4.6 Gradient PCR	140
5.2.4.7 Cloning of PCR, RT- PCR and gradient PCR products	140
5.2.4.8 Plasmid isolation and restriction digestion for confirmation of clones	140
5.2.5 Comparative and bioinformatic analyses	141
5.2.5.1 Primer designing	141
5.2.5.2 Protein domain architecture studies	142
5.2.5.3 Multiple sequence alignment of sequences	142
5.2.5.4 Construction of phylogenetic tree for deduction of evolutionary relationships	142
5.2.5.5 Homology- based modeling of the sequences and model- validation studies	142
5.2.6 Tissue- level expression profiling by semi- quantitative RT- PCR (sq RT- PCR)	144
5.3 Results	144
5.3.1 Partial clones of actin (SaACT)	146
5.3.2 Partial clone of cinnamyl alcohol dehydrogenase (SaCAD)	146
5.3.3 Partial clones of 1- deoxy- D- xylulose 5- phosphate synthase (SaDXS)	147
5.3.4 Partial clones of farnesyl pyrophosphate synthase (FPPS)	148
5.3.5 Partial clones of monoterpene synthase (MTPS)	148

5.3.6 Partial clones of sesquiterpene synthase (STPS)	148
5.3.7 Phylogenetic analysis of the clones	149
5.3.8 Bioinformatic analysis, homology- based modeling and structure- function relationships of the proteins	150
5.3.9 Tissue level expression profiling of the 6 genes	156
5.4 Discussion	156
Chapter 6 Conclusions and Future Scope	163
6.1 Conclusions	163
6.2 Future Scope	165
References	167
Appendix	In CD
Curriculum vitae	

Chapter 1

Introduction, Review of the Literature and Objectives of the Present Investigation

1.1 Introduction

The East Indian Sandalwood, *Santalum album* L., is a tropical, woody and perennial essential oil yielding tree. The fragrant heartwood accumulates the sandalwood oil (SWO), rich in sesquiterpenoids and is reported to have many medicinal properties. Epidemic phytoplasmal ‘spike’ disease leading to severe destruction, illegal poaching and over exploitation owing to increased global demand are the reasons of it being inducted into IUCN, Red List of Threatened Species (IUCN, 2006) as vulnerable, thus necessitating its *in vitro* micropropagation and biotechnological manipulation. The *Santalum album* wood and oil (rich in sesquiterpenoid santalols) are among the costliest plant products (wood ~ US \$ 180 kg⁻¹; oil ~US \$ 2600 l⁻¹). The estimated average global annual export is about 2, 000 tons of wood and 100 tons of oil (Gupta, 1998), against a projected demand of 10, 000 tons of wood, involving an international trade of about US \$ 360 million; thus implying a huge gap between demand and supply, largely due to dwindling natural population of the plant. In USA alone the total need is 22, 000 kgs yr⁻¹ (Burdock and Carabin, 2008). Sandalwood tree is valued for commercial, medicinal, traditional, spiritual, aesthetic and social importance. The traditional curative properties for acne, urino- genital tract infections, bronchitis, congestion etc. are known.

The number of plant species in India is estimated to be over 45, 000 representing about 7 % of the world’s flora. Medicinal plants, as a group, comprise approximately 8, 000 species and account for about 50 % of all the higher flowering plant species of India. India is one of the richest countries in the world as regards genetic resource of medicinal and aromatic plants. It constitutes 11 % of the total known world flora having medicinal properties (<http://www.inheritanceindia.co.in>). The commercial, traditional and social importances of this medicinal tree are:

1. Cosmetics, perfumes, soaps, musks, face packs for its fragrance, fixative property and its oil base fixes both dry and oily skin.
2. Handicrafts, luxury items and wooden furniture for domestic use due to durability and scent.
3. The unique fragrance of the oil is known as an aid to meditation, spiritual growth, emotionally it relaxes stress, soothes irritation, lifts depression and is aphrodisiac.
4. It has a long history in ethno pharmacology in India and elsewhere, for uses such as a tonic to the immune system, effective against strep throat, gastrointestinal ailments, skin infections, reducing vomiting, fever, thirst and so on, though the perfumery use has outweighed the medicinal uses.

No active principles were implicated in such healthcare purposes. However, in the last decade, the compounds from oil were shown to have antimicrobial, anticancer and antiviral properties. The above facts inspired us to the present study on bioprospecting of the phenylpropanoids and terpenoids. Apart from the healthcare angle, the accumulation of the oil in the heartwood in this plant generates natural curiosity of correlating the profiles of phenylpropanoids and terpenoids.

1.2 Review of literature

1.2.1 Taxonomy

The East Indian Sandalwood, belongs to the angiosperm class of ‘Magnoliopsida’, specifically to the ‘Rosids’, in the order ‘Santalales’, family ‘Santalaceae’, type genus ‘*Santalum*’ and species ‘*S. album*’. The common name is East Indian Sandalwood or White Sandalwood as well as Chandan in Hindi.

Tree, semi- parasitic in roots (host plants usually are *Cassia*, *Dalbergia*, *Azadirachta*, *Pongamia*, *Casuarina*, *Pterocarpus*, *Acacia* etc.). Leaves opposite, simple, decussate, shape varies among six morphological types- ovate, lanceolate, elliptic, linear, big and small. Inflorescence axillary or terminal cymose panicles. Flowers: small, purplish brown, unscented. Fruits: drupe, purplish when fully matured, single seeded, shape- round to oblong. Seed: polymorphic, lacking testa, dicotyledonous embryo, endosperm fleshy. Basic chromosome number of the genus is $x=10$, few are polyploids. Twenty different species, a few are commercially important; include *S. lanceolatum*, *S. yaasi*, *S. spicatum*, *S. austrocaledonicum*. In India, more than 90 % of sandalwood forest lies in Karnataka and Tamil Nadu covering an area of 8, 300 sq. km. Andhra Pradesh, Kerala, Maharashtra, Madhya Pradesh and Orissa are some other states where sandalwood trees are found (Figure 1.1). A total area of about 9, 600 sq. km. harbors sandalwood plantation (Rao and Bapat, 1992). *Santalum album* L. is distributed throughout Indonesia in the West, Portuguese island of Timor and on Celebes, Ceylon to Juan Fernandez Islands in the East and from Hawaiian Archipelago in the North to New Zealand in the South.

Figure 1.1 Distribution of East Indian sandalwood in Indian subcontinent (modified from Srinivasan et al., 1992)

Appendix F.3 Flowchart showing the Harborne protocol (Harborne, 1998).

CURRICULUM VITAE OF AUTHOR

Biswapriya Biswavas Misra was born on the 4th of April, 1982 in the ancient historical capital-city of Orissa state, Cuttack. He received his Bachelor's Honors degree in Botany from Sambalpur University in 2002 and Master's degree in Botany with specialization in Biotechnology and Plant Genetic Manipulation from Utkal University, Orissa, India in 2004. He topped in both the degrees and received University Gold Medals. He was a recipient of Junior and Senior Research Fellowships from Council of Scientific & Industrial Research, India and qualified in GATE. He pursued his doctoral studies in the field of Plant Biotechnology on the thesis entitled, "Prospecting of phenylpropanoids and terpenoids in East Indian Sandalwood (*Santalum album* L.)" under the supervision of Professor Satyahari Dey at the Department of Biotechnology, Indian Institute of Technology, Kharagpur, India. While continuing doctoral studies he got trained in plant tissue and organ culture, animal cell culture, spectroscopy and chromatography methods, metabolite profiling, microscopy and histochemistry, molecular biology, immunology, bioassays, bioinformatics as well as statistical analysis and computer skills. The sequences obtained from the cloning studies have been submitted with the NCBI GenBank Public Database. From the doctoral work, three Indian patents have been filed and four manuscripts are being communicated to peer reviewed journals. During this period, he got an opportunity to work at Eidgenössische Technische Hochschule (ETH), Zurich, Switzerland at as an exchange research student through Indo Swiss Bilateral Research Initiative (ISBRI), in a project entitled "*Arabidopsis* isoprenoid metabolism by means of protoplast transformation, loss-of-function mutant, genotyping and phenotyping and confocal based gene co localization studies" under the supervision of Prof. Wilhelm Gruissem and Dr. Eva Vranova, for 5 months starting August, 2009. During the doctoral studies his research activities involved extraction and characterization of sandalwood enzymes and metabolites, testing the biological activities and obtaining clones for several metabolically important biosynthetic pathway genes. His research interests include secondary metabolism, plant metabolic engineering, plant functional genomics, metabolomics, developmental biology and natural products research. More information regarding the author's research interests, and information included in Appendix in this thesis are found at the personal webpage, <http://sites.google.com/site/bbmisranow/>.