Immunolocalization of α-santalol in sandalwood

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(Received 1 November 2012; accepted 23 March 2014)

Alpha-santalol is a key constituent of sandalwood essential oil and is responsible for most of its biological activities. The heartwood of a mature East Indian sandalwood tree accumulates this sesquiterpenoid-rich oil. Although gas chromatography (GC) and GC–mass spectrometry (GC–MS)-based technologies are used to detect and quantify santalols from heartwoods and the essential oil, information on the sites of deposition of these molecules remains obscure. Recently, in vitro cells of sandalwood were shown to accumulate sandalwood oil constituents. However, no reports are available on the visualization of these small molecules in planta. Immunization of rabbits with a bovine serum albumin (BSA)-α-santalol conjugate resulted in the production of anti-α-santalol polyclonal antibody in six weeks, which showed high affinity and specificity. The success and extent of cross-linking of α-santalol with BSA was further confirmed by photometric, fluorometric and chromatographic methods. These polyclonal rabbit antibodies were used to immunolocalize α-santalol in sandalwood plant materials for the first time. Results indicate the localization of α-santalol to the vascular bundles of somatic embryos and leaves, whereas distribution was evident in secondary xylem, cortical parenchyma and epidermis of the mature stem. Furthermore, the polyclonal antibody is shown to be a useful tool in detection of both free and immobilized α-santalol for screening purposes.

Keywords: BSA; conjugate; EDC; ELISA; Santalum album L.; somatic embryo

1. Introduction

The East Indian Sandalwood tree Santalum album L. (family Santalaceae), accumulates a sesquiterpenoid-rich essential oil in the core of its heartwood. The yield from harvest-quality wood is typically 3–6% from thirty- to fifty-year-old mature trees, though sesquiterpenoids are also known to be present in immature heartwood [1]. Globally, the trade of sandalwood oil and wood is worth about US$ 600 million. Sandalwood oil constituents are antibacterial, antifungal [2], antioxidant [3], anti-cancer [4], anti-hypertensive, anti-pyretic, sedative [5], antiviral [6] and anti-Helicobacter pylori [7]. Hence, sandalwood oil finds numerous applications in traditional medicinal systems, cosmetics and aromatherapy. However, a mycoplasmal ‘spike’ disease, illegal poaching and over-exploitation to meet the rising global demands resulted in the tree being inducted into IUCN Red List of Threatened Species [8] as vulnerable. Thus, in vitro modes of micropropagation in sandalwood have provided immense scope through biotechnological means of conservation of the species [9]. Moreover, in vitro-grown callus is known to accumulate the essential oil constituents [10, 11].

Post-extraction, the methods for quantification and detection of sandalwood oil constituents from commercial oils [12] and heartwood samples essentially depend on gas chromatography (GC) [13] and GC–mass spectrometry (GC–MS) [14]. Furthermore, newer methods of detection have been proposed for evaluation of sandalwood oil constituents, such as Fourier transform infrared spectroscopy [15, 16]. The major essential oil components are sesquiterpenoid alcohols (C15H24O): cis-α-santalol (up to 45–55%), cis-β-santalol (up to 20–35%), α-trans-bergamotol and epo-cis-β-santalol, whereas minor constituents include trans-β-santalol and cis-lanceol; and sesquiterpene hydrocarbons (C15H24): α-santalene, β-santalene, α-bergamotene, epo-β-santalene, α-curcumene, β-curcumene, γ-curcumene, β-bisabolene and α-bisabolol [12, 14].

Although it is well known that the site of deposition of sandalwood oil is the heartwood, the exact location and tissue type involved have not been addressed till date, much owing to the difficulties associated with sampling the deepest cores of heartwood of mature tree in a non-destructive manner [14]. In addition, no information is available on the sites of accumulation or distribution of sandalwood oil constituents in the plant parts and tissues. Hence, this study was focused on developing polyclonal antibodies against the carrier protein-conjugated α-santalol to immuno-recognize and localize α-santalol [C15H24O, 220.35 Da, CAS No. 11031-45-1, FEMA No. 3006], the major constituent of

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sandalwood oil. Moreover, there are no reports available on the detection of sandalwood oil constituents in differentiated in vitro tissue, i.e. somatic embryos. To our knowledge, this is the first time immunodetection and visualization of α-santalol from in vitro and in vivo tissues has been attempted using confocal laser scanning microscopy (CLSM).

2. Experimental

2.1 Chemicals and reagents

1-Ethyl-3-(3’-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), Phytagel and 4’,6-diamidino-2-phenylindole (DAPI) were procured from Sigma (St. Louis, MO, USA); ethylene diamine (EDA), was obtained from SRL (Mumbai, India); goat anti-rabbit immunoglobulin (IgG) from MO, USA); ethylene diamine (EDA), was obtained from SRL (Mumbai, India); goat anti-rabbit immunoglobulin (IgG)–fluorescein isothiocyanate (FITC)-conjugated secondary antibody (Cat. No. FTC3) was obtained from Bangalore Genei (Bengaluru, India); and bovine serum albumin (BSA, Fraction V) was obtained from HiMedia (Mumbai, India).

Commercially available steam-distilled sandalwood oil was procured from CauveryTM (Government of Karnataka, Bengaluru, India) and was subjected to preparative scale high-performance thin-layer chromatography (HPTLC) to purify α-santalol in bulk (~122 mg). Furthermore, GC analysis was used to detect the purity of the preparations.

2.2 Plant materials

In vitro somatic embryos were obtained from a highly proliferating cell line (IITKGP/91) obtained from an elite Kerala (India) clone were maintained aseptically on solid Woody Plant Medium [17], supplemented with indole-3-acetic acid (IAA) (0.5 mg/L) + 6-benzylaminopurine (BAP) (0.5 mg/L), 4% sucrose, 0.35% Phytagel, at pH adjusted to 5.8 ± 0.5. The somatic embryos were harvested two weeks after induction into embryogenic media from the callus. The cultures were maintained in the dark at 25 ± 2°C with 50–60% relative humidity. Seedlings raised from somatic embryos were cultured in vitro on Murashige and Skoog (MS) medium [18] supplemented with IAA (1 mg/L), indole-3-butyric acid (IBA) (1 mg/L), gibberellic acid (GA) (0.5 mg/L), 3% sucrose and 0.35% Phytagel (Sigma Aldrich, USA). The seedlings were harvested after six weeks of induction of organogenesis and maintained under white fluorescent light of 1000–1500 lux (16-hour light/8-hour dark cycle) at 25 ± 2°C with 50–60% relative humidity. Young stems and leaves were obtained from a fifteen-year-old oil-yielding mature tree growing on the campus of the Department of Biotechnology of the Institute.

2.3 Antigen (α-santalol–BSA conjugate) preparation

EDC (60 mg, 60 mM), 1 mL EDA and α-santalol (60 mg, 80 mM) were added to 30% pyridine (5 mL) and 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) containing 0.9% NaCl (5 mL). The reaction mixture was added drop wise to MES buffer (2.5 mL) containing BSA (60 mg) and then stirred at 25°C for 6 hours. Subsequently, the mixture was dialyzed (10,000 kDa MWCO, SnakeSkin®, Thermo, USA) with five changes of H2O over two days at 4°C and lyophilized to give ~82 mg α-santalol–BSA conjugate, as estimated gravimetrically and by the Bradford method.

2.4 Confirmation of conjugation of BSA–α-santalol

2.4.1 UV–visible spectrophotometry

Samples were suitably dissolved, i.e. α-santalol in MeOH (100%) and either BSA or BSA–α-santalol conjugate in 50 mM Tris–HCl (pH 8), and spectra were recorded from 190 to 350 nm against suitable controls, in scanning mode using an UV–visible double-beam spectrophotometer (Spectrascan UV 2600 PC, Chemito, India).

2.4.2 Spectrofluorimetry

Either BSA or BSA–α-santalol conjugate in various comparable concentrations (1–100 mg/mL) were dissolved in 50 mM Tris–HCl, pH 8, and the fluorescence spectra were recorded using the excitation and emission wavelengths of 278 and 350 nm, respectively, for BSA. Data points obtained were plotted and analyzed using Origin software.

2.4.3 SDS–PAGE analysis

A 15% sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) [19] was run to confirm the conjugation of BSA with α-santalol, from its migration difference in comparison with BSA. Samples were run in non-denaturing conditions, using a sample-loading buffer without dithiothreitol/β-mercaptoethanol and avoiding boiling of the BSA and conjugated BSA.

2.5 Raising polyclonal antibodies in rabbits

2.5.1 Immunization of rabbits

Two female New Zealand white rabbits were housed in the Departments Animal Facility. The handling of the animals and experiments were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), New Delhi, India, and was permitted by the Animal Ethical Committee of Indian Institute of Technology
PBS, 0.02% NaN₃, pH 7.2

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The titer of the antisera was tested by indirect ELISA. The antisera was tested using a checkerboard non-competitive ELISA. Antibody titers of rabbit sera were determined by centrifugation to remove blood cells and particulate material. Ten days after the last booster, both the rabbits were then ex-sanguinated by heart puncture under general anesthesia. The serum was separated from blood cells by storage of the blood overnight at 4°C and centrifuged at 13,000 × g for 20 minutes. The crude serum obtained was purified by saturated ammonium sulfate (SAS) precipitation method [purified thrice using 50% and 33% (v/v) SAS, respectively] and NaN₃ (0.02%, w/w) added as a preservative, aliquoted and stored at −70°C until further use. This supernatant was used to determine their optimal dilution. The optimal dilutions were those that resulted in an absorbance of 1 at 450 nm, following incubation of the substrate for 30 minutes at 25°C. Antibody titers of rabbit sera were further performed in 1.5-mL micro-centrifuge tubes. Tissues were fixed in a mixture of 4% para-formaldehyde and 0.5% glutaraldehyde prepared in 0.1 M phosphate buffer, pH 7, for 3 hours. The materials were then rinsed thrice with 0.1 M phosphate buffer, pH 7, for 15 minutes each. Permeabilization was done for 5 minutes with PBST [0.025% Triton X-100 in PBS], followed by blocking [2% casein, 0.1% NaN₃, 0.05% polyvinylpyrrolidone (MW=10,000) and 0.05% Triton X-100] for 1 hour and a wash in PBST for 30 minutes. The tissue sections were then incubated with rabbit anti-α-santalol–BSA conjugate polyclonal antibody at a dilution of 1:500. This was followed by three rinses in PBST for 15 minutes each. The plant materials were incubated with goat anti-rabbit IgG–FITC-conjugated secondary antibody for 1 hour at 25°C, followed by three PBST washes each for 15 minutes. Materials were stained with DAPI for nuclear and xylem staining at a 1 μg/mL working concentration in PBS for 10 minutes and again washed once with PBST for 15 minutes. The plant sections were mounted in a mounting medium solution, from a commercial kit (Bangalore Genei, India) was added (50 μL per well), and the plates were incubated for another 45 minutes at 25°C. The color was developed by adding 75% H₂SO₄ stopping solution (50 μL per well), and absorbance of the enzyme activity product was measured at 450 nm. Absorbances were corrected by blank readings. The serum collected before immunization (pre-immunized serum) was used as the negative control and the antibody titer was defined as the reciprocal of the dilution that results in an absorbance value that is twice the background or the reciprocal of the dilution giving a half maximum binding.

2.6 Cross-reactivity of common sesquiterpenoid competitors

The optimized immunoassay was used to run cross-reactivity (CR) studies by using standard solutions of the sesquiterpenoids (including α-santalol, α-santalol–BSA conjugate), phenolics and other phytochemicals in MeOH stock solutions by competitive indirect ELISA. The tested compounds are listed in Supplementary Table S1. The CR was calculated as a percentage using the following formula: (IC₅₀ of the target analyte/IC₅₀ of the tested compound) × 100%.

2.7 Localization of α-santalol by confocal laser scanning microscopy (CLSM)

Freshly collected in vitro-grown somatic embryos and seedlings, and field-grown stems and leaves were carefully hand-cut with a thin razor blade, while keeping them hydrated all the time. All the obtained cross-sections were washed with water and all steps were performed in 1.5-mL micro-centrifuge tubes. Tissues were fixed in a mixture of 4% para-formaldehyde and 0.5% glutaraldehyde prepared in 0.1 M phosphate buffer, pH 7, at 4°C for 3 hours. The materials were then rinsed thrice with 0.1 M phosphate buffer, pH 7, for 15 minutes each. Permeabilization was done for 5 minutes with PBST [0.025% Triton X-100 in PBS], followed by blocking [2% casein, 0.1% NaN₃, 0.05% polyvinylpyrrolidone (MW=10,000) and 0.05% Triton X-100] for 1 hour and a wash in PBST for 30 minutes. The tissue sections were then incubated with rabbit anti-α-santalol–BSA conjugate polyclonal antibody at a dilution of the serum at 1:500. This was followed by three rinses in PBST for 15 minutes each. The plant materials were incubated with goat anti-rabbit IgG–FITC-conjugated secondary antibody for 1 hour at 25°C, followed by three PBST washes each for 15 minutes. Materials were stained with DAPI for nuclear and xylem staining at a 1 μg/mL working concentration in PBS for 10 minutes and again washed once with PBST for 15 minutes. The plant sections were mounted in a mounting medium.
composed of glycerol: PBS (1:1) on cover glass and observed with CLSM using an Olympus Fluoview FV 1000 with I X 81 inverted microscope fitted with a Multi-line Argon Laser at 488 nm excitation wavelength. The photomicrographs were further processed with Fluoview™ and Adobe Photoshop™ software. The antiserum was pre-incubated with an excess of α-santalol did not show any binding with sandalwood tissue sections, whereas untreated antiserum did not show any staining in cross-sections of tissues from Amaranthus tricolor (Grain amaranth) seedlings available in the laboratory (negative control).

3. Results

3.1 Confirmation of conjugation of α-santalol to BSA

3.1.1 UV–visible spectrophotometry

The hapten (α-santalol) was coupled to a carrier protein, i.e. BSA, to produce immunogenic constructions in order to obtain anti-α-santalol polyclonal antibodies. Spectra of hapten (α-santalol), BSA and α-santalol–BSA conjugate were recorded from 200 to 400 nm to identify the resultant artificial antigens. The shapes of the three curves were distinct. BSA showed a characteristic maximum absorption at 280 nm, owing to tryptophan residues, whereas α-santalol showed a characteristic maximum absorption at 226 nm owing to conjugated double bonds in the molecules. Addition of α-santalol to BSA, forming α-santalol–BSA conjugate, showed a shift in the wavelength to 275 nm, probably owing to masking of tryptophan residues by α-santalol groups. The peak value of hapten–BSA was larger than that of the hapten and carrier protein. All this indicates that the hapten and carrier protein coupled successfully and provided a qualitative characterization of the coupling efficiency (Figure 1A).

3.1.2 Spectrofluorimetry

Similarly, the spectrofluorimetric analysis showed a characteristic fluorescence spectrum of BSA (ex = 278 nm, em = 350 nm) attributed to the tryptophan residues (Figure 1B). Addition of α-santalol to BSA caused a decrease in the fluorescence intensity at 350 nm, and a concomitant increase at 360 nm, probably as a result of fluorescence resonance energy transfer (FRET) between excited tryptophan residues and the bound santalols. The appearance of an isoactinic point with emission at 380 nm indicated the existence of bound and free α-santalol in equilibrium.

3.1.3 SDS–PAGE analysis

As the conjugate is crucial for the success of immunization, an initial analysis of the conjugate was made with SDS–PAGE. Analysis of conjugated and free BSA by 15% SDS–PAGE revealed gel bands with slightly different molecular weights from their peak centroids. Small changes detected in the molecular weight, i.e. approximately 2 kDa, is well explained by the increased molecular weight of BSA owing to the bound α-santalol molecules (Figure 1C). Thus, the hapten density was determined as approximately ten molecules of α-santalol per molecule of BSA, but SDS-PAGE may not reflect the actual degree of hapten incorporation because it is insensitive to small changes in molecular weight after cross-linking reactions.

3.2 Production of polyclonal antibodies against α-santalol

Considering the three-dimensional structure of BSA, only twenty-six ε-NH2 groups of surface lysines (of fifty-nine lysine residues) of the BSA are theoretically available for coupling with hapten. In fact, at a relatively high conjugation ratio, few BSA and a high number of α-santalol molecules are available at the well surface for antibody binding, with a lower steric hindrance. The carrier protein BSA could have provided multiple α-santalol binding sites on its surface, thus creating diverse numbers and types of hapten. To develop an antibody that is specific to α-santalol, the design of the corresponding immunogen was important. To convert carboxylic acid groups on the carrier protein to primary amine groups, BSA was treated with an excess of EDC. The cationized BSA (cBSA) prepared has a higher linking capacity owing to more primary amine groups being available on cBSA than on BSA. Moreover, the use of cationized carrier proteins minimizes cross-linking and increases their pi to generate more immune response compared with their native forms. Thus, using α-santalol with its free hydroxyl (-OH) group as a hapten was a convenient way of coupling while leaving the open ring fully exposed for antibody recognition. An increase in antibody titer over the immunization period was determined using an antibody-capture, non-competitive ELISA [20]. The increase reflected a greater concentration of antibodies and/or a higher binding affinity over the immunization period. The highest titer was achieved in rabbits after the fifth immunization, i.e. at six weeks (Figure 1D). The six-week-old antiserum collected from the rabbits were used for all subsequent analyses.

3.3 Optimization of antibody and α-santalol–BSA conjugate concentrations

Preliminary experiments were performed to assess the optimal coating antigen (α-santalol)/antibodies ratio. Alpha-santalol–BSA and anti-α-santalol polyclonal
antibody concentrations were optimized by comparing dose–response curves obtained using different combinations of antigen and antibodies dilutions, according to an optimized experimental design (data not shown). The optimal concentrations of antibodies and solid-phase conjugates were determined by checkerboard ELISA. Increasing the coating antigen (α-santalol–BSA) above 5 μg/mL did not significantly increase the overall absorbance. A serum dilution of 1:1000 and 5 μg/mL coating solution were therefore selected for subsequent experiments. After two immunizations, the rabbits had shown the presence of antibodies that recognized α-santalol–BSA in a non-competitive ELISA. The titers were determined to be approximately 1:5000 (signal > 3 × background) using 5 μg/mL α-santalol–BSA (Figure 1E). A satisfactory compromise between the lowest limit of detection, the highest sensitivity (slope of the curve) and the widest linear dynamic range was obtained by using a 500 ng/mL α-santalol–BSA solution in the immobilization step and a 1:5000 (v/v) dilution of the antibody (data not shown).

3.4 Cross-reactivity (CR)

Several sandalwood oil constituents were evaluated for CR in this study, as they are structurally related to α-santalol and can compete for antibody binding. Thus, a competitive α-santalol ELISA (Supplementary Table S1) was used to determine the degree to which these constituents will bind and prevent its binding to immobilized α-santalol/BSA. Since the antibody was generated against the hapten α-santalol, this immunoassay showed broad specificity for free α-santalol (100%) as well as bound α-santalol–BSA conjugate (100%). Due to different stereochemistry of β-santalol (Supplementary Figure S1A), another major constituent of sandalwood oil showed only 9.39% CR. As expected, little CR was observed for α-bisabolol, β-caryophyllene, etc.

![Figure 1](image-url)
and trans-farnesol, all of which are C$_{15}$ sesquiterpenoids but do not possess 'santalane'-type backbones or stereochemistry. Unlike chromatographic separation and detection methods that can provide non-specific detection of the separated analytes, this immunoassay can be used to monitor α-santalol in either naturally occurring, free or immobilized bound forms. As expected, the IC$_{50}$ values of α-santalol in free and conjugated forms were 0.17 and 1.34 μg/mL, respectively. However, the CR of unrelated isoprenoids (squalene and cholesterol) and phenolics (p-coumaric acid, tannic, gallic acids and quercetin) ranged from 1.35% to 9.39%. Nevertheless, β-santalol at 1.81 μg/mL partially competes for binding, but approximately ten times as much β-santalol as α-santalol is needed for equivalent competition.

3.5 Immunolocalization of α-santalol in sandalwood developmental stages by confocal laser scanning microscopy (CLSM)

Immunocytochemistry was employed to ascertain the tissue localization of α-santalols in various tissues of the sandalwood tree. The FITC-tagged secondary anti-rabbit IgG antibody was used for localization of the primary anti-α-santalol–BSA conjugate antibody present in the target sites. A florescent stain DAPI was used to demarcate the nucleus, lignified xylem elements, collenchymatous strands, phloem fibers, ray parenchyma and cuticular tissues. In the cross-section of in vitro-grown somatic embryos in the torpedo stages, α-santalol was localized exclusively to the nascent and developing xylem bundles and epidermis (Figure 2A). In the cross-section of leaves of the oil-yielding mature tree, α-santalol was localized to both spongy and palisade parenchymas interspersed with cells emitting strong plastidial signals (emission of the bright red color, characteristic of chlorophyll autofluorescence) and around the vasculature (Figure 2B). In the cross-section of in vitro-grown seedlings, α-santalol was localized to the stele and parenchymatous cortex regions, but not in the collenchymatous strands (Figure 2C). In the cross-section of a stem from the oil-yielding mature tree, α-santalol was localized to the

![Image](image-url)
extensive parenchymatous ground and cortical tissues, vast secondary xylem and ray parenchymatous regions. Remarkably, α-santalol was never localized in collenchymatous strands (from either stem or leaves), where only DAPI-stained lignified collenchymatous cell boundaries and their nuclei were visible. Intense labeling was also observed in some ray parenchyma cells while the strongest signals were observed in the larger xylem vessels and tracheid elements of the field-grown tree stems (Figure 2D). The labeling was observed as spots in the cytoplasm and thus, the exact cellular and organelar locations could not be ascertained with available magnification and resolution of the images. No staining was observed when the sections were treated with the pre-immune serum (data not shown).

4. Discussion

Immunoaassays have been helpful in the detection of a plethora of substances both qualitatively and quantitatively. Antibodies demonstrate specific and sensitive binding to their antigens and are useful across several immunoassay formats. One major objective of this investigation was to develop an improved and easier method of detection for α-santalol, compared with the conventional analytical detection methods that are currently in use, i.e. GC [13] and GC–MS [12]. Moreover, an immunological tool would facilitate the development of an ELISA-based detection method that can offer a better selectivity for α-santalol in sandalwood oil and plant parts. Thus, we decided to immunize rabbits as the potential source of IgG antibodies for use in α-santalol immunoassay. Furthermore, ELISA-based methods have been developed for the immunodetection of herbicides [21] and fungicides [22]. Thus, at the first instance we intended to raise polyclonal antibodies in rabbits by allowing the α-santalol to conjugate with a suitable carrier molecule through mediation by a crosslinker. Small molecules (>5000 Da) are not effective immunogens [23], and thus α-santalol (MW: 220 Da) had to be conjugated to a carrier protein. BSA, a high molecular weight polypeptide carrier protein (66 kDa) with a large number of functional groups, was used as a conjugate for immunization.

By choosing a proper site of conjugation, it is possible to elicit the formation of antibodies with enhanced specificity towards selected parts of target molecules [24]. Our approach involved conjugation either by using a cross-linker EDC or by directly using functional groups in BSA. Following similar strategies, polyclonal and monoclonal antibodies have been developed against several terpenoid-origin molecules, i.e. gossypol, 2-methylisoborneol and camphor [25], and a host of small molecules [26]. The spectral characteristics of the conjugate, studied by UV–visible spectrophotometry and Spectrofluorimetry, revealed proper conjugation of α-santalol with BSA. Analysis by SDS–PAGE could distinguish this difference between native BSA and BSA–conjugated to α-santalol molecules. Furthermore, the ELISA checkerboard results suggested that the α-santalol–BSA conjugate at 1 μg/mL concentration at 1:5000 dilutions produced a good titer (~0.5 at 405 nm).

Polyclonal antibodies are important tools for subcellular co-localization of both a small molecule, i.e. sinigrin (a glucosinolate), and its biosynthetic enzyme, i.e. myrosinase in seedlings of Brassica juncea [27]. Thus, the results obtained here are merely indicative of the location of biosynthesis and/or accumulation of α-santalol. In this study, the crude antiserum containing the polyclonal antibodies were used as primary antibodies to immunolocalize α-santalol in several parts of the sandalwood tree. In fact, applying immunolabeling to microscopic sections of plant tissues, several phytomolecules have been localized in situ and their biosynthesis examined. For example, using antibodies specific to secoisolariciresinol diglucoside (a lignan), the developmental increase in lignan content was followed [28]. Additionally, quantification of a ginsenoside Rb1 (G-Rb1) derivative from Panax ginseng roots was performed using a double-antibody method by coupling of BSA and β-D-galactosidase [29]. Furthermore, monoclonal antibody-based ELISA are routinely used for the determination of polycyclic aromatic hydrocarbons [30] such as benzo[α]pyrene, a harmful carcinogen in human body fluids [31].

We observed that α-santalol was mostly localized to the vasculature in embryogenic and matured plant parts, but never in the collenchymatous patches/strands. Furthermore, the sesquiterpenoid compound, α-santalol, was localized to epidermis, ground parenchyma, secondary xylem and leaf mesophyll cells. Similarly, in cotton, the bulk of the terpenoids was localized in the epidermis and scattered cortical parenchyma [32]. It is well established that heartwood extractives such as lignans are biosynthesized in ray parenchyma cells and are embedded in secondary xylem tissue before being released from these cells and then being infused into neighboring tracheid cells [33]. The epidermal and vascular localization of α-santalol in the somatic embryo and tree stems are probably indicative of their roles in defense – a claim that necessitates further investigations.

5. Conclusion

Since immunoassays may be easily performed on large numbers of samples, they are ideal for application to the screening of large-scale populations within plantation and breeding units. Moreover, the presence of numerous geometric isomers of sesquiterpenoids in
sandalwood oil makes only GC or GC–MS techniques amenable to their proper separation and identification. Thus, the polyclonal antibodies raised against α-santalol confer a greater selectivity and sensitivity to the detection of major sesquiterpenoids in sandalwood oil. With recent progress in the screening of biological activities of α-santalol such as antineoplastic activity [34], the polyclonal antibodies would aid in the monitoring of the differential biosynthesis and deposition patterns of α-santalol in heartwood [35] in vitro cells and tissues of sandalwood in a developmental manner [36]. Furthermore, we are now currently developing a ‘dip-stick’ format assay that would allow both quantitative and qualitative evaluation of sandalwood oil constituents in oil and associated products.

Conflict of interest
The authors have declared that there is no conflict of interest.

Acknowledgements
Thanks are due to the anonymous reviewers for their constructive and critical comments. We also thank Dr. Tapas Kumar Maiti, Professor, Department of Biotechnology, IIT Kharagpur, for his helpful insights during the entire course of this study. BBM received the Junior and Senior Research Fellowships of the Council of Scientific & Industrial Research (CSIR), New Delhi, and Research Associateship conferred by the Department of Biotechnology (DBT), Government of India. The experimental work in *S. album* in the author’s laboratory was supported under the project: Prospecting of novel genes and molecules of *Santalum album* L. (NGM), sponsored by DBT, Government of India. The authors are co-inventors of pending patents filed by IIT Kharagpur that relates to the detection of α-santalol. Supplemental data for this article can be accessed

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