5.1. Introduction

The *Santalum album* Linn. (Santalaceae), commonly known as sandalwood, is a mid-sized evergreen hemi-root parasitic plant widely distributed in South Asia and Australia. The Indian sandalwood tree is known worldwide for its pleasant woody fragrance. The essential oil of sandalwood is usually obtained by steam distillation of chips and billets cut from the heartwood and are widely used in treatment of several human health diseases. The first committed step in Santalol biosynthesis is cyclization of farnesyl diphosphate (FPP) by Santalene synthase to yield α- and β-Santalenes, which will be converted to Santalols by the cytochrome P450 system. The sesquiterpene alcohols (Z)-α-Santalol 1 and (Z)-β-Santalol 2 together constitute over 80% of heartwood oil obtained from the well matured tree while heartwood oil of 14 year old Indian sandalwood tree contains 44.7–46.7% (Z)-α-Santalol and 20.8–22.2% (Z)- β-Santalol, which is in the range of current international standards for the Indian Sandalwood oil. Both (Z)-α-Santalol 1 and (Z)-β-Santalol 2 are responsible for most of the biological activities of the sandalwood oil and have attracted increasing attention for their neuroleptic properties and chemo-preventive effects in *in vitro* and *in vivo* bioassay systems.

Influenza virus is documented to cause epidemics and pandemics in human population over several centuries. Influenza A virus has several zoonite hosts, therefore cannot be eradicated from human populations. Despite widespread access to vaccines and antiviral therapies, influenza continues to be a major cause of morbidity and mortality. About 31,000 deaths each year in the US are associated with influenza infections. Influenza A viruses are respiratory pathogens that can raise to severe illness in humans. In the 20th century, three influenza pandemics occurred: the H1N1 Spanish Flu in 1918, the H2N2 Asian Flu in 1957 and the H3N2 Hong Kong Flu in 1968 (Kilbourne 2006). In developing countries the availability of modern
medicines is limited in supply. Therefore the traditional medicine is still the mainstay of health care and most drugs come from plants. Thus the search on new drugs is still continued and natural products from plants are kept on emerging in recent past years. Few substances are known to be an effective against of viral infections \textit{in vivo} (Balfour 1999).

Currently, two classes of anti-influenza agents have been reported for influenza management and are under consideration for stockpiling in the event of an influenza pandemic; one of which targets the M2 ion channels (e.g., amantadine and rimantadine) and the another one inhibits neuraminidase (e.g., oseltamivir and zanamivir). In fact, influenza A viruses still pose a major burden to human health and cannot be completely eradicated due to their large natural reservoir. Treatment with amantadine and their derivatives rapidly results in the emergence of resistant variants and hence is not recommended for the general and uncontrolled use (Hayden and Hay 1992). Oseltamivir is considered the drug of choice for patients with pandemic influenza for whom drug treatment is recommended because adamantanes seem to be ineffective against pandemic A/H1N1 influenza virus and zanamivir (the other available neuraminidase inhibitor) is contraindicated in people with underlying respiratory conditions and difficult to administer in younger children (Garman and Laver 2004). Emergence of resistance to oseltamivir in human influenza A viruses (Ison \textit{et al.}, 2006) and the H5N1 subtype in Vietnam is a cause for concern. However, resistance has not been reported for the other neuraminidase inhibitor, zanamivir. Nevertheless, expanding the range of antiviral drugs that effectively inhibit replications of the influenza A virus, or potentially act in synergy with neuraminidase inhibitors is a matter of urgency.

Medicinal plants have been used in treatment of various diseases. It is mainly used for ethno botanical and ethno pharmacological experiences of certain nation are used in the
treatment of wide range of diseases including treatments for the difficult to cure diseases such as cancer, AIDS, Alzheimer’s disease etc. Herbal medicines were represented as the most important field of traditional medicine all over the world. Hence, it is very essential to study the medicinal plants in order to promote their proper use and also to determine their potential as the primary source for the preparation of new drugs (Gajalakshmi et al., 2012). Today according to the World Health Organization reports, as many as 80% of the world's people depend on traditional medicine for their primary health care needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal spices for the treatment of various diseases (Amiri et al., 2012).

*Santalum Album* L (Sandal wood) is commonly known as Chandan, Srigandha. Naturally grown sandal wood can find in Karnataka, Tamilnadu, Andra, Kerala, Gujarat, Madhya Pradesh, and U.P. Manipura and in some other states. Chandan is known all over the world as East Indian sandal wood and is prized for its quality of oil and heart wood. Chandana is also considered as medicinal plant and is used on large scale in the manufacture of Ayurvadic Medicines. National Medicinal Plant Board, govt.of India had recognized as a Medicinal and Aromatic Plant.

Sandalwood, the dried wood of *Santalum album* L. has a long history of use in Oriental medicine as a sedative and is also used extensively in the Orient as incense. Chemical studies on sandalwood oil have resulted in the isolation and characterization of a large number of sesquiterpenes (Christenson, et al., 1981). Biological investigations of the oil have shown that it has an antibacterial effect (Okazaki, et al., 1953; Winter, 1958), diuretic action and an irritant action on the rabbit ear (Okanishi, 1928).

The essential oil emulsion or paste of sandalwood is routinely used in India as an ayurvedic medicine to inflammatory and eruptive skin diseases. Sandalwood oil treatment
significantly decreased papilloma incidence by 67% multiplicity by 96% and TPA-induced ODC activity by 70%. This oil could also be an effective chemo preventive agent against skin cancer (Dwivedi et al., 1997). The bioactive compounds (Z)-beta-Santalol, (Z)-lanceol were isolated from Santalum album have strong anti-\textit{H. pylori} activities against a clarithromycin-resistant strain (Ochi et al., 2005). Sandal wood oil consist of mainly $\alpha$ and $\beta$-Santalol. $\beta$-Santalol is an organic chemical compound and is a principal constituent of oil of sandalwood 20%, and also a possible viral inhibitor. Santalol, an active component of sandalwood oil, has been studied for its skin cancer preventive efficacy in murine models of skin carcinogenesis; employing human epidermoid carcinoma A-431 cells (Scartezzini et al., 2000). The potential effect of $\beta$-Santalol on the replication of influenza virus has not yet been systemically studied and limited basic information is available on this context. However no reports have been found demonstrating that the $\beta$-Santalol has anti-influenza activity, which prompted the present investigation.

5.2. Materials and methods

5.2.1. Plant material

High-quality sandalwood was purchased from a local market in India which the essential oil was collected by distillation and subsequent chromatography. It is a valuable and expensive material because it can only be obtained from mature sandalwood trees. The highest quality of Sandalwood trees for incense and perfume are grown in India (especially East India). Many investigations on the composition of sandalwood essential oils have been carried out, and more than 300 constituents have been identified. The main constituents are $\alpha$-Santalol and $\beta$-Santalol. These compounds have distinctive woody odors. Many studies have been done on sandalwood, and the structure-odor relationships of $\beta$-Santalol and its related compounds have been investigated in detail.
5.2.2. Viruses, cells and reagents

Influenza A/HK (H3N2) virus obtained from King Institute of Preventive Medicine & Research, Virology department, Chennai. Virus was propagated in Madin-Darby canine kidney (MDCK) cells at 37°C. MDCK cells were maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 0.01% antibiotic-antimycotic solution. Antibiotic-antimycotic solution, trypsin–EDTA, FBS and MEM were supplied by Himedia. All other chemicals were of reagent grade. Oseltamivir (Sigma, Aldrich) Stock solutions (10 mg/ml) of the antiviral compounds were made in dimethyl sulfoxide (DMSO) and were subsequently diluted in appropriate culture media. The final DMSO concentration was a maximum of 0.1%, which had no effect on the cell cultures. Therefore, 0.1% DMSO was also added to all no-drug control samples.

5.2.3. Hexane Extraction of Sandalwood Chips

In general, compounds with a formyl group (aldehyde and formate) are important odor constituents. These compounds, especially aldehydes, are common decomposition products of the corresponding carboxylic acids. Aldehydes, because they are prone to decomposition, are difficult to collect from an extract by chromatography or distillation. Chips of sandalwood (30-100 g) were extracted with hexane (300-1000 ml) at room temperature. Removal of the solvent by rotary evaporation at room temperature afforded reddish brown oil in 7.1% yield. The beta Santalol from hexane extract of sandalwood was isolated from fractions separated by distillation and subsequent chromatography.

5.2.4. High Pressure Liquid chromatography (HPLC) analysis

HPLC separates mixture of compounds on the basis of polarity. Polarity refers to the greater difference in electron affinity i.e. electro negativity between atoms in a covalent bond,
the more polar the bond. It was used to analyze, identify, purify and quantify compounds. It has a mobile phase, a stationary phase and detector. The mobile phase was continuously pumped at a fixed flow rate through the system and mixed by the pump. The injector was used to introduce a plug of a sample into the mobile phase without having to stop the mobile phase flow & without introducing air into the system. The mixture of components was carried in a narrow band to the top of the column. Some compounds in the sample mixture will have greater preference for stationary phase than the mobile phase and will be retained in the column longer.

All solvents used were of analytical chromatographic grade (Carlo Erba reagents). HPLC was performed with a HP Ti series 1050 liquid chromatograph, equipped with a photodiode array detector (DAD, HPseries 1050). Solutions of the tested oil (5% v/v in n-hexane or ethanol) was subjected to normal phase HPLC analysis carried out on a Phenomenex Hypersil 5 CN (5 µm, 25×4.6 mm) column using a mobile phase of n-hexane at a flow rate of 1.0 ml/min. The injector was a Rheodyne model valve with a 20 µl loop. UV detection (DAD) at two wavelengths (245 and 265 nm) was recorded. Eluate fractions obtained from HPLC analyses (n-hexane) were further subjected to GC-MS analysis after concentration under vacuum.

5.2.5. Gas Chromatography–Mass Spectrometry (GC–MS) analysis.

GC-MS is a technique performs sample identification and quantification according to mass and charge (m/z) and works on the principle that a mixture will separate into individual substances when heated. Gas Chromatography is a technique used to separate drugs that might be present in a sample. The sample was injected into a long tubular column, the chromatography column. The drugs are swept through the column by a stream of helium gas. Drugs in a sample are separated from each other because some take longer to pass through the column than others. The process was like a race around a track: at the beginning the racers are all together in a group
and at the end they are all separated with the fast ones finishing far in advance of the slow ones. A drug’s individual chemical characteristic determines how long it will take to go through the chromatography column. The time it takes for any given drug to travel the length of the column is referred to as its retention time (RT). The RT for a given drug is an identifying characteristic. As a drug exits the end of the GC column it was fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern.

The chemical composition of the essential oil was analyzed using GC–MS. The essential oil (10 μl) was dissolved in acetone (100 μl) and 1 μl of the solution was injected into a GC–MS (QP-2010, Shimadzu Co., Kyoto, Japan). The capillary column was Rtx-5MS (length=30 m, i.d=0.25 mm, thickness=0.25 μm). Helium was used as the carrier gas at a flow rate of 0.94 ml/min. The column inlet pressure was 55.8 kPa. The GC column oven temperature was increased from 60 to 170°C at a rate of 10°C/min, with a final hold time of 10 min. Injector and detector temperatures were maintained at 150°C. EI mode was at 70 eV, while mass spectra were recorded in the 45–450 amu range and ion source-temperature was 200°C. Essential oil components were quantified by relative percent peak area of TIC from the MS signal and identified by comparing their mass fragmentation pattern.

5.2.6. Assays of antiviral activity and cytotoxicity.

Assays of antiviral activity and cytotoxicity were evaluated by the SRB method using cytopathic effect (CPE) reduction recently reported (Choi et al. 2009). Oseltamivir was used as positive, and DMSO was used as negative control. The effect of β-Santalol on influenza virus-induced CPE was observed. Briefly, MDCK cells were seeded onto a 96-well culture plate at a concentration of 2×10^4 cells per well. Next day, medium was removed and washed with PBS. Then, 0.09 ml of diluted virus suspension and 0.01 ml of medium supplemented with trypsin–
EDTA containing β-Santalol of 100 μg/ml were added. After incubation at 37°C in 5% CO₂ for 2 days, the morphology of cells was observed under microscope of 32×10 magnifications (Lobamed, Germany), and images were recorded.

5.2.7. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis

RT-PCR combines cDNA synthesis from RNA templates with PCR to provide a rapid, sensitive method for analyzing gene expression. RT-PCR was used to detect or quantify the expression of mRNA, often from a small concentration of target RNA. MDCK cells were seeded onto a 96-well culture plate at a concentration of 2×10⁴ cells per well. After 24 hr, medium was removed and the cells were washed with PBS. Subsequently, 0.09 ml of diluted virus suspension and 0.01 ml of medium supplemented with trypsin–EDTA containing β-Santalol or oseltamivir of 100 μg/ml were added. After incubation at 37°C in 5%CO₂ for 48 hr, the next step was performed. Total RNA was extracted from cells as described elsewhere (Chomczynski and Sacchi 1987). The parallel expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was tested under the same PCR conditions as an internal standard. Randomly primed cDNA obtained by reverse transcription (RT)- PCR was amplified (Rochi, Germany) in a PCR mixture (50 μl) that contained membrane (M) or GAPDH gene (Housekeeping gene) primers: M gene, 5’-AGTGAGCGAGGACTGCAGCGT-3’ and 5’-TAGCYTTAGCYGTRGTGCTGGC-3’; GAPDH, 5’-CCCA-TCACCATCTTCCAGGAGC-3’ and 5’-CCAGTGAGCTTCCCTTCAGC-3’. The products were electrophoresed and visualized by ethidium bromide staining

5.3. Results

5.3.1. Essential oils analysis

An important task in natural products chemistry is the identification of new compounds with novel properties and structures. Moreover, in fragrance chemistry, the odorant constituents
of sandal wood were fractionated, evaluated and key compounds were identified in the present investigation that contribute much more towards the scent profiles of fragrance materials. In addition to the scent profiles, we traced the occurrence of β-Santalol a biomedically valuable compound and used to target the influenza virus in the present study.

The gas chromatogram showed nearly thirty five fractions with different retention time ranging from 0 to 35 minutes. Interestingly the fractions 4, 8, 10, 17, 19, 24, 25, 30 and 33 were observed to be more prominent peaks when compared to the rest of them. Thus the GC report clearly witnessed that these compounds were found to have more than 40% and upto 100% of relative abundance. The GC-MS analyses of the extracted sandal wood oil sample contained higher concentration of β-Santalol compound and was identified using their mass spectra and retention indices (RI) by comparing with the standard. In all of the triplicate samples, β-Santalol was found to be present as the main component that corresponded to about 80% of the oil content with minor fractions of other constituents of oil (Fig.5.1a). The minor fractions were reported as oil was found to contain also sesquiterpene hydrocarbons (ca. 20%, mainly α-copaene, germacrene D and α - caryophyllene), alcohols (ca. 12%, mainly linalool and α -terpineol) and monoterpenic hydrocarbons (ca. 7.5%, mainly myrcene, limonene and the two ocimene isomers), in addition to minor fractions of oxides, and or unidentified structures.

The Mass per charge (m/z) ratio was plotted against the relative abundance of the daughter ions produce from the sandal wood oil subjected to GC and showed the mass peaks which are very close to the standard beta Santalol compound and explored more than 90% similarity while comparing with the standard Mass spectra available in the chem.-library software of the instrument. The spectral data showed the peaks at 326, 360, 407, 643, 748 and 814 were significantly correspond to the mass values of beta Santalol (Fig.5.1b). Hence the GC-MS
analysis clearly authenticate that the compound extracted and purified in the present study is beta Santalol.

The HPLC analyses under normal phase conditions (CN-column and n-hexane as mobile phase) were allowed the oil components to separate into different groups of chemical classes of increasing polarity (mainly hydrocarbons, ethers, esters and alcohols). The composition of the HPLC-fractions was subsequently confirmed again by GC-MS analysis. Referring to the HPLC chromatogram (Fig. 5.1c), the highest intense was found by the GC-MS runs to include almost exclusively sesquiterpene (β-Santalol) and its derivatives of the oil.

5.3.2. Antiviral activity of β-Santalol against influenza A/HK (H3N2) virus

β-Santalol was investigated for its anti viral activity against influenza A/HK (H3N2) virus and MCDK cell viability. The antiviral assays demonstrated that β-Santalol possessed strong antiviral activity of about 86% against influenza A/HK (H3N2) virus at the concentration of 100 μg/ml and anti viral activity of about 40% at the same virus at the concentration 10 μg/ml (Fig. 5.2a). Oseltamivir also did show moderate anti viral activity of about 83% against influenza A/HK (H3N2) virus at the concentration of 100 μg/ml and weak anti viral activity of less than 37% at under of 10 μg/ml (Fig. 5.2a). β-Santalol and oseltamivir were not toxic to MDCK cells with cell viability of about 93% at the concentration of 100 μg/ml (Fig. 5.2b).

5.3.3. The effect of β-Santalol on influenza A/HK (H3N2) virus-induced CPE

After 2 day infections of MDCK cells with influenza A/HK (H3N2) virus, there was no difference between the mock cells (Fig. 5.3a) or cells treated with 100 μg/ml β-Santalol (Fig. 5.3c) or oseltamivir (Fig. 5.3e) in terms of typical spread-out shapes and normal morphology. The cell proliferation was not significantly affected under the 100 μg/ml concentrations of β-Santalol. Infection with influenza A/HK (H3N2) virus in the absence of β-Santalol resulted in a
severe CPE (Fig. 5.3b). The addition of β-Santalol on infected MDCK cells inhibited the formation of a visible CPE (Fig. 5.3d). However, the addition of oseltamivir in influenza A/HK (H3N2) virus infected MDCK cell was weakly prevented CPE (Fig. 5.3f). Thus, this result showed that the CPE of the virus infection was prevented by above mentioned concentrations of β-Santalol.

5.3.4. Effect of β-Santalol on synthesis of viral mRNAs

The viral mRNA synthesis assessed by PCR analysis of the M gene were inhibited completely at β-Santalol 100 μg/ml after 48 hr infection, while oseltamivir (100 μg/ml) exhibited a small amount of the viral mRNA synthesis product band at 48h after infection (Fig. 5.4). The amplification of housekeeping gene GAPDH from the same sample was positive in the PCR analyses (Fig. 5.4). These findings indicate that β-Santalol does decrease viral mRNA synthesis. The development of new antiviral agents for influenza is receiving much greater attention because of the frequent emergence of antiviral resistance during oseltamivir, its association with clinical failure in immuno-compromised hosts, and the emergence of new pandemic subtypes of influenza A virus (Beigel and Bray 2008; Hayden 2001; Ison et al. 2006). The present study describes the cytotoxicity and antiviral activity of β-Santalol. β-Santalol was shown to exhibit the anti-influenza virus activity against influenza A/HK (H3N2) virus reducing the formation of a visible CPE. These results are similar to the effects of quercetin 3-rhamnoside on influenza virus (Choi et al. 2009a, b). In conclusion β-Santalol is a mixture of small molecules that can efficiently inhibit influenza A/HK (H3N2) virus replication. Therefore, β-Santalol is an alternative to agents for treating influenza virus infections. Their potent anti-influenza virus activity in vitro warrants further studies to evaluate whether β-Santalol treatments can also result in anti viral activity in vivo.
5.4. Discussion

The reality of the long predicted global human influenza pandemic, the first in 41 years, has arrived. Outbreaks of a new form of influenza A (H1N1) causing illness in humans, commonly known as ‘swine flu’, occurred in Mexico and the United States in March and April 2009. The emergence of this virus, with a very high rate of human to human transmission, and its rapid spread around the world, led the World Health Organisation to declare a global pandemic (an epidemic that spreads over a wide geographical area, affecting a large proportion of the population), on June 11th. This new virus was originally called swine flu because laboratory testing showed that many of its genes were very similar to influenza viruses that normally occur in pigs in North America. Actual ‘swine flu’ is a highly contagious acute respiratory disease of pigs, caused by one of several swine influenza viruses. The H1N1 subtype of influenza A virus is the most common, but other subtypes also circulate in pigs (eg. H1N2, H3N1, H3N2). Hence the present study propose a suitable remedy to fight against the influenza infection. In addition to that the search of phytochemicals to target virus is more reliable and effective targeting steps in modern medical sciences. It is therefore essential that phytotherapists and preferably other clinicians have some knowledge of this subject, and appraise the evidence of possible usefulness of various herbal medicines, in order to help their patients and populations resist the dangers of the current pandemic, as best they can.

The tree *Santalum Album* found in southern India and Indonesia is called the “Royal Tree” in India (Fox, 2000). Emulsion, paste, or essential oil of sandalwood has been used for centuries in India for treatment of inflammatory and eruptive skin diseases (Banerjee et al., 1993). Ayurvedic physicians (traditional medical practitioners in India) treat numerous skin lesions in patients with sandalwood oil (Boutwell, 1984). The essential oil of sandal wood was
distilled from the small chips and billets cut out of the heartwood of SW. The oil is extremely viscid, of a light yellow color, and possesses a characteristic pleasant odor. The major constituent of oil is Santalol, a mixture of two isomers, α- and β-Santalol. Banerjee et al., 1993 reported that feeding of Santalwood oil caused an increase in glutathione S-transferase activity and acid soluble sulphhydril levels and suggested possible chemopreventive effects. Sandalwood oil inhibits the replication of Herpes simplex viruses-1 and 2 in vitro (Benencia and Courreges 1999).

The results from this investigation indicated that β-Santalol (a major component of sandalwood oil) inhibited the influenza viral replication in cultured MDCK cells. It showed a remarkable inhibition of viral replication (Fig 5.4). Several sandalwood extracts were examined for effects on hexobarbital -induced sleeping time, hypothermic activity, analgesic action, and spontaneous motility in mice. The active principles were isolated from sandalwood and identified as α and β-Santalols on the basis of comparison of physical and optical data. They have been reported as the major constituents of sandalwood oil, which is highly prized as a perfume and to have cosmetic as well as medicinal value (Christenson, et al., 1979).

On intraperitoneal administration to mice, α-Santalol showed more effects on hypothermia and reduction in spontaneous locomotor activity than β-Santalol, while β-Santalol had more analgesic activity than α-Santalol. Both compounds produced the same effects on hexobarbital-induced sleeping time by i.p. administration. α- and β-Santalols reduced both methamphetamine and apomorphine-induced activities However, hypnosis, muscle relaxation, reversal of reserpine-induced hypothermia and anticonvulsive effect were not produced by α- and β-Santalols in mice. These previous reports support that our study, β-Santalol have more attention to pharmacological activity.
Previous reports (Chaumont et al., 1989) stated that the Triterpenoid and palmitate has been isolated from sandalwood shows moderate biomedical applications. The tropical application of triterpenoid on fresh pupae of forest insects viz: Atteva fabriciella produced morphologically defective adults indicating growth inhibition activity of the terpenoid isolated from sandalwood. A detailed study was carried on seven essential oils and their constituents for their antifungal activities against eight strains known to be human pathogens. Sandalwood oil was found to be effective against Microsporum canis, Trichophyton mentagrophytes but ineffective against Candida albicans, Aspergillus niger (Chourasia et al., 1987).

With reference to antibacterial activities of sandalwood oil reveal that the efficacy of some Indian essential oils including Sandalwood oil against Bacillus anthracis (+), Bacillus mycoides(+), Bacillus pumilis(+), E.coli(-), Micrococcus glutamicus(+), Sarcina lutea (+), Salmonella paratyphi(-), Staphylococcus albus (+), Xanthomonas campestris (-) and Xanthomonas malvacearum (-) at different concentrations. Not only oil but also, the aqueous extract of air dried powdered bark in concentration of 25 to 100 µg/ml in phosphate buffer showed good inhibition against virulent species, Staphylococcus aureus. In our study strongly stated that the sandalol from sandal wood oil could efficiently cytopathic effect of influenza virus (fig 5.3).

To date, suspected, probable and confirmed cases have generally been treated with the antiviral drugs oseltamivir (Tamiflu®) and zanamivir (Relenza®). These drugs inhibit neuraminidase, an essential viral glycoprotein for virus replication and release, and thus block the release of the influenza virus from infected cells and inhibit its transmission to neighbouring cells. Tamiflu®, which is given orally, and Relenza®, which is administered by oral inhalation, is currently the only available drugs likely to help contain influenza A virus and reduce illness and
death (Baz et al., 2009; Le et al., 2009). Our findings are in agreement with the previous report to target the influenza virus at early and late phases of viral replication. However, these drugs need to reach the site(s) of any pandemic outbreak quickly and must be taken early following infection in order to be effective. While wealthier countries have reasonable stockpiles of Tamiflu® (whose expiry date was recently extended by 2 years in New Zealand) and Relenza®, the situation for most of the world’s population is considerably less reassuring. Global stocks of these drugs are currently estimated to be sufficient to treat only about 5% of the world’s population, and less if used prophylactically (Bright et al., 2005). While manufacturing capacity can expand following the onset of a pandemic to an output of sufficient drug to treat 400 million people, this is still only a fraction of possible global demand (Sato et al., 2005). A cheaper generic version of Tamiflu® (oseltamivir) has been developed by an Indian manufacturer, but patent protection issues have to date prevented its production.

Additionally, it is intrinsic to the nature of infectious microorganisms, that they have an incredible capacity to develop resistance to commonly used antiviral and antibacterial drugs, and the likelihood of this is increased, the more they are exposed to the drug(s) concerned. With use of Tamiflu® for seasonal influenza being common in Japan for many years, and on the increase in most other developed countries since 2006, it is no surprise that cases of resistance to it are increasing (Sacca et al., 2009). Thus while both oseltamivir and zanamivir seem to have some efficacy against the current form of H1N1 swine flu, it is unknown whether this will extend to future variants. Any gene swapping between this strain and strains of seasonal H1N1 viruses that are already resistant to oseltamivir will be worrying indeed. Like H5N1 avian influenza, the recent H1N1 swine influenza virus isolated from humans is resistant to the older antiviral drugs amantadine and rimantadine. Interestingly all the commercially available antibiotics as noticed
above are found to have less effective to in minimization of viral infection and comparatively more toxic to non targeted mammalian host cells. Therefore the present investigation addresses an innovative approach to target the influenza at *in vitro* level using the isolated beta Santalol.

The long history of use and folklore popularity of Garlic (*Allium sativum*) as a useful agent in the treatment of various infectious diseases including viruses, and general accessibility of most populations to this herb, also warrant further investigations. Another possible mode of preventing infection, involves inhibition of adsorption of virus particles at the viral entry route to the target cell surface in the bronchial epithelium. Many plant polyphenolic compounds have exhibited *in vitro* antiviral activities, probably due to non-specific binding to and agglutination of viral proteins such as haemagglutinin or neuraminidase. If administered in a timely manner to the site of viral infection in the respiratory tract, a localised antiviral activity could be useful.

Examples of such compounds include tannic acid, epigallocatechin gallate, theaflavin digallate and green tea catechins, all of which can inhibit influenza virus replication *in vitro*. Antiviral properties reported for tannin-rich plants, *Castanea* (Chestnut) and *Schinopsis* (Quebracho) species, *Potentilla tormentilla* (Tormentil) and *Geranium sanguineum*, also probably relate to a similar mechanism (Bodinet *et al.*, 1999; Zakay-Rones *et al.*, 1995). However, no reports are available to emphasise the significance role of sandal wood oil in curtailing the replication and multiplication of influenza viruses. Hence the data presented in this chapter reveals the antiviral effect of beta Santalol at molecular level.

A polyphenol-rich extract of the Mediterranean plant *Cistus incanus* (Pink Rockrose) has been shown to exert potent anti-influenza virus activity against highly pathogenic forms of H7N7 and H1N1 influenza A viruses both *in vitro* as well as in a mouse infection model. Several plant based medicines have been developed to block the influenza viral replication as described here.
under. A combination preparation of Andrographis and *Eleutherococcus senticosus*, has shown efficacy in both adults and children with early noncomplicated upper respiratory viral infection (Petereit *et al.*, 1991; Danne *et al.*, 1993). Quicker recovery and a lower risk of post-influenza complications, has also been reported in a Russian trial comparing this product to the antiviral drug amantadine. In addition to that the tuber of this native South African plant, has shown positive results in clinical trials involving patients with acute bronchitis (Simpson *et al.*, 2010), as well as reducing the severity of symptoms, and shortening the duration of the common cold (Kusche *et al.*, 1993).

Furthermore, it has been reported that the replication of Herpes simplex viruses is inhibited in the presence of the sandalwood oil. It may due to the presence of active compounds like α, β-sandalol. Although it effect was a dose-dependent effect and more pronounced against HSV-1. A slight diminution of the effect was observed at higher multiplicity of infections. The oil was not virucidal and showed no cytotoxicity to the cultured Vero cells. Simultaneously β-sandalol showed significant antiviral activity and no cytotoxicity to MDCK cells (Fig. 5.2). The present chapter findings concluded that the β-sandalol could efficiently inhibit the influenza viral replication in cultured MDCK cells. Thus, it is tempting to speculate that the anti viral activity of β-sandalol against influenza virus could be an indirect effect of its genomic RNA replication preventive mechanisms well as its necessary antigenic protein expression.

The present study clearly demonstrated the method for separation of β-santalols using preparative column chromatography with silver nitrate impregnated silica gel as the stationary phase and hexane and dichloromethane as mobile phases, respectively. The method developed enabled the quantitative separation of β-santalol, which are responsible for most of the biological activities observed with sandalwood oil. Limits of quantification (LoQ) relative to the FID
detector were measured for 14 important chemical constituents of heartwood oil of *S. album* using serial dilutions of the stock standard solutions and also demonstrated that the quality of commercial sandalwood oil can be assessed for the content of individual sesquiterpene alcohols regulated by International Organization for Standardization ISO 3518:2002 (E). Thus the present findings would seem a promising step to help prevent the onset of influenza infection at *in vitro* level.