



**ASSESSMENT OF HISTOCHEMICAL, PHYTOCHEMICAL AND
ANTIMICROBIAL ACTIVITY OF *PTEROCARPUS SANTALINUS* L
WOOD EXTRACTS**

P. Jyothi Chaitanya^{*}, R. Chandrashekar, N. Lakshmi Bhavani

Plant Tissue Culture and Plant Molecular Genetics Lab Department of Botany, University
College of science, Saifabad Osmania University, Hyderabad- 500004, India.

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***Correspondence for
Author**

P. Jyothi Chaitanya

Plant tissue culture and plant
molecular genetics lab
Department of Botany,
University College of
science, Saifabad Osmania
University, Hyderabad-
500004, India

ABSTRACT

Present study focused on the preliminary qualitative histochemical, phytochemical analysis and antimicrobial activity of wood extracts of *Pterocarpus santalinus* L. It is aimed to find out the Histochemical tests for wood authentication, phytochemical and antimicrobial analysis. Specific tests were conducted to identify each group of the phytochemicals of various extracts of wood of *Pterocarpus santalinus* L. Wood were extracted separately with methanol, ethanol, hot water, petroleum ether and chloroform that were screened for phytochemical

constituents. Among all the extracts tested ethanol and methanol extracts showed more phytochemicals than the others followed by petroleum ether, water and chloroform. Analysis revealed the presence of alkaloids, phenols, glycosides, flavonoids, triterpenoids, anthraquinones, steroids and tannins. Ethanol, methanol, petroleum ether, hot water and chloroform wood extracts were tested against selected bacteria and fungi. Ethanol, methanol and petroleum ether showed more inhibition zones against bacteria and fungi than the extracts of chloroform and hot water extracts. The analysis revealed the maximum activity of wood extracts against the *Salmonella typhi*, *E.coli*, *Streptococci*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. *Klebsiella pneumonia* is found to be inhibited by methanol and ethanol extracts only. Extracts of methanol showed broad spectrum inhibition zone against the fungal species *Aspergillus Niger*, *Fusarium*, *Colletotrichum* and *Rhizopus* than the ethanol, pet ether, chloroform and hot water. The broad spectrum inhibition zone exhibited by *Pterocarpus santalinus* L may be due to the various active constituents

present in it, which either attributed to their individual or combined action exhibit antibacterial activity and antifungal activity. Result indicated that the phytochemical extracts of *Pterocarpus santalinus* L wood exhibited dosage dependent antibacterial and antifungal activity.

KEYWORDS: Phytochemicals, *Pterocarpus santalinus* L, wood, antimicrobial activity.

INTRODUCTION

Pterocarpus santalinus L. (family Fabaceae), popularly known as red sanders, red sandal wood. It is a great economical and ecological important rare, threatened forest legume tree. This species occurs exclusively in a well defined forest tract of Andhra Pradesh in Southern India (Raju and Nagaraju 1999). Because of its more economical value it is growing everywhere in India. Trees are broadly classified as quality and non quality varieties based on texture and color of the wood. The quality trees are characterized by wavy grain wood texture with intense red color. In contrast, the non quality trees possess straight grain wood texture with light red color. Earlier reports indicated that the red sanders wood contains two red organic compounds called santalin having a quinonoid structure and desoxy santalin a supposed derivative of naphthaquinonoid (Ravidranath and Seshadri 1972). The quality wood is considered among the finest luxury woods in the world and highly useful for making high value musical instruments, cabinets, ornamental veneers, toys, and dolls. It is priced in the vicinity of US\$2000 per ton in the international market and is most notably exported from India to Japan and other countries (Raju and Nagaraju, 1999). The red color of the wood is used as a natural dye in pharmaceutical, paper, pulp, textile, tannery and food industries. The wood is also considered in indigenous medicine as an astringent, tonic, and diaphoretic and is useful to cure bilious infections and skin diseases. The bark of the tree also yields a kino similar to *Pterocarpus marsupium* (Anonymous 1969). Indiscriminate and illegal logging, low natural regeneration potential, narrow habitat specificity and microclimatic changes in the habitat resulted in the severe depletion of natural populations of *P. santalinus* L (Madhava Chetty and Rao 1990). Chemical constituents reported from bark, wood and leaves of this plant are alkaloids, phenols, glycosides, triterpenoids, tannins, steroids, santalin, stillbenes, pterocarpol, pterocardiolone. (Kodithuwakku *et al* 2011). The present work evaluates the phytochemical constituents of wood of *Pterocarpus santalinus* L. Traditionally parts of the plant are used to cure the skin disorders, fever, boils, improve sight, headache, gastric ulcers and scorpion sting (Chopra *et al.*, 1956). Literature study showed that the wood, leaves and

bark of this plant has more biological activity like antibacterial, antifungal, antidermatophytic, antidiabetic, antiinflammatory, antioxidant, antitumor, analgesic, antiseptic, and antihelmenthic (Manjunatha BK 2006). Antioxidant property was evaluated and reported by (Arokyarajet *al* 2008).

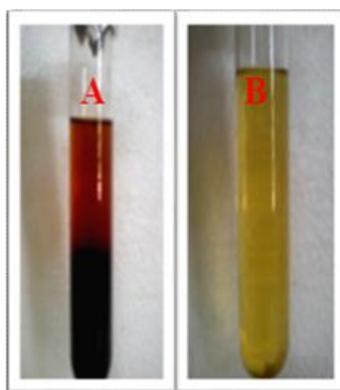
| S.No | Solvent | Colour | compound |
|------|---------|--------|------------|
| A | Ethanol | Red | Santalin A |
| B | Ether | Yellow | Santalin B |

MATERIAL AND METHODS

Plant Materials: The healthy and diseasedfree wood of *Pterocarpus santalinus* L plant material was collected from the four district region of Telangana, Andhra Pradesh, India in the month of February, 2012. The collected plant material was washed thoroughly in tap water, shade dried in open air separately. Powder of the wood is obtained by grinding them mechanically.

Histochemical tests methodology

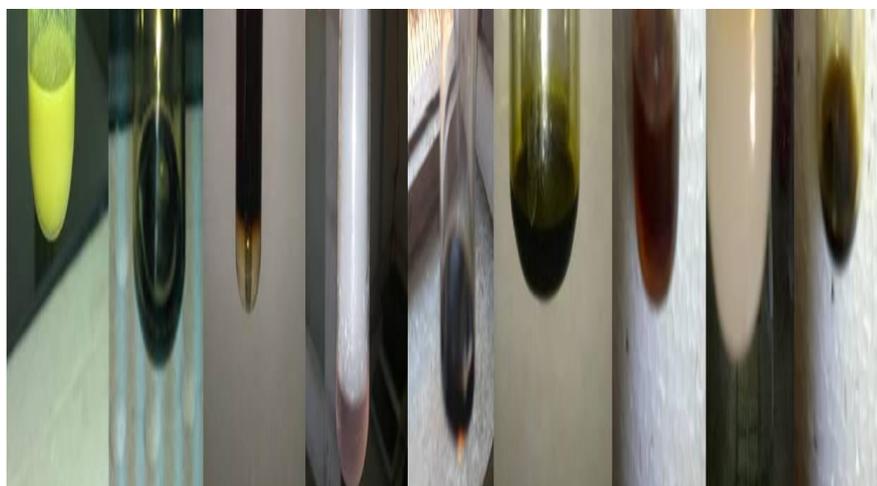
Wood of *Pterocarpus santalinus* contains santalin and desoxy santalin. When the wood is boiled at 226⁰C it gives blood red colour small crystals. These crystals are not solubulise in water, but soluble in alcohol. When wood powder is dissolved separately in alcohol gives red colour, in ether yellow colour and in caustic soda(NaoH) purple colour. (Bowyer, Alexander Robertson and Whalley, 1957).



Extraction of Plant Material

About 100 gm of each dried powder of the plant were soaked separately in 100 ml of different solvents like methanol, ethanol, chloroform, pet ether and hot water in conical flasks and then subjected to agitation on a rotary magnetic shaker for about 3 days. After 72 hours

filter the plant extracts, filtered with No 42 whatman filter paper separately. Concentrated extracts was preserved in sterilized air tight labeled bottles and preserved in refrigerator at 4°C until required for further use. The extract was filtered under reduced pressure using rotary flash evaporator and subjected for further preliminary phytochemical tests and antimicrobial activity. Different tests were conducted for the identification of phytochemicals is adopted by using the methods described by Edeogal *et al* (2005) and B. Thamilmalaiselvi *et al* (2011).



A B C D E F G H I

Phytochemical screening test of wood extracts of *Pterocarpus santalinus*L

- A) - Yellow precipitate formed confirms the tannins
- B) - Bluish green Phenols confirmation color test
- C) - Reddish colour terpenoids confirmation test
- D) - Color less solution Flavonoids confirmation test

- E) - Bluish red glycosides confirm color test
 F) - Bluish green conformation for steroids
 G)- Reddish yellow conformation for anthraquinones
 H) - white froth conforms saponins
 D)- Brick red colour indicates the presence of reducing sugar

TABLE1: Phytochemical screening test of wood extracts of *Pterocarpus santalinus* L

| Test name | Method | Conformation |
|--------------------|--|--|
| Alkaloids | To the 5ml of extract 5ml of 2N HCL is added and boiled and then the mixture is filtered. To the filterate a few drops of Mayer's reagent is added. | A cream colour precipitate was produced immediately indicating the presence of alkaloids. |
| Saponins. | Boil 5ml of extract in 10ml of distilled water in a test tube and is shaken vigorously for about 30 seconds. The test tube is allowed to settle for half an hour. | Formation of froth indicates the presence of saponins. |
| Tannins | Tannins are tested by adding a few drops of 1% lead acetate to 5 ml of plant extract. | Appearance of yellow precipitate indicates the presence of tannins. |
| Phenols | Phenols are tested by adding 2ml of ferric chloride solution to 2ml of plant extract. Appearance of bluish green colour solution indicates the presence of phenols. | Appearance of bluish green colour solution indicates the presence of phenols. |
| Steroids | Steroids 1ml extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added from the walls of the test tube. | Appearance of red colour in the upper layer and yellow with green fluorescence indicates the presence of steroids. |
| Cardiac glycosides | To 1ml of extract glacial acetic acid, few drops of ferric chloride and then finally concentrated sulphuric acid were added from the walls of the test tube. | Appearance of the reddish brown at the junction of two layers and the bluish green colour in the upper layer indicates the presence of cardiac glycosides. |
| Anthraquinones | 5ml extract was boiled with 10ml of sulphuric acid and filtered while hot. The filterate was shaken with 5ml of chloroform the chloroform layer was pipetted out into another test tube then 1ml of dilute ammonia is added. The resulting solution was observed for colour changes. | The change in colour indicates the presence of anthraquinones. |
| Flavonoids | To one ml of the extract, a few drops of dilute sodium hydroxide are added. | An intense yellow colour was produced in the plant extract, which became colorless on addition of few drops of dilute acid. This indicates the presence of flavonoids. |

| | | |
|-----------------------|---|--|
| Terpenoids. | 1ml of the extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid. | Formation of reddish colour indicates the presence of terpenoids. |
| Amino acids. | 1ml of the extract was treated with few drops of Ninhydrin reagent. | Appearance of purple colour indicates the presence of amino acids. |
| Reducing sugars | 1ml of extract was added 5 to 10 drops of Fehling's solution. Mixture was then subjected to boiling for 15 minutes. | Appearance of brick red precipitate indicates the presence of reducing sugars. |
| Monosaccharide's | To the 1ml of extract, 1ml of Barfoed's reagent was added and heated on water bath. | Formation of brown precipitate indicates the presence of monosaccharides. |
| Anthocyanins | To the 1ml of extract, 1ml of water was added and swirl the test tube then to the test tube add NaOH by drop wise. | Colour will change to blue green that eventually fades that indicates the presence of anthocyanins. |
| Cyanogenic glycosides | To the 1ml of extract add 6 drops of chloroform and then the tube was stoppered with a cork containing a strip of picrate impregnated paper hanging down from the stopper. Incubated the tube for 2 hrs | A colour change of the paper, from yellow to brown red, indicated the presence of Cyanogenic glycosides. |

TABLE 1: Phytochemical screening test of wood extracts of *Pterocarpus santalinus* L

| S.NO | phytochemicals | Methanol | Ethanol | Chloroform | Pet ether | water |
|------|-----------------------|----------|---------|------------|-----------|-------|
| 1 | Tannins | +++ | +++ | - | + | + |
| 2 | Phenols | +++ | +++ | - | ++ | - |
| 3 | Saponins | ++ | ++ | - | + | - |
| 4 | Alkaloids | ++ | + | - | + | - |
| 5 | Flavonoids | +++ | ++ | + | + | + |
| 6 | Anthraquinones | ++ | ++ | + | + | - |
| 7 | Amino acids | - | - | - | - | - |
| 8 | Carbohydrates | +++ | +++ | - | - | - |
| 9 | Terpenoids | +++ | +++ | - | + | - |
| 10 | Cardiac glycosides | ++ | ++ | - | + | - |
| 11 | Steroids | ++ | ++ | + | + | + |
| 12 | Anthocyanins | - | - | - | - | - |
| 13 | Cyanogenic glycosides | - | - | - | - | - |

(+++)= strongly present, (+) = poorly present

(++)= moderately present (-) = absent

Antibacterial activity

Nutrient agar medium is employed for the antibacterial screening test. The nutrient agar medium plates were prepared by pouring 15ml of nutrient agar media into sterile Petri plates. Twenty four hours old cultures of the organisms to be tested were used. Inoculate the plates with test organism. The plates were allowed to stand for one hour for prediffusion of extracts to occur in agar disc diffusion method. The plates were then incubated at 37°C for 24hours.

Antifungal activity

The extracts of leaf and bark were screened for antifungal activity by agar disc diffusion method (Perez et al., 1990) with. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molten PDA and poured on to a petri dish by pour plate technique. In agar disc diffusion method different concentrations of extracts were introduced medium. Incubation period of 24-48hours at was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition.

Preparation of concentrations

Methanolic, ethanolic, pet ether, chloroform and hot water extracts of wood *Pterocarpus santalinus* L were prepared as a different concentrations (100µg/ml, 300µg/ml, and 500µg/ml) to get the final drug concentration 15µg/well, 25µg/well, and 75µg/well respectively, (DMSO) and control (streptomycin 10µg/ml for bacteria and ketoconazole 10µg/ml for fungi). Various Concentrations of extracts were prepared by using no1 whatman filter paper discs with 9mm diameter were prepared and concentrations of the wood extracts were impinged into the sterile filter paper discs sterilize by hot air oven. Then, the discs had been used as a drug to test the wood extracts antimicrobial activity.

Test organisms

Microbial cultures obtained from the Department of Microbiology, University College of science, Osmania University, Hyderabad, INDIA. Among seven bacterial species investigated four gram negative bacteria (*E.coli*, *Pseudomonas*, *Salmonella typhi*, *Klebsiella pneumoniae*), gram positive bacteria (*Staphylococci*, *Bacillus subtilis*, *Bacillus cereus*) and certain fungal species such as *Aspergillus Niger*, *Aspergillus flavus*, *Fusarium*, *Colletotrichum*, *Rhizopus* and *Mucor*. Were carefully identified using standard

microbiological methods. All the bacterial and fungal species were maintained at 4°C Nutrient agar and Potato Dextrose agar slants respectively.

Table 2: Anti bacterial activity of wood extracts of *Pterocarpus santalinus L*

| Solvents | Con. (µl) | Zone of inhibition in mm | | | | | | | |
|------------|-----------|--------------------------|---------------------|---------------------|-----------------|-----------------|----------------------|----------------------|--------------------|
| | | <i>E.coli</i> | <i>K. pneumonia</i> | <i>Streptococci</i> | <i>S. typhi</i> | <i>B.cereus</i> | <i>P. aeruginosa</i> | <i>Staphylococci</i> | <i>B. subtilis</i> |
| Methanol | 5 | 14 | 3 | 15 | 7 | 9.6 | 6.3 | 14.3 | 9.8 |
| | 10 | 16 | 3 | 18 | 8 | 13 | 10.5 | 16 | 15.3 |
| | 15 | 19 | 8 | 20 | 11 | 15.1 | 16.8 | 17.5 | 18.1 |
| | Control* | 20.6 | 8 | 22 | 13.2 | 17 | 20 | 18.7 | 19.2 |
| Ethanol | 5 | 4.5 | 2 | 8.7 | 3 | 7.5 | 5.9 | 5 | 8.9 |
| | 10 | 6 | 3 | 10 | 5 | 10 | 9.6 | 6 | 13.8 |
| | 15 | 8 | 11 | 18.5 | 8 | 15 | 7.9 | 8 | 17 |
| | Control* | 20.6 | 8 | 22 | 13.2 | 17 | 15 | 18.7 | 19.2 |
| Pet ether | 5 | 3 | - | 2.4 | 2.5 | 2.8 | 3 | 1 | 4.4 |
| | 10 | 4.1 | - | 3 | 4 | 5 | 3.4 | 2.3 | 8.7 |
| | 15 | 5 | - | 4.5 | 6.5 | 6.3 | 4.7 | 3 | 9.5 |
| | Control* | 20.6 | 8 | 22 | 13.2 | 17 | 15 | 18.7 | 19.2 |
| chloroform | 5 | 1.5 | - | 0.5 | 1.3 | 3 | 1.6 | 0.5 | 2.9 |
| | 10 | 2.5 | - | 3 | 2 | 4 | 2 | 2 | 4.9 |
| | 15 | 3 | - | 3.8 | 3.5 | 4.6 | 3 | 2.5 | 5.0 |
| | Control* | 20.6 | 8 | 22 | 13.2 | 17 | 15 | 18.7 | 19.2 |

*Added Streptomycin 10µg/ml concentration in mm (milli meter)

Table 3: Anti fungal activity of wood extracts of *Pterocarpus santalinus L*

| Extracts | Concentration (µg/ml) | Zone of inhibition in mm | | | | |
|----------|-----------------------|--------------------------|-----------------------|-----------------|-----------------|--------------|
| | | <i>Aspergillus niger</i> | <i>Colletotrichum</i> | <i>Fusarium</i> | <i>Rhizopus</i> | <i>Mucor</i> |
| Methanol | 5 | 6 | 6 | 7 | 4 | 6.3 |
| | 10 | 7.2 | 8 | 8 | 4.4 | 8 |
| | 15 | 8 | 8.9 | 8.6 | 5.9 | 8.1 |
| | Control* | 10 | 9.8 | 9.1 | 7 | 9 |
| Ethanol | 5 | 6.5 | 3 | 5 | 3 | 3.1 |
| | 10 | 7.8 | 4 | 6 | 4 | 4 |
| | 15 | 8.8 | 7.5 | 8 | 3.9 | 4.9 |

| | | | | | | |
|------------|----------|-----|-----|-----|-----|-----|
| | Control* | 10 | 9.8 | 9.1 | 7 | 9 |
| Pet ether | 25 | 3.8 | 2 | 2.8 | 2 | 0.5 |
| | 50 | 5.2 | 4 | 3 | 3 | 1.8 |
| | 75 | 6 | 5 | 4 | 3.9 | 2.6 |
| | Control* | 10 | 9.8 | 9.1 | 7 | 9 |
| Chloroform | 25 | 2.5 | 2 | 1.3 | - | - |
| | 50 | 4.2 | 3.5 | 2 | - | - |
| | 75 | 4.9 | 3.9 | 2.9 | - | - |
| | Control* | 10 | 9.8 | 9.1 | 7 | 9 |

*Added Ketocazole 10µg/ml concentration in mm (milli meter)

RESULTS

For the wood authentication wood powder is boiled at 226°C it gives blood red colour small crystals. When crystals these crystals dissolve separately in alcohol gives red colour, in ether yellow colour are not soluble in water, but soluble in alcohol. Indicates that the wood is fine wood for work. From the table 2 the wood extracts revealed the presence of tannins, phenols, saponins, carbohydrates commonly from methanol, ethanol and water that were not observed in chloroform and pet ether. Only the flavonoids, anthraquinones and steroids are commonly found in methanol, ethanol, pet ether, chloroform and hot water.

Table 2 explains the Antibacterial activity of *Pterocarpus santalinus* L wood extracts was assayed. The data revealed that significant reduction in growth of bacteria and fungi was observed with wood extracts of methanol, ethanol, pet ether, chloroform and water. And all the extracts showed significant differences in their efficacy. Among all the extracts methanolic extracts of wood were more prominent activity than the ethanol, pet ether, chloroform and water. Methanol, ethanol and pet ether extracts of wood showed broad inhibition zone on all the bacteria tested than hot water and chloroform. The extracts of methanol and ethanol of wood showed maximum activity even at lower concentrations. Methanol extracts of wood showed broad spectrum inhibition zone against the bacteria *Pseudomonas aeruginosa*, *E.coli*, *Salmonella typhi*, *Streptococci*, *Bacillus cereus* and *Bacillus subtilis*. Whereas ethanol extracts showed maximum antibacterial activity in the order of *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumonia*, *E.coli*, *Streptococci* and *Pseudomonas aeruginosa*. *Klebsiella pneumonia* is found to be inhibited by methanol and ethanol extracts only.

Table 3 explained about the Antifungal activity of the wood extracts. Extracts of methanol showed broad spectrum inhibition zone against the fungal species *Aspergillus Niger*, *Fusarium*, *Colletotrichum* and *Rhizopus* than the ethanol, pet ether, chloroform and hot water. The broad spectrum inhibition zone exhibited by *Pterocarpus santalinus L* may be attributed to the various active constituents present in it, which either due to their individual or combined action exhibit antibacterial activity and antifungal activity. Result indicated that the phytochemical extracts of *Pterocarpus santalinus L* wood exhibited dosage dependent antibacterial and antifungal activity.

DISCUSSION

The result shows that most of the phytochemicals which are present in the wood extracts solubilised abundantly in polar solvents and less in water. Most of the extracts showed the similar properties to the screening tests. Similarly the qualitative phytochemicals such as carbohydrates, steroids, anthocyanins, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides and glycerides were reported by (Narayan *et al.*, 2007). Triterpene is reported to be present in the callus of stem cuttings (Krishnaveni and Srinivasa Rao, 2000b). Isoflavonoids, terpenoids, and related phenolic compounds, β -sistosterol, lupeol, epicatechin were reported by (Kesari *et al.*, 2004). Terpenoids are attributed for analgesic and anti-inflammatory activities and flavonoids are have been reported to possess many useful properties, including anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumour activity (B. Havesteen *et al* 1990) and (J.B. Harborne *et al* 2000). Anthocyanins help the human immune system to work more efficiently to protect against viral infections. Decreasing the ability of the influenza viruses itself to get into the human cell or to be related from infected cells or by having a viricide effect and used as a better source for stomachache and in the treatment of diarrhoea (A.L. Liu, *et al.*, 2009). Glycosides, flavonoids and alkaloids have hypoglycemic activities (B. Oliver 1980). Tannins inhibit the growth of many fungi, yeasts, bacteria and viruses and also have the wound healing property (K.T. Chung, T.Y. Wong 1998). Saponins have beneficial health effects, traditionally saponins have been extensively used as detergents, as pesticides and in addition to their industrial applications as foaming and surface active agents (J. Shi, K. Arunasalam *et al* 2004). Steroids used in pharmacy due to their relationship with sex hormones (R. Santhi, G. Lakshmi *et al* 2011) phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics (V.K. Gupta, *et al* 2010) and they can protect the human body from the

oxidative stress which may cause many disease, including cancer, cardiovascular problems and ageing (K. Robards, P.D. Prernzler *et al*, 1999). Due to the presence of phytochemicals in the wood of the *Pterocarpus santalinus* L have shown antimicrobial, antifungal, antidiabetic and anticancer activities. The present studies have justified the wood of the *Pterocarpus santalinus* L possess antimicrobial, antifungal activities based upon their dosage.

REFERENCES

1. A.L. Liu, *et al.*, 2009, *Planta Med*, 2009; 75: 337-9.
2. A test book of B.Sc Botany final year paper 3 Taxonomy, Palaeontology, Embryology, Medicinal important plants. Edited by Dr.M.Radha Krishnaiah and written by Dr.B.K.Vijay Kumar *et al* Telugu Akademi First edition 2008 page number 236.
3. Anonymous 1969, the wealth of India, publication and information directorate (CSIR), New Delhi,.
4. Arokiyaraj S, Martin S, Perinbam K, Marie Arockianathan P, and Beatrice V 2008 Free radical scavenging activity and HPTLC finger print *Pterocarpus santalinus* L an in vitro study. *Indian J Sci Technol* 1, 1-13.
5. B. Sandhya, S. Thomas, W. Isabel, R. Shenbagarathai, *Complementary and alternative medicines*, 2006; 3: 101-114.
6. B. Thamilmara Selvi, S. Ahamed John and G. Theivandran 2011 preliminary phytochemical investigation and antimicrobial activity of *Sphaeranthus indicus* Linn. *Ad Plant Sci*, 2011; 24 (I): 81-85.
7. Chopra RN, Nayar SL and Chopra IC 1956 *Glossary of Indian medicinal plants*. India, CSIR, p: 171.
8. D.E. Okwu, C. Josiah, 2006, *Afri J Biotech*, 2006; 5: 357-361.
9. Edeogal HO, OKWUDE and Mbaebie Bo 2005 phytochemical constituents of some Nigerian medicinal plants. *African J. Biotechnol*, 2005; 4: 685-688.
10. G.V. Satyavati, A.K. Gupta, N. Tandon, *Medicinal plants of India*, Indian Council of Medical Research, New Delhi, India, 1987.
11. Kesari AN, Gupta RK, and Watal G 2004 Two aurone glycosides from heart wood of *Pterocarpus santalinus* L. *Phytochemistry*, 2004 ; 65: 3125-3129.
12. Krishnaveni KS and Srinivasa Rao JV 2000b A new triterpene from callus of *Pterocarpus santalinus*. *Fitoter*, 2000; 24: 167-171.
13. Kodithuwakku Kankanange Indika Upali Arunakumara, Buddhi Charana Walpola, Siripalas Subasinghe and min- Ho Yon 2011 *Pterocarpus santalinus* Linn. f.

- (Rathhandun): A Review of Its Botany uses, phytochemistry and pharmacology J Korean Soc, Appl. Biol.Chem, 2011; 54 (4): 495-500.
14. K. Robards, P.D. Premzler, G. Tucker, P. Swatsitang, W. Glover, FoodChem, 1999; 66: 401-36.
 15. Manjunatha BK 2006 Antibacterial activity of *Pterocarpus santalinus*. IndianJ.Pharma.Sci, 2006; 68: 115-116.
 16. S. Narayan, R.S. Devi, G.Vani, Devi C.S.S., 2007 Effect of *Pterocarpus santalinus* extract on the gastropathology elicited by a hypertensive drug in Wistar rats, Pharm. Biol, 2007; 45 (6) 468–474.
 17. Raju KK and Nagaraju A 1998 Geobotany of red sanders (*Pterocarpus santalinus*) a case study from the southeasternportion of Andra Pradesh. Environ Geol, 1998; 37: 340-345.
 18. Ravindranath B, Seshadri TR., 1972 Chemistry of santalinpigments I. Structure of santalinpermethyl ether. Tetrahedr Lett, 1972; 13: 1201–1204.
 19. R. Ilavarasan, M. Vasudevan, S. Anbazhagan, S. Venkataraman,2003a, J Ethnopharmacol, 87: 227-230.
 20. R. Santhi, G. Lakshmi, A.M. Priyadharshini, L. Anandaraj, 2011,Inter Res J Pharm, 2: 131-135.
 21. R. Verpoorte,1998,Chemodiversity and the Biological Role of Secondary metabolites, some thoughts for selecting plant material for drug development. Proc PhytochemSoc Europe, Kluwer Publishers, 43; P. 11-24.
 22. T. Satyanarayana, T. Saritha, M. Balaji, A. Ramesh, M.K. Boini, Saudi, 2004, Pharma J, 2004; 12:107-111.
 23. S.D. Sabnis, M. Daniel, A. 1990 Phytochemical approach to economic Botany, Kalyani Publishers, New Delhi, 1990; 15: 65.
 24. B. Havesteen, 1990, Eur J ClinMicrobiol Infect Dis, 9: 455-61.
 25. J. Shi, K. Arunasalam, D. Yeung, Y. Kakuda, G. Mittal, Y. Jiang, 2004,J Med Food, 2004; 7: 67-78.
 26. V.K. Gupta, G.D. Singh, S. Singh, A. Kaul, Medicinal Plants: Phytochemistry,
 27. Pharmacology and Therapeutics, Daya Publishing House, Delhi, 2010.
 28. P.B. Mallikharjuna, L.N. Rajanna, Y.N. Seetharam, G.K. Sharanabasappa, E-J Chem , 2007; 4: 510-518.
 29. W.J. Bowyer, Alexander Robertson and W.B. Whalley, 1957, the chemistry of the insoluble red woods Part VII. The synthesis of coumarino (3':4'-3 : 2)coumarones J. Chem. Soc., 1957; 542-544.