WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 08, 582-590. Research Article ISSN 2277–7105

IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM *PTEROCARPUS SANTALINUS* HEART WOOD USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND THEIR ANTIMICROBIAL ACTIVITY

Chandra Sekhar Challa[1], Saradamma Bulle[2], Lokesh T., Devanna Nayakanti[4] and Varadacharyulu Nallanchakravarthula[5*]

[1]Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, 515 002.
[2,3,5]Department of Biochemistry, Sri Krishnadevaraya University, Anantapur, 515 003.
[4]Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, 515 002, Andhra Pradesh, India.

Article Received on
27 Feb. 2018,
Revised on 19 March 2018,
Accepted on 09 April 2018
DOI: 10.20959/wjpr20188-11672

***Corresponding Author**
Varadacharyulu Nallanchakravarthula
Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, 515 002.Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, 515 002.

ABSTRACT

This study aims at investigating the secondary metaboliotes and antimicrobial activity of *Pterocarpus santalinus* L. Heartwood. The sample of *Pterocarpus santalinus* L. Heartwood was analyzed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) for secondary metabolites characterizations such as of flavonoids, saponins and steroidal sapogenins and others phenolic compounds. The results revealed that more peaks corresponding to major compounds. The compounds Pregnon-20- 0ne, CycloHexane, 1-ethyl- 1-methyl,2,4- bis(1-methyl,ethanyl), 4,5,7-trihydroxy isoflavone, Phytol, Caryophelleneoxide were identified in the peaks samples. The blue-colored spots were present at sample with two others colored spots (violet and yellow) were presented. It suggested that in vitro antimicrobial activity would be based on compounds. Sometime the compounds eluted at 5.0 and 10.0 min contribute to in vitro antimicrobial activity. The results revealed again the presence of the Terpinoids in *Pterocarpus santalinus* L., made for the characterization by HPLC and TLC probables flavonoids and presence of Terpinoids.

KEYWORDS: Antimicrobial activity, Thin layer chromatography, high performance liquid chromatography, *Pterocarpus santalinus* L., Terpinoids.

INTRODUCTION

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs.[1-3] Before onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. With introduction of scientific procedures the researchers, were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active. Aconitine, Atisine, Lobeline, Nicotine, Strychnine, Digoxin, Atropine, Morphine are some common examples.

The efficacy of some herbal products is beyond doubt, the most recent examples being Silybum marianum (silymarin), Artemisia annua (artemesinin) and Taxusbaccata (taxol). On the other hand, randomized, controlled trials have proved the efficacy of some established remedies, for instance, Ginkgo biloba for tinnitus, Hypericum perforatum is a reputed remedy for depression. In Hypericum some researchers are of the view that hypericin is the active principle of the herb and some believe that hyperforin is responsible for antidepressant action of the herb.[4-6] Recently research has supported biological activities of some medicinal herbs. Diabetes is such a segment where researchers are expecting new molecules from herbs that can provide us with tools for fighting this dreaded disease.

METHODOLOGY

Isolation of the constituents from *Pterocarpus santalinus* Linn

One part of Methanolic extract of Heartwood of *Pterocarpus santalinus* Linn was extracted with methanol-n-butanol (1:9). The extract was concentrated and purification was carried out by column chromatography using silica gel of mesh size 200-400 and n-butanol-ethyl acetate-water (1:1:1) as solvent system. Various fractions were collected and concentrated where by the Terpinoids separated as a crystalline solid. It was filtered, washed and recrystallized from n-butanol methanol-water system. The Terpinoid was readily soluble in water and lower alcohols but insoluble in acetone and ethyl acetate. The compound gave a single spot, RrO.3

in solvent system as mentioned above. The obtained Terpinoid was subjected to hydrolysis. A solution of the Terpinoid (0.5g) in 3 N HCl (50ml) was boiled under reflux for 2 hrs. After cooling, this was filtered and repeatedly crystallized from methanol to get colourless rectangular plates.[7, 8]

Characterization of the isolated constituents

TLC was carried out using Silica gel (Merck, Darmstadt, Germany) and commercially available readymade aluminum foiled sheets with silver nitrate impregnated silica gel (Merck, Darmstadt, Germany). Column chromatography was carried out on Silica gel (Merck, 60-120 and 70-230 mesh). All the chemicals and reagents used were obtained in high purity either from S.D. fine chemicals Pvt. Ltd., Bombay, India or E.Merck Pvt. Ltd., Bombay, India.

Methanolic extract of Heartwood of *Pterocarpus santalinus* Linn

The methanol extract (5g) of Heartwood was washed with acetone and allowed to settle. After 2 hrs the acetone soluble fraction was separated and the insoluble fraction was resuspended and washed again with fresh acetone two to three times. The acetone soluble fraction was chromatographed on a silica gel column using chloroform and acetone in the ratio of 6:4, The eluted fractions were collected at an interval of 5 ml each and were monitored by thin layer chromatography. The fraction one recovered in higher concentration was recrystallized from acetone to get a whitish compound. Dark brown residue (l0g) of methanol extract of stem was separated into a major fraction by chromatography on Silica gel with methanol/water (9.5:0.5). The elution was collected and profiled by TLC showed single spot. Then the fraction was subjected to HPLC showed single peak. The recovered compound was washed with cold methanol and filtered. This compound was labeled.[9]

HPLC

Working solutions were prepared with a concentration of 100µg/ml and 10µg/ml. The internal standard solution, 1,7-diaminoheptan (C7H18N2) was also purchased from Sigma Aldrich Company. The concentration stock solution was prepared at 1mg/ml concentration and the working solution at 100µg/ml. Installations and equipment: homogenisation type blender, Kern analytical balance, Silent CrusherM, centrifuge EBA 21, filter paper of Φ=55 mm, syringe filters having porosity of 0.45µm, agitator REAX control, ultrasonic water tank Aquawave TM, incubator BMT INCUCELL 55, water cleaning system EASY pure RoDi, filtering system with vacuum pump. The HPLC analysis system consists in: pump, column

thermostat, UV-VIS detector with diode array, computer system, and printer. Chromatography column are BDS Hypersil C18 250 x 4.6 mm, having the particles size of 5 µm and Hypersil Gold precolumns 10 x 2.1 mm. In order to make different concentrations (from 0.1 up to 7 µg/ml), we prepared the standard working solutions of 100 µg/ml and 10µg/ml concentrations as well as the known internal standard working solution. Then we added different volumes of perchloric acid in order to obtain a final volume of 0.5 ml. Quantitative measurements were performed depending on the internal standard, using the chromatography peaks obtained for each biogenic amine. The absorbance of derivatised compounds was measured at 270 nm and the peaks were integrated with CromQuest software. Each compound concentration was expressed in µg/ml, and the compound content were expressed in mg/kg. The results obtained are of 10 determinations; the mean values were calculated with Microsoft Excel software from Microsoft Office suite.[10]

Antibacterial Activity

Test Microorganisms and Growth Media

Staphylococcus aureus (MTCC 3160), *Bacillus cereus* (MTCC 1305) *E.Coli* (MTCC 443) and *Pseudomonas aureoginosa* (MTCC 2453) were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Department of Microbiology, Osmania University, were used for evaluating antibacterial activity. The bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar. The bacteria were grown on Mueller-Hinton agar plates at 37°C.

Determination of zone of inhibition method

Preparation of Discs

Whatman No.1 filter paper discs of 5mm diameter were autoclaved by keeping in a clean and dry Petri plate. The discs were soaked in compound solutions for 5 hours were taken as test material. After 5 hours the discs were shade dried. The concentrations of compound solutions per disc are accounted for 0.1 grams/1ml. Subsequently they were carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, Hexane, benzene and distilled water are prepared and used as control.

Testing of antibacterial activity

To test the antibacterial activity, LB agar medium was prepared and the medium was sterilized at 121°c for 30 mins. The agar plates were prepared by pouring about 10ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to

solidify the medium. 1ml of inocculum (containing suspension) of *Staphylococcus aureus*, *Bacillus subtilis*, *E.Coli* and *Klebsiella pneumoniae* was poured on to the plates separately containing solidified agar media. The prepared sterile filter paper discs were impregnated with the compound solutions and shaken thoroughly and these test plates incubated for a period of 48 hrs in BOD at 37ºc for the development of inhibitory zones and the average of 2 independent readings for each organism in different compound solutions were recorded.[11-13]

Measuring the diameter of inhibition zone

The inhibition zones were measured after 1 day at 37ºc for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler. Five paper discs placed in one Petri plate (Table 1).

RESULTS AND DISCUSSION

Results from the analysis of methanol extracts from *Pterocarpus santalinus L* extracts are illustrated in Figures 1.

Fig 1: TLC analysis of *Pterocarpus santalinus.*

Figure 1 illustrated Terpinoids obtained in *Pterocarpus santalinus* plant using methanol extracts from Heartwood after TLC investigation. All samples of *Pterocarpus santalinus* showed the presence of Terpinoids. These Terpinoids revealed by blue spots (all samples) and violet, green or yellow-green spots depending on that degree of unsaturation of the molecules (Figure 1). In samples, blue spots are more accentuated than of others colored spots (violet, yellow, green and yellow-green). The violet spot in sample may be due to lipids and the green spot corresponded to that obtained by Uematsu et al. (2004). These authors

have identified the blue spot compounds as hydroxymethylfurfral and its ethyl adducts. The same authors reported that yellowgreen colored spot obtained with standard sarsasapogenin was characteristic of steroidal sapogenin after application of anisaldehyd reagent. The violet colored spot from Kimwenza's sample was obtained also by Yung et al. (2005) with the butanol extract from aqueous layer of aerials portions of *Pleurospermum kamtschaticum* after several extraction procedures.[14-16]

High performance liquid chromatography

The results obtained by high performance liquid chromatography analysis of methanol extracts of *Pterocarpus santalinus* were presented in Figure 2. For comparison of chromatograms of methanol extract from *Pterocarpus santalinus* obtained by HPLC, it was revealed that the number of major peaks differs. If consider as the major peak with a relative area equal to or greater than 2.5 samples have got more than seven major peaks with their respective relative areas 6.904, 10.578, 10.625, 6.865, 3.425, 7.742, 0.583, 0.277, 5.011, 31.69 which eluted respectively at 3.183, 3.566, 4.108, 4.158, 4.560, 4.960, 5.555, 5.780, 9.050, 9.780 (Figure 2). The compounds Pregnon-20- 0ne (Peak 2), CycloHexane, 1-ethyl- 1-methyl,2,4- bis(1-methyl,ethanyl) (Peak 4), 4,5,7-trihydroxy isoflavone (Peak 6), Phytol (Peak 7), Caryophelleneoxide (Peak 10) were identified in the peaks samples.[17-19]

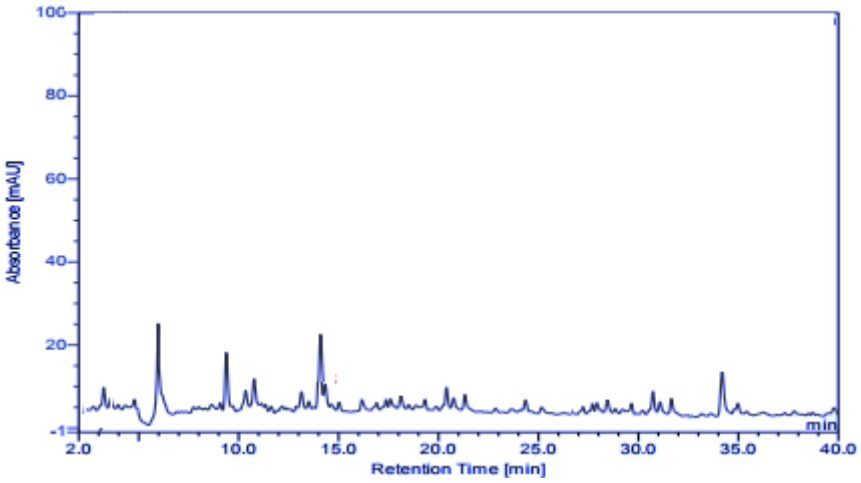

Fig 2: HPLC sample analysis of methanolic extract of *Pterocarpus santalinus*.

Table 1: Peak Analysis of *Pterocarpus santalinus extract.*

Peak#	Ret.Time	Area	Height	Area%	Height%
1	3.25	21421243	256545	2.426	4.348
2	4.23	2153931	2548626	5.225	10.3
3	6.02	12372428	34858	13.152	5.204
4	9.54	543615	153939	3.578	8.25
5	10.5	332129	267821	3.215	5.436
6	11.02	10143927	267586	2.456	6.276
7	13.5	2713821	21594	1.265	0.423
8	13.9	2143366	2727	1.235	0.154
9	14.21	5671627	196917	13.216	4.143
10	15.03	4804329	2833665	2.32	12.265
11	16.86	636465	3261187	2.362	11.543
12	17.34	36143366	364946	4.204	8.54
13	20.64	71627	365134	8.325	5.854
14	20.95	4804386	216976	8.326	6.645
15	21.38	107499	148837	4.235	0.423
16	24.35	172413	143526	4.425	0.198
17	30.89	543624	26638	8.345	4.349
18	34.5	2332129	2128	11.69	5.649
Total		107111925	11113650	100	100

Antimicrobial Activity

By comparing all the zones of inhibition values it can be concluded that *Klebsiella pneumoniae* and *E.coli* were sensitive even in low concentration. Now in the present study the used plant extract was found as antimicrobial agents and inhibits the growth of *Staphylococcus aureus* and *E.coli* effectively at all concentrations.[20]

Table 3: Antibacterial Bioactivite of Compounds.

Compound No	**Gram positive bacteria**		**Gram negative bacteria**	
	Staphylococcus aureus	***Bacillus subtilis***	***E.Coli***	***Klebsiella pneumoniae***
	Zone of inhibition in mm [b]			
Pregnon-20- 0ne	3	3	5	4
CycloHexane, 1-ethyl-1-methyl,2,4- bis (1-methyl,ethanyl)	4	3	3	5
4,5,7-trihydroxy isoflavone	5	3	6	3
Phytol	2	2	2	2
Caryophelleneoxide	1	1	1	1
Ciprofloxacin [a]	5	5	7	6

a. Concentration: 4 mg/mL^{-1} of DMSO; b. Values, including diameter of the well (8 mm), are means of three replicates; c. No activity.

CONCLUSION

Through the present work, we found that effectively the metabolite profiles of *Pterocarpus santalinus.* All extracts from *Pterocarpus santalinus* Heartwood were exhibited in the HPLC profiles of methanolic extracts indicated that *Pterocarpus santalinus* was able to synthesize Terpinoid constituents in *Pterocarpus santalinus* Heartwood. Results obtained in the present study confirm the difference of in vitro antimicrobial activity of *Pterocarpus santalinus* according to its geographical location. Those results are confirmed by TLC and the extracts showed presence of flavonoids compounds and Terpinoids. These compounds, flavonoids, saponins and Terpinoids, would be explained the many effects which are played by *Pterocarpus santalinus* in the treatments of several diseases. Thus, studies could be continued for the characterization of flavonoids or others compounds responsible of antimicrobial activity and Terpinoids who would be presents in *Pterocarpus santalinus*.

REFERENCES

1. Stuffness M and Douros J. Current status of the NCI plant and animal product program.Nat Prod, 1998; 45(1): 1-14.
2. Okigbo RN, Eme UE and Ogbogu S. Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnol. MolBiol Rev, 2008; 3: 127-34.
3. Olayiowola A. WHOs traditional medicine programme: progress and perspective. WHO Chron, 1984; 38(2): 76-81.
4. Grover JK and Yadav SP. Pharmacological actions and potential uses of Momordicacharantia: a review. J Ethnopharmacol, 2004; 93: 123-32.
5. Smith JR, Karunaratne NS and Mahindapala R. Rapid inventory of wild medicinal plant populations in Sri Lanka. BiolConcerv, 2006; 132: 22-32.
6. Lavin M, Pennington RT, Klitgaard BB, Sprent JI, de Lima HC &Gasson PE. "The dalbergioid legumes (Fabaceae): delimitation of a pantropical monophyletic clade". Am J Bot, 2001; 88(3): 503–33.
7. International Legume Database & Information Service (ILDIS). Genus Pterocarpus. Version, Nov 2005; 10: 01.
8. World Agroforestry Centre (WAC). AgroForestryTree Database-Pterocarpus soyauxii, 2008.
9. Rao SP and Raju AJS. Pollination ecology of the Pterocarpus santalinus (Fabaceae), an endemic and endangered tree species. CurrtSci India, 2002; 83: 1144-48.

10. Rudd V E. In a Revised Handbook of the Flora of Ceylon, CRC Press, Boca Raton, FL, USA, 1991; 108-381.
11. Raju KK and Nagaraju A. Geobotany of red sanders (Pterocarpussantalinus)- a case study from the southeastern portion of Andra Pradesh. Environ Geol, 1998; 37: 340-45.
12. Selvam AB. Pharmacognosy of Negative Listed Plants, 2012; 171-82.
13. Mulliken T and Crofton P. Review of the Status, Harvest, Trade and Management of Seven Asian CITES-listed Medicinal and Aromatic Plant Species German Federal Agency for Nature Conservation, Germany, 2008; 71-92.
14. Ravikumar and Ved. 100 red listed medicinal plants of conservation concern in Southern India, Bangalore: Foundation for Revitalisation of Local Health Traditions, 2000; 1st ed, p 308-12.
15. Jain SK &Sastry AR K. Threatened Plants of India. A State-of-the Art Report, 1980; 42.
16. Dhanabal P, Kannan SE and Bhojraj S. Protective and therapeutic effects of the Indian medicinal plant Pterocarpus santalinus on D-galactosamine-induced liver damage. Asian J Trad Med, 2007; 2: 51-57.
17. PurnachandraRao S and Solomon Raju AJ. Pollination ecology of the red sanders Pterocarpus santalinus (Fabaceae), an endemic and endangered tree species. CurrSci India, 2002; 83: 1144-48.
18. Anderson GJ, Bernardello G, Stuessy TF and Crawford DJ. Breeding system and pollination of selected plants endemic to Juan Fernández Islands. Am J Bot, 2001; 88: 220-33.
19. Rajeswari V and Paliwal K. In vitro plant regeneration of Pterocarpus santalinus(L.f.) from cotyledonary nodes. Indian J Biotechnol, 2008; 7: 541-46.
20. Kurjogi, M.; Satapute, P.; Jogaiah, S.; Abdelrahman, M.; Daddam, J.R.; Ramu, V.; Tran, L.-S.P. Computational Modeling of the Staphylococcal Enterotoxins and Their Interaction with Natural Antitoxin Compounds. *Int. J. Mol. Sci*, 2018; *19:* 133.