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Short Communication

A comparative study on antimicrobial activity of *Pterocarpus santalinus* L.f. plant parts

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Abstract

In recent days pathogenic microbes are becoming more drug resistance resulting in a great risk of their inhibition. So there is a need for us to explore for efficient alternate natural antimicrobial sources for better prevention of emerging novel infectious diseases. Hence a phytochemical analysis and comparative in vitro antimicrobial test was done on the methanol (Me) extracts of Pterocarpus santalinus L.f. plant parts in Kirby – Bauer disk diffusion method against four types of harmful bacteria like Pseudomonas aeruginosa, β - Streptococcus pneumonia, Escherichia coli and Salmonella enterica with Cefixime as synthetic antibacterial standard. In this assay Me extract of leaves and flowers exhibited good antimicrobial potential against all bacterial species with maximum inhibition zone (MIZ) in the range of 24.0 – 38.6mm.

Keywords: *Pterocarpus santalinus* L.f., phytochemical analysis, antimicrobial, antibacterial, Kirby – Bauer disc diffusion method, *Pseudomonas aeruginosa, Escherichia coli, Salmonella enteric,* β - *Streptococcus pneumonia* Cefixime, maximum inhibition zone (MIZ) and methanol (Me).

Introduction

New diseases are emerging currently due to drug resistant strains of microbes which resulted in the major deaths in human beings[1]. These microbes are highly irresistable[2] and their spreading is hard to curb. So there is a need to invent new drugs which can act efficiently controlling resistant strains without any side effects[3]. But synthetic drugs somehow show more or less side effects resulting in other health ailments. Hence natural antimicrobial sources are the only option for minimising side effects of artificial synthetic drugs[4].

Usually plants have a wide range of phytochemicals, which are responsible for their therapeutic values[5]. Majority of present drugs were discovered from plant sources only[6]. *P.santalinus* L.f. is a rare medicinal plant with limited growth in humid regions[7]. This plant has many pharmacological therapeutic qualities which include hepato-protective[8,9], antihyperglycemic[10], antidysenteric[11], antidiabetic[12], antifungal[13] and antimicrobial[14]. In this regard a comparative antimicrobial study was took up to assess the presence of antimicrobial potential in Me extracts of different plant parts of *Pterocarpus santalinus* L.f. against four pathogenic bacterial strains in Kirby – Bauer disk diffusion method[15], where Cefixime has acted as synthetic antibiotic standard.

Earlier an antibacterial study was done on stem bark and leaves of this plant[14] which revealed good antimicrobial activity against many pathogens. Another antibacterial study was also done on the callus of *Pterocarpus santalinus*[16].

Materials and methods

Collection: Different parts of *Pterocarpus santalinus* L.f plant i.e., leaves, flowers, fruits and roots were procured from Seshadri hills, Tirupati, Andhra Pradesh, India. The plant parts collected from *Pterocarpus santalinus* L.f plant were shown from Figures-1, 2, 3 and 4. Plant authentication of *Pterocarpus santalinus* L.f. was done by Professor B. Ravi Prasad Rao, a plant sampling has been kept in herbarium of Botany Dept., with a voucher no- 48799 in Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.

Figure-1: Pterocarpus santalinus L.f. leaves.

Figure-2: Pterocarpus santalinus L.f. flowers.

Figure-3: Pterocarpus santalinus L.f. fruits.

Figure-4: Pterocarpus santalinus L.f. roots.

Extraction: *Pterocarpus santalinus* L.f. plant parts were washed under water carefully and dried in shade totally, then pounded into powder. 500gm of powder of each plant part was extracted with 1500ml of Me by soaking it for 24hrs with intermittent stirring. By using Whatman filter paper the filtrate was filtered and again extracted with the same solvent till the solution become colourless. The solvent in solution was recycled by using Heidolph Rota-Evaporator, under vacuum to give crude extract. Likewise each plant part was extracted with Me in the same procedure shown in Figure-5.

Figure-5: Cold extraction of Pterocarpus santailnus L.f. Plant parts with Methanol.

The yields of plant parts and extraction efficiencies of solvents were tabulated as Table-1.

Table-1: Yields of *Pterocarpus santalinus* L.f. plant parts Me extracts and extraction efficiencies of the solvents

Plant part	Yield (grams)	Extraction efficiency (%)
Leaf	210.0	42.0
Flower	185.0	37.0
Fruit	140.0	28.0
Root	206.5	41.3

Qualitative Phytochemical screening[17]: A qualitative phytochemical analysis was done on Me extracts of *Pterocarpus santalinus* L.f. plant parts which shown the occurrence of secondary metabolites existing in them and the details were shown in Table-2.

Table-2: Phytochemical analysis of *Pterocarpus santalinus* Lf. Plant parts Me extracts.

Phytochemical	Qualitative test	*Pterocarpus santalinus* Lf. Plant part			
		Leaf	Flower	Fruit	Root
Alkaloids	Mayers Reagent	+	+	+	+
	Wagners Reagent	+	+	+	+
	Hagers Reagent	+	+	+	+
	Dragendorffs Reagent	+	+	+	+
Carbohydrates and glycosides	Molishs Test	+	+	+	+
	Fehlings Test	+	+	+	+
	Barfoeds Test	+	+	+	+
	Benedicts Test	+	+	+	+
	Borntragers Test	+	-	+	-
	Legals test	+	-	+	-
Saponins	Foam test	+	+	-	+
Proteins and Amino Acids	Millons Reagent	+	+	-	-
	Biurett Reagent	+	+	-	+
	Ninhdrin Reagent	+	-	-	-
Phytosteroids	Libermann's-Buchard's Test	+	+	-	+
Oils and fats	Spot Test	-	-	-	-
	Saponification Test	-	-	-	-
Phenolics and Flavonoid compounds	FerricChloride Test	+	+	+	+
	Gelatin Test	+	+	+	+
	LeadAcetate Test	+	+	+	+
	Alkaline Reagent	+	+	+	+
	Magnesium & HCl Reduction	+	+	+	+
Gums & Mucilages	Alcohol- 95% test	-	-	-	-

(+) denotes positive, (–) denotes negative.

Antimicrobial assay by Kirby - Bauer disc diffusion susceptibility test: An *in vitro* antimicrobial assay was done by adopting the Kirby - Bauer disk diffusion susceptibility test as per the National Committee for Clinical Laboratory Standard protocol for aerobic testing. This test was done against four dangerous bacteria like *Pseudomonas aeruginosa, Escherichia coli,* β - *Streptococcus pneumonia,* and *Salmonella enterica*.

Procedure: The microbe to be tested was cultured two times on agar; few colonies were transferred aseptically into each test tube separately having 10ml of sterilized nutrient fluid. Incubation of these test tubes was done for 8-12hours around 37°C to obtain log phase growth. Consequently, dilution of these inoculates was done with sterilized deionised water to attain a density of almost 0.5 McFarland standard turbidity scale i.e., 1x106 cfu/ml. 100µl of the bacteria was aseptically moved to the sterilized nutrient agar discs, where as every disc contains 20-25ml of the sterilized medium, which was spread by a sterilized L-shaped glass rod. Then seeding was done by letting the bacteria to settle on the agar medium for few minutes. A bore of 6mm diameter was made by a sterilized cork borer, and then 100 ml of 1µg/1µl concentration of Me extract of plant part (leaf/flower/ root/fruit) was shifted aseptically into that bore. In the same disc synthetic standard 100 ml of Cefixime of 1µg/1µl concentration was added as standard reference (+ve control) and Dimethyl sulphoxide (DMSO) was added as blank (-ve control). Me extracts of leaf, flower, root and fruit of *Pterocarpus santalinus* L.f. were tested in this method in different sterile nutrient agar plates in triplicates. Incubation of these plates was done for a period of 24 hours near 37˚C, which was shown in Figure-6 and Figure-7.

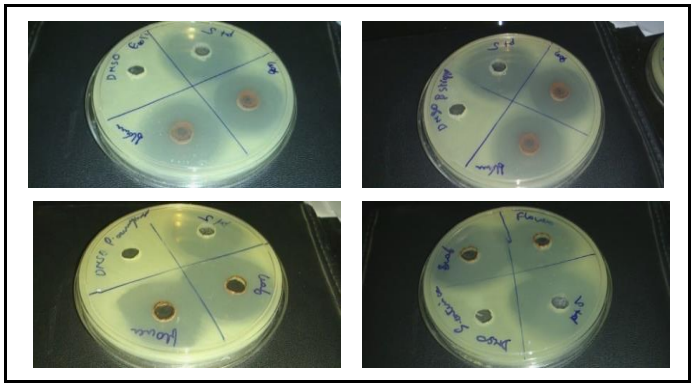

Figure-6: Inhibition Zone of Pterocarpus santailnus L.f. leaf and flower extracts.

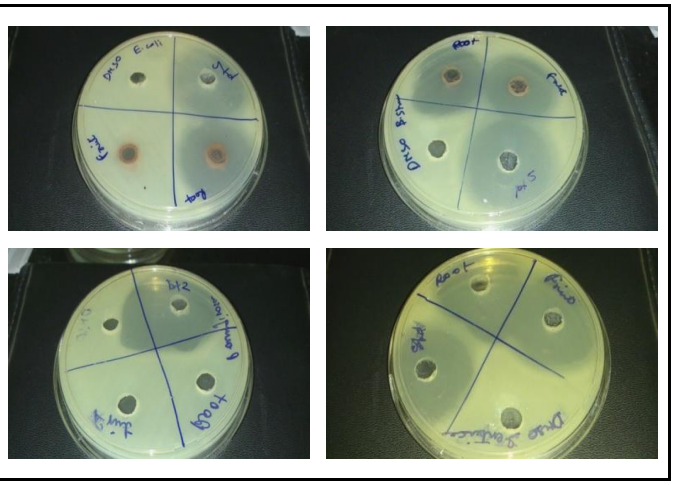

Figure-7: Inhibition Zone of Pterocarpus santailnus L.f. fruit and root extracts.

The mean values of inhibition zones along with standard deviation were measured and tabulated in Table-3.

Results and discussion

Yields of extracts and extraction efficiencies of the solvents: The extraction efficiencies of solvents and yields of extracts were shown in Table-1, in which Me leaf extract showed highest yield of 210gms and methanol solvent showed highest extraction efficiency of 42%.

Qualitative Phytochemical screening: In qualitative phytochemical screening Me leaf extracts showed the presence of extensive phytochemicals in it. The floral and root extracts revealed the occurrence of all secondary metabolites except amino acids, oils, fats, mucilage and gums. But the Me fruit extract showed the absence of various phytochemicals like saponins, proteins, amino acids, phytosteroids, fats, oils, gums and mucilages.

Antimicrobial assay by Kirby - Bauer disc diffusion susceptibility test: A relative *in-vitro* antibacterial assay on leaf, flower, fruit and root Me extracts of *Pterocarpus santalinus* L.f. was done against four bacterial species i.e., β - *Streptococcus pneumonia, Escherichia coli, Salmonella enterica* and *Pseudomonas aeruginosa.* The inhibition zone was calculated in millimetre using standard Hi -media scale and readings were recorded and tabulated in Table-3, which showed that, leaf and flower Me extracts were antibacterial against all bacterial species by showing MIZ in the range of 24.0-38.6mm. Whereas root Me extract exhibited antibacterial potential against all species except *P.aeruginosa.* But the fruit Me extract displayed antibacterial potential against only two bacterial species viz., β-*Streptococcus pneumonia* and *Salmonella enterica.* But all Me extracts of flower, fruit, root and leaf showed less antibacterial activity than synthetic antibacterial standard Cefixime.

Conclusion

By the qualitative phytochemical analysis and comparative *in vitro* antimicrobial study on Me extracts of *Pterocarpus santalinus* L.f. plant parts, we can conclude that the Me crude extracts of leaf and floral has showed the occurrence of almost all phytochemicals and showed good antibacterial potential against all human pathogenic bacterial strains than fruit and root Me crude extracts. The antimicrobial potential of the floral and leaf Me extracts can be endorsed to the occurrence of copious phytochemicals like glucosides, alkaloids, phenolics[18], phytosteroids and flavonoids[19] etc. Hence further extensive investigation on flowers and leaves of this plant can be recommended.

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Table-3: Zone of Inhibition of Me extracts of *Pterocarpus santalinus* L.f. plant parts.

Test Organism	Zone of Inhibition in mm (Mean ± *Std. Dev)				
	*Std. Ref	Me extracts of *Pterocarpus santalinus* L.f. plant			
	Cefixime (1µl/1ml)	Leaf (1µl/1ml)	Flower (1µl/1ml)	Fruit (1µl/1ml)	Root (1µl/1ml)
Escherichia coli	37.33 ± 0.72	28.83 ± 0.16	28.00 ± 0.28	*na	28.83 ± 0.16
β-Streptococcus pneumonia	34.33 ± 0.44	24.16 ± 0.16	24.5 ± 0.28	30.16 ± 0.44	27.83 ± 0.16
Pseudomonas aeruginosa	38.83 ± 0.16	30.00 ± 0.28	29.16 ± 0.16	na	Na
Salmonella enterica	41.83 ± 0.16	38.66 ± 0.33	37.83 ± 0.60	36.83 ± 0.16	38.50 ± 0.28

*Std. Dev = Standard deviation, Std. Ref = Standard reference, na = not active at 1µl/1ml concentration.

References

1. Mitscher, L.A. (2008). Coevolution: Mankind and microbes. *Journal of Natural Products*, 71(3), 497-509.

2. Jarvis, L.M. (2008). Imminent threat. *Chemical and Engineering News*, 86(15), 21–24.

3. Shakeri, A., Hazeri, N., Vlizadeh, J., Ghasemi, A. and Tavallaei, F.Z. (2012). Phytochemical screening, antimicrobial and antioxidant activities of *Anabasis aphylla* L. extracts. *Kragujevac J. Sci*, 34, 71-78.

4. Parimala, M. and Shoba, F.G. (2014). In vitro antimicrobial activity and HPTLC analysis of hydroalcoholic seed extract of *Nymphaea nouchali*. Burm.f. *BMC Complement Altern Me*, 14(361), 1-9.

5. Vashist, H. and Jindal, A. (2012). Antimicrobial activities of medicinal plants-review. *Int J Res Pharm Biomed Sci*, 3(1), 222-230.

6. Rosakutty, P.J. and Roslin, A.S. (2012). Isolation and characterization of an antimicrobial compound from the traditional medicinal plant *Pittosporum tetraspermum* Wight & Arn. *Int J Med Arom Plant*, 2(1), 141-150.

7. Rao, S.P. and Raju, A.J.S. (2002). Pollination ecology of the red sanders *Pterocarpus santalinus* (Fabaceae), an endemic and endangered tree species. *Currt Sci India*, 83(9), 1144-1148.

8. Dhanabal, P., Kannan, S.E. and Bhojraj, S. (2007) Protective and therapeutic effects of the Indian medicinal plant *Pterocarpus santalinus* on D-galactosamine-induced liver damage. *Asian J Trad Med*, 2(2), 51-57.

9. Bhattacharya, D., Mukherji, R., Pandit, S., Das, N. and Sur, T.K. (2003). Prevention of carbon tetra chloride induced hepatotoxicity in rats by Himoliv, a polyherbal formulation. *Indian J Pharmacol*, 35(3), 183-185.

10. Kameswara Rao, B., Giri, R., Kesavulu, M.M. and Apparao, C. (2001). Effect of oral administration of bark extracts of *Pterocarpus santalinus* on blood glucose level in experimental animals. *J Ethnopharmacol*, 74(1), 69-74.

11. Kondeti, V.K., Kameswara Rao, B., Maddirala, D.R., Thur, S.K.M., Fatima, S.S., Kasetti, R.B. and Appa Rao, C. (2010). Effect of Pterocarpus santalinus barl, on blood glucose, serum lipids, plasma insulin and hepatic carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats. *Food Chem Toxicol*, 48(5), 1281-1287.

12. Nagaraju, N., Prasad, M., Gopalakrishna, G. and Rao, K.N. (1991). Blood sugar lowering effect of *Pterocarpus santalinus* (Red Sanders) wood extract in different ratmodels. *Int J Pharmacogn*, 29(2), 141-144.

13. Mohandass, K., Suguna Devi and Devanesan. (2010). Antifungal activity of *Pterocarpus santalinus* an in vitro study. *Biomedical & Pharmacology Journal*, 3(1), 107-110.

14. Manjunatha, B.K. (2006). Antibacterial activity of *Pterocarpus santalinus*. *Ind. J. Pharm. Sci*, 68(1), 115-116.

15. Jan Hudzicki (2009). Kirby – Bauer Disk Diffusion Susceptibility Test Protocol. American *Society for Microbiology*, pp.1-23.

16. Ashrafee, T.S., Rahman, M.M., Anindita Chakraborthy and Prodhan, S.H. (2014). Antibacterial potentiality of Red sandalwood callus against pathogenic isolates of Aeromonas and Pseudomonas. *Universal Journal of Plant Science*, 2(4), 86-91.

17. Raaman, N. (2006). Phytochemical techniques. New India Publishing Agency, India, pp.19-24.

18. Mason, T.L. and Wasserman, B.P. (1987). Inactivation of Red Beet beta glucan synthase by native and oxidized phenlic compounds. *Phytochemistry*, 26(8), 2197-2202.

19. Al-Abdl N.M., Zurainee Mohamed Nor., Marzida Mansor., Fadzly Azhar., Hasan, M.S. and Mustafa Kassim. (2015). Antioxidant, antibacterial activity and phytochemical characterization of *Melaleuca cajuputi* extract. *BMC Complementary and Alternative Medicine*, 15(1), 385, 1-13.