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STUDIES ON PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIMICROBIAL STUDIES OF SANTALUM ALBUM BARK EXTRACT

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ABSTRACT

Phytochemical screening and antioxidant properties of the *Santalum album* bark extract was studied. *Santalum album* bark material was dried, powdered and used for a phytochemical screening. In the present study the photochemical compunds of *Santalum album* were identified using different extracts. Different fractions of *Santalum album* for free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) showed remarkable scavenging activity. Ethanolic extract showed the highest scavenging activity followed by Ethyl acetate. Ethanolic extract of the bark recorded the highest antimicrobial activity and achieved the highest activity index among all the extracts.

KEYWORDS: *Santalum album*, Antioxidant activity, anti-microbial activity, phytochemical screening.

INTRODUCTION

The idea of using tonic remedies to restore health and balance in a person is an ancient idea. In Ayurveda, the qualities of the chemical drugs influence the health of other tissues of the body. Hence the medicine that improves the quality of Rasayanas strengthens the health of all the tissues of the body. These Rasayana plants have the properties like strengthening life, improving the brain power, preventing age, preventing diseases and re-establishing youth[1,2] all of which imply that they increase the resistance of the body against any onslaught. According to modern medicine, traditional agents used against disorders with no pathophysiological connection and these appear to exhibit ability to combat stress related diseases. In traditional medicine several plants have been studied, which were used as medicine earlier due to their adaptogenic and rejuvenating properties.[3] Increasing

nonspecific resistance of an organism towards the influences of various origins has importance in developing analogues with anti-stress activity. The concept of an “adaptogen” was relatively a new way of explaining a type of method of curing found in traditional Ayurvedic (Rasayana), Tibetan, Chinese (Qi tonic), Cherokee and African (Manyasi) medicine. The first success on the pathway of medicinal treatment on increase of nonspecific resistance was the finding of similar properties in benzimidazol derivatives. Lazarev and his collaborators in 1958 found 2-benzylbenzimidazol (dibazol) was effective in medicine to increase nonspecific resistance to different influences and for damage to various regions in nervous system.[4,6] The theoretical basis for separation of a new group of medicinal substances was laid down by Lazarev[7, 8] phrased the concept of "a state of nonspecifically increased resistance" of the organism (SNIR). The medicinal compounds due to which SNIR occurs were named as "adaptogens.[9] In 1969, Brekhman and Dardymov explained the general pharmacological properties of adaptogenic substances.[10] Medicinal plants such as *Rhodiola rosea, Panax ginseng, Raponticum carthamoides* and *Eleutherococcus senticosus* were found to fulfill the criteria given by Brekhman and Dardymov and were called as adaptogens. These scientific studies opened a vast research area in abroad and also in India and thereafter a notified work was done on plants such as *Rhodiola rosea* and *Acanthopanax sessiliflorum*[11] from Russia, *Cicer arietinum* and *Albizzia julibrissin* and from Japan, *Codonopsis pilosula*[12] and *Panax ginseng* from China as well as *Alium sativum*[13, 14], *Phyllanthus emblica*[15], *Hoppea dichotoma*[16], *Ocimum sanctum*[17,19], *Tinospora cordifolia*[20] and *Withania somnifera*[21,23] from India. Many of these plants were selected on the basis of their therapeutic properties in Ayurvedic medicines. Herbs which were used as tonics, rejuvenators, vitalizers and restorers were thought to be capable of modulating stress related changes, might be by increasing the ability to resist stressors adapt and nonspecifically to surroundings. Many theories have been proposed to explain the effects of adaptogenic substances. Theory proposed by Dardymov and Kirkorian[24] argued that adaptogens is due to their free radical scavenging and antioxidant effects.

MATERIAL AND METHODS

PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL STUDIES OF SANTALUM ALBUM

PLANT MATERIALS

From the Ethno botanical studies *Santalum album* plant material was selected and dried, powdered and used for a phytochemical screening. Bark of *Santalum album* was selected for further studies.

PHYTOCHEMICAL INVESTIGATION

Bark of *Santalum album* was collected from Tribal areas of Anantapuram district and identified by Dr. C. Prabhakar Raju, Associate Professor and Botanist, Department of Botany, S.S.B.N Degree and P.G College, Anantapuram, India. Vocher specimen Herbarium accession No. 312 for *Santalum album* was deposited in the herbarium, Department of Botany, S.S.B.N Degree and P.G College, Anantapuram, Andhra Pradesh.

The shade dried powdered material of *Santalum album* was weighed and extracted using 50% ethanol (hydro alcoholic mixture) at 60°C and methanol at 50°C in soxhlet apparatus and distilled water (aqueous extract) at 100°C for 18 h by hot reflux extraction method. The aqueous, ethanolic and methanolic plant extracts of *Santalum album* was then filtered and concentrated using rotary vacuum evaporator. The dried plant extracts were stored in amber colored wide mouth bottles under refrigeration (4°C) and were used for phytochemical and pharmacological investigations.

To prepare ethanolic extract the plant material powder (1 kg) was soaked in ethanol (3 volumes) in a glass jar for 2 days at room temperature and the solvent was filtered. This was repeated 3 to 4 times until the filtrate gave no coloration. The filtrate was concentrated under reduced pressure in the Buchirotavapour R-200 and finally freeze dried. The yield of the extract was concentrated in a rotary vacuum evaporator to yield crude extract, which was used in bioassays. The plant extracts throughout the study were abbreviated as *Santalum album (SAET).*

Qualitative phytochemical studies

The qualitative phytochemical screenings of extracts were carried out to detect the presence of various plant constituents and Physicochemical parameters such as pH, consistency, color, Ash value and percent yield (% w/w) were determined for all plant extracts.

Detection of Phytosterols and triterpenoids

Libermann-Burchard's test

Extracts were treated with chloroform and filtered. Then few drops of acetic anhydride were added to the filtrate, boiled and cooled and then concentrated H_2SO_4 was added, shaken and allowed to stand. Formation of brown ring at the junction indicates the presence of phytosterols.

Salkowski test

Extracts were treated with chloroform and filtered. Then few drops of acetic anhydride was added to the filtrate, boiled and cooled and then conc. H_2SO_4 was added, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

Detection of Saponins

Froth test Extracts were diluted with distilled water to 20 ml and shaken for 15 min. Development of 1 cm layer of foam shows the presence of saponins.

Detection of alkaloids

Small portions of the solvent free extract were treated separately with few drops of dilute hydrochloric acid and filtered. Filtrates were tested carefully with alkaloid reagents such as Wagner's reagent (rosy chestnut ppt.), Dragandroff's reagent (Orange cocoa ppt.), Hager's reagent (yellow ppt) and Mayer's reagent (cream ppt.).

Mayer's test

Filtrates were treated with Mayer's reagent (Potassium mercuric iodide). Formation of a cream colored precipitate indicates the presence of alkaloids.

Dragendorff`s test

Filtrates were treated with Dragendorff's reagent (solution of Potassium bismuth iodide). Development of orange precipitate confirms the presence of alkaloids.

Wagner's test

Filtrates were treated with Wagner's reagent (Iodine in Potassium iodide). Development of brown/reddish precipitate specifies the presence of alkaloids.

Hager's test

Filtrates were treated with Hager's reagent (saturated Picric acid solution). Formation of yellow precipitate indicates the presence of alkaloids.

Detection of carbohydrates

Small quantity of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was confirmed for the presence of carbohydrates.

Molisch's test

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Development of the violet ring at the junction confirms the presence of carbohydrates.

Fehling's test

Filtrates were hydrolyzed with dil. HCl neutralized with alkali and heated with Fehling's A and B solutions. Development of red precipitate shows the presence of reducing sugars.

Benedict's test

Filtrates were treated with Benedict's reagent and heated gently. Red precipitate shows the presence of reducing sugars.

Detection of Flavonoids

Ferric chloride test

Extracts were treated with 3-4 drops of ferric chloride (FeCl3) solution. Development of bluish black color shows the presence of phenols.

Shinoda test (Magnesium hydrochloric acid)

Extracts were treated with few magnesium turnings and concentrated hydrochloric acid. Flavanoids produce magenta, crimson red color or occasionally green to blue color.

Lead acetate test

Extracts were treated with a few drops of lead acetate solution. Development of yellow color precipitate shows the presence of flavonoids.

Alkaline reagent test

Extracts were treated with a few drops of sodium hydroxide solution. Development of intense yellow color, which becomes colorless on addition of dilute acid, shows the presence of flavonoids.

Detection of Phenolics and Tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Development of white precipitate indicates the presence of tannins.

Vanillin hydrochloric acid test

Extracts were treated with a few drops of vanillin hydrochloric acid reagent. The formation of pinkish red color indicates the presence of phenolic compounds.

Detection of Anthraquinone Glycosides

Modified Borntrager'stest

Extracts were treated with ferric chloride ($FeCl_3$) solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was taken out and reacted with ammonia solution. Development of pink colour in the ammonical layer shows the presence of anthranol glycosides.

***IN-VITRO* EFFICACY EVALUATION**

Dried plant ethanolic extracts were dissolved in the respective solvents at the stoc0.8ml of concentration of 1mg/ml. The appropriate dilutions of the stock solutions were made and used for the *in vitro* antioxidant assays.

DPPH radical scavenging activity

To 0.1 ml of ethanolic solution of DPPH an equal volume of plant extract was added at different concentrations in methanol. Equal volume of methanol was added to control. Above mixture was kept at room temperature for 20 minutes for incubation. Absorbance was recorded at 517 nm. Scavenging capacity was calculated by monitoring the decrease in absorbance at 517 nm. The antioxidant activity of plant extract was expressed as IC50.

Percentage Inhibition was measured by using formula;

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide from sodium nitroprusside in aqueous solution at pH interacts with oxygen to generate nitrite ions, which were measured by the Griess reaction. The reaction mixture (3 ml) containing sodium nitroprusside (10mM) in phosphate-buffered saline and various concentrations of Medicinal plants OS Aqueous, hydroalcoholic and methanolic extracts at different concentrations were incubated at 25ºC for 150 min. A 0.5-ml aliquot of the incubated sample was removed and 0.5 ml Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylene di amine dihydrochloride in 2% phosphoric acid) was added. The absorbance of purple chromophore formed during diazotization of nitrite along with suphanilamide and subsequent coupling with napthylethelenediamine was measured at 546 nm.

Percent inhibition was measured by comparing the absorbance values of test samples as per the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

Antimicrobial Activity

Test Microorganisms and Growth Media

Staphylococcus aureus (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 10311) and fungal strain *Aspergillus niger* (MTCC 1785) were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Department of Microbiology, Osmania University, were used for evaluating antimicrobial activity. The fungal and bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium following refrigeration storage at 4°C. The bacteria were grown on Mueller-Hinton agar plates at 37°C, whereas the fungi were grown in dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°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 plant extracts for 5 hours were taken as test material. After 5 hours the discs were shade dried. The concentrations of plant extracts 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 antimicrobial activity

To test the antimicrobial activity, LB agar medium was prepared and the medium was sterilized at 121°c for 30 min's. 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 inoculum (containing suspension) of *P.aeruginosa* and *staphylococcus aureus* was poured on to the plates separately containing solidified agar media. The prepared sterile filter paper discs were impregnated with the extracts and shaken thoroughly and this 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 extracts were recorded.

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.

RESULTS AND DISCUSSION

The screening of *Santalum album* extracts of different solvents was performed to test for the presence of steroids, triterpenes, saponins, alkaloids, carbohydrates, flavanoids, tannins, glycosides. The phytochemical screening of *Santalum album* bark ethanolic extract revealed the presence of steroids, triterpenes, saponins, alkaloids, carbohydrates, flavanoids, tannins and glycosides, while other solvents showed negative results for some of the phytochemicals (Table 1).

In the present study, *Santalum album* of phytochemicals followed the order: ethanol extract > aqueous extract > ethyl acetate extract > hexane extract. According to the results *Santalum album* ethanolic extract (SAET) at the dose of 300 mg/kg bw exhibited maximum protective activity.

Table 1: Phytochemical Screening of Hexane, Ethyl acetate, Ethanolic and Aqueous extract *Santalum album*.

S.No	Secondary metabolites	Hexane	Ethyl acetate	Ethanolic	Aqueous
1	Steroids	+	+	+	+
2	Triterpenes	-	++	+	-
3	Saponins	+	-	+	-
4	Tri terpinoidal saponins	+	-	-	++
5	Alkaloids	+	++	+	+
6	Carbohydrates	++	+	+	+
7	Flavonoids	+	+	++	+
8	Tannins	+	+	+	+
9	Glycosides	+	++	-	+
10	Polyphenols	++	+	+	++

Different fractions of *Santalum album* for free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) showed remarkable scavenging activities in Table 2. Ethanolic extract showed the highest scavenging activity followed by Ethyl acetate. DPPH scavenging activity was significantly correlated with phenolics and flavonoids in different extracts.

Table 2: Antioxidant activity of plant solvent extracts based on their polarity.

Extracts	Concentration of extract in PPM	% of DPPH free radical Scavenging activity
Hexane	50 100 150 200 400	30% 50% 60% 65% 70%
Ethyl acetate	50 100 150 200 400	50% 60% 68% 72% 78%
Ethanol	50 100 150 200 400	55% 68% 72% 78% 85%
Aqueous	50 100 150 200 400	48% 54% 60% 64% 67%

Antimicrobial activity

The antimicrobial activity index of extracts of *Hemidesmus indicus* roots at different concentrations was also investigated and is detailed in Table 3. Ethanolic extract of the roots recorded the highest antimicrobial activity and achieved the highest activity index among all the extracts. The difference in the activity indices may be due to different phytoconstituents present in the individual extracts. This is because different solvents have different degrees of solubility for different phytoconstituents.

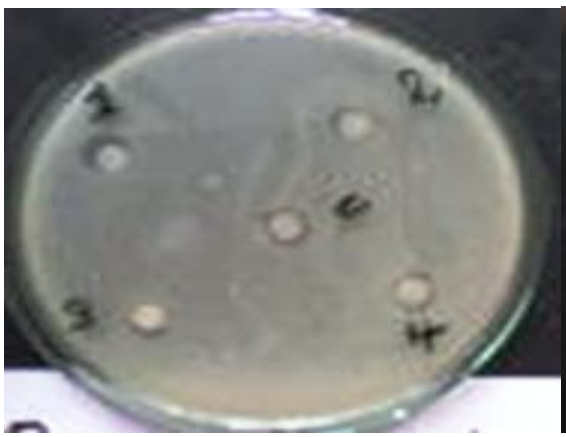

Figure 1: ***Pseudomonas aeruginosa.***

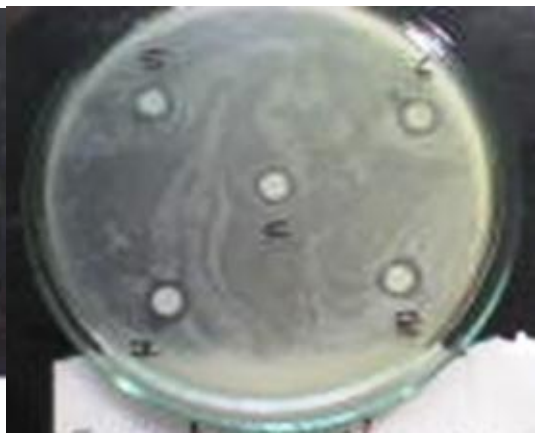

Figure 2: ***Staphylococcus aureus.***

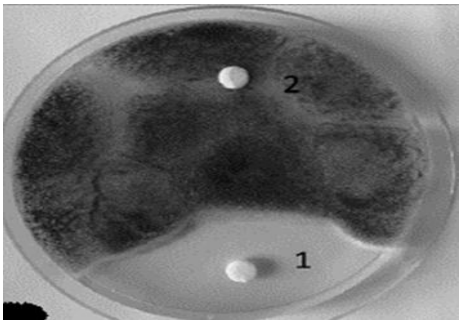

Figure 3: *Aspergillus niger.*

Table 3: Inhibitory activities of leaf extract of *Santalum album* on microorganisms.

Plants	Zone of inhibition (MIC)		
	Pseudomonas *aeruginosa* (-ve) (mm)	**Staphylococcus aureus (+ve) (mm)**	**Aspergillus niger (mm)**
Santalum album	2.4	2.6	1.5

CONCLUSION

In the present study it was found that *Santalum album bark* extract has an excellent antimicrobial activity. The pathogenic bacteria like Pseudomonas aeruginosa, Staphylococcus aureus and fungus Aspergillus niger were inhibited in presence of the leaf extracts of *Santalum album* ethanolic extract. Therefore the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant is controlling pathogenic bacteria.

REFFERENCES

1. Sharma, P. Chikitsasthana. In, Charaka Samhita, Chapter 6; Stanzas, 1983; 7,8: p3-4.
2. Ghanekar, BG. Sutrasthana. In, Sushrut Samhita, Chapter 1; Stanza, 1981; 7: p.3.
3. Rege N., Thatte U. and Dahanukar S., Adaptogenic property of six rasayana herbs used in Ayurvedic medicine. Phytotherapy Research, 1999; 13: 275.
4. Lazarev, NV., 7th All-union congress of physiology., biochemistry., pharmacology, Medgiz, Moscow, 1947; 579.
5. Rozin, MA. Medicinal remedies for restoration therapy and prophyl axis of diseases of the nervous system, Izdanie Voenno-morskoy Medicinskoy Acad., Leningrad, 1951; 51.
6. Anosov NN., Prozerin, ezerin, dibazol and their use in neuropathology Medgiz, Leningrad, 1956; 196.
7. Lazarev NV., Ljublina EI., Rozin MA., Patol. Fiziol. Eksperim. Terapia, 1959; 3: 16- 21.
8. Panossian A., Wikman G., Wagner H., Plant Adaptogens III, Earlier and More Recent Aspects and Concepts On Their Mode of Action. Phytomedicine, 1999; 6: 287-299.
9. Brekhman II., and Dardymov IV. New substances of plant origin which increase non-specific resistance. Annual Review of Pharmacology, 1969; 9: 419-430.

10. Wagner H., Norr H. and Winterhoff H. Plant adaptogens. Phytomedicine, 1994; 1: 63-76.
11. Yongxin Z, Jun D, Tao W, Haji AA Investigating the antioxidant and Acetylcholinesterase inhibition activities of Gossypium herbaceam. Molecules, 2013; 18: 951-962.
12. Singh, N., Nath, R., Lata, A. et al. Withania somnifera (Ashwagandha),a rejuvenating herbal drug which enhances survival during stress (an adaptogen). Inte national Journal of Crude Drug Research, 1982; 20: 29-35.
13. Bhattacharya SK., Goel RK., Kaur R. and Ghosal SAntistress activity of sitoindosides VII and VIII, new acylsteryglucosides from Withania somnifera. Phytotherapy. Research, 1987; 1: 32-37.
14. Ghosal, S., Lal, J., Srivastava, R. et al. Immunomodulatory and CNS effects of sitoindosides IX and V, two new glycowithanolides from Withania somnifera. Phytotherapy. Research, 1989; 3: 201-206.
15. Bhargava, KP. and Singh, N. Anti-stress activity of Ocimum sanctum Linn. Indian Journal of Medical. Research, 1981; 73: 443-451.
16. Singh, N., Verma, P., Mishra, N. and Nath, R. A comparative evaluation of some antistress agents of plant origin. Indian Journal of Pharmacology, 1991; 23: 99-103.
17. Sen, P. Therapeutic potentials of Tulsi: From experiences to facts, Drugs: News and Views, 1993; 1: 15-20.
18. Ghosal, S., Jaiswal, DD., Singh, SK. et al. Dichotosin and dichotosinin, 2 adaptogenic glucosyloxy rasayans from Hoppea dichotoma. Phytochemistry, 1985; 24: 831-833.
19. Essman, EJ. The medical uses of herbs. Fitoterapia, 1984; 55: 279-289.
20. Ushijima, M., Sumioka, I., Kakimoto, M. et al. Effect of garlic and garlic preparations on physiological and psychological stress in mice. Phytotherapy. Research, 1997; 11: 226-230.
21. Patil M., Patki P., Kamath HV. and Patwardhan B. Antistress activity of Tinospora cordifolia (Willd) Miers. Indian Drugs, 1997; 34: 211-215.
22. Katiyar CK., Brindavanam NB., Tiwari P. and Narayana, DBA. Immunomodulator products from Ayurveda: Current status and future perspectives. In, Immunomodulation, ed. by S. Upadhyay, Narosa Publishing House, New Delhi, 1997; 163-187.
23. Davydov M., Krikorian AD. Eleutherococcus senticosus (Rupr. & Maxim.) Maxim (Araliaceae) as an adaptogen: a closer look Journal of Ethnopharmacology, 2000; 72: 345–393.