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Evaluation of Preliminary Parameter and Antimicrobial Potential of *Pterocarpus Santalinus*

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Abstract: ***The study's aimed to evaluate preliminary parameter and Antimicrobial potential of Pterocarpus santalinus. Standardization using various analytical techniques was also performed. Pterocarpus santalinus was collected and studied for preliminary analysis, and anti - microbial activity along with the minimum inhibitory concentration, using the standard protocol. The antimicrobial potential was evaluated by well diffusion assay. The presence of variety of phytoconstituents was discovered during phytochemical analysis. The results of TPC and TFC of hydroalcoholic extract was 0.935mg/gm and 0.900 mg/gm respectively. Antimicrobial results indicated that at 100µg/ml the extract showed zone of inhibition of 11.2mm and 12.4 mm against bacillus subtilis and P. aeruginosa bacteria respectively with the MIC value of 0.154nm and 0.171nm respectively at 50µg/ml which was comparable with the standard drugs. The findings of this study may be useful in establishing botanical and analytical grades for the root of Pterocarpus santalinus.***

Keywords: *Pterocarpus santalinus.;* cold maceration extraction; phytoconstituents; antimicrobial; Minimum inhibitory concentration

1. Introduction

Plants as natural products are a valuable source of bioactive compounds and have been used for medicinal purposes in all over the world. Recently, scientific attention to oriental medicine has increased in context of the discovery of novel drugs for treating various diseases including cancer and diabetes. (1) (2) The World Health Organization (WHO) endorses the evaluation of the potential benefits of plants as effective therapeutic agents, especially in areas where there is a lack of safe modern drugs. (3) One - third of total drugs (35%) in the USA and 80% of drugs used in fast - developing countries such as China and India are derivatives of phytoextracts. (4) (5) India has a rich heritage of medicinal plants of wide diversity, which are used by the local population and traditional healers for the treatment of several diseases. One such plant is *Pterocarpus santalinus*, which is widely used for the treatment of various ailments due to its extensive medicinal properties.

P. santalinus is a small - to - medium–sized deciduous tree belonging to the Fabaceae family. It is widely distributed in the tropical regions of the world, especially in India, Sri Lanka, Taiwan, and China. Earlier reviews explored the phytochemistry, pharmacology, and ethnomedicinal values of *P. santalinus*. (6) (7) Bioactive compounds present in the plant's heartwood have been shown to have a wide range of biological activities, suggesting the potential of *P. santalinus* for the treatment of various diseases. *In vitro* and *in vivo* studies showed that the heartwood and bark have exhibited antidiabetic, antioxidant, anti - inflammatory, and hepatoprotective activities. (8) (9) In Ayurveda, an Indian system of traditional medicine, it is mentioned that the heartwood of the plant is used as external application for treating inflammation, diabetes, headache, skin diseases, and jaundice, and in wound - healing. (10)

Antibacterial drugs are the greatest contribution of the 20th century in developing countries, where infective diseases predominate. With advancement in medicinal chemistry, a wide range of natural, semi synthetic and synthetic antimicrobials are available for treating infectious diseases in human and animal. Natural antibacterial agents are called as ‘antibiotics’. These are substances produced by microorganisms, which suppress the growth or kill other microorganisms at very low concentrations. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. (11) Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs.

Despite the availability of a range of synthetic antibiotics, the infectious diseases continue to be a major health problem worldwide. The development of widespread antibiotic resistance among pathogens and undesirable side effects associated with the continued use of synthetic drugs has stimulated a renewed interest in the alternative therapeutics. Therefore, it is essential to search substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. To overcome the problems associated with antimicrobials, medicinal plants would be the best source to obtain a variety of drugs as an alternative treatment for diseases. (12) Therefore, the study was focused on the extraction, phytochemical screening and antimicrobial activity of the leaves of *P. santalinus extract*.

2. Materials and Methods

Chemicals and Reagents

Methanol, ethanol and Petroleum ether were procured from Qualigens Fine Chemicals, Mumbai, India. Follinciocalteu’s reagent, Ascorbic acid and quercetin were procured from Loba chemie Pvt. Ltd., Mumbai. All of the other reagents, solvents, and chemicals used were laboratory grade.

Plant Material

The leaves of *Pterocarpus santalinus* were collected from Govt. Home Science College Hoshangabad (Botany

Department). The leaves of the *Pterocarpus santalinus* were washed, shade dried and powdered. The specimen was authenticated by submitting it to the Saifia Science College, Bhopal, Madhya Pradesh. authenticated the entire plant in the lab of Saifia Science College, Bhopal and voucher no. is 146/Saif. /sci/clg. /Bpl.

Extraction

Cold Maceration

Crude material of plant was extracted by using cold maceration method; plant samples were collected, washed, and dried properly. Dried powder of plant sample (150 gms) were extracted with hydroalcholic solvents (70: 30: Methanol: Water) and pet. ether and allow to stand for 4 - 5 days each. The extract of the plant was filtered to remove all unextractable matter. Extract was transferred to beaker and evaporated; excessive moisture was removed and extract was collected in an air tight container. (13) Extraction yield of all extracts were calculated. **[Table 1]**

Qualitative Phytochemical Estimation of Extracts

Phytochemical screening was performed on the extracts of the leaves of *Pterocarpus santalinus* (obtained in solvents such as methanol: water) to determine the presence of phytochemicals such as carbohydrates, alkaloids, flavonoids, glycosides, proteins & amino acids, saponins, triterpenoids and steroids, tannins and other phenolic compounds. [**Table 3]** These plant extracts were obtained using Cold Maceration extraction techniques. To identify the constituents in the plant extracts, specific qualitative phytochemical tests were performed. (13)

Quantitative Phytochemical Estimation

Total Phenolic Contents

The total phenol content of the extract was determined by the modified folin - ciocalteu method.10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10 - 50µg/ml was prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.2 ml of extract and each standard was mixed with 1 ml of Folin - Ciocalteu reagent (previously diluted with distilled water 1: 10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer. (14)

Total Flavonoid Content (TFC) Estimation

Determination of total flavonoids content was based on aluminium chloride method.10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5 - 25µg/ml were prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm. (15)

Anti - Microbial Activity

Well Diffusion Method

Well diffusion method was used for the determination of antimicrobial activity of the extract. For the preparation of nutrient Media, 28 g of nutrient agar media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121ºC at 15 lbs pressure for 15 minutes. After sterilization, media was allowed to be cool but not solidify. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

Culture of bacterial strains was spread on the nutrient agar media (NAM). Then 1ml of test sample (*Pterocarpus santalinus* extract) was taken directly from the stock. The drug Amoxicillin and Ofloxacin 10mg/10ml was used as a reference drug. After inoculation of test plates, plates were punched to make the well of 6mm diameter with the help of sterile cork borer. Four wells were made in each plate. Four different concentrations of each extract, 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml is poured into the well in assay plates. The other well was filled with standard antibiotic. The plates were incubated for 18 - 24 hours at 37°C, and all plates were observed for the zone of inhibition; diameter of these zones was measured in millimetres by using ruler. (12) (16) The zone for inhibition was measured in millimeters and compared with standard drug. [Table 5]

Minimum Inhibitory Concentration (MICs)

Determinations of the MICs of test sample (*Pterocarpus santalinus* extract) against the strains above were determined by broth dilution using two fold serial dilutions in a Nutrient Broth medium. Firstly two - fold serial dilution of plant extracts were prepared in sterilized test tube beginning from 1: 1 undiluted and 1: 2, 1: 4, 1: 8, 1: 16, 1: 32, 1: 64, 1: 128, 1: 256, 1: 512. Then the bacterial inoculums of one gram positive *(Bacillus subtilis*) and one gram negative (*Pseudomonas aeruginosa*) was prepared by adjusting the turbidity to 0.5 McFarland. Further dilution of 0.5 McFarland suspensions was carried out 1: 10 with sterilized nutrient broth to obtain an inoculum of 10^8 CFU/ml. The MIC was carried out in ten sets of sterile test tubes. Briefly, the stock solution of test sample (*Pterocarpus santalinus* extract) was prepared in methanol and ethyl acetate to ensure complete solubilisation in a concentration of 1 mg/ml. A total of 3 ml of Nutrient Broth was dispensed in 1st test tubes to test tube 10. Test sample (*Pterocarpus santalinus* extract) (300 µl) was added to the first tube. The solution was serially diluted from tube 1 to tube 10 while 300 µl was discarded from tube 10.200 µl of bacterial suspension was added to all dilution ranges from tube 1 to tube 10. Overnight bacterial suspension (200 µl) was dispensed in tube 11 and 3 ml of sterile broth was added to serve as positive control or growth control while 3 ml of sterile Nutrient Broth in tube 12 served as (negative control or sterility control). The tubes were incubated at 37°C for 24 h. After incubation, absorbance of each tube was measured using UV - Visible spectrophotometer (Systronics 2202) at 640nm wavelength. The aforementioned procedure was performed for each microbial strain. The concentration of sample and standard which inhibited 50 percent of bacterial growth was determined for all microorganisms. [Table 6]

3. Results and Discussion

Percentage Yield

The % yield of crude extracts ranged from 0.260 to 10.97 percent, depending on the solvent. When the % yields of the materials with two different solvents were compared, hydroalcoholic extract gave the highest yield (10.97 percent), while pt. ether provide 0.260 percent. [**Table1]**.

Table 1: Extractive values of *Pterocarpus santalinus*

S. No.	Extracts	% Yield* (W/W)
1.	Pet. Ether	0.260%
2.	Hydroalcoholic	10.97%

Qualitative Analysis of Phytochemicals

The phytochemical composition of different *Pterocarpus santalinus* leaves extract was evaluated using qualitative tests. The presence of phytochemicals can be seen in the extracts. [**Table2]**.

Curves generated from the area under the peak in TPC assays were used to obtain linear equations and linear regression (R^2) of various concentrations of standards. When the secondary metabolites in different extracts were counted, it was discovered that hydroalcohalic extracts had the highest number of secondary metabolites The number of SMs in extract of pet. Ether extract and hydroalcoholic extract is listed below in table 2.

Table 2: Result of phytochemical screening of extract of *Pterocarpus santalinus*

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Hydroalcoholic extract
Alkaloids		
Dragendroff's test	Absent	Absent
Mayer's reagent test	Absent	Absent
Wagner's reagent test	Absent	Absent
Hager's reagent test	Absent	Absent
Glycoside		
Borntrager test	Absent	Absent
Killer - Killiani test	Absent	Absent
Carbohydrates		
Molish's test	Absent	Absent
Fehling's test	Absent	Absent
Benedict's test	Absent	Absent
Barfoed's test	Absent	Absent
Flavonoids		
Shinoda's Test	Absent	Present
Tannin and Phenolic Compounds		
Ferric Chloride test	Absent	Present
Gelatin test	Present	Present
Saponin		
Froth test	Present	Present
Test for Triterpenoids and Steroids		
Salkowski's test	Present	Present

Qualitative phytochemical screening of the leaves extract revealed that alkaloids, carbohydrates and tannins were absent in both pet. Ether and hydroalcoholic extracts. Flavonoids and tannins were present in hydroalcoholic extract. Saponnins and tri - terpinoids are present in both hydroalcoholic and pet ether extracts.

Quantative Analysis of Phytochemical

Total Phenolic Content

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$y = 0.014x + 0.004$, $R^2 = 0.999$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table 3: Preparation of Calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	10	0.145
2	20	0.302
3	30	0.442
4	40	0.569
5	50	0.721

(n=3, Mean±SD)

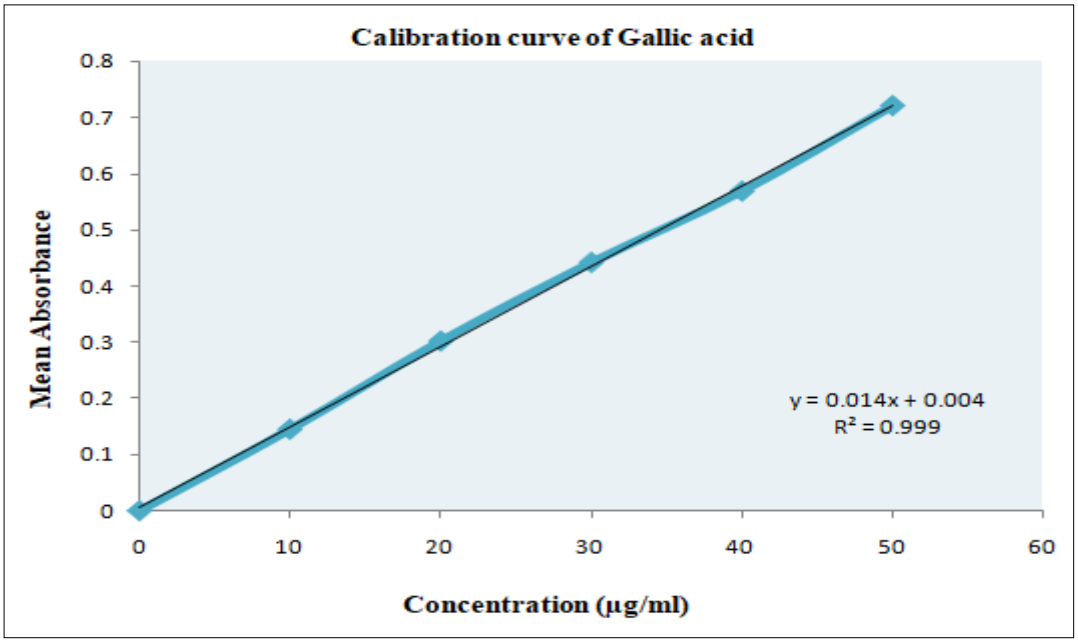


Figure 1: Graph of calibration curve of Gallic acid

Total Flavonoids Content

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **$y = 0.021x + 0.008$, $R^2=0.999$**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 4: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.123
2	10	0.225
3	15	0.337
4	20	0.44
5	25	0.541

(n=3, Mean±SD)

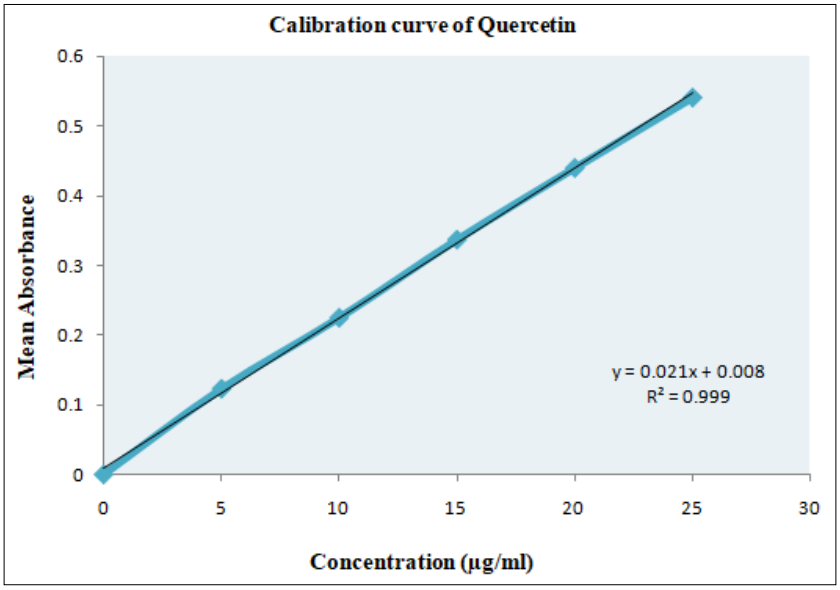


Figure 2: Graph of calibration curve of Quercetin

Table 5: Estimation of total phenol and flavonoids content of *Pterocarpus santalinus*

S. No.	Extract	Total phenol content	Total flavonoids Content
1.	Hydroalcoholic	0.935mg/ 100 mg	0.900mg/ 100 mg

Antimicrobial Potential

Well Diffusion Method

The extract were prepared into (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml) concentration and they were subjected to antimicrobial test using diffusion method in presence of an antibiotic (Amoxicillin and Ofloxacin 100mg/10ml) in two bacterial strains *B. subtilis* and *P. aureoginosa*. The results of antimicrobial test are given in table 6. At 100 µg/ml, the extract showed best zones of inhibition of 11.2 mm and 12.4 mm in diameter against gram positive *B. subtilis* and gram negative *P. aureoginosa* bacteria compared to standard drug (Amoxicillin) which showed 28.2 mm zones of inhibition in diameters against gram positive bacterias and Ofloxacin which showed 32.4 mm zones of inhibition in diameters against gram negative bacterias. The solvents used for solubility purpose not exploited any antimicrobial activity.

Table 6: Results of antimicrobial activity of *Pterocarpus santalinus* extracts.

Sample	Concentration (µg/ml)	Zone of inhibition in diameter (mm)	
		Bacillus subtilis	*P. aureoginosa*
Extract	25	0	0
	50	0	0
	75	0	6.5
	100	11.2	12.4
Reference standard		Gram positive	Gram negative
	Amoxicillin	28.2	
	Ofloxacin		32.4

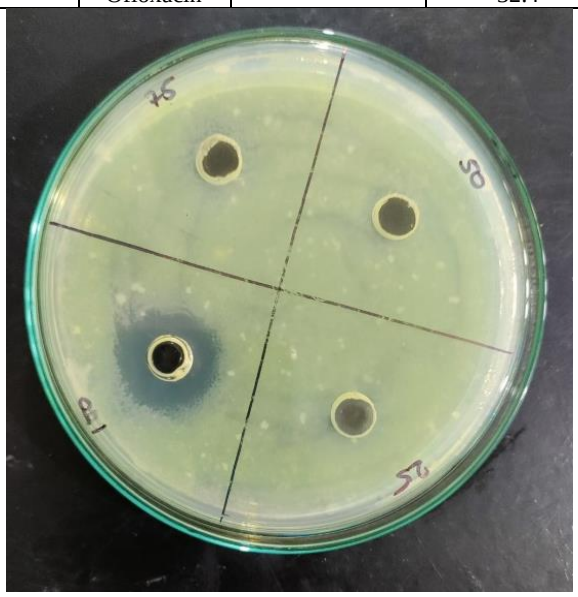


Figure 3: Antimicrobial activity of *Pterocarpus santalinus* extract against *gram positive bacteria (Bacillus aubtilis)*

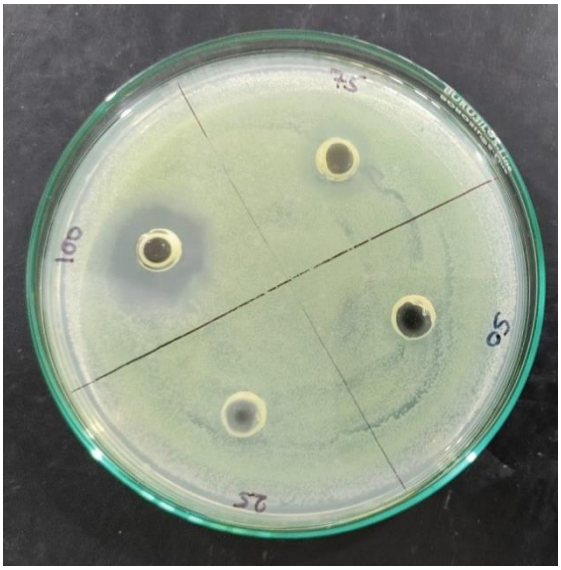
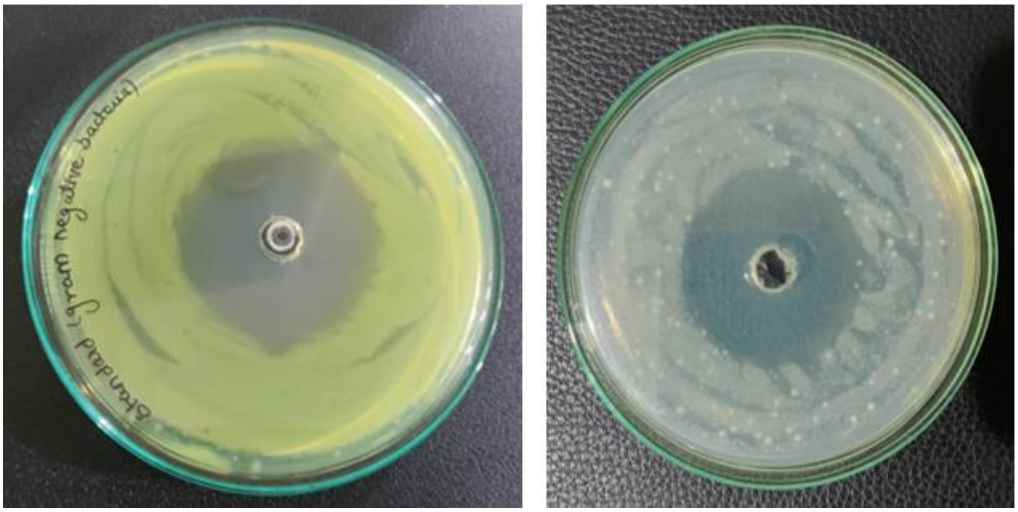


Figure 4: Antimicrobial activity of *Pterocarpus santalinus* extract against *gram negative bacteria (P. aureoginosa)*



(a) **(b)**

Figure 5: (a) Antimicrobial activity *of* standard drug against Gram negative (b) Antimicrobial activity standard drug against Gram positive

Minimum Inhibitory Concentration

As can be seen in table 7, different concentration (100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.12µg/ml, 1.56µg/ml, 0.78µg/ml, 0.39µg/ml and 0.19µg/ml) of extracts were investigated to determine their MICs against test microorganisms. The Minimum Inhibitory Concentration of phytosomes against *B. subtilis* and *P. aeruginosa* observed at 50µg/ml is 0.154 and 0.171 respectively. The Minimum Inhibitory Concentration of standard Ofloxacin and Amoxicillin was found to 0.191 and 0.161 at 0.39 and 0.19 µg/ml against *B. subtilis* and *P. aeruginosa* bacteria which is comparable to the plant extract

Table 7: Results of minimum inhibitory concentration of *Pterocarpus santalinus* extracts against gram positive and gram negative bacteria

Formulation		Minimum Inhibitory Concentration (µg/ml)	
		Bacillus subtilis	*P. aeruginosa*
Extract		0.154	0.171
Reference standard		Gram positive	Gram negative
	Amoxicillin	0.191	
	Ofloxacin		0.161

4. Conclusion

The cold maceration method was used to obtain extract of the leaves of the plant *Pterocarpus santalinus*. The yield of compounds was higher in the polar solvent (hydroalcoholic), and the probable reason may be a higher concentration of polar constituents. The total polyhenolic and TFC of hydroalcoholic extract were determined. In general, more polar solvents had higher TPC and TFC values. Based on the observations, in - vitro anti - microbial activity of plant extract against gram positive bacteria (*Bacillus subtilis)* and gram negative (*P. aeruginosa*) bacteria was found to be 11.2 mm and 12.4 mm respectively at 100 µg/ml. The minimum inhibitory concentration is the lowest concentration if an antimicrobial agents like plant extract or bacteriostatic or bactericidal or an antifungal agent that inhibit the growth of microbial pathogen after overnight incubation. The MIC of extract is 0.154nm and 0.171nm respectively at 50 µg/ml, compared with the standard Amoxicillin and Ofloxacin which was found to be 0.191 and 0.161 at 0.39 and 0.19 µg/ml against *B. subtilis* and *P. aeruginosa* bacteria. It is concluded that *Pterocarpus santalinus* leaves are proven to

be a very useful medicinal plant as an antibacterial agent and showed antimicrobial activity even at low concentration. Other pharmacological properties and its mechanisms of action of *Pterocarpus santalinus* leaves are yet to be explored.

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