International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.5, pp 353-359, 2016

Phytochemial and Antimicrobial Studies of the Methanol Extract and Less Polar Solvent Fractions of *Pterocarpus santalinoides* Leaves

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Abstract : The methanol extract of *Pterocarpus santalinoides* leaves were investigated for phytochemical studies and antibacterial assay using *Staphylococcus aureus* and *Escherichia coli.* . The leaves were extracted with 7.0 L methanol by cold maceration at room temperature for seven day sand the extract used for the phytochemical analysis and antibiotic assay. Phytochemical analysis of the methanol extract revealed the presence of alkaloids and terpenoids in high abundance; while carbohydrates are in moderate abundance. Saponins and tannins are present in little abundance. The antimicrobial assay of the Methanol Extract of the n-hexane fraction showed that the extract could not inhibit the growth of *Escherichia coli* only. The Minimum Inhibitory Concentration of the Methanol Extract of n-hexane fraction against *Staphylococcus aureus is* 4.58 mg/mL, and the Minimum Inhibitory Concentration of the Methanol Extract of $CHCl_3$ fraction against *Staphylococcus aureus and Escherichia coli* are 2.9559 mg/mL and 2.7478 mg/mL respectively.
Keywords: *Pterocarpus santalinoides;* methanol extract; minimal inhibitory concentration, E. coli: *Escherichiacoli.* S. aureus: *Staphylococcus aureus.*

1.0. Introduction

Local herbalists have depended on medicinal plants as a reliable means of treating ailments[1]. Medicinal plants could heal or minimize diseases infections or sufferings[2,12-20]. Locally known as **utụrụkpa,** the fresh leaves of *Pterocarpus santalinoides* are consumed locally as vegetable in soups by the Igbos of South Eastern Nigeria and are reputed to be useful in the treatment of diarrhea and other gastrointestinal disorders[3]. Medicinal plants were known to be the precursor to modern medicine[4]. Herbal remedies are obtained from a wide variety of natural resources including plant leaves, bark, berries, flowers, and roots[5]. Herbal medicine remains a popular alternative throughout China and the Far East, and is growing in popularity throughout the United States[6]. Many of these indigenous medicinal plants are used as spices and food[7]. They are also sometimes added as supplements to food given to pregnant and nursing mothers for medicinal purposes[8]. Medicinal plants are of great importance to the health of individuals and communities[9]. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body[10]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds[11]. This study investigates the fundamental scientific bases for the use of a Nigeria medicinal plant (*Pterocarpus santalinoids*) by defining and quantifying the percentage of crude phytochemical constituents present in this plant that can aid curing.

Materials and Methods

2.1 Plant Collection

The leaves of *Pterocarpus santalinoides* were collected at Ngwuru, Nsukka Local Government Area in Enugu state of Nigeria between the months of November and December and authenticated in Department of Botany of university of Nigeria, Nsukka.

Fig. 1: Sample of plants collected

2.2 Preparation of Plant Material

The leaves were air-dried and pulverized using Thomas-Wiley Laboratory Mill, Model 4. The powdered leaves (1000 g) were extracted with 7.0 L of methanol by maceration at room temperature for seven days. The extract were filtered with chess cloth, and then filtered again with filter paper to obtain clear filtrate. The clear filtrate was concentrated with a rotary evaporator and stored in the refrigerator throughout the experiments.

2.3 Phytochemical Screening of the Methanol Extract

Phytochemical analysis of the plant material, extract and fractions was performed using standard methods. They were screened for the presence of the following:

Alkaloids: A little quantity of the sample was boiled with 15 mL of 5 % H_2SO_4 in ethanol. The mixture was cooled and filtered. To a 2 mL of the filtrate was added 2 drops of Dragendorff's reagent. Formation of reddish-brown precipitate indicated the presence of alkaloids.

Tannins: A little quantity of the sample was boiled with 50 mL of water and filtered. Few drops of ferric chloride solution were added to 5.0 mL of the filtrate. A greenish-black precipitate observed indicates the presence of tannins.

Saponins: A little quantity of the sample was extracted with about 2.0 mL of distilled water by boiling in a water bath for 2 minutes. It was filtered and the extract (2.0 mL) was diluted with water and shaken vigorously. Frothing which persisted on warming indicates the presence of saponins.

Carbohydrates (Molisch's Test): A little quantity of the sample was shaken with water and filtered. To 2.0 mL of the filtrate in a test-tube was added two drops of α-naphthol solution followed by 1.0 mL of concentrated H_2SO_4 to form two layers. A colour change to brown at the interface of two layers indicates the presence of carbohydrates.

Steroids: A little quantity of the sample was refluxed for few minutes and filtered. The filtrate was concentrated to 2.5 mL by boiling in a water bath and then followed by addition of 5.0 mL of hot water. The mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate was then extracted with 2.5 mL of chloroform. To 0.5 mL of the chloroform extract in the test tube was added 1.0 mL of concentrated H_2SO_4 to form two layers. A reddish-brown at the interface shows the presence of steroids.

Terpenoids (Salkowski test): 5.0 mL of each extract was mixed in 2.0 mL of chloroform, and concentrated H_2SO_4 (3.0 mL) was carefully added to form a layer. A reddish brown colouration of the inter face showed positive results for the presence of terpenoids.

Antibiotic Assay

The Minimum Inhibitory Concentration (MIC) was determined using agar diffusion method.

Antimicrobial Activity

100 mg of extract was dissolved with 2.0 mL of DMSO or Tween 80 to get 50 mg/mL concentration which served as stock. 1.0 mL of the stock was taken and serially diluted in double dilution up to 6 tubes.

-Nutrient Agar was prepared according to manufacturers direction.
-MacFarland standard of 24 hours *Escherichia coli* and *Staphylococcus aureus* was prepared with sterile normal saline.

0.1 mL of each organism was smeared over the face of its respective nutrient agar plate. Sterile cork borer was used to bore hole on the nutrient agar plate. The diluted extract was put into its respective hole on the agar plate and was allowed to stay on the bench for prediffusion time of 1 hour and incubated for 24 hours. The zone of inhibition was measured and the inhibition zone diameter was recorded.

3.0. Results and Discussion

3.1 Results

Table 3.1: Results of Phytochemical Assay of the Methanol Extract of *Pterocarpus santalinoides*

Phytoconstituents	Relative Abundance
Alkaloids	+++
Tannins	+
Saponins	+
Glycosides	-
Carbohydrates	++
Steroids	-
Resins	-
Proteins	-
Acidic compounds	-
Flavonoids	-
Terpenoids	+++
Reducing sugars	-
Fats & Oils	-

Keys: +++ = Abundantly present; ++ = Moderately present; + = Present; - = Absent

Table 3.2: Result of MIC of methanol extract of *Pterocarpus santalinoides* (n-Hexane fraction) against some bacteria at a concentration of 50mg/mL

Organism	***Mean IZD (mm)* ± *SEM***						
Concentration (mg/mL)	50	25	12.5	6.25	3.125	1.5625	0.78125
Staphylococcus aureus	3.02 ± 0.33	2.12 ± 0.57	2.02 ± 0.33	1.51 ± 0.33	-	-	-
Escherichia coli	-	-	-	-	-	-	-

Key: - = No activity or growth not inhibited.

Table 3.3: Logarithm of concentration of the methanol extract of *Pterocarpus santalinoides* (n-Hexane fraction) and mean IZD squared.

	Mean IZD^2 (mm)						
Organisms	1.69897	1.39794	1.09691	0.79588	0.49485	0.19382	-0.10721
Staphylococcus aureus	10.24	4.41	4.2	2.28	-	-	-
Escherichia coli	-	-	-	-	-	-	-

Table 3.4: Result of MIC of methanol extract of *Pterocarpus santalinoids* (CH_3Cl fraction) against some bacteria at a concentration of 50 mg/mL

	Mean IZD (mm)* ± *SEM						
Organism	50	25	**12.5**	**6.25**	3.125	1.5625	0.78125
Staphylococcus aureus	4.15±0.33	4.14±0.33	5.1±0.00	4.51±0.57	4.05±0.33	4.01±0.33	5.05±0.57
Escherichia coli	2.1±0.33	3.05±0.57	2.01±0.00	0	0	0	0

Key: - = No activity or growth not inhibited.

Table 3.5: Logarithm of concentration of the methanol extract of *Pterocarpus santalinoides* (CH_3Cl fraction) and mean IZD squared.

	Mean IZD^2 (mm^2)						
Organism	1.69897	1.39794	1.09691	0.79588	0.49485	0.19382	-0.10721
Staphylococcus aureus	17.2225	17.1396	26.01	20.3401	16.4025	16.0801	25.5025
Escherichia coli	4.41	9.3025	4.0401	-	-	-	-

Keys: - = no microbial activity

Table 3.6: MIC of Amoxicillin sodium (IZD)

	Conc. (mg/mL)/IZD (mm)				
Organism	4	0.4	0.2	0.05	0.025
S. aureus	30	29	27	25	23
E. coli 23	21	20	10	+	

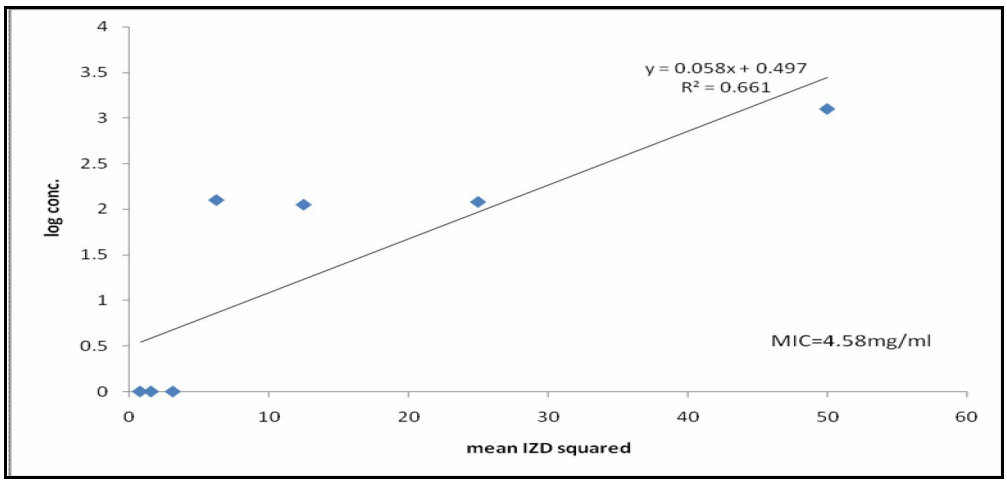

Fig. 3.01: Antimicrobial assay of *Pterocarpus santalinoides* (n-hexane fraction) against *Staphylococcus aureus.*

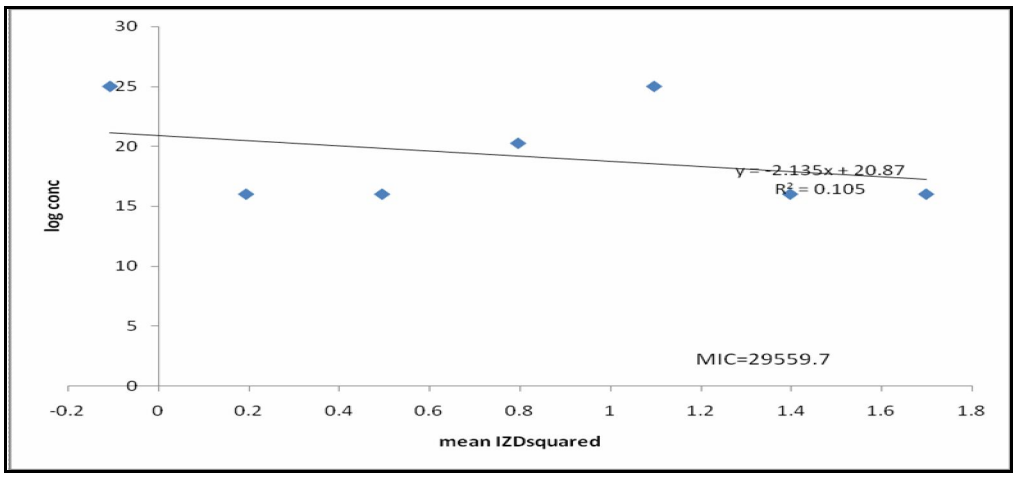

Fig. 3.02: Antimicrobial assay of *Pterocarpus santalinoides* ($CHCl_3$ fraction) against *Staphylococcus aureus*.

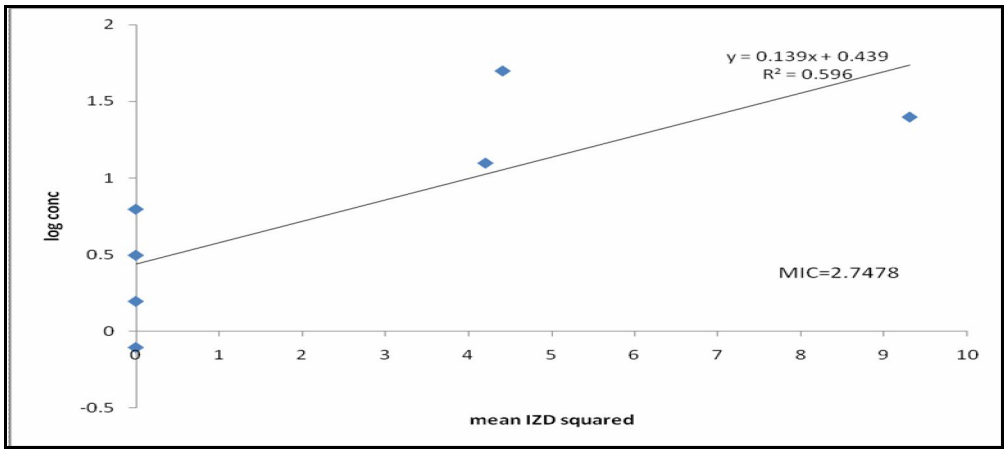

Fig. 3.03: Antimicrobial assay of *Pterocarpus santalinoides* ($CHCl_3$ fraction) against *Escherichiacol*

3.2 Discussion

Phytochemical studies of the methanol extract of *Pterocarpus santalinoids* revealed that the crude extract contained alkaloids, tannins, saponins, carbohydrates and terpenoids.

Table 3.1; Alkaloids were found to be abundantly present and are known to have physiological properties e.g. lowering of human blood pressure. The extract showed appreciable anti-microbial activities on bacteria indicating that *Pterocarpus santalinoides* is rich in terpenoids which is of great medicinal importance. Carbohydrates were moderately present whereas tannins, saponins were found to be present.

Tables 3.2; The results on sensitivity tests reveal that the less polar solvent fraction (n-Hexane fraction) of *Pterocarpus santalinoides* has broad spectrum anti-bacterial activity at the various concentrations except at concentrations of 3.125 mg/mL, 1.5625 mg/mL and 0.78125 mg/mL respectively. The fraction had a broader activity on *S. aureus bacterium* at a concentration of 50 mg/mL. The broad spectrum is observed where the IZD is about 3.02 mm at 50 mg/mL and its MIC is observed to be 4.58mg/mL from **fig. 3.01,** whereas it is observed that this fraction showed no activity against *E. coli*.

Table 3.3; The results on sensitivity tests reveals that the less polar solvent fraction (CH_3Cl fraction) of *Pterocarpus santalinoides* has broad spectrum anti-bacterial activity at the various concentrations. The fraction had a broader activity on *bacteria* at a concentration of 50 mg/mL. The broader spectrum is observed with *S. aureus* where the IZD is about 4.15 mm at 50 mg/mL and from **fig. 3.02,** the MIC is observed to be 29559.7 mg/mL showing that a much larger amount of the fraction is required to inhibit the growth of the bacteria and lesser spectrum activity is observed against *E. coli* which inhibited the organism at IZD of about 2.1 mm at a concentration of 50 mg/mL, but showed no anti-bacterial activity at concentrations of 6.25 mg/mL, 3.125 mg/mL, 1.5625 mg/mL and 0.78125 mg/mL. The MIC is observed to be 2.7478 mg/mL from **fig. 3.03.**

Table 3.4; shows the result of the MIC of the control drug [Amoxicillin sodium], it was observed to show a very good broad spectrum activity against bacteria. This can be attributed to the pure form of the drug as compared to that of the less polar solvent fraction of *Pterocarpus santalinoides* which showed reduced spectrum activity against bacteria.

From all these information (phytochemical results and the antimicrobial assay results) put together; we can justify the use of the leaves by traditional medicine practitioners in curing diseases and other medical purposes.

4.0 Conclusion

The claims of the use of *Pterocarpus santalinoids* in ethnomedicines can be justified by the fact that it contains important therapeutic phytochemicals and shows broad spectrum activities against bacterial micro-organisms. Moreover, owing to the results in (**Tables 4.1, 4.2 and 4.4**) and the natural form of the extract, we can draw a conclusion that this plant has improved physiological effects, less toxological effects and fewer side effects to man as compared to other synthetic drugs and the leaves can be used in curing some bacterial infections.

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