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Phytochemical and Antimicrobial Constituents of the Leaf Extracts of *Pterocarpus Santalinoides* (Nturukpa), a Nigerian Common Food and Herb

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Abstract: Plants have formed the basis of traditional medicine system which has been used for thousands of years. In Nigeria *Pterocarpus santalinoides* is used as food as well as medicine. The study was carried out to assess the antimicrobial potential and phytochemical composition of Nturukpa (*Pterocarpus santalinoides*) leaf extracts on selected test organisms. Fresh leaf samples were collected and screened using standard methods for phytochemicals using methanol and aqueous solvents. Antimicrobial activity of two extracts (Methanol and Aqueous) was performed using Agar well diffusion method against the selected test organisms at different concentrations. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal/Fungicidal concentration (MBC/MFC) of the extracts were also carried out. Phytochemical analysis indicated the presence of alkaloids, tannins, flavonoids, proteins, glycoside, saponin, steroid, terpernoid and phenol in both extracts. The methanolic extract demonstrated a higher antimicrobial activity than the aqueous extract. The zones of inhibition for the methanolic extract of the leaf range from 11.00mm – 23.67mm while the zones of inhibition for the aqueous extract of the leaf range from 6.00mm – 16.33mm. The Minimum Inhibitory Concentration (MIC) for the aqueous extract was 50% for all test organisms but had no Minimum Bacteriocidal and Fungicidal concentration (MBC/MFC) effect while the MIC for the methanolic extract on the test organism was at 25% concentration except for *Staphylococcus aureus* that was at 50% concentration and its Minimum Bactericidal and Fungicidal Concentration was at 50% for *Escherichia coli* and *Candida albicans.* Statistical test using ANOVA showed that there was significance difference ($P<0.05$) in the zones of inhibition at different concentration between the Methanolic and Aqueous extract of the leaf. The results of the investigation clearly indicated that *Pterocarpus santalinoides* leaf extracts have a potential antimicrobial activity against various microorganisms due to the presence of various phytochemicals. The demonstration of antimicrobial activity of *Pterocarpus santalinoides* leaf against the test organisms is an indication that there is possibility of sourcing alternative antibiotic substances in this plant for the development of newer antimicrobial agents and further studies should be carried out on the safety and efficacy of this plant extract to establish whether they can offer therapeutic benefit alone or in synergy with other conventional antimicrobials.

Keywords: Phytochemical analysis, *Pterocarpus santalinoides,* Antimicrobial.

1. Introduction

Nigeria is blessed with many medicinal plants whose roots, barks, seeds and leaves are used for the treatment of many diseases. Medicinal plants are plants which contain substances that can be used

for therapeutic purposes or which are precursors for the synthesis of useful drugs. Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care (Akinyemi, 2000). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on or in the human body.

Pterocarpus santalinoides belongs to the family *Fabaceae*, it is commonly known as Nturukpa (Igbos); gunduru or gyadar kurmi (Hausa); maganchi (Nupe); ikyarakya or kereke (Tiv); gbengbe (Yoruba); okumeze (Edo); nja (Efik) (Kaey, 1989) and ugbam piegwu or uturukpa (Igede) (Igoli, 2003). The antidiarrheal activity of this plant has been investigated in an attempt to verify its traditional medical management of diarrhoea (Nworu *et al.*, 2009; Anowi *et al.*, 2012a; Eze *et al.*, 2012).

The plant species of the genus *Pterocarpus* have been shown to produce valuable phytochemical classes including flavonoids, isoflavonoids, pterocarpans, aurones, lignans, stilbenes, sterols, triterpenes and sesquiterpenes, which are known for their effectiveness in treatment of certain diseases. The isoflavonoids, a major phytochemical class in *Pterocarpus* genus, have multi-biological activities on cell functions, including activation of estrogen receptors, anti-inflammatory, chemopreventive, antioxidant, antiproliferative, antihemolytic, xenobiotic metabolism modulator (Medjakovic *et al.*, 2010; Arora *et al.*, 1998). Another important phytochemical class in this genus is the pterocarpans, which has antiviral, cytotoxic, antimitotic activities (Falcao *et al.*, 2005). These extracts inhibited growth of test organisms, and implies antimicrobial activity on *Escherichia coli, Salmonella typhi, Staphylococcus aureus and Candida albicans*.

Phytochemical screening of *Pterocarpus santalinoides* revealed that it has a high level of alkaloids among other phytochemical components which includes; tannin, steroid, flavonoid, carotenoid and anthocyanin. (Onyeka and Nwambekwe, 2007).

In Nigeria, *Pterocarpus santalinoides* (Nturukpa) is used as food and medicine. The tender leaves are used as vegetable in preparing soup while the stem bark is used in making pepper soup. The plants are used for treating rheumatism, diarrhoea, dysentery, cough, asthma, diabetes, malaria, elephantitis, cold and others, (Okwu and Ekeke, 2003). The use of the leaves in treating skin diseases such as eczema, candidiasis, and acne have been reported (Adetunji, 2009). The use of the concoction made from its root in treating asthmatic patients have also been reported (Igoli *et al.*, 2005). The stem bark extract is also used in treatment of cough and diabetes. The leaves are used in veterinary medicine to reduce abdominal pain in goat and also menses in female (Igoli *et al.*, 2005; Ama, 2010).

The aim is to determine the phytochemical compositions, antimicrobial activities of the *Pterocarpus santalinoides* leaf in order to determine their medicinal value.

2. Materials And Methods

Sample Collection

Plant Sample Collection

Fresh leaves of *Pterocarpus santalinoides* were collected from Mr. Eje Igodo's Compound, Anyioga Ikachi-Oju Local Government Area, Benue State, Nigeria in February, 2021. The plant leaves were identified in the Department of Botany, University of Agriculture, Makurdi, Benue State, Nigeria.

Plant Sample Preparation

The leaves were sorted, washed and air dried at room temperature in the laboratory for two weeks. The dried leaves were grinded into powder using mortar and pestle and filtered using a sieve. The powder was then stored in an airtight container for further extraction.

Preparation of Crude Extract

The method of (Idris *et al*., 2009) was used. One hundred gram (100g) of the powdered leaf was weighed and soaked in 500ml each of distilled water and Methanol for three days (72 hours) and was

constantly shaken. The extract was filtered using a Whatman No. 1 filter paper to remove the residue. The filtrate was evaporated at 50^{o}C in a water bath to get the crude extract.

The crude aqueous and methanol extract was stored in a sample bottle until required and was used for phytochemical and antimicrobial analysis.

Phytochemical Screening

The aqueous and methanolic leaf extracts of *Pterocarpus santalinoides* were phytochemically screened qualitatively for the presence of alkaloids, flavonoids, saponins, tannins, steroids, quinones, proteins, glycosides, phenols and terpenoids using standard methods (Ngbele *et al*., 2008).

Test for Glycosides

A 5ml of dilute H_2SO_4 was added to 1ml of the plant extract in a 100ml flask. It was boiled for 15 minutes, it was cooled and neutralised with 10% NaOH. 1ml of Fehling solution A and B was added to the neutralised solution and a brick red precipitate of reducing sugar indicated the presence of glycoside.

Test for Steroids

One gram (1g) of the plant extract was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated H_2SO_4 acid was added along the sides of the test tube. The appearance of a green colour indicated the presence of steroids.

Test for Saponins (Frothing Test)

About 2ml of the extracts were shaken vigorously with distilled water, a foamy leather formation indicated the presence of saponins.

Test for Quinones

About 2ml of the extract was mixed with concentrated sulphuric acid, blue-green or red colour indicated the presence of quinones.

Test for Phenol

A few drops of Ferric Chloride solution was added to 2ml of the extract in a test tube, the presence of a green colour indicated the presence of phenol.

Test for Terpernoids

A few drops of chloroform were reacted with 2ml of the extract and a few drop of concentrated sulphuric acid (H_2SO_4) were added to form a layer. A reddish brown precipitate produced immediately indicated the presence of terpernoids.

Test for Proteins

The plant extract was treated with 2 to 3 drops of concentrated Nitric acid. Yellow colour precipitation in the test tube indicated the presence of protein.

Test for Flavonoids (Ferric Chloride Test)

2 to 4 drops of Ferric chloride was added to 2ml of the extract, a blackish red colour shows the presence of flavonoid.

Test for Tannins

About 3 drops of 0.1% Ferric chloride was added to 1ml of the extract, a brownish green or blue black colour indicated the presence of tannin.

Test for Alkaloids (Mayer's Test)

About 2ml of the extract was added to a few drops of 2NHCL, an aqueous layer formed which was decanted and one or two drops of Mayer's reagent was added. Formation of white turbidity or precipitate indicated the presence of alkaloids.

Antimicrobial Activity of the Plant Extract

Test Organisms

Cold stored Agar slant cultures of already identified *Escherichia coli* for Gram negative, *Stapylococcus aureus* for Gram positive *and Candida albicans* for fungi were obtained from the Microbiology Laboratory of the Federal University of Agriculture, Makurdi, Benue State.

Viability test of each organism was carried out by resuscitating the organisms in buffered peptone broth and thereafter subcultured unto nutrient agar medium and incubated at 37^{o}C for 24 hours for bacteria isolates and 72 hours for fungi. The probable identity of the clinically sourced isolates was further confirmed by exposing the cultures to an array of biochemical test which include coagulase production, catalase, methyl red, indole, citrate utilization and oxidase test as described by (Collins *et al.*, 2004; Sharma, 2009). The results of the biochemical reactions elicited by the test isolates were compared with standard identification keys as described by (Collins *et al*., 2004). The fungi isolates were subcultured on Sabouraud Dextrose Agar (SDA) to check the purity.

Preparation of Concentrations of the Plant Extracts

One gram (1g) each of the aqueous and methanolic extracts were added to two millilitres (2mls) of distilled water and methanol respectively to give a concentration of 500mg/ml, and others concentrations of 250mg/ml, 200mg/ml, 125mg/ml and 100mg/ml were prepared by double broth dilution method as described by (Udochukwu *et al.*, 2015).

Determination of the Antibacterial Properties of the Extracts

Susceptibility testing was carried out using agar well diffusion method according to the recommendation of (NCFCL, 2000). Mueller-Hinton Agar was prepared according to the manufacturer's direction. It was sterilized by autoclaving for 15 minutes at 120^{o}C.

In this method, 0.5 MacFarland turbidity standards of 24 hours *Escherichia coli* and *Staphylococcus aureus* was prepared in a normal saline broth. About 0.5ml each of the organism from the 24 hours normal saline broth was pipette onto a petri dish after which the prepared Muller-Hinton agar was pour plated, it was swirled evenly and was allowed to solidify.

After the cultured plates have gelled, a sterile cork borer (4mm) was used to bore wells on the surface of the agar plate and was labelled. About 0.2ml of the different concentrations of each extract was transferred into the wells using a Pasteur pipette. The wells were sufficiently spaced to prevent the resulting zone of inhibition from overlapping. Ciprofloxacin (500mg/ml) was used as control. The experiment was performed in triplicates and the resulting zones of inhibitions were measured as the diameter of the well using a ruler calibrated in millimeters. The average of the reading was taken to be the zone of inhibition of the isolates in question to that particular concentration.

Determination of Anti-Fungal Properties of the Extracts

The antifungal susceptibility test was carried out, and the agar well diffusion method was used here also. The substrate, Sabouraud dextrose agar (SDA) was prepared according to manufacturer's direction and was sterilized by autoclaving at 120^{o}C for 15minutes. In this method, the innocular was prepared by inoculating the test organism in a normal saline broth and was allowed to stand for three days (72 hours). The culture was diluted to 0.5 MacFarland turbidity standards. About 0.5ml of the cultured *Candida albicans* was pipette onto a petri dish after which the prepared Sabouraud dextrose agar was pour plated, it was swirled evenly and was allowed to solidify.

After the cultured plates have gelled, a sterile cork borer (4mm) was used to bore wells on the surface of the agar plate and was labelled. About 0.2ml of the different concentrations of each extract was transferred into the wells using a Pasteur pipette. The wells were sufficiently spaced to prevent the resulting zone of inhibition from overlapping. Fluconazole (100mg/ml) was used as control. The experiment was performed in triplicates and the resulting zones of inhibitions were measured as the diameter of the well using a ruler calibrated in millimeters. The average of the reading was taken to be the zone of inhibition of the isolates in question at that particular concentration.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined according to the macro broth dilution technique as described by (Baron and Feingold, 1990). Standardized suspension of test organisms were inoculated into a double fold serial dilution of the extract in a normal saline broth in a series of four sterile test tubes. The mixtures in sterile test tubes were incubated at 37^{o}C for 24 hours for bacteria and 72 hours for fungi and observed for turbidity (signifying growth) or absence of it (signifying inhibition). The minimum inhibitory concentration was recorded as the least concentration of the extract solution that inhibited microbial growth.

Minimum Bactericidal/Fungicidal Concentration

The MBC/MFC of the respective extracts was determined by the procedure described by (Baron and Feingold, 1990). An aliquot of the test mixture was taken from the MIC tube that showed no visible growth and was subultured onto a freshly prepared Nutrient agar plate which was prepared according to manufacturer's direction and was later incubated at 37^{o}C for 24 hours for bacteria and 72 hours for fungi. The minimal bactericidal or fungicidal concentration was recorded as the least concentration of extract that showed no bacterial or fungal growth.

Statistical Analysis

Data were analysed for mean and standard deviation. Difference in parameter was tested for statistical difference at $p<0.05$ using ANOVA. All the analysis was done using a statistical package service solution (SPSS) version 21.

3. Result

The result of the phytochemical analysis of the aqueous and methanolic leaf extracts of *Pterocarpus santalinoides* is shown on Table 1. The presence of alkaloid, tannin, protein, glycoside, flavonoid, steroid, phenols, saponins, terpernoids were recorded while quinone was absent in both aqueous and methanolic leaf extract.

Table 2 presents the zone of inhibition in (mm) of the methanolic leaf extract of *Pterocarpus santalinoides. Escherichia coli* exhibited the highest activity across the different range of concentration while *candida albicans* showed the least activity across the different concentrations used. Statistical analysis indicates that there was significant difference.

The zone of inhibition in (mm) of the aqueous leaf extract of *Pterocarpus santalinoides* is shown on Table 3. The result shows that there was significant difference $P< 0.003$ in the zones of inhibitions among the different concentrations. *Staphylococcus aureus* had the highest zones of inhibition across the different range of concentrations while *Candida albicans* showed the least zones of inhibitions across the different concentrations used.

Table 4 shows the result of the Minimum Inhibitory Concentration (MIC) and the Minimum Bacteriocidal/Fungicidal Concentration (MBC/MFC) of Methanol and Aqueous Leaf Extract of *Pterocarpus santalinoides* on the test organisms. It was observed that the MIC was 50% for all the test organisms with aqueous extract and 25% for *Escherichia coli* and *Candida albicans* except for *Staphylococcus aureus* that was observed at 50% concentration. The MBC and MFC of Methanol extracts on *Escherichia coli* and *Candida albican* was 50% concentration while *Staphylococcus aureus* had no Minimum Bactericidal Concentration of the methanol extract. The aqueous extract had no MBC and MFC effect on the test organism.

Table 1: Phytochemical screening of the Aqueous and Methanolic Leaf-Extract of *Pterocarpus santalinoides*

Phytochemical constituent	Aqueous extract	Methanolic extract
Alkaloid	+	+
Glycoside	+	+
Flavonoids	+	+
Phenols	+	+

Saponins	+	+
Terpernoids	+	+
Quinones	-	-
Steroids	+	+
Tannins	+	+
Proteins	+	+

Key:

Present = (+)

Absent = (-)

Table 2: Zones of Inhibition (mm) of the Methanolic Leaf Extract of *Pterocarpus santalinoides* on Selected Test Organisms

Test organisms	Concentrations (mg/ml)						
	500	**250**	**200**	**125**	**100**	**Control (Ciprofloxacin)**	**Control (Fluconazole)**
S. aureus	21.67 ± 2.08	16.33 ± 2.52	14.00 ± 2.65	13.00 ± 2.65	11.00 ± 1.00	24.00 ± 1.73	-
E. coli	23.67 ± 4.73	19.33 ± 2.08	18.00 ±1.00	14.00 ± 1.00	13.00 ± 1.00	20.00 ± 0.00	-
C. albicans	18.33 ± 0.58	15.67 ± 1.53	15.67 ± 0.58	14.67 ± 1.53	12.00 ± 2.65	-	19.67 ± 2.52

(P < 0.05) Df = 4

Table 3: Zone of Inhibition (mm) of the Aqueous Leaf Extract of *Pterocarpus santalinoides* on the Selected Test Organisms

Test organisms	Concentrations (mg/ml)						
	500	**250**	**200**	**125**	**100**	**Control (Ciprofloxacin)**	**Control (Fluconazole)**
S. aureus	16.33 ± 0.58	14.33 ± 0.58	13.33 ± 1.16	12.00 ± 2.65	9.67 ± 0.58	24.00 ± 1.73	-
E. coli	14.67 ± 0.58	13.00 ± 1.00	12.33 ± 2.08	10.67 ± 1.16	9.33 ± 0.58	20.00 ± 0.00	-
C. albicans	11.00 ± 1.00	9.33 ± 0.58	8.67 ± 0.58	7.67 ± 2.08	6.00 ± 1.65	-	19.67 ± 2.52

(P < 0.05) Df = 4

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal/Fungicidal Concentration (MBC/MFC) of the Methanol and Aqueous Leaf Extracts of *Pterocarpus santalinoides* on selected Test Organisms

Test organisms	Methanol		Aqueous	
	MIC	**MBC/MFC**	**MIC**	**MBC/MFC**
Staphylococcus aureus	50	-	50	-
Escherichia coli	25	50	50	-
Candida albicans	25	50	50	-

4. Discussion

In this study, the *in vitro* activity of the methanol and aqueous leaf extracts of *Pterocarpus santalinoides* was investigated against Gram negative *Escherichia coli*, Gram positive *Staphylococcus aureus* and Fungus *Candida albicans.* These pathogens are responsible for a number

of diseases. For instance, Gram positive bacteria cause disease such as boil, sore, abscesses, food poisoning, burns, wounds, skin disorder, etc. Gram negative bacteria cause typhoid fever, urinary tract infections (UTIs), diarrhoea, etc. (Tor-Anyiin *et al*., 2006). *Candida albicans*, one of the few species of the genus *Candida* that causes human infection candidiasis, is opportunistic pathogenic yeast. That is, they can become pathogenic in immunocompromised individuals under variety of conditions (Erdogan and Rao, 2015). Both extracts showed antimicrobial effect on the test organism at different concentrations. The antimicrobial properties of these extracts may be due to the different phytochemical constituents. This finding agrees with report of Kachawa, (2012) who reported the antimicrobial activity of the methanol stem extract of *Pterocarpus marsupium* and also on methanol leaf extract of *Pterocarpus santalinoides* (Anowi *et al*., 2012b).

This study also showed the phytochemical constituents of the aqueous and methanol leaf extracts of the plant. These phytochemical constituent include alkaloid, tannin, protein, glycoside, flavonoid, steroid, phenols, saponins, terpernoids. This result corroborates with the findings of Odeh and Tor-Anyiin, (2014) who reported the phytochemical constituent of the leaf extract of *Pterocarpus santalinoides* and these phytoconstituents have shown varying degrees of antimicrobial effects on different microorganisms and also have pharmacological and medicinal importance to humans (Yasir *et al.*, 2010). Alkaloids can act as anti-malarial, anticancer, antiasthma and antibacterial pharmacological constituent to humans. Tannins on the other hand have been used to combat diarrhoea (Idris *et al*., 2009). Saponins have gained ground in dietary supplements and nutraceuticals (Akinpelu *et al.*, 2014), they have also been used to lower blood cholesterol level and also has anticancer, anti-inflammatory agents and exhibit diverse physiological response in animal (Ndukwe and Ikpeama, 2013). Glycosides are known for their antibiotic properties. Flavonoids are reported to have antibacterial, anti-inflammatory, anticancer, antifungal, anti-allergic and diuretic properties (Kar, 2007).

There was significant difference in the zones of inhibition at different concentration between the methanolic and the aqueous extract of the leaf. This study demonstrated that the methanolic extracts were more effective than the aqueous extracts. This may be due to the better solubility of the active components in organic solvents (Umeh *et al.*, 2005). Different solvent have different polarities, hence different degrees of solubility for the various phytoconstituents. This result agrees with the reports of Cowan (2002) that the alcoholic solvents like methanol and ethanol are more soluble than other solvents such as water in extracting components of medicinal plants.

The Aqueous leaf extract of *Pterocarpus santalinoides* had the highest Minimum Inhibitory Concentration (MIC) with no Minimum Bactericidal/Fungicidal Concentration for the test organisms while the methanol leaf extract had the least Minimum inhibitory concentration and it also had a bacteriocidal/fungicidal effect of *Escherichia coli* and *Candida albicans.* The MIC result showed that increasing concentration has an increasing efficiency in inhibiting the test organisms. The methanol extract had the highest zone of inhibition with *Escherichia coli* and it had more activity than the activity of Ciprofloxacin as standard used at 500mg/ml.

Conclusion

Pterocarpus santalinoides leaf extracts contain phytochemicals such as alkaloids, protein, glycoside, tannin, saponin, steroid, alkaloid, phenol, flavonoid which constitutes the medicinal properties of the plant.

The aqueous and methanolic extracts showed antimicrobial activities at different concentrations against the test organisms. The extracts, particularly the methanol extract had the highest effect and as such can be exploited as a possible antimicrobial agent for the management of infectious pathogenic diseases. *Pterocarpus santalinoides* can be further utilized in pharmaceutical industries for the manufacturing of drugs for treatment of various diseases and ailments.

Recommendations

From the research study carried out, it is recommended that;

1. The methanol extract, having shown the highest antimicrobial activities on all test organisms at different concentrations, be recommended for its exploitation in the management of infectious diseases.
2. Further research should be carried out on medicinal benefit of this plant in the treatment of tuberculosis that are resistant to some synthetic drugs.
3. Further studies should be carried out on the safety and efficacy of this plant extract to establish whether they can offer therapeutic benefits either alone or in synergy with other conventional antimicrobials.
4. More research gearing towards harnessing other part of the plant as biocontrol, for pest and insects is recommended.

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