PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF THE STEM BARK OF PTEROCARPUS SANTALINOIDES, (NTURU UKPA)

Eze, S.O¹, Cornelius, C¹. and Okereke H.C²

¹Department of Chemistry, & ²Department of Microbiology, ^{1& 2}Abia State University, Uturu Abia State, NIGERIA. <u>sundayoeze@yahoo.com</u>

ABSTRACT

Sequel to the use of the stem bark of Pterocarpus Santalinoides (Nturu Ukpa) in traditional medicine the phytochemical and antimicrobial analysis of the stem back was undertaken. Qualitative and quantitative evaluation of the phytochemicals were made using standard methods. The microbiological analysis of water and ethanolic extracts of the sample were performed on some common microorganisms such as Salmonella species, Klebsiella Pneumonia, Escherichia Coli, Pseudomonas Aerugunosa using the disc diffusion method for the screening using nutrient agar for the in vitro antimicrobial activities. The results showed that the stem bark of Pterocarpus Santalinoides (Nturu Ukpa) is rich in bioactive substances such as alkaloids 1.01+0.05, flavonoids 6.7+0.02, tannins 8.53+0.48, saponins, 1.7+0.08, phenolics, 31.45+0.15, cyanogenic glycosides $1.52 \times 10^{-4} + 1.73 \times 10^{-6}$. The antimicrobial analysis showed that the ethanolic extract was active against Klebsiella pneumonia, Salmonella spp. and Escherichia coli with percentage inhibitions of 25.8+0.83, 25.8+8.3×10⁻¹ and 15.8+8.3×10⁻¹ .respectively.The ethanolic extract did not show activity on staphococcus aureus and pseudomonas aeruginosa. The water extract was active against Pseudomonas aeuriginosa 19.2 + 8.4×10^{-1} . The results indicate that the plant stem bark have potential medicinal uses and the ethanolic and water extracts can protect against some common microorganisms.

Keywords: Phytochemicals, Pterocarpus santalinoides, stembark, antimicrobial, medicinal plants.

INTRODUCTION

Pterocarpus species belong to the family *fabaceae* and they occur through- out the tropics, (Ogan, 2004) the Nigerian species are trees with bright yellow flowers and usually have alternate leaflets (Osuagwu *et al*, 2007). The fruit pod has an usual irregular shape, (Adetunji 2007). It is a shade tree commonly found along riverine forest in Africa and tropical South America (Galant 1972). The plant can also help in erosion control because of the type of root system as well as nitrogen fixation (Tian *et al*, 1992).

In Nigeria many indigenous plants including *Pterocarpus Santalinoides are* used as food or medicine. The tender leaves are used as vegetables in soup making while the stem bark is used in making pepper soup. Plants are used in treating rheumatism, diarrhea dysentery, cough, asthma, diabetes, malaria, elephantiasis, cold and others (Okwu, and Ekeke, 2003, Shulz,*et al*, 2001

In Southern Nigeria the tender stem bark and leaves extract of *Pterocarpus santalinoides* usually called "Nturu Ukpa" in Igbo language is used in stopping stooling and vomiting in both children and adults. The nitrogen fixation ability of the plant roots has been reported (Tian *et al1992*)

The use of the leaves in treating skin diseases such as eczema, Candidiasis, and acnes reported (Adesina, 1982) use of the concoctions made from its roots in treating asthmatic patients have been reported (Adesina, 1982).

It is used in treating diarrhea which is a major cause of death as it has a proven antienteropooling activity in traditional medicine, Nworu, *et al*, 2009. The anti-malaria activity has been reported also as well as use of the stem bark decoction in treating infertility in females (Alexis et al, 2000). The bark extracts is used in treatment of cough and diabetes. The leaves are used in Veterine medicine to reduce abdominal pains in goats (Igoli et al, 2005, Ama, 2010).

MATERIALS AND METHODS

The stem of *Pterocarpus santalinoides* is thin and flanking in small patches slashes yellowish white exuding drops of red gum (Keay, 1989). The bark contain tanning which can be used as a stomach cache remedy (Okwu 2004). It has been used in herbal medicine in the treatment of arthritis. It has a marked Physiological activity on animals through its leaves as anti-analgesic antispasmodic and bactericidal medical agents as well as in the treatment of high blood pressure, (Igoli et al, 2003, Alexis et al, 2000) also reported the use of the plant extra it's against *Plasmodium falciparium* FCBT in Colombia and FCM29 in Cameroon chloroquine resistance strain on a Nigerian Chloroquine sedative strain as well as the use of the decortion made from the stem bark for treating infertility in women. Lawal, et al, 2010.)

The root extracts is used in the cure of internal heat and scarlet fever. The roots also contain glycosides that neutralize blood sugar thereby helping in the cure of diabetes while the stem bark extract is used for treatment of cough (.Ama, 2010)

The stem bark of *Pterocarpus Santalinoides* was obtained from healthy plants in Osusu Aba in Aba North LGA, Abia State Nigeria.

The plant was identified by Prof C.I. Ogbonnaya of the Plant Science and Biotechnology Department, Abia State University, Uturu, Nigeria.

The chemicals- ethanol (May and Baker, England), diethyl ether (BDH England), n-butanol and hydrochloric acid (BDH-England), Sodium chloride and copper(II) sulphate (Merck USA), ferric chloride and aqueous ammonia (BDH England),Potassium Ferro cyanide, potassium hydroxide and petroleum ether (BDH England) methanol (May and Baker, England) amyl alcohol (Merck, USA) sodium carbonate and sodium hydroxide (Merck USA), potassium permanganate and conc. H_2SO_4 ,(BDH,England) were used as such without further purification.

SAMPLE PREPARATION

The stem bark were cut into smaller bits with a sharp knife and air dried for 7 days, the dried samples were ground into powder using a corona grinding machine and stored in air tight sample plastic bottles for analysis.

Phytochemical screening and quantification for tanning, Flavonoides, saponins, and alkaloids, Phenolics and cyanogenic glycosides were evaluated using standard methods according to (Harbone 1973, Harbone 1988, Dietland^a, 2009 Dietland^b, 2009 and Elvis, 2003)

ANTIMICROBIAL ANALYSIS

Ethanol and water extracts of the samples were prepared by cold perolation method. 2g of the powdered samples were soaked in 20ml of water and ethanol respectively for 48 hours with intermittent shaking. The Suspensions were filtered through Whatmann No 1 filter paper. Extracts were stored at 4° c for further use.

Culture medium used for the anti-bacterial activity test was nutrient agar. The milk colored powder has the formulation- 5g/L, peptone agar-agar; 3g/L sodium chloride and 3g beef extracts.

The medium was prepared by dissolving 32g of the powder in 100ml of deionized water. This was allowed to soak for 10mins and swirled to mix thoroughly sterilized by autoclaving at 121°C for 15mins. The medium was cooled to 47° C and mixed thoroughly before pouring into petri dishes. (Eloff, 1998). Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for the experiment were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient agar broth (NAB) for bacterial that were incubated without agitation for 24 hours at 37° C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 2.0x10⁶ colony forming units (cfu/ml) for the bacterial strains (Eloff 1998).

The samples were screened and assessed for microbial inhibition for *salmonella* species, *Klebsiella pneumoniae*, *Eschesichia coli*, *Staphylococcus aureus*, *and Pseudomonas aeringinisa*. The agar disc diffusion method was used and the zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the stem bark of pteropus santalinoides is shown in table 1 below.

| Phytochemical Test | Result |
|-----------------------|--------|
| Alkaloids | + |
| Flavonoids | + |
| Tannins | + |
| Saponins | + |
| Phenolics | + |
| Cyanogenic glycosides | + |

| Table 1. Results of Thy tochenical Screening |
|--|
|--|

The Quantitative results of the phytochemical tests is shown in table 2

| Phytochemical | Percentage Composition | |
|-----------------------|---|--|
| Alkaloids | 1.1 ± 0.05 | |
| Flavonoids | 6.7 ± 0.02 | |
| Tannins | 8.53 ± 0.48 | |
| Saponins | 1.70 ± 0.08 | |
| Phenolics | 31.45 ± 0.15 | |
| Cyanogenic glycosides | $1.52 \times 10^{-4} \pm 1.73 \times 10^{-6}$ | |

Table 2. Percentage phytochemical composition of the stem bark of phytochemical <u>P</u>Santalinoides

Each data is a mean of triplicate analysis $\pm S_x$ where S_x = standard error of the mean.

The result of Microbial screening of the stem bark of *P. Santalinoides* is shown table 3 and 4 the quantitative results is shown in tables 5 and 6 for the water and ethanolic extracts.

| Bacteria | Extract | | |
|------------------------|---------|-------|--|
| | Ethanol | Water | |
| Salmonella spp | + | - | |
| Klebsiella pneumoniae | + | - | |
| Escherichia coli | + | - | |
| Staphylococcus aureus | - | - | |
| Pseudomonas aeruginosa | - | + | |

 Table 3. Antimicrobial screening of the water and ethanolic extracts of the stem bark of *P. santalinoides*

 Table 4. Percentage inhibition of the ethanolic and water extracts of the stem bark

 of *Pterocarpus santalinoides*

| Bacteria | Ethanol extract | Water extract |
|------------------------|-------------------------------|---------------|
| Salmonella spp. | $25.8 \pm 8.3 \times 10^{-1}$ | - |
| Klebsiella pneumoniae | 25.8±0.83 | - |
| Escherichia coli | 15.8 ±8.3 x 10 ⁻¹ | - |
| Staphylococcus aureus | - | - |
| Pseudomonas Aeruginosa | - | - |

The phytochemical screening test shows that the stem bark of *Pteropcarpus Santalinoides* contains all the Phytochemicals tested including alkaloids, Flavonoides, tannings, Saponins, Phenolics and cyanogenic glycosides. These are essential compounds required in various herbs used for medicinal purposes. Akortha and Nwachukwu (2009) has earlier worked on the antimicrobial effect *of vernonia amygdalina* (bitter leaf) extract on antibiotic resistant strains of *Escherichia Coli, Klebsiella species, Pseudomonas aeruginosa, Roteus Morganii*, and *staphylococcus aureus*.

The water extract of the stem bark has the highest effect on pseudomonas aeruginosa than the ethanol extract. Pseudomonas aeruginosa is said to be responsible for nosocomial infections such as pneumonia, urinary tract infections (UTIs) and bacteriamia.(Nostro et al, 2000).

Leaves of this plant are used as green vegetable, vegetable spice in soup, especially in the popular bitter leaf soup. The bitter leaf wastes extract is taken as tonic to prevent certain illnesses. This result of this work is similar to that of the bitter leaf. The Zones of inhibition resulting from the Ethanol and water extracts of the *Pterocarpus santalinoides* ranged from 1mm -25mm. The extract that gave the best zone of inhibition is the ethanol extract against salmonella species and Klebsiella Pneumoniae. (Akortha and Nwachukwu (2009) The Antimicrobial effect of Vernonia amygdalina (bitter leaf) extract on antibiotic resistant strains of E. coli, Klebsiella species, Pseudomonas aeruginosa, Proteus morganii and staphylococcus aureus. The organisms used for the antimicrobial screening are well known pathogens and these include salmonella species which causes gastroenteritis, Escherichia coli which causes some food borne intoxications & UTI, Staphylococcus aureus which causes wound infections, Klebsiella pneumoniae the pathogen for pneumonia and pseudomonas aeruginosa which causes some skin infections in other words P. Santalinoides have good nutritional qualities as well as good chemotherapeutic values because of its ability to inhibit most pathogens when ethanol and water extracts are used (Iroha el al, 2008) Invang ad Adegoke, (2008).

CONCLUSION

This work lends credence to the fact that these extracts have good phytochemical and antimicrobial qualities and justify the use in traditional medicine.

ACKNOWLGEMENTS

The authors are grateful to the laboratory staff of the Department of Microbiology University of Nigeria, Nsukka for assisting with the microbial analysis.

REFERENCES

Adesina, S.K. (1982). "Studies in the Nigeria Herbal medicinal plants" *International Journal of crude Drug Reserves*, 20, (2), 93-100

Adetunji J.A. (2007). "Reviewing *Pterocarpus species* and their distribution" African *Journal of Traditional, Complementary and Alternative Medicine*, 4, (2) 23-36

Afri. J. Trad. Cam. 2, (2), 134-152

Akortha, E.E and Nwachukwu, N.C. (2009). The Antimicrobial effect of Vernonia amygdalina (bitter leaf) extract on antibiotic resistant strains of *E.coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Proteus morganii* and *Staphylococcus eureus*. International Journal of Biotechnology and Allied sciences <u>4 (1)</u>: 408-412.

Alexis, V, Mustofa F, Benoit, V.P, Yves, P. and Michele M. (2000) "Antiplasmodial activity of plant extracts used in west African traditional medicine" *J. of Ethno pharmacology*, *73*, (1-2), 145-151

Ama, C.A. (2010). Traditional use perocarpus santalinoides parts , Chief Dr Ama C. Ama Trado-Medical Centre, Amajieke, Owerri, Imo State, Nigeria.

Antimicrobial Properties and preliminary phytochemical screening of *Chromonella odoranta* (Siam or Sapysa weed) leaf, Clarendon press, Oxford, p.210

Dietland, M.S. (2009). "Test for Cyanogernic compounds in Plants" <u>Hand book</u> on Chemical Ecology, (Simple Field and laboratory Exercises) part 1 1st edition. New York: Springer. pp.69-73

Dietland, M.S. (2009). *Handbook on Chemical Ecology,(Simple Field and laboratory: Exercise)*, *Part 2*, 1st edition. New York: Springer. pp.80-95.

Harbone J.B (1988). Analysis of non-Nutritive components of plants. *Introduction of Ecological Biochemistry*, 3rd ed. London: Academic Press.

Harbone, J.B. (1973). "Determination of Phytohemical", *Phytochemical Methods*,1st edition. London: Chapman and Hall Ltd. pp.111-113

Igoli J.O. Ogaji O.G, Tor, A.A. and Igoli, N.P. (2005). Traditional medicinal practice amongst the Igede people of Nigeria, Part II, *International Journal of Biotechnology and Allied sciences*, 4,(4),408-412

Inyang C.U and Adegoke , A.A. (2008). Antimicrobial properties ad preliminary phytochemical screening of *chromolaena odorata* (Siam or Sapysa weed)leaf. *Nigeria Journal of Microbiology*, 22, 1652-1659

Iroha, I.R, Oji, A.E, Afiukwu T.N and Nwuzo A.C. (2008). Antimicrobial activity of Extracts of *Garcinia Kola* against resistant extended Beta Lactamase Producing *Escherichia coli* and *Klebsiella Pneumoniae*. *Nigerian Journal of Microbiology*, 22, 1693-1697.

Iroha, I.R., Oji A.E. Afiukwa, T.N and Nwezo, A.C. (2008). Antimicrobial activity of extracts of *Garcina kola* against resistant extended Beta Laefarmase producing *Escherichia coli* and *Klebsiella Pneumoniae, J. Sustain. Agric. Environ* <u>6</u>, (1) 30-34

Keay, R.W. (1989). *Pterocarpus species*, *Trees of Nigeria*, *Vol III*, Clarendon Press, Oxford, p.210

Kelvin, R. (2003). "Strategies for the Determination of Bioactive constituents in plants, fruits and vegetables, *Journal of Chromatography*, *A*, *1000*, (1-2) 657 -691

Lawal I.O, Uzokwe N.E, Igbanugo, B.I. Adio, A.F., Awosan, E.A. Faloye, B. and Adesoga, A.A (2010) "Ethno- medicinal information on plants" *African Journals of Pharmacy and Pharmacology*, 4,(1), 001-007

Nostro, A., German, M.P., Angelo, V.D., Morino, A. and Cannatelli, M.A. (2000). Extraction Methods and Bioauthgraphy for Evaluation of Medicinal Plant antimicrobial Activity, Left. Appl. Microbial. 30,(1), 379-384

Okwu D.E. and Ekeke 2. (2003). Phytochemical screening and the Medical composition of chewing sticks in South Eastern Nigeria, *Global Journal of Pure and Applied sciences*, 9(1), 235-238.

Okwu, D. E. (2004). Phytochemical and Vitamins content of indeginous species in South Eastern Nigeria, *J. Sustain. Agric. Environ.* 6(1) 30-34

Organ, M.T. "Trees of Nigeria" *Journal of Complementary and integrative medicine* 123, (1), 125-129.

Osuagwu, G.G.E, Okwulehie, J. and Emenike, J.O. (2007). "Phytochemical and mineral content of the leaves of four *Nigeria Pterocarpus (2 ACQ) species*" International *Journal of Molecular Medicine and Advance Sciences*, 3(1), 6-11

Shulz ,V., Rudelf, H. and Mark, B. (2001). "Medicinal plants Phytoremedies and Phototherapy and their Rational phototherapy" *A Physician's Guide to Herbal medicine*,. 4th edition, Berlin, New York: Springer Publisher. pp.1-39

Tian G., Knag, B. and Buisard L. (1992). "Chemical composition on N, Ca and Mg release during incubation of leaves from selected Agro- Forestry fallow plant species *Biogeochemistry Journal*,6,(2), 13 -19