International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 3 Issue 4 || April 2014 || PP.17-22

Preliminary study of the Antibacterial and Analgesic effect of the Leaf Extract of Pterocarpus santalinoides L'Hér. Ex DC.

[1,]Umeh S.O, [2,]Umerie S.C, [3,] Emelugo B.N, [4,] Nwobi S.C,
[1,]*Dept. of Applied Microbiology and Brewing, NAU, Awka, Anambra State, Nig.*
[2,3]*Dept. of Applied Biochemistry, NAU, Awka, Anambra State, Nig.*
[4,]*Dept. of Polymer and Textile Engr., NAU, Awka, Anambra State, Nig*.

ABSTRACT: *Phytochemical screening, antibacterial and analgesic activities of the ethanolic extract of Pterocarpus santalinoides L'Hér. ex DC leaves were carried out in this study. Phytochemical analysis of the plant extract revealed that the crude extract contains alkaloids, saponins, tannins, cardiac and cyanogenic glycosides, flavonoids, terpenoids, carbohydrates and protein. Trace elements tested using the Atomic absorption spectrophotometer showed the presence of iron, potassium, phosphorous, magnesium, manganese and calcium. Antibacterial screening of the crude extract showed inhibitory activity against some gram positive and gram negative bacteria (Staphylococcus aureus, Escherichia coli, Salmonella sp, Enterobacter sp and Bacillus sp). Analgesic activity of the crude ethanolic extract on albino mice, using tail-flick method and hot-plate (20 - 40^oC) method showed that the plant leaves extract exhibit analgesic effects. The analgesic effect when compared with the control drug, morphine, showed that the extract dose of 27.5 mg/g gave the same analgesia as 1.5 mg/g of morphine using the tail-flick and hot-plate methods.*

Key words: *P. santalinoides, analgesic activity, medicinal plants, albino mice, hot-plate method, tail-flick method.*

I. INTRODUCTION

Osborne in 1934 initiated screening program for the therapeutic effects of plants. His work on 2,300 plant species showed that 63 genera had activities against some microbes like *Pseudomonas aeruginosa* and *Escherichia coli* (Ayesu, 1978). Today, the use of natural crude drugs of botanical origin (phytotherapy) in treatment, cure, prevention and control of disease are being reconsidered. The recent trend is a return to the world of plants for the treatment of different sicknesses and ailment (Umerie *et al*., 2007). Many plants with useful pharmaceutical constituents have not been studied. There is need therefore to study such plants.

P. santalinoides L'Hér. ex DC (winged fruit) belongs to the family *Fabaceae* and sub-family Papilionoideae consisting of large genera of diverse species (Dutta, 1989). It is a buttress tree (9-12 m tall) seen around wet places like riverbanks and forests and widely distributed in the tropics of Africa and South America. In Nigeria, it is found mostly in the Cross river, Anambra and Imo states. The stem is often short, branched and evergreen. The bark is thin, flaking off in small packs and when cut exuding drops of red gummy fluid (Keay, 1989). The leaves has slender glabrous common stalk of about 10-12 cm long with 5- 9 (odd) leaflets. The plant flowers around December through March to June. Flowers are golden yellow in auxiliary racemes and panicles about the same size as the leaves. Fruiting is around March to April. The fruit is light brown with glabrous seed measuring about 3-6 cm across. The fruit contains soft fleshing narrow wing which extends round the body, with a corky and knobby flesh surrounding a hard woody shell (Keay, 1989).

In the South-Eastern Nigeria, particularly among the 'Igbo' communities, it is called '*Nturukpa*'. The leaf is consumed as vegetables and its medicinal use among the 'Igbos' include; the cure of stroke, diarrhea, dysentery, fever and pains, etc (Ajiwe et al., 2008). In order to ascertain these claims by the local users and some ethnomedical practitioners, the present work therefore investigated the phytochemical contents, antibacterial and analgesic activities of the ethanolic leaf extract of *Pterocarpus santalinoides.*

Materials

Collection and preparation of the plant material

Fresh leaves of *Pterocarpus santalinoides* were collected from a farm at Umuchu, Anambra state, south eastern Nigeria and identified in the Department of Botany, Nnamdi Azikiwe University Awka. The leaves were air-dried for 3 weeks and pulverized using a sterile manual Corona grinding machine. 200g of the powder was macerated in 3 liters of absolute ethanol for 72 hours with intermittent stirring to aid extraction. The mixture was sieved through a cotton wool plugged funnel to obtain a clear filtrate. A semi-solid extract, free from chlorophyll was obtained in vacuo using a rotary evaporator at 55^oC.

Experimental Animals

Adult male albino mice (18 – 22 g) were used for the experiment. They were obtained from the Department of Zoology, Nnamdi Azikiwe University, Awka, Nigeria. They were housed and fed in the laboratory for 5 days before the experiment.

Bacterial stock cultures

Pure stock cultures of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella sp*, *Enterobacter sp*, *Klebsiella sp*, *Proteus sp*, *Streptococcus sp* and *Bacillus sp*) were obtained from the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria.

Methods

Extract analysis

Phytochemical screening of the crude ethanolic extract was carried out using the methods of Harborne (1998) and Evans (2002). Atomic absorption spectroscopy (AAS) method as outlined by Willard et al (1984) was used to determine the mineral elements in the plant extract. Moisture and ash contents were determined using the AOAC method of 1980.

Reaction of different reagents on the alkaloid extracted

The colour change of morphine with other reagents was employed to confirm the type of alkaloid present in the *P. santalinoides* leaf extract using the method of Umerie *et al*. (2007).

Drug preparation

The plant extract was prepared in normal saline in the ratio 1:2 w/v. Morphine sulphate (Ms), which served as a control drug was also prepared in the ratio 1:10 w/v. A solution of 0.6% acetic acid was prepared as recommended by Koster *et al*. (1959) and used by Umerie *et al*. (2007).

Effective dose (ED) and Acute toxicity assays

The method of Takemori and Portoghese (1987) and adopted by Umerie *et al*. (2007) was used with slight modifications. The effective dose ED was determined as the mean dose administered to the surviving viable mice after the test period. The lethal dose (LD_{50}) of the extract was calculated as the geometric mean of the maximum dose that caused 0 % death and the minimum dose that caused 100 % death (Lorke, 1983; Umerie *et al*., 2007).

Analgesic assay

The hot-plate method and the tail-flick method were used to check the analgesic activity of the plant extract.

A total of 30 mice were used. Fifteen mice were used for the hot - plate assay and the other fifteen mice were used for the tail-flick assay. The 15 mice in each method were divided into 3 groups of 5 mice each. Two groups received treatment while the other group served as control. All the 30 mice were first injected with 0.3 ml of the 0.6 % acetic acid solution intra peritoneally (ip) and the extract was given to 2 groups while Ms was given to the control group subcutaneously after 10 minutes.

The hot-plate assay

The methods described by Vohora (1980) and Twycross (1984) were used. After two hours of administering the graded concentrations of the extract solution, the mice were placed on a hot plate whose temperature was electrically maintained at 20 – 40^{o}C, the time taken for the mice to withdraw its tail and jump out of the hot - plate was noted.

The tail-flick assay

The tail-flick method as described by Hayashi and Takemori (1971), Takemori and Portoghese (1987) and Umerie *et al*., (2007) was used. Ten minutes after the administering of the graded concentrations of the extract solution to the mice, the time taken to voluntarily flick their tails were observed and noted.

Control

From the two groups, the ten mice that were used as control were treated with acetic acid solution. Increasing doses of Ms Solution were given. Five mice were examined under hot -plate assay and five others for tail – flick assay.

Antibacterial screening
Anti bacterial screening was done using the method of Bryant (1972) as described by Ajiwe *et al*., (2008).

Results and Discussion
The characteristics of the fresh and dried leaves powder were presented in Table 1. The leaf is green when fresh with a moisture content of 77.2%. Its taste is astringent when fresh and salty when dried. It has an inoffensive odour.

Phytochemical analysis of the crude extracts showed the presence of saponin, alkaloid, tannins, flavonoids, cardiac glycosides, cyanogenic glycosides, terpenoides, carbohydrates and proteins as shown in Table 2.

Reacting the crude extract of *P. santalinoides* L'Hér. ex DC leaf with different reagents revealed the alkaloid to be morphine-type (Table 3). Melting point of the alkaloidal salt was found to be between 228 – 248^{o}C. This was very close to the value for pure alkaloid (254^{o}C) as found by Finar (2002). The slight disparity in the melting point could be as a result of impurities. The prominent peaks of the infra-red (IR) spectrum of the alkaloidal salt were detected at the frequencies of 3310, 3260, 2840, 1850, 1819, and 740 cm $^{-1}$. The bands showed the presence of -OH (phenol) group, N-H (amines), C-H (alkyl group), five-member cyclic anhydride and the presence of monosubstituted aromatic rings with 5-adjacent H- atoms respectively. The IR spectral data highlighted the presence of functional groups strongly suggestive of morphine-type alkaloids.

Result of the antibacterial activity showed the leaf extract to be active against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella sp*, *Enterobacter aerogenes* and *Bacillus sp* (Table 4). The mineral elements of the extract were iron, potassium, phosphorous, manganese, magnesium and calcium as shown in Table 5 while the results of the analgesic activities were as presented in Tables 6 and 7.

Table 1: Characteristic features of the *Pterocarpus santalinoides* leaves

Parameters	Observation
Color	Green when fresh
Moisture content	77.2%
Ash content	4.0%
Texture (dried leaf powder)	Smooth powdery
Taste	Astringent (fresh), Salty (dried powder)
Odour	Inoffensive (fresh), Mild (dried powder)

Table 2: Phytochemical profile of the crude ethanolic extract of the leaf

Parameter	Relative abundance
Alkaloids	++
Saponins	++
Tannins	+
Resins	-
Cardiac glycosides	++
Cyanogenic glycosides	+
Flavonoids	++
Terpenoids	+
Carbohydrates	++
Steroids	-
Protein	+

Key: ++ high concentration
+ Moderate concentration
- Absent

Table 3: Reaction of different reagents on the crude extract of *Pterocarpus santalinoides* leaves

Reagents	Color on reaction	Remarks
Concentrated H_2SO_4	Pink, violet then brown On warming	Morphine suspected
Concentrated H_2SO_4 + little Powdered K_2CrO_7	Greenish brown	Morphine or Codeine
Erdmann's reagent Concentrated H_2SO_4 + 0.5% HNO_3)	Pink	Morphine present
Powdered cane sugar moistened with Concentrated H_2SO_4	Red	Morphine confirmed

Table 4: Result of the antibacterial activity of the leaves extract

Bacteria	Activity	
Staphylococcus aureus	+	
Escherichia coli	+	
Salmonella sp	+	
Enterobacter sp	+	
Klebsiella sp	-	
Proteus sp		-
Streptococcus sp	-	
Bacillus sp		+

Key: + active
- Not active

Table 5: Mineral elements of the leaves extract of *P. santalinoides*

Metals		Presence
Iron	+	
Sodium		-
Potassium	+	
Phosphorous	+	
Magnesium	+	
Cobalt		-
Lead		-
Copper		-
Manganese	+	
Calcium		+

Key: + present
- not present

Table 6: Tail – flick assay of Morphine and *P. santalinoides* extract on acetic acid treated mice

Morphine Dose (mg/g)	Reaction time (hr)	*P. santalinoides* extract Dose (mg/g)	Reaction time (hr)
1.0	4	7.5	died
1.5	2	12.5	7
2.0	1	17.5	5
2.5	0.67	22.5	3 .30
3.0	died	27.5	2

Table 7: Hot – plate assay of Morphine and *P. santalinoides* extract on acetic acid treated mice

Morphine Dose (mg/g)	Reaction time (hr)	*P. santalinoides* extract Dose (mg/g)	Reaction time (hr)
1.0	6	7.5	died
1.5	4	12.5	10
2.0	2	17.5	8
2.5	1	22.5	7
3.0	died	27.5	4

The study has successfully looked into the pharmaceutical constituents, analgesic and antibacterial activities of the leaves extract of *P. santalinoides* used in folkloric medicine for the treatment of several ailments like diarrhea, dysentery, pain and fever.

The phytochemical screening of the crude extract showed the presence of alkaloids, saponin, tannins, flavonoids, proteins, cardiac glycosides, cyanogenic glycosides, terpenoids, protein and carbohydrates (Table 2). Further characterization of the alkaloid showed the alkaloid as morphine- type alkaloid (Table 3).

Saponins and flavonoids are generally haemolytic, anticancer and anti-inflammatory compounds and not analgesic (Evans, 2002; Ajali, 2004 and Umerie *et al.* 2007). Steroidal saponins have the ability of drastic reduction in cholesterol levels and raises high density lipoprotein (HDL) (Umerie et al. 2007). The presence of protein in the extract shows the leaves as a good vegetable which can help in the body building and replacement of worn out cells (Ajiwe et al. 2008).

The extract also contains cardiac glycosides and cyanogenic glycosides. Glycosides are known to posses anti-neoplastic properties (Kar, 2007). Cyanogenic glycosides are known to be active against slugs and snails (Harbone, 1998, Umerie, *et al*., 2007) and also Cardiac glycosides show cardiotonic effect and have the potential for management and control of cardiac arrest.

However, none of these phytocompounds except alkaloids have been implicated in analgesic activities. Morphine and morphine-type alkaloids are classed as narcotic or opiate analgesics and are strong agonists (Way *et al*., 2001). These substances mediate their action by binding to opiate receptors in the central nervous system, causing inhibition of ascending pain pathways and altering the perception of and response to pain, thus producing generalized central nervous system depression (Takemori and Portoghese 1987; Umerie *et al*., 2007).

Antibacterial screening of the extract showed that it has high activity against both Gram positive and Gram negative bacterial species (Table 4). Atomic absorption spectrophotometer results showed the micro elemental composition of the extract (Table 5). The plant extract contain useful elements that help in body building like calcium, iron, sodium, phosphorus etc. None of the heavy metals tested were present in the extract and this makes the fresh leaves recommended as vegetables without any fear of toxicity.

Intraperitoneal administration of 0.3 ml of 0.6% acetic acid solution to the mice resulted in the writhing of the abdominal muscle and stretching of the hind limb showing pain (Twycross,1984).

The group of mice that received 3.0 mg/g of morphine died due to overdose. During the effective dose and lethal dose determination, those that received 7.5 and 11.0 mg/g of the extract died after 48 hours while those given 17.5 and 20.5 mg/g remained weak and lifeless. Those given 22.5 and 27.5 mg/g recovered and remain viable in shorter time. The effective dose (ED) of the plant extract was then obtained as 25.0 mg/g body weight. The lethal dose (LD_{50}) of the extract was obtained as 14.5 mg/g.

In both the tail – flick and hot – plate assays, analgesia were achieved with *Pterocarpus santalinoides* leaves extract. The dose of 27.5 mg/g gave a comparable analgesic effect obtained with 1.5 mg/g of Ms in both the tail-flick assay and the hot-plate assay (Tables 6 and 7). The narrow range between the LD_{50} (14.5 mg/g) and the ED (25 mg/g) suggests the narrow margin of safety. Low doses of morphine drug and most of its relatives have high affectivity and persistent administration of doses as low as 10 mg would result in addiction and physical dependence (Way *et al*., 2001; Umerie *et al*., 2007). Overdose of morphine drug results in death in man and most other species (Goldstein *et al*., 1974) as seen with 3.0 mg in laboratory mice.

In conclusion, the results of this study confirm the claim of ethnomedical healers and other local users that the leaf extracts of *P. santalinoides* exhibit analgesic effect and may be effective in the treatment of fever and pain. The analgesic effect can be as a result of the morphine-type alkaloid present in the extract. The plant can be a potential source of morphine since morphine is derived from natural sources mainly plants (Kar, 2007).

REFERENCES

[1] Ajali U, 2004. Saponins. In: Chemistry of Bio – compounds. First ed. Rhyce Karex Publishers, Enugu, Nigeria. Pp167 – 175.

[2] Association of Official Analytical Chemists (AOAC), 1980. Methods of analysis 13th ed. AOAC Publishers, Washington D.C., pp 128 – 134.

[3] Ajiwe VIE, CI Agupugo, SO Umeh and HO Nnabuenyi, 2008. The Preliminary study of the Pharmaceutical constituents of Pterocarpus soyauxi (Oha – ocha) leaf. *Journal of the Chemical Society of Nigeria (Anachem)* 3 (2): 501 – 511.

[4] Ayesu ES, 1978. Medicinal Plants of West Africa. Reference publications Inc. Michigan USA, pp 235

[5] Bryant MC, 1972. Antibiotics and their Laboratory control 2nd ed. Butterworth's, London. Pp85.

[6] Dutta AC, 1989. Textbook of Botany for degree students. Oxford University press London, 2nd ed pp 580 -81

[7] Evans WC, 2002. Trease and Evans Pharmacognosy. 15th ed Saunders Company Ltd, Edinburgh pp56

[8] Finar IL, 2002. Alkaloids. In :Organic Chemistry Vol 2. Stereochemistry and the Chemistry of Natural Products, 5th ed Pearson Education Ltd, Delhi, India, pp 696 – 768.

[9] Goldstein A, L Aronow and S Kalman, 1974. Drug Tolerance and Physical Dependence. In: Principles of drug action: the Basis of Pharmacognosy, 2nd ed. John Wiley and sons Inc, New York. Pp 569 – 621.

[10] Harborne JB 1998. Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall, London, UK. Pp 1-48

[11] Hayashi G and AE Takemori, 1971.The Type of Analgesic Receptor Interaction Involved in certain Analgesic assays. *European Journal of Pharmacology*. 1: 63 – 66.

[12] Kar A, 2007. Narcotic Analgesics (Opiate Analgesics). In: Medicinal Chemistry, 4th ed. New Age International publishers, Ltd., New Delhi pp 304 – 341.

[13] Keay RW 1989. Trees of Nigeria. Oxford University press, New York. vol. 1 pp 262 -68.

[14] Koster R, M Anderson and EJ deBeer 1959. Acetic acid for Analgesic Screening. *Fed. Proc* 18: 412.

[15] Lorke D, 1983. A new approach to Practical Acute Toxicity Testing. *Arch of Toxicology*. 53: 273 – 289.

[16] Takemori EA and PS Portoghese, 1987. Evidence for the interaction of morphine with Kappa and Delta Opoid, receptors to induce analgesia in beta – funaltrexamine – treated mice. *The Journal of Pharmacology and Experimental Therapeutics* 243: 91 – 94.

[17] Twycross RG, 1984. Use of Analgesics to achieve Analgesia. *Journal of the Royal College of Physicians of London*, 18: 32 – 35.

[18] Umerie SC, SO Umeh and SC Nwobi, 2007. Phytochemical analysis and Analgesic studies On *Scoparia dulcis* (*Scrophulariaceae*). *Natural and Applied Sciences Journal*, 8: (1), 66 – 72.

[19] Vohora SB, 1980. Antipyretic, Analgesic and Antimicrobial Studies on *Sismbrium irio. Plant medica* 38 (3): 255- 259.

[20] Way WL, HL Fields and MA Schumacher, 2001. Opoid Analgesics and Antagonists. In: Basic and Clinical Pharmacology. 8th ed, Katzung, B.G. McGraw – Hill Companies, inc. New York. Pp 512 – 531

[21] Willard H; L Merritt and J Dean, 1984. Instrumental Method of Analysis, 6th ed. Van Nostrand New York; pp 420 – 423.