Journal of Medical and Applied Biosciences *Volume 4, September 2012*

www.cenresinpub.org
ISSN 2277-0054

PHYTO-CHEMICAL SCREENING AND ANTI-BIOTICS POTENTIALS OF *PYCNANTHUS ANGOLENSIS* (WELW.) WARB. (MYRISTICACEAE) BARK JUICE

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ABSTRACT

Chloroform and methanol extracts of the bark of *Pycnanthus angolensis* (Myristicaceae) were investigated with the aim of establishing its acclaimed potency as a blood clotting and wound healing agent (Anti-bacterial). Preliminary phyto-chemical screening of the juice extracted from the bark using chloroform/methanol (1:2) showed that it contained flavonoids, anthraquinones, tannins and saponins. Agar diffusion studies revealed that bark juice chloroform /methanol extract caused inhibition of *Salmonella typhii, Pseudomonas aeruginosa* and *Escherichia coli* but do not inhibit *Bacillus subtilis* at all concentrations. *P. aeruginosa* and *E.coli* produced inhibition zones of 17$\pm$ 0.2 and 18 $\pm$ 0.1respectively as the highest, these values were however significantly different from the values obtained from the control antibiotics levofloxacin tablet (KNISS India) 5mg /ml ($P< 0.05$), which yielded 19$\pm$0.1 and 20 $\pm$ 0.2 respectively for *P. aeruginosa* and *E. coli.* The result are consistent with the local use of the bark juice in stopping blood from wounds as well as healing up wounds, since these bacteria are mainly found in wounds.

Keywords: *Phytochemical screening, Anti-biotics, Pycnanthus angolensis, Bark juice, Bacteria, Levofloxacin.*

INTRODUCTION

Pycnanthus angolensis (Welw.) Warb. (Myristicaceae) belongs to family of plants known for their numerous fruit trees, fragrant spicy, whose dried fruit are used as condiment (Burkill, 2000). The plants is commonly known as "Carra board" in the Cameroon where it is split into rough planks for house building. In Nigeria, it is called by various local names such as "Akwa-mili" in 1bo, "Akomu" in Yoruba, "Kpokogi" in Nupe and "Umoghan" in Bini. It is a medium-sized forest tree with slender branches bunched at the top in several whorls, slightly dropping at the ends. The densely clustered fruits are like Nutmeg hence its name as ''African Nutmeg" .Leaves are 7-12 inches long by 2 – 3.5 inches broad with margin nearly parallel (Hutchings, 1996). It flowers from December to March. These flowers are borne in densely cluster at the end. The bole is straight, cylindrical without buttress. Bark grey, exuding a sticky honey – coloured juice turning red. Geographically the plant is found low land rain forest especially in secondary forest of Africa like Guinea, Uganda, Angola, Southern part of Nigeria, Cameron, Senegal, DR Congo, Tanzania and Zambia. The plant has been used for various purposes in Africa. A yellow reddish brown fat called "Combo butter" or "Angola tallow" is extracted from the seed and use for illumination and in soap making (World Agro Centre, 2006). Other uses include: *Bark:* for treatment of skin infection, as purgative, cleanse milk of lactating mothers and treat cough and chest complain. In Ghana the bark is use to treat anemia, in Cote d' Ivoire as a poison for antidote and against ascites and leprosy, in

Congo DR, the bark is used to solve infertility problems as well treat gonorrhea and in Sao' tome it is used to treat malaria. *Roots:* in Cote d' Ivoire mixture of roots and other parts treat schistosomiasis (Vabi and Mala'a, 1995). Antimicrobial and antihelminthc properties of the leaves stem and roots had been reported (Onocha and Otunka, 2010). The leaves also possess antioxidant and anti -inflammatory activities. (Asianowa and Acquaye, 2005). The aim of this study is therefore, to determine the phytochemical screening and antibiotics potentials of the plant's juice on wound bacterial.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The bark of *P. angolensis* containing the reddish juice were collected from a forest in Kachia Local Government Area of Kaduna State, Nigeria, and identified by Mallam Musa of the Herbarium unit Department of Biological Sciences Ahmadu Bello University, Zaria. A voucher number of *1751* was identified for the plant where it was deposited. The barks were air-dried for seven days and pounded using local mortar. The powdered barks, 1kg (1000g) of the plant were successfully extracted with n-hexane, chloroform and Chloroform/Methanol (1:4) for 48 hours respectively. The filtrates were concentrated by heating in water bath. Percentage yield was calculated and then stored in desiccators for further use.

Preliminary Phytochemical Screening of Extract

The methods by Evans (2006) were used to test for the presence of tannins, saponins, alkaloids, flavonoids, anthracenes and terpenes. Analysis of the phytochemicals were carried out using thin layer chromatographic techniques (Touchstone, 1992) as well as column chromatography in order to determine the fraction that is responsible for the activity of the bark juice of *P. angolensis*.

Test for Saponins

The extract test positive to both frothing and Haemolysis test (Evans, 2006).

Test for Flavonoids

0.5g of extract in NaOH solution test positive also Shinoda's test.

Test for Tannins

The extracts when soaked in Gold-beaters skin give positive color of black with the skin and green color with ferric chloride.

Test for Alkaloids

0.5g of the extract in the following reagents gives positive results: Dragendorff's, Meyer, Wagner, picric acid and tannic acid.

Test for Anthraquinones

The extract gives positive result to both Borntrager's test and modified Borntrager's test (MBT)

Test for Cardiac Glycosides

0.5g of the bark juice extract in 2ml chloroform and H_2S0_4 gives no color change.

Test for Terpenes

Some extracts were treated with Lieberman's reagents, there were no color change.

Chromatographic Analysis by TLC and Column Chromatography

Detecting reagents were used to locate the type of compound in each spot and various fractions of between 100 and 200 samples were collected from the column. These fractions were test for antibacterial properties on the selected microbes, using methods by Bauer *et al.* (1996).

Antibacterial Assay Using Antibiotics Susceptibility Testing

Four bacteria were clinically isolated from the patients at Medical Microbiology Department, Ahmadu Bello University, Zaria. The bacteria are: *Salmonella typhii*, *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. Isolates were checked for purity and maintained in slant of nutrient agar preserved at 5^0C to get needed activities. Antibiotics/ Susceptibility testing was carried out by well diffusion (Zwadyk, 1972). An overnight broth culture of $1x10^7$ CFU of each bacterium was used to seed sterile molten agar medium maintained at 45^0C. Five wells of 6mm diameter were bored using sterile cork borer after seeded plates had solidified.0.1ml of the extract was poured into the wells using sterile syringe, and controls were set up containing solvent and levofloxacin tablet (5mg/lm) with the bacteria, each plate had wells filled with methanol or chloroform as well as levofloxacin tablet (KNISS India). Diameters of zone of inhibition were measuring using transparent ruler. Diameters greater than or equal to 12mm were considered active. The experiment was done in triplicates and zones of inhibition (mm) were expressed as the mean/standard errors of means. Significant difference between the extract and the controls were determined using t-test at $P< 0.05$. Based on the outcome of the above steps, susceptibility testing was done using the fractions collected and testing on the bacteria each to know the component that is responsible for the activity of the extract (Bauer *et al.,* 1996).

Table1: Phytochemical Screening of Bark juice Using Chloroform/Methanol (1:4)

Constituents	Test	Observations	Inference
Saponins	Frothing	30 min frothing	+
	Hemamolysis	RBC haemolysed	+
Flavonoids	NaOH	Yellow coloration	+
	Shinoda's	Orange coloration	+
Tannins	Gold beaters	Black coloration	+

Table: 1 Contd.

	$FeCl_3$	Green coloration	+
Alkaloids	Dragendorff's	Rose -red coloration	+
	Mayer	Whitish coloration	+

	Wagner	Cloudy ppt	+
	Picric acid	Yellow ppt	+
	Tannic acid	Dark- black color	+
Cardiac Glycosides	Salkowski	No color change	-
	Kella killiani	No color change	-
	Kaddes's	No color change	-
Anthracenes	Borntrager	Pink-red	+
	MBT	Yellow	+
Terpenes	Lieberman	No color change	-

+ = positive (presence), - = Negative (absence), BMT = modified Borntrager's test.

Table 2: Antibacterial / Susceptibility Testing of Bark Juice of *P. angolensis*

Plant Extract	Conc. (mg/ml)	Mean±SE *S. typhii*	Mean±SE *P.aeruginosa*	Mean±SE *E.coli*	Mean±SE *B.subtilis*
Bark Juice	20	11±0.1	10±0.2	-	-
	25	11±0.1	12±0.1	13±0.2	-
	30	13±0.3	15±0.6	14±0.1	-
	35	16±0.1	16±0.6	17±0.2	18±0.3
	40	18±0.2	19±0.2	18±0.1	19±0.2
	Levofloxacin^{R}	21±0.4	23±0.1	20±0.2	20±0.1

Methanol was used for dissolving the extract as well as the control, n= 3, Levofloxacin^{R} 5mg/ml (KNISS India), P<0.05.

Table 3: Antibiotics Susceptibility Testing Using Fractions (with Activities) Collected in any Two Bacteria with Highest Mean ± SE

Bacteria	Conc. (mg/ml)	Mean diameter zone of inhibition + SE (mm).			
		Flavonoids P1	Anthracenes P2	Alkaloids P3	Tannins P4
P. aeruginosa	20	12±0.2	3±0.01	2± 0.01	-
	25	13±0.1	8±0.01	3±0.02	-
	30	15±0.6	9±0.1	5±0.01	-
	35	15±0.6	9±0.1	5±0.01	5±0.01
	40	17±0.2	9±01	6±0.02	6±0.02
E. coli	20	12±0.1	6±0.02	8±02	-
	25	13±0.1	7±0.01	8±0.2	-
	30	15±0.2	8±0.01	9±0.1	-
	35	17±0.1	9±0.1	9±0.1	-
	40	19±0.2	9±0.1	8±0.2	-

- (means not susceptible or no activity), P1 – P4 (Portions 1-4), NaOH (spraying reagent for flavonoids), mean± SE ≤ 12 mm = inactive on the bacteria.

RESULTS AND DISCUSSION

The results showed that the plant bark juice chloroform/methanol extract contains mainly glycosides (Table 1). Antibiotics susceptibility testing showed that the extract was very potent on *P. aeruginosa, S. typhii* and *E. coli* (Table 2). These bacteria are often found on wounds and caused prolonged healing of wounds. The inhibition rates were however, concentration dependent, because increase in concentration of the extract also leads to increase in diameter zones of inhibition which is significantly different from the control as seen from the results (Table 2). It is very accurate to say at this junction, that the antibacterial activity (that is wound healing ability) of the bark juice chloroform /methanol (1:4) extract is due to the presence of flavonoids in the plant (Table 3) since diameter zones of inhibition were greater than 12 mm. The study therefore, showed that the bark juice extract possess anti-bleeding and wound healing activities (antibiotics potentials) and can be used to stop bleeding wounds as well as heal up wounds, and flavonoids in the extract are responsible for this activity.

ACKNOWLEDGEMENTS

I thank very immensely my friend who had helped me in making this work a success Samuel Ndidi Uche of the Department of Biochemistry Ahmadu Bello University Zaria, Nigeria.

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