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**Archives of Applied Science Research, 2010, 2 (4): 186-190**

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**ISSN 0975-508X**
**CODEN (USA) AASRC9**

# Biological activities of extracts of *Pycnanthus angolensis* (Welw.)Warb

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## ABSTRACT

*Chloroform and methanolic extracts of the leaves, roots and stem of Pycnanthus angolensis (Myristicaceae) were investigated with the goal of establishing its acclaimed potency as an anthelmintic and antimicrobial agent. The result of the agar diffusion studies revealed that the leaf methanol extract caused inhibition against two of the 5 bacterial strains namely, Salmonella typhii and Pseudomonas aeruginosa used for the study. It also exhibited marked inhibition against the three fungal strains used for the study, the order of sensitivity being Aspergillus niger > Candida albicans > Dermatophyte sp. The chloroform extract of the leaves and methanol extracts of the leaves and stem exhibited considerable anthelmintic activities invitro using Fasciola gigantica, Taenia solium and Pheritima pasthuma. The sensitivity was concentration related and comparable to that of the reference compound piperazine citrate. These results are consistent with the folklore use of the plant in treatment of helmintic and microbial infections.*

**Key words:** *Pycnanthus angolensis*, anthelmintic, antimicrobial, Myristicaceae.

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## INTRODUCTION

*Pycnanthus angolensis* (welw.) warb (Myristicaceae), reputed for its analgesic, stomachic, aperative, carmimative, anti-inflammatory, haemostatic and antimicrobial actions belong to a family known for its numerous fruit trees, fragrant spicy plants whose dried fruits are used as condiment [1,2].

The plant known as wild African nutmeg is a lowland tree forest native to West and East Africa. It has also been reported to be useful for treatment of female sterility, gonorrhoeal infertility, rhinopharyngeal and broncho pneumonia and as a poison antidote [1,3]. A collyrium of the latex is used for treatment of various eye troubles especially cataract, filaria in the eye and for schistosomiasis [2]. Allantoin [4], isoflavonoids [5] and dihydroguaiacetic acid [6] have been isolated from the bark while some terpenoid type quinones with hypoglycaemic activity have been isolated from the bark and leaves [7,8].

With the aim of establishing its acclaimed potency as an anthelmintic and antimicrobial agent and in continuation of studies in our Laboratory of antimicrobial components and derivatives

from Nigerian medicinal plants [9-11], we now report the biological activities of extracts of *Pycnanthus angolensis.*

## MATERIALS AND METHODS

### Collection, Authentication and Extraction of Plant Material

Different parts of *P. angolensis* (root, stem and leaves) were collected from Barth road, University of Ibadan. A voucher specimen (FHI 1064) was identified by Mr. Felix Usang of Forest Research Institute of Nigeria (FRIN) where it is deposited. The air dried roots (1kg), stem (1.2kg) and leaves (900g) of *P .angolensis* were extracted successively with hexane, chloroform and methanol for 48 hours respectively, and the resulting plant extracts were stored in the refrigerator prior to use.

### Antimicrobial assay

Clinical strains of human pathogenic microorganisms made up of 5 bacteria and 3 fungi namely : *Staphylococcus aureus, Bacillus subtilis, Salmonella typhii, Escherica coli, Pseudomonas aeruginosa, Aspergillus niger, Candida albicans* and *Dermatophyte sp*. (listed in Table 1) were obtained from the Microbiological laboratory unit of Pharmaceutical Microbiology Department, University of Ibadan. The cultures were maintained on nutrient agar slants and preserved at 4 – 5 $^{o}$ C until needed for antimicrobial activity. Antimicrobial activity was carried out by the cup agar broth diffusion method [12]. An overnight broth culture of 1-2 x $10^{7}$ CFU of each bacterium was used to seed sterile molten agar medium maintained at 45$^{o}$ C. Sterile tryptone soya agar plate was similarly seeded with fungi. Seven wells respectively, were bored in each plate (7mm, diameter) with an aseptic cork borer when seeded plates had solidified. Different concentrations of the extracts (1ml portions) were introduced into the wells with the aid of a Pastuer pipette and controls were set up containing solvent and ampicillin solution (12.5 µg / ml). Diameters of zones of inhibition were determined after incubating plates at 37$^{o}$ C for 24 hr (bacteria) and at 25$^{o}$ C for 72h (fungi). When seeded with bacteria, each plate had wells filled with methanol (or chloroform) as well as ampicillin and for fungi, tioconazole was filled in one of the wells also. Diameters of zones of inhibition ≥ 10mm were considered active. Antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as the mean and standard errors on means. Student's "T" test was used to test probability at $P < 0.05$.

**Table 1: Antimicrobial activity of Leaf Methanol Extract of *Pycnanthus angolensis***

| Plant Extract | Conc[a] (mg/ml) | Mean Diameters of zones of Inhibition of bacteria in mm (+SEM[b]) | | | | | Mean Diameters of zones of Inhibition of fungi inmm (+SEM[b]) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *B. subtilis* | *S. typhii* | *S. aureus* | *E. coli* | *P. aeruginosa* | Conc[a] (mg/ml) | A. niger | C. albicans | *D .spp* |
| Leaf extract | 25 | - | 13±0.2 | - | - | 9±0.2 | 25 | 16±0.5 | 10±0.9 | 10±0.3 |
| | 50 | - | 13±0.3 | - | - | 9±0.5 | 50 | 18±0.2 | 10±0.5 | 10±0.1 |
| | 75 | - | 15±0.1 | - | - | 15±0.6 | 75 | 20±0.1 | 15±0.5 | 10±0.1 |
| | 100 | - | 17±0.4 | - | - | 15±0.2 | 100 | 20±0.1 | 18±0.1 | 12±0.3 |
| | 125 | 10±0.5 | 17±0.2 | 15±0.1 | - | 15±0.3 | 125 | 22±0.2 | 20±0.5 | 12±0.2 |
| | Amp | 15±0.5 | 19±0.1 | 27±0.2 | 13±0.2 | 18±0.1 | Tioconazole | 12±0.5 | 16±0.8 | - |

*[a] Methanol was used for dissolving the extracts as well as the control;[b] N=3*

Nutrient broth, nutrient agar, sabourand dextrose agar (SDA), tryptone soya agar (Oxford Laboratories, U.K.) were used in the assays. Methanol (and chloroform) (Merck) was also used in solublising the extracts \ drugs and as a negative control in the assays.

Ampicillin, 12.5µg\ml (Lab Oftalmiso, Spain), tioconazole cream 12.5µg\ml (Pfizer Inc., New York) were included as standard reference drugs in the study.

**Anthelmintic assay**

Helminths used for the assay include *Fasciola gigantica* (liver fluke mean weight of 0.05-0.07g), Taenia *solium* (tapeworm, 2.4 -2.8g) and *Pheritimia pasthuma* (earthworm, 0.5 - 0.6g). *P. pasthuma* was collected from the Awba dam and the water logged areas of staff school, both within the campus of University of Ibadan (UI) while the other two worm types were obtained from freshly slaughtered cows in the Bodija abattoir, in Ibadan metropolis.. All three worm types were authenticated at the Parasitology Research Unit, Zoology Department, UI. Anthelmintic assay was carried out by previous method [13]. Two worms (same type) were both placed in 9cm petri dishes in solution of crude extracts in six different concentrations (10, 20, 30, 50, 80 and 100mg/ml in distilled water), respectively. Mean times for paralysis (P, in minutes) were recorded when no movement of any sort could be observed, except when the worms were shaken vigorously. Times of death of worms (D, in minutes) were also recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in water ($50^0$c). Piperazine citrate (10mg/ml) was included as control. The assay was done in triplicate for each concentration of extract, reference and control. Six extracts namely, chloroform extracts of leaves (LC),roots (RC) and stem (SC), respectively and methanolic extracts leaves (LM),roots (RM) and stem (SM), respectively of the plant (as listed in Table 2) were used for this assay.

**Table 2 :Anthelmintic activity of Extracts of *Pycnanthus angolensis***

| S/N | Plant Extracts | Yield (%) | Conc[a]. (mg/ml) | Time of Paralysis (P) and Death (D) of worms in minutes (+SEM[b]) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *T. solium* | | *F. gigantica* | | *P. pasthuma* | |
| | | | | P | D | P | D | P | D |
| 1. | LC | 5.9 | 10 | 4.5±0.5 | 6.0±0.3 | 5.1±0.6 | 6.3±0.2 | 23±0.1 | 70±0.2 |
| | | | 20 | 4.2±0.3 | 5.5±0.2 | 4.3±0.1 | 6.0±0.9 | 20±0.3 | 67±0.9 |
| | | | 30 | 4.0±0.2 | 5.0±0.6 | 4.0±0.2 | 5.8±0.5 | 18±0.9 | 65±0.1 |
| | | | 50 | 3.8±0.5 | 4.5±0.5 | 3.8±0.5 | 5.5±0.9 | 15±0.2 | 58±0.3 |
| | | | 80 | 3.1±0.4 | 4.4±0.2 | 3.6±0.2 | 5.0±0.1 | 15±0.5 | 49±0.2 |
| | | | 100 | 1.5±0.3 | 2.0±0.1 | 2.8±0.1 | 4.1±0.9 | 10±0.1 | 40±0.1 |
| 2. | LM | 8.9 | 10 | 5.1±0.1 | 8.6±0.2 | 5.1±0.1 | 8.6±0.5 | 45±0.2 | 60±0.8 |
| | | | 20 | 4.9±0.3 | 7.7±0.6 | 5.0±0.3 | 8.0±0.2 | 42±0.5 | 55±0.5 |
| | | | 30 | 4.8±0.2 | 7.7±0.3 | 4.3±0.4 | 7.8±0.1 | 40±0.3 | 50±0.3 |
| | | | 50 | 4.0±0.9 | 7.5±0.2 | 3.6±0.2 | 7.6±0.1 | 38±0.8 | 44±0.5 |
| | | | 80 | 3.0±0.9 | 7.3±0.1 | 2.8±0.5 | 7.4±0.2 | 31±0.5 | 45±0.5 |
| | | | 100 | 2.3±0.4 | 7.1±0.2 | 2.3±0.3 | 7.2±0.2 | 32±0.9 | 60±0.5 |
| 3. | RC | 8.9 | 10 | 23±0.1 | 52±0.2 | 36±0.5 | 30±0.3 | 33±0.9 | 45±0.2 |
| | | | 20 | 22±0.3 | 49±0.1 | 27±0.7 | 24±0.6 | 28±0.5 | 42±0.1 |
| | | | 30 | 20±0.8 | 46±0.3 | 25±0.2 | 18±0.5 | 26±0.3 | 40±0.3 |
| | | | 50 | 18±0.2 | 38±0.3 | 21±0.5 | 18±0.3 | 23±0.8 | 36±0.3 |
| | | | 80 | 15±0.5 | 31±0.2 | 20±0.4 | 15±0.2 | 22±0.2 | 35±0.2 |
| | | | 100 | 13±0.1 | 26±0.2 | 17±0.5 | 13±0.3 | 20±0.1 | 30±0.3 |
| 4. | RM | 9.9 | 10 | 34±0.9 | 38±0.2 | 15.4±0.2 | 18.5±0.2 | 39±0.1 | 45±0.5 |
| | | | 20 | 31±0.5 | 30±0.2 | 11.5±0.5 | 16.5±0.6 | 30±0.2 | 38±0.3 |
| | | | 30 | 28±0.3 | 31±0.3 | 8.0±0.5 | 15.0±0.5 | 26±0.5 | 32±0.2 |
| | | | 50 | 13±0.8 | 26±0.6 | 5.4±0.1 | 13.5±0.2 | 24±0.3 | 27±0.5 |
| | | | 80 | 10±0.1 | 24±0.5 | 5.3±0.1 | 11.5±0.2 | 15±0.1 | 20±0.9 |
| | | | 100 | 5±0.2 | 18±0.5 | 3.5±0.2 | 7.4±0.3 | 9±0.1 | 15±0.1 |
| 5. | SC | 6.8 | 10 | 29±0.5 | 60±0.1 | 8.3±0.9 | 23±0.5 | 80±0.2 | 105±0.1 |
| | | | 20 | 25±0.4 | 57±0.5 | 7.1±0.5 | 22±0.5 | 75±0.1 | 100±0.3 |
| | | | 30 | 23±0.5 | 55±0.2 | 4.5±0.5 | 20±0.4 | 70±0.3 | 98±0.8 |
| | | | 50 | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 80<br>100 | 19±0.2<br>18±0.1<br>15±0.1 | 52±0.7<br>49±0.3<br>46±0.2 | 4.3±0.1<br>4.2±0.9<br>3.1±0.5 | 18±0.3<br>15±0.2<br>14±0.1 | 70±0.3<br>68±0.4<br>62±0.2 | 90±0.5<br>90±0.2<br>90±0.1 |
| 6. | SM | 4.5 | 10<br>20<br>30<br>50<br>80<br>100 | 7.1±0.9<br>6.1±0.5<br>3.4±0.3<br>1.2±0.8<br>1.0±0.2<br>1.0±0.1 | 57±0.5<br>40±0.8<br>37±0.2<br>15±0.3<br>12±0.2<br>8±0.2 | 4.5±0.5<br>4.3±0.7<br>3.3±0.2<br>3.0±0.5<br>2.6±0.4<br>2.0±0.4 | 8.1±0.1<br>7.1±0.5<br>7.0±0.3<br>6.6±0.5<br>6.0±0.4<br>3.9±0.1 | 39±0.5<br>30±0.7<br>26±0.2<br>21±0.5<br>15±0.4<br>9±0.1 | 45±0.5<br>35±0.3<br>32±0.2<br>27±0.5<br>20±0.1<br>15±0.9 |
| 7. | Piperazine citrate | | 10 | 1.6±0.05 | 40±0.05 | 1±0.2 | 3±0.05 | 20±0.2 | 60±0.5 |

*P<0.05*

[a] *control worms (in distilled water) for:*

(i) *T. Solium lived till the next 24 hours.*

(ii) *F. gigantica were alive for 5 hours.*

(iii) *P. pasthuma were alive up till 48 hours.*

[b] *N =3*

## RESULTS AND DISCUSSION

900g of air dried powdered leaves of *Pycnanthus angolensis* produced 4.8, 5.9, and 8.9 % of hexane, chloroform and methanol extracts respectively, while 1.2kg of *stem* afforded hexane (3.8%),chloroform(6.8%) and methanol (9%) extracts. The roots (1kg) yielded 5.5, 8.9, and 9.9% of hexane, chloroform and methanol extracts, respectively.

The result of the agar diffusion studies revealed that the leaf methanol extract caused inhibition against two of the 5 bacterial strains namely, *Salmonella typhii* and *Pseudomonas aeruginosa* used for the study as shown in Table 1. It also exhibited marked inhibition against the three fungal strains used for the study. The activity is comparable to the reference tioconazole trosyd, the order of sensitivity being *Aspergillus niger > Candida albicans > Dermatophyte sp.*

Results of the anthelmintic assay are given in Table 2. The chloroform extract of the leaves and methanol extracts of the leaves and stem exhibited considerable anthelmintic activities invitro using *Fasciola gigantica, Taenia solium* and *Pheritima pasthuma*. The sensitivity was concentration related and comparable to that of the reference compound piperazine citrate.

The present findings demonstrate that the leaf and stem methanolic extracts of *P. angolensis* possesses good anthelmintic activity while the leaf methanolic extract is antimicrobial in action. The antimicrobial and anthelmintic activities of *Pycnanthus angolensis* have hitherto not been investigated and the results obtained from the present study establishes to a large extent, its acclaimed potency as an anthelmintic and antimicrobial agent.

Conclusively, there is always the constant need for development of new drugs due to increasing number of antibiotic resistant organism. *Pycnanthus angolensis* thus provide a good opportunity for drug development.

**Acknowledgements**

PAO acknowledges with thanks the travel grant (2008/2009) and the Senate Research Grant (SRG/FSC/2000/22A) from the University of Ibadan, Ibadan.

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