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Full Length Research Article

# Antibacterial and Anti-Inflammatory Activities of *Anacardium occidentale* Leaves and Bark Extracts

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**ABSTRACT:** *Anacardium occidentale* is a local medicinal plant used in ethno medicine for the treatment of diarrhea, constipation,pain and inflammation. The aqueous and ethanolic extracts of this plant parts were assessed for anti-inflammatory and antibacterial activities using experimental animal model and agar disc diffusion methods respectively. Results show that the ethanolic extract of the plant were more efficacious than the aqueous extract in inhibiting the carrageenan induced paw oedema in rats in a non dose-dependent manner( P>0.05).No significant difference was found between the ethanolic extract of the leaves and bark (P>0.05). Also, the antibacterial activity was apparently higher in ethanolic extract than in aqueous extract for both leaves and barkwith the bark extract displaying a significantly (P<0.05) higher activity compared to the leaves extract. The results of this study therefore justify the use of this plant in the treatment of inflammation and bacterial infections.
**Key words**: Antibacterial, Anti inflammatory, *Anacardium occidentale*

## INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008) and a principal source of pharmaceutical drugs and agents (Ogundipe *et al*., 1998).It is estimated that today,plant materials are present in or have provided the models for 50% of Western drugs (Robbers, 1996).The primary benefit of using plant derived medicine is that they are relatively safer than synthetic alternatives,offering profound therapeutic benefits and more affordable treatments (Ajali and Okoye, 2009).The search for better alternative anti-inflammatory and antimicrobial drugs from the bounties of our vegetation is thus a worthwhile venture. *A. occidentale* is a tree that grows up to 1.5M in height with thick tortous trunk and woody branches. It belongs to the family Anacardiaceae is native to Brazil and has a great economic and medicinal value (Rajesh *et al*., 2009). *A. occidentale* is commonly called Cashew in English, "Kashu" in Hausa, "Okpokpo" in Ibo and Kaju in Yoruba (Arekemase *et al*., 2011).

This plant is a multipurpose tree whose leaves, stems and bark extracts are used extensively for the treatment of diarrhea, dysentery and Colonic pain (Sadiq*et al*.,2009).It has also been reported to possess anti-diabetic, anti-inflammatory and anti-ulcerogenic properties (Akinpelu,2001).Some of these ethno medicinal uses could be attributed to the antimicrobial, analgesic and anti-inflammatory properties of the plant. This research was therefore carried out to determine the antibacterial and anti-inflammatory activities of *Anacardium occidentale.*

## MATERIALS AND METHODS

### Collection of Plant Materials

Fresh leaves and barks of *A. occidentale* were collected from Ilaro, Ogun State, Southern Nigeria. The plants were authenticated by a taxonomist at the Forestry Research Institute of Nigeria (FRIN) and were designated L1 and B1 on the basis of the parts of the plant collected. L1 and B1 represent leaves and bark of *A.occidentale* respectively.

### Preparation of Plant Extract

The leaves and bark of *A. occidentale* were air-dried for 3 weeks. Each of the samples was powdered using mortar and pestle into fine powder. Cold extraction of the plant was made as described by Thomas *et al*. (2012).The flasks were manually agitated at 2hours intervals for 5 days. All extracts were then filtered with Whatman no. 2 filter paper (Marjorie, 1999). The filtrates were later concentrated to dryness with the aid of a rotary evaporator (Thomas *et al*.,2012).

### Collection of Test Bacteria

Authentic pure cultures of human pathogenic bacteria like *Escherichia coli*, *Proteus vulgaris, Pseudomonas aeruginosa*, *Staphylococcus aureus, Enterococcus faecalis*, and *Salmonella typhi* were collected from the Molecular Biology & Biotechnology Division of the

Nigeria institute of Medical Research (NIMR) Lagos,Nigeria.These isolates were further confirmed using standard microbiological techniques according to (Efuntoye *et al*., 2010).

**Antibacterial Activity of Plant Extracts**

Susceptibility of the test bacterial isolates to the extracts was determined by standard disc diffusion assay (Rioss *et al.*, 1998). Well dried Mueller Hinton Agar plates were seeded with 24hour old culture of bacterial strains. The inoculums size was adjusted to achieve a final concentration of $10^5$cfu/ml after dilution from $10^8$cfu/ml (equivalent of 0.5Mcfarland standards).The sterile Whatman filter paper discs (5mm in diameter) impregnated with plant extract were placed on the surface of the culture plates and incubated at 37$^o$C for 24 hours and diameter of zone of inhibition were measured in mm. Discs with ethanol and sterile distilled water were used as control.

**Anti-inflammatory Activity of Plant Extracts**

Carrageenan-Induced rat paw oedema was used as a model of inflammation. Acute inflammation in the rats was produced according to the method described by Winter *et al.* (1962). Five groups of rats each containing five animals per group were used for the study. Group 1 served as the control receiving normal saline 5ml/kg, animals in groups 2 were given 10mg/kg indomethacin orally while group 3, 4 and 5 received the plant extracts at doses of 100,200 and 400mg/kg respectively. Animals were given a saline solution, indomethacin and the appropriate dose of the extract depending on the group, 1 hour before administration of an intradermal injection of carrageenan (0.1ml of a 1% solution in 0.9% saline solution) into the plantar region of the right hand paw.

The paw size was measured before injection of Carrageenan and every hour for a period of 6 hours. Measurement of paw size was carried out by measuring the circumference with a meter rule. The average increase in paw size of each group was calculated and compared with the control (normal saline) and indomethacin groups.

The percentage inhibition was calculated thus:
% inhibition = St- So/St

Where
St = the mean paw size for control group
So = the mean paw size obtained for each group

**Statistical Analysis**

All data were expressed as Mean ±SEM and both the student "t" test and ANOVA were applied to determine significant difference between the control group and mice treated with the test compound.. The level of significance was taken to be $P<0.05$.

**RESULTS**

Table 1 and 2 depict the antibacterial activity of the extract of *Anacardium occidentale* leaves and barks respectively. Both extracts show significant antibacterial activity but to varied degrees. The relative measurement of the antibacterial activity of the ethanolic extract of *A. occidentale* reveal significant statistical disparity between the leaves and the bark extracts (t value= 4.24, $p<0.05$) (table 3). Tables 4 and 5 represent the anti-inflammatory activities of aqueous and ethanolic extracts of the leaves and bark of *A. occidentale* respectively. Table 6 shows the comparative analyses of the ethanolic extracts of *A. occidentale* leaves and bark. No significant difference was observed between the anti inflammatory activity of ethanolic extracts of *A. occidentale* leaves and bark (t = -0.99, $P>0.05$). Table 7 shows the effect of different concentrations of ethanolic extract on the anti-inflammatory activity of *A. occidentale*. No apparent variation was found between the different concentrations examined (F value = 4.40, $P>0.05$).

**Table 1:** Antibacterial activity of *A. occidentale* leaves extract

| Plant designate | Test Organisms | Zone of inhibition (mm) Ethanol extract | Aqueous extract |
|---|---|---|---|
| *Anacardium occidentale* | EC | 12±0.90 | 8±0.01 |
| | PV | 10±1.20 | 8±0.21 |
| | PA | 9±1.00 | 6±0.21 |
| | SA | 12±0.00 | 8±0.00 |
| | EF | 7±0.00 | 5±0.00 |
| | ST | 8±0.42 | 5±1.00 |
| | EF | - | - |
| | PV | - | - |
| Distilled Water | SA | - | - |
| | EF | - | - |
| | ST | - | - |
| | EC | - | - |
| | PV | - | - |
| 50% Ethanol | PA | - | - |
| | SA | - | - |
| | EF | - | - |
| | ST | - | - |

EC = *Escherichia coli,* PV = *Proteus vulgaris,* PA = *Pseudomonas aeruginosa*, SA = *Staphylococcus aureus,* EF = *Enterococcus faeealis,* ST = *Salmonella typhi*

**Table 2:** Antibacterial activity of *A. occidentale* bark extract

| Plant designate | Test organisms | Zone of inhibition (mm) Ethanol extract | Aqueous extract |
|---|---|---|---|
| *Anacardium occidentale* | EC | 16±0.00 | 10±0.00 |
| | PV | 14±0.90 | 8±0.10 |
| | PA | 14±0.90 | 10±0.80 |
| | SA | 12±0.00 | 8±0.00 |
| | EF | 15±1.20 | 9±0.10 |
| | ST | 13±0.00 | 8±0.83 |
| | EF | 0.00±0.00 | 0.00±0.00 |
| | PV | 0.00±0.00 | 0.00±0.00 |
| Distilled Water | SA | 0.00±0.00 | 0.00±0.00 |
| | EF | 0.00±0.00 | 0.00±0.00 |
| | ST | 0.00±0.00 | 0.00±0.00 |
| | EC | 0.00±0.00 | 0.00±0.00 |
| | PV | 0.00±0.00 | 0.00±0.00 |
| 50% Ethanol | PA | 0.00±0.00 | 0.00±0.00 |
| | SA | 0.00±0.00 | 0.00±0.00 |
| | EF | 0.00±0.00 | 0.00±0.00 |
| | ST | 0.00±0.00 | 0.00±0.00 |

Key: EC = *Escherichia coli*; PV = *Proteus vulgaris*; PA = *Pseudomonas aeruginosa*; SA = *Staphylococcus aureus*; EF = *Enterococcus faecalis*; SA = *Staphylococcus aureus*

**Table 3:** Relative measurement of the antibacterial activity of the ethanolic extract of *A. occidentale*

| | | | Zone of inhibition(mm) | |
|---|---|---|---|---|
| Ethanolic extracts | n | Mean ± SEM | t-value | P-value |
| Leaves extract | 2 | 9.67±0.84 | 4.24 | <0.05 |
| Bark extract | 2 | 14.00+0.58 | | |

**Table 4:** Anti-inflammatory activity of aqueous and ethanolic extract of the leaves of *A. occidentale*

| Extracts | Doses (mg/1kg) | change in Paw oedema (Mean ± SEM) (mm) | % Oedema inhibition relative to control at the 6th hour |
|---|---|---|---|
| control (normal saline, 0.9% | 0.6ml | 3.82 ± 1.87 | - |
| | 10mg | 2.19 ± 0.12 | 42.80 |
| Aqueous extract | 100 | 2.40 ± 0.02 | 37.20 |
| | 200 | 2.34 ± 0.00 | 38.74 |
| | 400 | 2.30 ± 0.91 | 39.80 |
| Ethanolic extract | 100 | 1.90 ± 0.00 | 50.26 |
| | 200 | 1.33 ± 0.41 | 65.18 |
| | 400 | 1.28 ± 0.31 | 66.50 |

**Table 5:** Anti-inflammatory activity of aqueous and ethanolic extract of the bark of*A. occidentale*

| Extracts Oedema | Dose | Changes in paw(Means± SEM) (mm) | % Oedema inhibition relative to control at the 4th hour |
|---|---|---|---|
| Control (normal Saline, 0.9%) | 0.6ml | 3.82 ± 1.87 | - |
| Indometacin | 10mg | 2.00 ± 0.00 | 47.6 |
| Aqueous extract | 100 | 2.10 ± 0.00 | 45.03 |
| | 200 | 1.98 ± 0.20 | 48.17 |
| | 400 | 1.80 ± 0.10 | 52.88 |
| Ethanolic extract | 100 | 1.50 ± 0.00 | 60.73 |
| | 200 | 1.21 ± 0.10 | 68.32 |
| | 400 | 1.12± 0.00 | 70.68 |

**Table 6:** Comparative Analyses of EthanolicExtracts of A.*occidentale*Leaves and Bark.

| *Anacardium occidentale* Extracts | n | % oedema inhibition relative to Control at the 4 hour (Mean ± SEM) mm | t-value | p-value |
|---|---|---|---|---|
| Leaves | 3 | 60.65 ± 5.21 | 0.99 | >0.05 |
| Bark | 3 | 66.58 ± 3.00 | | |

**Table 7:** Effect of Concentrations of EthanolicExtract on the Anti-inflammatory Activity of *A.occidentale*

| Concentrations (mg/ml) | n | % oedema inhibition (mm)(Mean ± SEM) | t-value | p-value |
|---|---|---|---|---|
| 400 | 2 | 68.8 ± 2.95 | 4.40 | >0.05 |
| 200 | 2 | 66.8 ± 1.57 | | |
| 100 | 2 | 55.5 ± 5.24 | | |

## DISCUSSION

Results of this study have shown that both aqueous and ethanolic extracts of *A. occidentale* posses antibacterial activities. This observation may be due to the presence of bioactive substances in both the leaves and the bark extracts (Faruq *et al*., 2004, Ogukwe*et al*., 2004) even though the ethanolic extract was more efficacious than the aqueous extract. The higher activity of the ethanolic extract observed in this study may be due to the ability of the active ingredient present in *A. occidentale* to dissolve more in ethanol than in water (Obi and Onuhia, 2000). Ethanol has been shown to be a stronger extracting solvent than water (Abulude *et al*., 2009). Ethanol in addition to achieving better extraction may also enhance the efficiency of the active ingredients (Omojasola and Awe, 2004).

The ethanolic bark extracts are significantly more efficacious than the ethanolic leaves extract in terms of antibacterial activity. This may not be unconnected to the fact that the bioactive ingredients present in the bark were more soluble in ethanol than that of the leaves.

The anti-inflammatory activities of the aqueous and ethanolic extract of *A. occidentale* were also evaluated using carrageenan-induced rat paw oedema method (Winter *et al*., 1962, Adeyemi *et al*., 2002).

The ethanolic extracts were more effective in reducing the oedema than the aqueous extracts but no significant difference was observed between the ethanolic bark and leaves extract. Therefore either the leaves or the bark extract can be used for treating inflammation. Equilibrium of anti-inflammatory activities between the lowest and highest concentrations of *A. occidentale* clearly shows that the anti-inflammatory quality of the active constituent of the plant occurs in a non dose specific manner. In conclusion, the outcome of this study has demonstrated the antibacterial and anti-inflammatory activities of concentrates from *A. occidentale* .

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