

**EVALUATION OF THE ANTI-INFLAMMATORY, ANTIMICROBIAL  
AND ANTIOXIDANT PROPERTIES OF AQUEOUS CRUDE GEL  
EXTRACT OF *ALOE BUETTNERI***

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**ABSTRACT**

*Aloe vera (Aloe buettneri)* is an ornamental and medicinal plant. It can be used in the management of disease conditions such as inflammation, headache, diabetes etc. In this study, Anti-inflammatory, antimicrobial and antioxidant properties of the aqueous crude gel extract of *Aloe buettneri* was evaluated. The anti-inflammatory activity was determined using wistar albino rats which were divided into seven groups of four wistar rats each. Inflammation was induced by injecting 1% egg albumen in the left hind paw of wistar albino rats. Aqueous crude gel extract was fed orally to the experimental and control rats groups to investigate its effects on the paw joint oedema by measuring

the paw circumference with vernier calliper. The positive control group was given indomethacin which served as the standard. The result showed that indomethacin has the highest inhibition value which was significant ( $p < 0.05$ ) after 180 minutes ( $2 \pm 0.40$ ). Treatment groups C, D, E also significantly ( $p < 0.05$ ) repressed paw swelling. The antimicrobial activity was determined using the agar diffusion method against pathogenic microbial isolates such as; *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Klebsiella pneumonia* and *Samonella typhi*. The values of inhibition of microbial growth by the aqueous crude gel extract showed that the extract has antimicrobial potency. The antioxidant activity was determined using the following methods; nitric oxide scavenging activity, DPPH activity and anti-lipid peroxidation method. The highest antioxidant activity was at 200mg for each method of analysis and the values showed significant antioxidant properties. The obtained

results therefore suggest that the aqueous crude gel extract of *Aloe buettneri* can be used in the treatment of inflammation, oxidative damage and infections.

**KEYWORDS:** Antioxidant, *Aloe Buettneri*, Anti-inflammation, Antimicrobial, Egg Albumen, Paw Joint Oedema.

## INTRODUCTION

Inflammation is a normal protective response of living tissue to injury caused by physical trauma, Hypersensitivity, noxious chemicals or Etiologic agents. It involves a well organized cascade of fluid and cellular changes within living tissue (Kumar *et al.*, 2013). It is characterized by redness (rubor), swollen joint that is warm to touch, joint pain, its stiffness and loss of joint function (Shrestha *et al.*, 2013).

Inflammation is either acute or chronic inflammation. Acute inflammation may be an initial response of the body to harmful stimuli. In chronic inflammation, the inflammatory response is out of proportion resulting in damage to the body (Pilotto *et al.*, 2010).

Biochemical reactions in the body generate reactive oxygen species (ROS) which can damage important biomolecules, leading to several disease conditions. The harmful action of the free radicals can be blocked by antioxidants which scavenge the free radicals and nullify their damaging effect on cellular constituents (Praveena and Pradeep, 2012).

Dietary antioxidants can stimulate cellular defenses and help to prevent cellular components against oxidative damage. In addition they have been used in the food industry to prolong shelf life as they inhibit lipid oxidation. Majority of the antioxidants from plants are secondary metabolites like phenolics and flavonoids that have been reported to be potent free radical scavengers (Mathew and Abraham, 2006).

Plants generally have secondary metabolites which are important sources of biocides and many other pharmaceutical drugs (Naili *et al.*, 2010). Medicinal plants are important in pharmacological research and drug development (Li and Vederas, 2009).

*Aloe buettneri* is a succulent plant with thick and fleshy leaves arranged in a rosette. The leaves grow to about 40–80 cm long, 8–9 cm broad. The leaves are rimmed by alternating paired and solitary teeth and come together to form an underground bulb-like base making the plant appear stemless (Burkill, 1995).

The leaf sap is used to treat intestinal and urogenital problems (Ferro *et al.*, 2003). The dried powdered leaves are taken to treat malaria (Suhaj, 2006). A leaf decoction is drunk to cure cough (Hartmann, 2007). A decoction of the chopped whole plant is taken to treat venereal diseases (Ferro *et al.*, 2003). A leaf decoction is applied for treating cancer and Rheumatism is treated with leaf ash. The leaves are applied externally for all kinds of skin trouble such as burns, wounds, insect bites and Guinea worm sores (Agarry *et al.*, 2005).

The aim of this study was to evaluate the anti-inflammatory, antimicrobial and antioxidant potency of aqueous crude gel extract of *Aloe Buettneri*.

## **MATERIALS AND METHODS**

### **COLLECTION OF PLANT MATERIAL**

The freshly harvested leaf of *Aloe buettneri* plant was collected from a garden at Amaoba-Ime, Oboro in Ikwuano LGA of Abia State. The plant was botanically authenticated by Prof. G.G.E Osuagwu of the department of plant science and biotechnology, Michael Okpara University of Agriculture Umudike. The plant was rinsed severally with clean tap water to remove dust particles and debris.

### **PREPARATION OF EXTRACT**

The crude gel was collected by peeling out the outer cuticle and cutting out aseptically into small pieces. They were grinded and weighed. The gel was then mixed with distilled water (1:5 w/v) and then homogenized to create a homogenate. The sample was freshly prepared every time before use. It contained all the ingredient of the crude gel in the same proportion as it appears in the leaf.

### **EXPERIMENTAL ANIMALS**

Twenty eight (28) wistar albino rats weighing between 120-200g were obtained from the department of zoology, university of Nigeria, Nsukka and kept in the animal house of the biochemistry department of Michael Okpara University of Agriculture, Umudike. The animals were allowed access to feed and water *ad libitum* and were allowed two weeks of acclimatization before the commencement of the experiment. The animals were kept in a well-ventilated aluminum cages at room temperature and under natural light/darkness cycles. They were maintained in accordance with the recommendation of the Guild for the care and use of laboratory animals (1985).

### ACUTE TOXICITY TEST

The acute toxicity of the extract was done using white mice of both sexes. The animals were of average weight of 33g. Five groups of four animals each were used. The plant extract was administered to the animals intra-peritoneally at the doses of 0.2mg/kg, 125mg/kg, 250mg/kg, 500mg/kg and 1000mg/kg body weight respectively. The animals were observed for a maximum of 72 hours separately.

### EXPERIMENTAL INDUCTION OF INFLAMMATION

Albumin was administered on the right palm of the wistar albino rats to induce inflammation. The wistar albino rats were grouped into seven groups.

Group one was administered 0.5ml of 1000mg of aqueous gel extract of *Aloe buettneri*.  
Group two was administered 0.4ml of 1000mg of aqueous gel extract of *Aloe buettneri*.  
Group three was administered 0.3ml of 1000mg of aqueous gel extract of *Aloe buettneri*.  
Group four was administered 0.2ml of 1000mg of aqueous gel extract of *Aloe buettneri*.  
Group five was administered 0.1ml of 1000mg of aqueous gel extract of *Aloe buettneri*.  
Group six was given 0.5mg/ml of indometacine which serve as positive control.  
Group seven was given 0.5mg/ml of normal saline which serve as a negative control.

The inflammation was checked at a regular interval of 30 minutes for 180 minutes. The result of the inflammation was recorded for the various groups at their respective time interval.

### EVALUATION OF *IN VITRO* ANTIOXIDANT ASSAY

**DPPH radical scavenging assay:** 2,2-diphenyl-1-picrylhydrazine (DPPH) scavenging activity was quantified in the presence of stable DPPH radical on the basis of 2,2-diphenyl-1-picrylhydrazine (DPPH) assay system (Mensor *et al.*, 2001 as cited by Omodamiro, 2010).

#### ➤ Preparation of DPPH solution

- 1mmol/L of DPPH = 0.394g OF DPPH
- 0.5mMol = 0.197g(197mg)
- 1000ml = 197mg
- 150ml = ×

$$\times = \frac{150 \times 197}{1000} = 29.55\text{mg}$$

2ml of *Aloe buettneri* extract dissolved in ethanol was mixed at different concentrations (12.5 – 200µg/ml) with 1ml of DPPH solutions in test tube and incubated for 30minutes in the dark at room temperature. 1ml of ethanol + 2ml of test extract were used as negative control. The degree of discoloration indicates the scavenging efficacy of the extracts and absorbance was measured at 517nm. The experiment was performed in triplicate and percentage of scavenging activity was calculated using the following equation

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

### **NITRIC OXIDE INHIBITION ACTIVITY**

The nitric oxide scavenging activity was conducted based on the Greiss assay method (1858) which involves generating nitric oxide from sodium nitroprusside by the Greiss reaction.

2.0ml of 10mM sodium nitroprusside and 5.0ml of phosphate buffer were mixed with 0.5ml of different concentrations (12.5-200µg/ml) of plant extract and incubated at 25°C for 150 minutes. The samples were run as above but the blank was replaced with the same amount of water. After the incubation period, 2ml of the incubated sample was added to 2ml of Greiss reagent (1% sulphanilamide, 0.1% α-naphthyl-ethyldiaminedihydrochloride and 3% phosphoric acid) and then incubated for a period of 30 minutes. The absorbance of the pink chromophore formed by the diazotization of nitrite with α-naphthyl-ethyl diaminedihydrochloride was measured at 540nm. Ascorbic acid was used as positive control. The experiment was performed in triplicate and the capacity to scavenge the nitric oxide was calculated using the following calculation

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

### **ANTI-LIPID PEROXIDATION ACTIVITY**

The determination of anti-lipid peroxidation activity was according to the method of Dinakaran *et al.*, (2011). The ethanolic extract of *Aloe buettneri* were used at different concentrations (200, 100, 50, 25 and 12.5µg/ml) individually. 3ml of liver homogenate was added to 100µl of 15mM ferric chloride and was shaken for 30 minutes. From collected mixture, 100µl was added with 1ml of different concentrations of plant extract individually in different test tubes. Ascorbic acid was used as the standard (100µg/ml). All the test tubes were incubated for four (4) hours at 37°C.

After incubation, 1.1ml of 30% trichloroacetic acid (TCA) and 1.1ml of 0.65% thiobarbituric acid (TBA) were added to all tubes containing the mixture. After 30minutes of incubation in a shaking water bath and subsequent cooling in ice-cold water for 10 minutes, the tubes were centrifuged at 800g for 15 minutes. The absorbance was measured at 530nm. The percentage inhibition of lipid peroxidation was calculated by using the equation below

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### DETERMINATION OF ANTIBACTERIAL ACTIVITY

The antibacterial activity of the aqueous crude gel extract of *Aloe buettneri* was evaluated using microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Salmonella typhi*. The ability of the extract to inhibit growth of the clinically significant bacteria was determined using 8mm diameter hole in agar-diffusion technique. Sterile glass pipettes of 8mm diameter were used to make holes on prepared agar medium. A plate was prepared, each containing five holes of 8mm diameter for the different concentrations of the extract, for a specific isolate. A control hole where the solvent used for extraction was added to each of the petri dish. In total, five petri dishes were used and the plates were incubated at 37C for 24 hours in the incubator. Following incubation in the diameter of the zone of inhibition was recorded.

### STATISTICAL ANALYSIS

The results were analysis for statically significance by one way ANOVA, DUNCAN multiple range comparison using SPSS version 22.0. All data were expressed as mean  $\pm$ SD and  $P < 0.05$  values were considered significant.

### RESULTS

**Table 1: Result of anti-inflammation activity of *Aloe buettenri* on oedema induced wistar rat per time interval**

|   | CONCENTRATION      | 0MIN         | 30MINS       | 60MINS       | 120MIN       | 150MINS      | 180MINS      |
|---|--------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| A | 5mg (Indomethacin) | 2 $\pm$ 0.46 | 2 $\pm$ 0.44 | 2 $\pm$ 0.41 | 2 $\pm$ 0.41 | 2 $\pm$ 0.42 | 2 $\pm$ 0.40 |
| B | N. saline          | 2 $\pm$ 0.42 | 2 $\pm$ 0.42 | 2 $\pm$ 0.41 | 2 $\pm$ 0.41 | 2 $\pm$ 0.43 | 2 $\pm$ 0.45 |
| C | 500mg              | 2 $\pm$ 0.42 | 2 $\pm$ 0.41 | 2 $\pm$ 0.46 | 2 $\pm$ 0.45 | 2 $\pm$ 0.43 | 2 $\pm$ 0.40 |
| D | 400mg              | 2 $\pm$ 0.48 | 2 $\pm$ 0.51 | 2 $\pm$ 0.41 | 2 $\pm$ 0.40 | 2 $\pm$ 0.40 | 2 $\pm$ 0.40 |
| E | 300mg              | 2 $\pm$ 0.42 | 2 $\pm$ 0.42 | 2 $\pm$ 0.41 | 2 $\pm$ 0.41 | 2 $\pm$ 0.40 | 2 $\pm$ 0.40 |
| F | 200mg              | 2 $\pm$ 0.47 | 2 $\pm$ 0.46 | 2 $\pm$ 0.45 | 2 $\pm$ 0.45 | 2 $\pm$ 0.45 | 2 $\pm$ 0.44 |
| G | 100mg              | 2 $\pm$ 0.53 | 2 $\pm$ 0.50 | 2 $\pm$ 0.50 | 2 $\pm$ 0.50 | 2 $\pm$ 0.49 | 2 $\pm$ 0.47 |

Values are written as MEAN  $\pm$  S.D.

Values having different superscripts are significantly ( $P \leq 0.05$ ) different from each other.

**Table 2: Minimum Inhibitory Concentration (MIC)**

| TEST ORGANISMS                 | 500mg/ml   | 250mg/ml   | 125mg/ml   | 62.5mg/ml        |
|--------------------------------|------------|------------|------------|------------------|
| <i>Salmonella typhi</i>        | 29.00±0.71 | 22.00±1.41 | 18.50±0.71 | 7.50±0.71 < 62.5 |
| <i>Escherichia coli</i>        | 27.00±1.41 | 17.00±1.41 | 9.50±0.71  | 6.50±0.71 < 62.5 |
| <i>Staphylococcus aureus</i>   | 8.00±1.41  | 4.50±0.71  | 0.50±0.71  | 0.50±0.71 < 250  |
| <i>Klebsiella pneumonia</i>    | 27.00±1.41 | 14.00±1.41 | 8.50±0.71  | 2.50±0.71 < 125  |
| <i>Streptococcus pneumonia</i> | 15.50±0.71 | 7.50±0.71  | 1.00±1.41  | 0.00±0.00 < 250  |

Values are written as MEAN ± S.D.

Values having different superscripts are significantly ( $P \leq 0.05$ ) different from each other.

**Table 3: Antimicrobial results for plant extract**

| Test organisms                 | 1000mg/ml  | Standard (ciprofloxacin) |
|--------------------------------|------------|--------------------------|
| <i>Salmonella typhi</i>        | 32.50±0.71 | 52.00±1.41               |
| <i>Escherichia coli</i>        | 39.00±1.41 | 63.00±1.41               |
| <i>Streptococcus aureus</i>    | 17.00±1.41 | 35.00±0.71               |
| <i>Klebsiella pneumonia</i>    | 39.00±1.41 | 57.00±1.41               |
| <i>Streptococcus pneumonia</i> | 29.00±1.41 | 49.00±1.41               |

Values are written as MEAN ± S.D.

Values having different superscripts are significantly ( $P \leq 0.05$ ) different from each other.

**Table 4: DPPH activity on the gel extract of *Aloe buettneri***

| GROUP                        | MEAN INHIBITION OF DPPH | (IC50) Value |
|------------------------------|-------------------------|--------------|
| 200mg/ml                     | 87.64±2.94 b            |              |
| 100mg/ml                     | 78.31±2.81 c            |              |
| 50mg/ml                      | 39.55±2.83 d            | 89.58559     |
| 25mg/ml                      | 9.28±2.91 e             |              |
| 12.5mg/ml                    | 8.24±5.72 e             |              |
| Ascorbic acid STD (100mg/ml) | 97.72±0.83a             |              |

Values are written as MEAN ± S.D.

Values having different superscripts are significantly ( $P \leq 0.05$ ) different from each other.

**Table 5: Anti lipid peroxidation activity on *Aloe buettneri* gel extract**

| GROUP                        | MEAN OF ALP | STANDARD (IC50) |
|------------------------------|-------------|-----------------|
| 200mg/ml                     | 82.33±3.87  |                 |
| 100mg/ml                     | 61.88±4.47  |                 |
| 50mg/ml                      | 31.60±10.45 | 97.33           |
| 25mg/ml                      | 28.26±13.88 |                 |
| 12.5mg/ml                    | 9.74±1.54   |                 |
| Ascorbic acid STD (100mg/ml) | 99.29±0.13  |                 |

Values are written as MEAN ± S.D.

Values having different superscripts are significantly ( $P \leq 0.05$ ) different from each other.

**Table 6: Nitric oxide scavenging activity on *Aloe buettneri* gel extract**

| GROUP                        | MEAN OF NOA  | STANDARD (IC50) |
|------------------------------|--------------|-----------------|
| 200mg/ml                     | 72.26±3.30b  |                 |
| 100mg/ml                     | 59.13±4.63c  |                 |
| 50mg/ml                      | 41.57±1.45d  | 100.45          |
| 25mg/ml                      | 26.20±2.13e  |                 |
| 12.5mg/ml                    | 19.90±7.13e  |                 |
| Ascorbic acid STD (100mg/ml) | 98.15±0.290a |                 |

Values are written as MEAN ± S.D.

Values having different superscripts are significantly ( $P \leq 0.05$ ) different from each other.

## DISCUSSION

The aqueous crude gel extract *Aloe buettneri* demonstrated a dose dependent antimicrobial activity on the test microorganisms. Different concentrations of; 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml of the extract were prepared and tested against human pathogen such as; *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Streptococcus pneumonia*. The result of the antimicrobial activity of *Aloe buettneri* was shown in table 3. The aqueous extract was shown to display an antimicrobial activity against the organisms, with the zone of inhibition ranging from 0.1mm to 39mm. among the bacterial isolate *Klebsiella pneumonia* revealed the highest antimicrobial activity of 39.00±1. The zone of inhibition of the aqueous extract showed non-significant ( $p > 0.05$ ) different when compared with that of the standard drug ciprofloxacin.

The significant antimicrobial activity of *Aloe buettneri* could be attributed to its phenolic content and the presence of salicylic acid. These properties have been detected earlier by researchers (David *et al.*, 2011). Plant phenolic compounds have been implicated in antibacterial properties in plant (Adesegun and Rotibi, 1998). They react with protein like microbial enzymes through hydrogen bonding and can cause iron deprivation leading to the death of the microorganism (Scalbert, 1991).

Egg albumen is widely used to induce acute inflammation (Manueli *et al.*, 1994). Thus albumen-induced rat paw oedema is a suitable test for determining anti-inflammatory action of drugs and has been frequently used to assess the anti oedematous effect of natural products (Manueli *et al.*, 1994). The development of oedema in the paw of the rat after the injection of albumen is a biphasic response. The initial phase is due to the release of histamine and serotonin and the maintenance of the oedema during the plateau is caused by kinin like substances (Shrestha *et al.*, 2013). The second phase of oedema is due to the release of



prostaglandins, protease and lysozyme, that is mediated by bradykinin, leucotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Brito *et al.*, 1998). Histamine and serotonin are important inflammatory mediators and they are potent vasodilator substances as well as they increase the vascular permeability (Shrestha *et al.*, 2013). The result of albumen induce oedema indicates that *Aloe buettneri* plays a crucial role against inflammation. It could be argued that the suppression of the first phase may be due to inhibition in the release of mediators, such as histamine and serotonin and the action in the second phase may be explained by an inhibition of cyclooxygenase. Thus, it may be suggested that treatment with *Aloe buettneri* may prevent the inflammatory action of albumen by decreasing PGE2 level, which may be due to the presence of vitamin B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, C,  $\beta$ -carotene, choline, folic acid,  $\alpha$ -tocopherol in the flesh of Aloe vera (Ferro *et al.*, 2003). Reduction in the level of prostaglandins might be due to the phenolic compound present in the *Aloe buettneri* which resulted in the reduction of paw oedema.

Anti-inflammatory activity of *A. buettneri* is also attributed to the inhibition of arachidonic acid pathway through cyclooxygenase. Also, the aqueous extract of Aloe gel is reported to have inhibited the production of prostaglandins E<sub>2</sub> from arachidonic acid *in vitro*. Prostaglandins tend to stimulate nerves that signal pain to the brain and are involved in the swelling of the blood vessels at the injured site, opening space in the capillary walls for the white blood cells to enter (Peng *et al.*, 2009). Therefore, reduction in the level of prostaglandins might be due to the phenolic compound present in the *Aloe buettneri* which resulted in the reduction of paw oedema.

*Aloe buettneri* crude gel extract showed antioxidant properties when analyzed taking in consideration the following activities: DPPH radical scavenging activity, anti-lipid peroxidation and nitric oxide inhibition activity with respect to their absorbance when read on a spectrophotometer. Different concentrations of the aqueous extract such as 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml were used and ascorbic acid served as the standard. The percentage of the activities showed that the antioxidant property of the aqueous crude gel extract of *Aloe buettneri* is dose dependent. The result showed that increase in antioxidant activity increases with increase in dose. The antioxidant effect of the extract may be due to the presence of some compounds such as: polyphenol, indoles and alkaloids (Peng *et al.*, 2009). These compounds reduce lipid peroxidation and free radicals like MDA.

## CONCLUSION

The result of this work support the folklore claim that *Aloe buettneri* has medicinal properties because this study showed that *Aloe buettneri* aqueous crude gel extract has the efficacy in reducing inflammation, inhibiting bacteria and is a good antioxidant.

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