Phytocomponents and effect of aqueous extract of the leaves of *Alstonia boonei* (de wild) on serum electrolyte levels in wistar rats

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**ABSTRACT**

*Alstonia boonei*, common name cheese wood contains major phytochemical compounds used for healing. This study evaluated the effects of the aqueous leaves extract of *A. boonei* on renal function of male and female wistar rats. Twenty-four (24) male and female wistar rats weighing between 120-230 g were randomly assigned into eight (8) groups (A - H) of three (3) rats per group. Groups (A and E) were used as male and female controls respectively while male groups (B – D) and female groups (F – H) served as the test groups and received 500, 1000 and 2500 mg/kg of the extract respectively. After twenty-eight days of extract administration all the animals were fasted for twenty-four hours and sacrificed. Blood was collected by cardiac puncture for analysis of serum electrolytes. The results revealed insignificant difference (P > 0.05) in the serum levels for males and females at the doses tested except level of urea in female rats, it also revealed significant (P < 0.05) reduction in serum sodium and potassium in male animals at doses tested. GC-MS analysis revealed the presence of medicinally important phytochemicals such as hydroquinone for lightening the dark patches of skin caused by pregnancy birth control pills and Oleic acid used as a pharmaceutical solvent for the production of soaps, cosmetics, ointments, lubricants and serves as an emulsifier and assist in the absorption of some drugs by the skin. *Alstonia boonei* leaf must carefully be exploited medicinally because of the increase in urea levels for female rats and decrease in potassium and sodium in male rats at doses used.

**Keywords:** Metabolic products, Electrolytes, phytochemicals, therapeutic agents.

1. **INTRODUCTION**

Traditional medicine has more dominance worldwide and the use is on the increase (Patrick-Iwuanyanwu *et al.*, 2012). Medicinal plants are vital in the maintenance of human health all over the world. About 70–95 % of the population rely on traditional medicine systems, particularly plant based to meet their primary health care needs (Adinortey *et al.*, 2012). Plants commonly used in traditional medicine are promoted as natural and harmless. Therefore, medicinal plants must be used with caution since they are potentially harmful at high doses and can interact with modern drugs (Maud *et al.*, 2014). The consumption of a variety of local herbs and vegetables by man contribute significantly to the improvement of his health either by prevention or cure of diseases (Afolabi *et al.*, 2014).

*Alstonia boonei* is a large evergreen tree of the family Apocynaceae. Found in tropics and rain forests of West and Central Africa. The roots, leaves, stem bark, latex, flowers and fruits are
used extensively for medicinal purposes (Akoto and Opoku, 2015; Nathaniel et al., 2010). It has been reported that alcoholic or aqueous preparations of the parts of the plant, especially the stem bark, are effective in the treatment of febrile illness, jaundice, rheumatism, malaria, fever, intestinal helminthes, and hypertension as well as an antivenom against snake bite (Nathaniel et al., 2010). The phytochemicals in the stem bark of A. boonei are saponins, alkaloids, tannins, and cardiac glycosides (Awodele et al., 2010). The bark contains some chemical compounds of the indole alkaloid group namely alstonine, porphine and alstonidine as well as triterpenoids (Nathaniel et al., 2010).

The three botanical synonyms of Alstonia boonei in literature are; Alstonia congensis, Alstonia macrophylla, Alstonia scholaris (Akoto and Opoku, 2015). The plant is known in English as Alstonia, cheese wood, Pattern-wood; Egbu (Igbo); Ahun (Yoruba).

The kidney being the chief regulator of all the body fluids is primarily responsible for maintaining homeostasis or equilibrium of fluids and electrolytes of the body. The determination of some waste metabolic products excreted exclusively via the kidneys provides useful information about the health status of the kidneys. Such metabolites include urea and creatinine. A study of the effect(s) of the aqueous extract of the leaves of A. boonei on kidney is essential because of the cardinal roles the organ plays in plasma clearance, detoxification, homeostasis and excretion of xenobiotics.

2. MATERIALS AND METHODS

2.1. Collection of Plant and Authentication
Fresh leaves of Alstonia boonei were collected from the forest of Obayantor community in Ikpoba Okha Local Government Area of Edo State, Nigeria and authenticated in the Department of Plant Biology and Biotechnology, University of Benin with voucher number UBH5343.

2.1.1. Preparation of Plant Extract
The leaves were picked, washed in clean water and air-dried. The dried leaves were ground to powder using an electric grinder. Six hundred grammes (600 grams) was weighed and soaked in 6.0 litres of sterile distilled water for 72 hrs. The extract was filtered and concentrated at a temperature regulated water bath set at 80 °C. Thereafter the extract was preserved for further use.

Experimental animals: Twenty-four males and females wistar rats weighing between 120-230 g were used for this study. The animals were housed in cages made of wooden frames and metal netting, acclimatized for 14 days in the animal house and maintained under standard conditions. The animals were randomly divided into 8 groups (A-H) of three (3) rats per group. Animals in groups A and E were not administered with the aqueous extracts and were used as males and females controls respectively. The rats were fed with rat pellet and tap water with 12-hours light/dark cycle. The cages were cleaned every morning and disinfected at intervals of 3 days. Body weight was taken at intervals of three days during administration of the extract. The control groups received 0.5 ml of sterile distilled water. While those in groups B to D and groups F to H were administered various concentrations of the aqueous extract of A. boonei leaves.
Animal Groupings for Sub Acute Toxicity Test:

The aqueous extract of *A. boonei* leaves was formulated in distilled water corresponding to the dosages 500, 1000, and 2500 mg kg\(^{-1}\) body weights of males and females’ rats. The extract was administered for 28 days using orogastric tube attached to a 1ml syringe at a single dose per day according to the following groupings:

**Group A:** Each male control rat received 0.5 ml of sterile distilled water.

**Group B:** Each male rat received 500 mg/kg body wt. of aqueous extract of *A. boonei* leaves.

**Group C:** Each male rat received 1000 mg/kg body wt. of aqueous extract of *A. boonei* leaves.

**Group D:** Each male rat received 2500 mg/kg body wt. of aqueous extract of *A. boonei* leaves.

**Group E:** Each female control rat received 0.5 ml of sterile distilled water.

**Group F:** Each female rat received 500 mg/kg body wt. of aqueous extract of *A. boonei* leaves.

**Group G:** Each female rat received 1000 mg/kg body wt. of aqueous extract of *A. boonei* leaves.

**Group H:** Each female rat received 2500 mg/kg body wt. of aqueous extract of *A. boonei* leaves.

At the end of 28 days, rats were fasted for 12 hours, the body weights were recorded and thereafter sacrificed humanely by cervical dislocation, blood was collected by cardiac puncture for serum analysis.

2.2. Quantitative assay kits

The assay kits for creatinine, urea, bicarbonate, Sodium, Chloride and potassium made by Randox were procured from Pyrex Ltd., Benin, City, Edo State, Nigeria. All other chemicals and reagents used were of analytical grade.

2.2.1. Biochemical Analysis

Creatinine, urea, bicarbonate, chloride, sodium and potassium concentrations were estimated according to the manufacturer’s instruction on the diagnostic assay kits. The rats were sacrificed by cervical dislocation and arteriovenous blood was collected. Serum was then collected from the blood at room temperature by centrifugation at 4000 rev/min for 10 mins.

**Determination of Extract Yield**

The percentage yield of the aqueous extract of *A. boonei* leaves was determined by weighing the pulverized leaf powder before extraction and the concentrated extract was obtained after extraction and then calculated using the formula:

\[
\text{Percentage Yield of extract} = \frac{\text{Weight of extract}}{\text{weight of leaves powder}} \times 100
\]

\[
= \frac{140g}{600g} \times 100
\]

Percentage yield of extract = 23.33 %.

**Determination of Relative Organ weights**

Relative weight of organ (%) = \(\frac{\text{organ weight}}{\text{Body weight}}\) \times 100

**Phytocomponents analysis with Gas Chromatograph-Mass Spectrophotometer (GC-MS)**

The aqueous extract of leaves of *A. boonei* was analyzed with GC-MS using GC Clarus 500 Perlin Elma system comprising an auto-sampler and gas chromatography (GC) interfaced to a mass spectrophotometer (MS) instrument. The oven temperature was programmed at 110 °C (isothermal for 2 min) with an increase of 10 °C/min, to 200 °C then 5 °C/min to 280 °C, and ended with a 9 °C/min isothermal at 280 °C.
Statistical Analysis: The results were analyzed with Duncan multiple range test and all data were expressed as mean ± standard error of mean. Differences between groups were considered at 95% confidence limit and probability level of 0.05 taken as significant.

3. RESULTS

The percentage yield of extract was calculated as 23.33%.

The results of phytocomponents of aqueous leaf extract of *Alstonia boonei* is presented in Table 1. The results of the effects of 28 days daily administration of aqueous extract of the leaves of *Alstonia boonei* on levels of serum potassium, sodium, bicarbonate, chloride, creatinine and urea, of Male Wistar Rats is presented in Table 2.

Table 3 shows the results of the effects of 28 days daily administration of aqueous extract of the leaves of *Alstonia boonei* levels of serum potassium, sodium, bicarbonate, chloride, creatinine, and urea, of female Wistar Rats.

Table 4 shows the results of effects of aqueous extract of the leaves of *Alstonia boonei* on body weight, organ weight and relative organ weight of male Wistar Rats.

The results of effects of aqueous extract of the leaves of *Alstonia boonei* on body weight, organ weight and relative organ weight of female Wistar Rats is presented in Table 5, while Table 6 shows the medicinal uses of phytocomponents of aqueous leaf extract of *Alstonia boonei*.

![Chromatogram of isolated phytocomponents of aqueous extract of the leaves of Alstonia boonei.](image)

**Figure 1:** Chromatogram of isolated phytocomponents of aqueous extract of the leaves of *Alstonia boonei*. 
| SN | Name of Compound | NP | MF | MW | RT | PA | % PA | Molecular Structure |
|----|------------------|----|----|----|----|----|------|---------------------|
| 1  | Beta., beta.-Galactonic phenylhydrazide | Unsaturated | C₁₃H₁₈N₂O₆ | 286 | 4.051 | 1861862 | 0.10 |
| 2  | 8-Methylenecyclooctene-3,4-diol | Unsaturated | C₈H₁₄O₂ | 154 | 7.899 | 1203800 | 0.06 |
| 3  | Hydroquinone | Unsaturated | C₆H₆O₂ | 110 | 8.167 | 2445019 | 0.13 |
| 4  | 2-Undecenoic acid | Unsaturated | C₁₁H₂₀O₂ | 184 | 11.461 | 133262219 | 7.11 |
| 5  | 3-O-Methyl-d-glucose | Unsaturated | C₂₁H₄₀O₄ | 356 | 18.153 | 119125174 | 6.35 |
| 6  | L-(+)-Ascorbic acid 2,6-dihexadecanoate | Unsaturated | C₃₈H₆₈O₈ | 652 | 14.134 | 75996392 | 4.04 |
| 7  | Oleic Acid | Unsaturated | C₁₈H₃₄O₂ | 282 | 15.529 | 898864484 | 47.94 |
| 8  | 9-Octadecenoic acid (Z)-, 2, 3-dihydroxypropyl ester | Unsaturated | C₁₅H₂₈O | 224 | 18.803 | 25507137 | 1.36 |
| 9  | 2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (E)-(+/-/-) | Unsaturated | C₁₃H₂₆O | 224 | 18.803 | 25507137 | 1.36 |

**NOTE:** NP - Natural product; MF - Molecular formula; MW - Molecular weight; RT - Retention Time; PA - Peak Area; % PA - Percentage Peak Area.
Table 2: Effects of 28 days daily administration of aqueous extract of the leaves of *Alstonia boonei* on levels of serum Potassium, Sodium, Bicarbonate, Chloride, Creatinine, and Urea, of male Wistar Rats.

| Parameters       | 500       | 1000      | 2500      | Control     |
|------------------|-----------|-----------|-----------|-------------|
| **K⁺ (mmol/L)**  | 5.53±0.95<sup>a</sup> | 5.40±0.35<sup>a</sup> | 7.00±0.69<sup>a</sup> | 10.30±1.95<sup>b</sup> |
| **Na⁺ (mmol/L)** | 129.00±5.67<sup>ab</sup> | 131.00±0.29<sup>ab</sup> | 133.00±0.58<sup>abc</sup> | 146.00±3.48<sup>c</sup> |
| **HCO₃⁻ (mmol/L)** | 11.67±1.67<sup>ab</sup> | 11.50±0.29<sup>ab</sup> | 19.00±0.58<sup>d</sup> | 17.67±1.45<sup>cd</sup> |
| **Cl⁻ (mmol/L)**  | 86.00±3.06<sup>ab</sup> | 91.00±0.58<sup>abc</sup> | 95.00±0.58<sup>abc</sup> | 101.00±6.36<sup>c</sup> |
| **Cr (mg/dL)**   | 1.55±0.75<sup>a</sup> | 2.03±0.75<sup>a</sup> | 1.38±0.54<sup>a</sup> | 1.06±0.24<sup>a</sup> |
| **Urea (mg/dL)** | 49.97±8.90<sup>c</sup> | 25.85±1.86<sup>ab</sup> | 47.17±3.73<sup>c</sup> | 38.40±3.71<sup>bc</sup> |

Values are Means ± Standard error of mean of the three Replicate determinations. Similar letters indicate means which are not significantly different from each other.

Table 3: Effects of 28 days daily administration of aqueous extract of the leaves of *Alstonia boonei* on levels of serum Potassium, Sodium, Bicarbonate, Chloride, Creatinine, and Urea, of Female Wistar Rats.

| Parameters       | 500       | 1000      | 2500      | Control     |
|------------------|-----------|-----------|-----------|-------------|
| **K⁺ (mmol/L)**  | 64.43±1.35<sup>a</sup> | 4.45±0.26<sup>a</sup> | 7.55±0.49<sup>b</sup> | 4.70±0.12<sup>a</sup> |
| **Na⁺ (mmol/L)** | 119.00±7.17<sup>a</sup> | 130.00±6.93<sup>ab</sup> | 134.00±1.73<sup>bc</sup> | 132.00±1.73<sup>ab</sup> |
| **HCO₃⁻ (mmol/L)** | 13.67±1.33<sup>bc</sup> | 9.00±0.58<sup>a</sup> | 14.00±0.58<sup>bc</sup> | 15.00±2.89<sup>bcd</sup> |
| **Cl⁻ (mmol/L)**  | 86.67±1.76<sup>abc</sup> | 84.50±9.53<sup>a</sup> | 94.67±1.45<sup>abc</sup> | 99.00±0.58<sup>bcd</sup> |
| **Cr (mg/dL)**   | 1.81±0.86<sup>a</sup> | 1.67±0.63<sup>a</sup> | 2.61±0.00<sup>a</sup> | 1.52±0.46<sup>a</sup> |
| **Urea (mg/dL)** | 27.57±4.95<sup>ab</sup> | 46.85±4.66<sup>c</sup> | 38.45±3.17<sup>bc</sup> | 20.36±0.19<sup>a</sup> |

Values are Means ± Standard Error of Mean of the three Replicate determinations. Similar letters indicate means which are not significantly different from each other.

P < 0.05 - Significant
P > 0.05 - Not Significant
Table 4: Effects of Aqueous Extract of the leaves of *Alstonia boonei* on Body weight (g), Organ weight, and Relative Organ weight of Male Wistar Rats.

| Parameters            | Daily Dosage (mg/kg) |
|-----------------------|----------------------|
|                       | 500                  |
| Body weight (g)       | 178.33±33.83<sup>a</sup> |
| Organ weight (g)      | 0.99±0.13<sup>ab</sup> |
| Relative organ weight (%) | 0.62±0.16<sup>a</sup> |
|                       | 1000                 |
| Body weight (g)       | 235.00±8.66<sup>b</sup> |
| Organ weight (g)      | 1.19±0.03<sup>b</sup> |
| Relative organ weight (%) | 0.51±0.03<sup>a</sup> |
|                       | 2500                 |
| Body weight (g)       | 182.00±14.43<sup>a</sup> |
| Organ weight (g)      | 1.01±0.02<sup>ab</sup> |
| Relative organ weight (%) | 0.55±0.04<sup>a</sup> |
|                       | Control              |
| Body weight (g)       | 210.00±22.55<sup>ab</sup> |
| Organ weight (g)      | 1.03±0.11<sup>ab</sup> |
| Relative organ weight (%) | 0.49±0.00<sup>a</sup> |

Values are Means ± Standard error of the three Replicate determinations. Similar letters indicate means which are not significantly different from each other.

P < 0.05       -      Significant
P > 0.05       -      Not Significant

Table 5: Effects of aqueous extract of the leaves of *Alstonia boonei* on Body weight, Organ weight, and Relative Organ weight of Female Wistar Rats.

| Parameters            | Daily Dosage (mg/kg) |
|-----------------------|----------------------|
|                       | 500                  |
| Body weight (g)       | 191.67±6.67<sup>ab</sup> |
| Organ weight (g)      | 1.01±0.02<sup>ab</sup> |
| Relative organ weight (%) | 0.53±0.03<sup>a</sup> |
|                       | 1000                 |
| Body weight (g)       | 172.67±7.22<sup>a</sup> |
| Organ weight (g)      | 0.93±0.06<sup>a</sup> |
| Relative organ weight (%) | 0.54±0.01<sup>a</sup> |
|                       | 2500                 |
| Body weight (g)       | 175.00±5.77<sup>a</sup> |
| Organ weight (g)      | 0.96±0.01<sup>a</sup> |
| Relative organ weight (%) | 0.55±0.03<sup>a</sup> |
|                       | Control              |
| Body weight (g)       | 200.00±2.89<sup>ab</sup> |
| Organ weight (g)      | 1.09±0.00<sup>ab</sup> |
| Relative organ weight (%) | 0.54±0.01<sup>a</sup> |

Values are Means ± Standard error of the three Replicate determinations. Similar letters indicate means which are not significantly different from each other.

P < 0.05       -      Significant
P > 0.05       -      Not Significant
Table 6: Medicinal uses of some phytocomponents of aqueous extract of the leaves of *Alstonia boonei*

| Phytocomponents                        | Medicinal Uses                                                                                                                                                                                                 |
|----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Hydroquinone                           | Used to lighten dark patches on skin (also called hyperpigmentation) caused by pregnancy, birth control pills, hormone medication or injury to the skin.                                                         |
| 2-Undercylenic Acid                    | Used to treat skin fungal infections such as athletes’ foot and jock itch.                                                                                                                                 |
| 3-O-Methyl-D-Glucose                   | Often used to study blood-brain barrier transport and the distribution space of hexoses in brain. It is also used as a marker to assess glucose transport by evaluating its uptake within various cells and organ systems. |
| Oleic Acid                             | Used as a component in many foods, in the form of its triglycerides. It is a component of normal human diet as a part of animal fats and vegetable oils. Oleic acid is also used as a pharmaceutical solvent which is used in the production of soaps, cosmetics, ointments, lubricants and its serves as an emulsifier and to assist absorption of some drugs by the skin. |
| 9-Octadecenoic acid (Z) - 2, 3-dihydroxypropyl ester | In industry, it is used as a lubricant and lubricant additive and surface-active agent, while consumers also use it in food packing and as lubricant and greases. |
| l-(+)-Ascorbic acid 2,6-dihexadecanoate | Used in the treatment of wound in herbal medicine. Ascorbic acid in the body helps in absorption from the intestine. It is required for connective tissue metabolism especially the bones and teeth. It is used as an anti-stress agent and protect against cold, chill and dumps. It prevents muscle fatigue and scurvy which is characterized by hemorrhage, bleeding gums, fragile bones, anemia, joint pain and defects in skeletal calcification (Aja *et al.*, 2014). |
Figure 2: Effects of Aqueous extract of the leaves of *Alstonia boonei* on serum Potassium (K⁺) of male and female Wistar rats. *Bars with similar alphabets are not significantly different from each other (P > 0.05)*

Figure 3: Effects of Aqueous extract of the leaves of *Alstonia boonei* on serum Sodium (Na⁺) of male and female Wistar rats. *Bars with different alphabets are significantly different from each other (P < 0.05)*
Figure 4: Effects of Aqueous extract of the leaves of *Alstonia boonei* on serum Bicarbonate (HCO\textsubscript{3}-) of male and female Wistar rats. *Bars with different alphabets are significantly different from each other (P <0.05)*

Figure 5: Effects of Aqueous extract of the leaves of *Alstonia boonei* on serum Chloride (Cl\textsuperscript{-}) of male and female Wistar rats. *Bars with different alphabets are significantly different from each other (P <0.05).*
Figure 6: Effects of Aqueous extract of the leaves of *Alstonia boonei* on serum Creatinine (Cr) of male and female Wistar rats. *Bars with different alphabets are significantly different from each other (P < 0.05).*

![Figure 6: Effects of Aqueous extract of the leaves of *Alstonia boonei* on serum Creatinine (Cr) of male and female Wistar rats.](image)

Figure 7: Effects of Aqueous extract of the leaves of *Alstonia boonei* on serum Urea of male and female Wistar rats. *Bars with different alphabets are significantly different from each other (P < 0.05).*

![Figure 7: Effects of Aqueous extract of the leaves of *Alstonia boonei* on serum Urea of male and female Wistar rats.](image)
**Figure 8:** Effect of aqueous extract of the leaves of *Alstonia boonei* on body weight (g) of male and female Wistar rats.

*Bars with different alphabets are significantly different from each other (P < 0.05)*

**Figure 9:** Effect of aqueous extract of the leaves of *Alstonia boonei* on organ weight (g) of male and female Wistar rats.

*Bars with different alphabets are significantly different from each other (P < 0.05)*
Figure 10: Effect of aqueous extract of the leaves of *Alstonia boonei* on relative organ weight of male and female Wistar rats.

*Bars with similar alphabets are not significantly different from each other (P > 0.05)*
4. DISCUSSION

Changes in the level of serum potassium are known to have serious health implications such as hypokalaemia, which is the presence of abnormally low levels of potassium in the blood which occurs in dehydration (Afolabi et al., 2014). Hypokalaemia can lead to muscular weakness, hypotonia and cardiac arrhythmias, while hyperkalaemia predisposes to cardiac arrest (Afolabi et al., 2014; Enemor and Okaka, 2013).

In this study, the significant (P < 0.05) decreases in serum potassium level observed in male wistar rats at all the doses of 500 mg/kg, 1000 mg/kg and 2500 mg/kg (5.53±0.95, 5.40±0.35, 7.00±0.69 mmol/L respectively) shows that the extract at these concentrations altered the serum potassium levels when compared with the result of the control (10.30±1.95 mmol/L) (Table 2). This means that the extract at these concentrations may cause hypokalaemia. While the insignificant (P > 0.05) decrease in serum potassium level observed in female wistar rats at the dose of 1000 mg/kg (4.45±0.26 mmol/L) shows that the extract at this concentration did not alter the serum potassium levels when compared with the result of the control (4.70±0.12 mmol/L) and may not cause hypokalaemia (Table 3).

Sodium is the major electrolyte in the extracellular fluid, and it has been implicated in the pathogenesis of hypertension (Afolabi et al., 2014). When the Sodium level is high, there is increased retention of water by the kidneys through the renin-angiotensin system, this helps to increase the blood volume and ultimately leads to hypertension. Also, high levels of sodium in the blood causes the cells to be dehydrated an this can lead to coma or death (Afolabi et al., 2014; Kebe et al., 2013). Increases or decreases in sodium concentration is a major contributor to fluctuations of blood pressure (Afolabi et al., 2014; Enemor and Okaka, 2013).

In this study, the significant decrease (P < 0.05) in serum sodium level observed in male wistar rats at the doses of (500, and 1000 mg/kg) (129.00±5.67, 131.00±0.29 mmol/L respectively) except at the dose of 2500 mg/kg when compared with control (146.00±3.48 mmol/L) showed that the aqueous leaf extract of *Alstonia boonei* has antihypertensive effect at the highest dose of 2500 mg/kg (Table 2). While the insignificant (P > 0.05) decrease in serum sodium level at the dose of (500 and 1000 mg/kg) (119.00±7.17, 130.00±6.93 mmol/L respectively) and the mild increase at the highest dose of (2500 mg/kg) (134.00±1.73 mmol/L) observed in female wistar rats when compared with the control (132.00±1.73 mmol/L) also indicated that the aqueous extract of the leaves of *Alstonia boonei* is antihypertensive at this dose (Table 3).

Also, there was a significant difference (P < 0.05) in serum Na⁺ levels for male wistar rats except at the highest dose of 2500 mg/kg after administration of the aqueous extract of the leaves of *Alstonia boonei* for 28 days. Even though the Na⁺ level decreased significantly (P < 0.05) in male wistar rats at a dose of 500 mg/kg and 2500 mg/kg, the level was brought down to a value that is significantly (P < 0.05) close to control at the highest dose of 2500 mg/kg. This suggested that the extract causes sodium level to be maintained at normal level thus preventing hypertension. This implies that the aqueous extract of the leaves of *Alstonia boonei* at the highest dose of 2500 mg/kg might have anti-hypertensive effect. This showed similar findings with the work by Afolabi et al., 2014.

Serum bicarbonate ions act as a buffer to maintain the normal levels of acidity (pH) in blood and other fluids in the body. The measurement of serum bicarbonate levels is used to determine the pH of the blood and body fluids (Afolabi et al., 2014).

In this study, there was no significant difference (P > 0.05) between the serum bicarbonate at the highest dose of (2500 mg/kg) (19.00±0.58 mmol/L) when compared with the control
(17.67±1.45 mmol/L) and urea levels at all the doses of (500, 1000, and 2500 mg/kg) (49.97±8.90, 25.85±1.86, 47.17±3.73 mg/dL) in male wistar rats when compared with the control group (38.40±3.71 mg/dL).

The presence of increasing concentration of creatinine in the blood is used in the evaluation of the effects of chemicals on the kidney function and it also serves as an indicator of the glomerular filtration rate of the kidney (Afolabi et al., 2014). Elevation in creatinine levels is majorly observed if there is marked damage to functional nephrons (Afolabi et al., 2014; Mukinda and Eagle, 2012). Serum creatinine level is also known to increase with the use of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB).

In this study, the insignificant increase (P > 0.05) in serum creatinine levels observed in both male and female wistar rats may be because the aqueous extract of the leaves of *Alstonia boonei* does not mimic either ACE inhibitors or ARB. Afolabi et al. 2014 reported that various species of *Alstonia* are highly rich in alkaloids, steroids and triterpenoids, and phenolic compounds which may contribute to the toxicity of *Alstonia boonei*.

Therefore, the insignificant (P > 0.05) increase in serum creatinine level observed in the male and female wistar rats after administration of aqueous extract of the leaves of *Alstonia boonei* for 28 days may be due to the toxic components of the extract which were detoxified in the liver and excreted via the kidneys. Urea and creatinine levels are used to assess renal function (Shatoor, 2011). An increase in the levels of these parameters means impairment in kidney function.

In this study, the absence of significant differences in serum creatinine of male wistar rats at all the doses of (500, 1000, 2500 mg/dL) (1.55±0.76, 2.03±0.75 and 1.38±0.54 mg/dL) when compared with the control (1.06±0.24 mg/dL), serum urea observed in male at all the doses of (500, 1000, and 2500 mg/dL) (49.97±8.90, 25.85±1.86 and 47.17±3.73 mg/dL) when compared with the control (38.40±3.71 mg/dL) and female wistar rats except serum urea level in female at the doses of (1000, and 2500 mg/kg) (46.85±4.66 and 38.45±3.17 mg/dL) when compared with the control (20.36±0.19 mg/dL) that the aqueous extract of the leaves of *Alstonia boonei* has no harmful effect on the kidney (Table 2 and 3).

In this study, there was a significant (P < 0.05) alteration in the serum levels of electrolytes analyzed (sodium and potassium) in male wistar rats with the exception of serum sodium at the dose of 2500 mg/kg (133.00±0.58 mmol/L). This is an indication that the integrity of the kidney in male wistar rats was compromised by the extract administration. This report was however different in female animals (Table 2 and 3).

For serum potassium (K⁺) level, there was a significant difference (P < 0.05) between the entire treatments’ groups and the control group for male Wistar rats. In serum K⁺ level for female wistar rats, there was no significant difference (P > 0.05) between control and the treatment groups except at the highest dose of 2500 mg/kg which was significantly different (P < 0.05) from the control.

In serum level of sodium (Na⁺), there was a significant difference (P < 0.05) between the control and the treatment groups for male wistar rats except at the dose of 2500 mg/kg which was not significantly different (P > 0.05) from the control. While in serum Na⁺ level for females, there was no significant difference (P > 0.05) between the control and the entire treatment groups.

Serum level of bicarbonates (HCO₃⁻) in male wistar rats showed that there was a significant difference (P < 0.05) between the control and the treatment groups except at the dose of 2500 mg/kg which was not significantly different (P > 0.05) from the control group. In serum level of HCO₃⁻ for females, there was no significant difference (P > 0.05) between the control and the
treatment groups except at the dose of 1000 mg/kg which indicated significantly different (P < 0.05) from the control, 500 and 2500 mg/kg.

There was no significant difference (P > 0.05) in serum chloride (Cl⁻) level in male wistar rats when compared with the control except at the dose of 500 mg/kg which was significantly different (P < 0.05) from the control. Also, there was no significant different (P > 0.05) in serum chloride level for female wistar rats when compared with control except at the dose of 1000 mg/kg which was significantly different (P < 0.05) from the control.

The determination of some waste metabolic products excreted exclusively via the kidneys provides useful information about the health status of the kidneys. Such metabolites are urea and creatinine (Awodele et al., 2010). There was no significant difference (P > 0.05) in serum creatinine level for male and female wistar rats in all the treatments when compared with their respective controls.

Also, no significant difference (P > 0.05) in serum urea level for male wistar rats in all treatments when compared with the control, but 1000 mg/kg was significantly different (P < 0.05) from 500 mg/kg and 2500 mg/kg when compared with each other.

There was a significant difference (P < 0.05) in serum urea level in female wistar rats when compared with control except at the lowest dose of 500 mg/kg which was not significantly different (P > 0.05) from the control group and highest dose of 2500 mg/kg.

In this study, there was no significant difference (P > 0.05) between body weights, organ weights, and relative organ weights of male and female wistar rats at all the doses tested when compared with their respective controls. Therefore, aqueous leaf extract of Alstonia boonei at all the doses used in this study has no effect on body weights, organ weights, and relative organ weights of male and female wistar rats.

GC-MS analysis of phytocomponents of the aqueous extract of A. boonei leaves contained medicinally important properties which include:

- **Hydroquinone** with a molecular formula of C₆H₆O₂ and molecular weight of 110 g used to lighten the dark patches of skin (also called hyperpigmentation, melasma e.t.c) caused by pregnancy birth control pills, hormone medication or injury to the skin. The 2-Undecylenic Acid with a molecular formula of C₁₁H₂₀O₂ and molecular weight of 184 g is used to treat skin fungal infections such as athletes’ foot and jock itch.

- **The 3-O-Methyl-D-Glucose** with a molecular formula of C₇H₁₄O₆ and molecular weight of 194 g is often used to study blood-brain barrier transport and the distribution space of hexoses in brain, it is also use as a marker to assess glucose transport by evaluating its uptake within various cells and organ systems.

- **Oleic Acid** which has a molecular formula of C₁₈H₃₄O₂ and molecular weight of 282 g is used as a component in many foods, in the form of its triglycerides. It is a component of normal human diet and as a part of animal fats and vegetable oils. Oleic acid is also used as a pharmaceutical solvent for the production of soaps, cosmetics, ointments, lubricants and serves as an emulsifier and to assist absorption of some drugs by the skin.

- **The 9-Octadecenoic acid (Z) -,2, 3-dihydroxypropyl ester** with a molecular formula of C₂₁H₄₀O₄ and molecular weight of 356 g, is used as a lubricant and lubricant additive and surface-active agent, while consumers also use it in food packing and as greases, while l-(+)-Ascorbic acid 2, 6-dihexadecanoate with molecular formula of C₃₈H₆₈O₈ and molecular weight of 652 g is used in the treatment of wound in herbal medicine and in the body it helps in absorption from the intestine.
5. CONCLUSION
The results obtained in this study indicated that the various concentrations of the extract of *A. boonei* has no side effect on the functions of the kidney, the lowest dose of the leaves extract caused significant changes in male rats as observed in few of the serum electrolytes assayed such as bicarbonate (HCO$_3^-$) and chloride (Cl$^-$). However, at higher doses the levels of the electrolytes were brought significantly close to those observed in the control. This implies that the extract has some toxicity at a lower dose which is reversed at higher doses of the extract. Aqueous leaves extract of *Alstonia boonei* at all the doses used in this study has no significant effect on body, organ and relative organ weights of both male and female wistar rats used. The analysis of aqueous leaves extract of *A. boonei* revealed the presence of medicinally important phytocompounds making it a good source of medicine for health benefits.

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