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Phytochemical Screening, Mineral Content, Antioxidant Potential and Antibacterial Activity of the Leaves Extract of *Alstonia boonei*

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ABSTRACT

This study evaluated the phytochemical constituents, antioxidant potential, mineral content and antibacterial activity of the ethyl acetate extract of *Alstonia boonei* leaves. The results of the phytochemical screening revealed the presence of phenolics, eugenols, steroids, alkaloids and reducing sugars. While the mineral content for sodium, potassium, calcium, magnesium, copper iron and zinc were found to be5.61 mg/kg, 120.63 mg/kg, 15.61 mg/kg, 2.63 mg/kg, 0.01 mg/kg, 7.15 mg/kg and 1.62 mg/kg respectively. The antioxidant potential examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay at 250, 200, 150, 100 and 50 µg/ml gave the IC_{50} of 2.89 µg/ml and 2.52 µg/ml for the extract and standard, respectively. Determination of the zone of inhibition of the bacterial isolates using agar well diffusion method revealed that the isolates showed varying sensitivity towards the extract. *Pseudomonas aeruginosa* had the highest value at 35 mm and *subtilis* had the lowest sensitivity value at 26.5 mm. The minimum inhibitory concentration (MIC) values were found to be 25 mg/ml for *Pseudomonas aeruginosa* and 12.5 mg/ml for *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus and Bacillus subtilis*. The minimum bactericidal concentration (MBC) values were found to be 12.5 mg/ml for *Klebsiella pneumoniae*, 50 mg/ml for *Escherichia coli* and *Staphylococcus aureus*, 25 mg/ml for *Pseudomonas aeruginosa* while *Bacillus subtilis* was above 50mg/ml. The results provided a useful insight into the medicinal uses of the plant extract against oxidative stress and microbial infections.

Keywords: *Alstonia boonei*, antibacterial, antioxidant, mineral, phytochemical

INTRODUCTION

Plants continue to be the sources of traditional medicine for the treatment of different illnesses due to their various pharmacological effects (Olajide *et al.*, 2000; Weshche *et al*., 1990; Taiwo *et al.*, 1998; Elisabetsky and Costa-Campos, 2006; Ogbeide *et al*., 2020; Rasool, 2012; Ogbeide *et al*., 2018).

Alstonia boonei belongs to the *Apocynaceae* family, It is indigenous to West Africa with certain varieties also found in Ethiopia and Tanzania (Hills, 2019). It is a common, large deciduous medicinal tree found in the lowlands and rain-forest areas of Nigeria as well as in various parts of Angola, Central African Republic, Ghana, Democratic Republic of Congo, Cote d'Ivoire (Adotey *et al*., 2012). It is a tree that grows up to 35 meters high, it buttresses deep-fluted high and narrow. It white latexes are copious. The leaves are in whorls at nodes, oblanceolate, apex rounded to acuminate, lateral vein prominent almost at right angle to midrib. The flowers are white with lax terminal cymes. The fruits are paired with slender follicle up to 16 cm long with brown floss at each end. *A. boonei* is known commonly in Nigeria as Ahun in Yoruba, Egbu in Igbo, Ukpo in Efik, Ukhu in Benin and Ojegbukhu in Esan (Maurice, 2014).

The stem bark of *A. boonei* is used treat fever, painful micturition, insomnia, malaria and chronic diarrhea, rheumatic pains, as anti-venom for snake bites and in the treatment of arrow poisoning (Majekodunmi *et al*., 2008; Tepongning *et al*., 2011). The stem bark extracts of *A. boonei* is used to induce labour, remove retained placenta and also in the management of post-partum haemorrhage (Uzor *et al*., 2017).In some parts of West and Central Africa, a mash of the leaves of *A. boonei* are applied topically to reduce swellings and for the treatment of sores, rheumatic pains, muscular pains and hypertension. A decoction of the leaves is also used in the treatment of resistant malaria (Omoya and Oyebola, 2019). The root bark of *A. boonei* has been used, over the years, in the treatment of rheumatic and breast pain (Osadebe, 2003). Its latex is usually boiled in water and drunk as remedy for fever in children, as a stimulant for lactation and also taken as a laxative (Adotey *et al*., 2012).

This work focused on the qualitative screening of the phytochemicals, mineral content, antioxidant potential and antimicrobial activity of the ethyl acetate extract of *A. boonei*.

MATERIALS AND METHODS

Collection and Preparation of Plant Sample

Fresh leaves of *Alstonia boonei* were obtained from the botanical garden, University of Benin, Benin City, Edo State, Nigeria. It was identified and authenticated at the herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin with voucher specimen number UBH-A591. The fresh leaves of *A. boonei* were washed, chopped into pieces and air-dried for three weeks and milled to a coarse powder using a blender. The powdered sample (0.25 kg) was macerated with ethyl acetate (1.5 L) with intermittent stirring and shaking manually. After 72 h, the mixture was filtered using fine linen and the residue was re-macerated for another 72 h with 870 ml of the solvent and filtered. The filtrate was concentrated using rotary evaporator at 50^{o}C to afford the extract. The percentage yield was calculated using equation 1;

$$\%yield = \frac{Weight\ of\ extract}{Weight\ of\ sample} \times \frac{100}{1} \qquad (1)$$

Qualitative Phytochemical Screening

Phytochemical screening of the leaf extract were performed using standard procedures (Alamzed *et al*., 2013; Thusa and Mulmi, 2017; Talukdar and Chaudhary, 2010).

Determination of Mineral Content

1 g of the sample was placed in a Kjeldahl flask treated with 10 ml of mixed acid (Nitric acid and Perchloric acid mixture, 3:1). The flask and its content were mildly heated for about 20 minutes at a temperature of 40^{o}C and then increased to about 100^{o}C for another 20 minutes. After cooling, 20 ml distilled water was added and filtered into a standard flask. It was then made up to the 100 ml mark with distilled water. The elements sodium and potassium were assayed using Flame Photometer while calcium, magnesium, iron, copper, and zinc were assayed using Atomic Absorption Spectrophotometer.

Determination of Antioxidant Potential

The antioxidant potential of the extract was determined using DPPH radical scavenging method with a little modification (Habila *et al.,* 2010; Jimoh *et al.,* 2010).

Preparation of Stock Solution:

A twofold dilution series of the extract was prepared to achieve a decreasing concentration resulting in 250 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml. 0.045g of DPPH powder was dissolved in methanol (20 ml) and made up to the 100 ml mark of the standard flask with methanol.0.25 g Ascorbic acid powder was dissolved in distilled water (40ml) and made up to 100 ml mark with distilled water and this was further subjected to dilution to achieve a decreasing concentration same as those aforementioned above.

A 0.1mM methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used in this study. With the aid of a micropipette 2 ml of the DPPH solution was added to 2 ml of the leaf extract at concentrations of 250, 200, 150, 100 and 50 mg/ml in different test tubes. 2 ml of the DPPH/methanol solution was added to 2 ml of the Ascorbic acid/water solution in a test tube across the five different aforementioned concentrations. Then a 2 ml of the DPPH/methanol solution was put in a test tube. The various test tubes were agitated intensely and left to stand in the dark for 30 minutes. Thereafter, each absorbance was recorded at 517 nm. The % DPPH scavenging activity was plotted against the concentration of the sample and from the graph, the 50% inhibition (IC_{50}) was obtained. The ability of the extracts to scavenge DPPH radical were calculated by using equation 2;

$$\%\boldsymbol{Free\ Radical\ Scavenging\ Activity} = \frac{\boldsymbol{Abs\ of\ Control - Abs\ of\ Sample}}{\boldsymbol{Abs\ of\ Control}} - \frac{\mathbf{100}}{\mathbf{1}} \qquad (\mathbf{2})$$

Antimicrobial Assay

Sourcing of Microorganisms

The clinical isolates of *Staphylococcus aureus*, *Bacilus substilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were sourced from the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

Preparation of Bacterial Isolates

The bacterial strains were maintained on a Mueller-Hinton agar medium at 40^{0}C where a loopful of each bacterial strain was inoculated into 50 ml of sterile nutrient broth in 100 ml conical flask. And the flask was incubated on a rotary shaker for 24 hr to activate the strain.

Preparation of Stock Solution

1g of the leaves extract was dissolved using tween 20 (3 ml), to give a stock solution of 250 mg/ml. Then 0.2 ml of the stock solution (50 mg/ml) concentration was taken.

Determination of Zone of Inhibition

Antibacterial activity of ethylacetate leaf extract were tested using agar well diffusion method. Thus 0.2 ml of bacteria were aseptically introduced and spread using cotton swabs on the surface of sterile Muller-Hinton agar plates. A 6.0 mm diameter well was aseptically punched with a sterile cock borer on each plate. And 0.2 ml of the extract was introduced into the wells in the plates using a micro pipette. A positive control well too

was made with 0.2 ml of Gentamycin (Antibiotic). The plates were then incubated at 37^0C for 24 hr. And the diameter of the zone of inhibition around each well was measured with a graduated meter rule for antibacterial activity. The width of the inhibition zone gives an indication of the relative activity of the extract against the various test micro-organisms (Opoku and Akoto, 2014).

Minimum Inhibitory Concentration (MIC)

The MIC was defined as the lowest concentration that completely inhibited the visible growth of microorganisms for 24 hrs after incubation. The minimum inhibitory concentration was carried out using agar well diffusion method. 1g of the extract was diluted with 1ml tween 20 to get a 1000 mg/ml concentration. This was added to 19 ml molten Muller-Hinton agar to form a 1 in 20 dilution, resulting in a final concentration of 50 mg/ml. This was done for 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml (Afolayan and Meyer, 1997). A sterilized wire loop was used to streak the culture bacteria on the already solidified petri dishes containing the Muller-Hinton agar. All the agar plates were incubated at a temperature of 37°C for 18-24 hours (Afolayan and Meyer, 1997). And the MIC was calculated as the lowest concentration at which a clear zone of inhibition was observed.

Minimum Bactericidal Concentration (MBC)

The MBC, defined as the lowest concentration at which a bacteria isolate can be killed, was determined by re-culturing (sub culturing) broth dilutions that inhibited the growth of the bacteria isolates (i.e after MIC determination). All plates were incubated at 18-24hr and at 37^0C.

RESULTS AND DISCUSSION

Percentage Yield of Extract

The ethylacetate extract of the leaves of *Alstonia boonei* (Table 1) gave the percentage yield of 4.58.

Table 1: Percentage yield of the Ethyl acetate Leaves Extract of *Alstonia boonei*

Extract	Yield (%)
Ethyl acetate	4.58

Qualitative Phytochemical Screening

The ethyl acetate leaves extract of *A. boonei* revealed the presence of reducing sugar, alkaloids, phenols, eugenols and steroids (Table 2).

Table 2: Phytochemical Screening of Ethyl acetate Leaves Extract of *Alstonia boonei*

S/N	Constituents	Results
1	Glycoside	–
2	Saponins	–
3	Phenolics	+
4	Terpenoids	–
5	Eugenols	+
6	Steroids	+
7	Alkaloids	+
8	Flavonoids	–
9	Tannins	–
10	Reducing Sugars	+

Mineral Content

Of the seven elements investigated, potassium was found to have the highest concentration while copper was the lowest (Table 3). The increase in relative order of abundance of elements in *A. boonei* leaves extract was in the order of Cu<Zn<Mg<Na<Fe<Ca<K.

Table 3: Mineral content of Ethyl acetate Leaves Extract of *Alstonia boonei*

Elements	Leaf Extract (mg/kg)	FAO/WHO Permissible limit (mg/kg)
Sodium	5.61	-
Potassium	120.63	-
Calcium	15.61	-
Magnesium	2.36	-
Copper	0.01	10
Iron	7.15	20

Zinc	1.62	50

Antioxidant Potential

The DPPH assay is a simple and reproductive test commonly used to determine the antioxidant potential of natural plants and compounds (Hsu *et al*., 2012). DPPH free radical scavenging activity of the *A. boonei* leaves extract was found to be high at concentrations of 200 and 250 µg/ml, mild for 150 µg/ml and weak at100 µg/ml and 50 µg/ml. At 250 µg/ml the scavenging effect of the extract was 43.87% which was comparable to that of the reference standard (Ascorbic acid) with scavenging effect of 48.32%. The extract showed an appreciable and concentration dependent free radical scavenging activity. The IC_{50} value of the extract was found to be 2.89 µg/ml and that of the standard was 2.52 µg/ml. These values indicated that the standard had a higher activity than the leaves extract since the lower the IC_{50} value, the higher the activity.

Table 4: Antioxidant Potential of Ethyl acetate Leaves extract of *A. boonei*

Concentration (ug/ml)	Ethyl acetate extract (%)	Ascorbic acid (%)
250	43.87	48.32
200	43.42	33.16
150	22.23	31.81
100	6.39	30.77
50	3.48	13.61

Antibacterial activity

The antibacterial activity of the ethyl acetate leaves extract of *A. boonei* at a concentration of50mg/ml (Table 5) displayed significant activity against *Pseudomonas aeruginosa* (35mm*), klebsiella pneumoniae* (30mm), *Escherichia coli* (29mm), *Staphylococcus aureus* (27mm) and *Bacillus subtilis*(26.5mm). This means that all the bacteria isolates were all sensitive to the leaves extract as per the NCCLS guidelines (NCCLS, 2021).

Table 5: Antibacterial Activity of Ethyl acetate Leaves Extract of *Alstonia boonei.*

Bacterial Isolates	50mg/ml	Gen 80µg
	mm	
Escherichia coli	29	24
Staphylococcus Aureus	27	25
Bacillus Subtilis	26.5	25
Klebsiella pnuemoniae	30	20
Pseudomonas aeruginosa	35	18

Determination of Minimum Inhibitory Concentration

The results showed that *E. coli, S. aureus, B. subtilis, K. pnuemoniae* were all inhibited by the extract at 12.5 mg/ml and *P. aeruginosa* was inhibited at 25 mg/ml. This agreed with the work of Kokkaiah *et al.* (2017) who reported that ethanolic leaves extract of *A. boonei* also had MIC values for *S. aureus* at 6.25 mg/ml while that of *P. aeruginosa* was at 12.5 mg/ml.

Table 6: Minimum Inhibitory Concentration of Ethyl acetate Leaves Extract of *Alstonia boonei*

Bacterial Isolates	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
		Zones of Inhibition		
Escherichia coli	NG	NG	NG	G
Staphylococcus aureus	NG	NG	NG	G
Bacillus subtilis	NG	NG	NG	G
Klebsiella pnuemoniae	NG	NG	NG	G
Pseudomonas aeruginosa	NG	NG	G	G

Key G=Growth NG=No Growth

Determination of Minimum Bactericidal Concentration

Table 7 showed that *Escherichia coli* and *Staphylococcus aureus* have their minimum bactericidal concentration at 50 mg/ml while *Kiebsiella pneumoniae* at 12.5 mg/ml and *Pseudomonas aeruginosa* at 25 mg/ml.

Table 7: Minimum Bactericidal Concentration of Ethyl acetate eaves Extract of *Alstonia boonei*

Bacterial Isolate	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
Escherichia coli	NG	G	G	G
Staphylococcus aureus	NG	G	G	G
Bacillus subtilis	G	G	G	G
Klebsiella pneumoniae	NG	NG	NG	G
Pseudomonas aeruginosa	NG	NG	G	G

Key G=Growth NG=No Growth

CONCLUSION

The ethyl acetate extract of the leaves of *Alstonia boonei* was obtained by maceration of the powdered material at room temperature. Qualitative phytochemical screening of the extract revealed the presence of phenolics, eugenols, steroids, alkaloids, and reducing sugars. While the mineral content of the extract demonstrated that the essential minerals were present within the permissible limit. Evaluation of the antioxidant potential of the extract using DPPH free radical scavenging assay indicated that the leaves extract had high activity at concentrations of 200 µg/ml and 250 µg/ml, mild for 150 µg/ml and weak at 100 µg/ml and 50 µg/ml. The antibacterial activity of the extract showed that *E. coli, S. aureus, B. subtilis, K. pnuemoniae* were all inhibited at 12.5 mg/ml and *P. aeruginosa* was inhibited at 25 mg/ml. Furthermore, *E. coli and S. aureus* have their minimum bactericidal concentration at 50 mg/ml while *K. pneumoniae* at 12.5 mg/ml and *P. aeruginosa* at 25 mg/ml.

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