

Antibacterial activity of partitioned methanolic extract of *Alstonia boonei* (De Wild) against some medically important pathogens

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ABSTRACT

Methanolic extract of *Alstonia boonei* (De Wild) has been reported to be very effective against bacterial pathogens. Four solvents (hexane, chloroform, ethyl acetate and distilled water) were used to partition the concentrated crude methanolic extract. The fractions were further diluted by dimethyl sulphoxide (DMSO). The concentrations used were 100, 50 and 25 mg/ml. Standard method was used to test the antibacterial properties of the fractions against nine clinically important pathogens. Hexane fraction showed no inhibition on *Enterococcus faecalis* but inhibit other test organisms at 25 mg/ml. *Staphylococcus aureus* was resistant to aqueous fraction at a very low concentration (25 mg/ml). *Shigella dysenteriae* and *Escherichia coli* were most susceptible to aqueous fraction with zone of inhibition ranging between 18 and 5 mm. Chloroform fraction was the most effective out of the four fractions. *Streptococcus* sp, *Staphylococcus aureus* *Proteus mirabilis* and *Escherichia coli* were resistant to Ethyl acetate fraction at 25 mg/ml. Ethyl acetate had the least activity on the test organisms. The minimum inhibitory concentration (MIC) ranged between 0 and 25 mg/ml.

Key words: *Alstonia boonei*, methanolic extract, pathogens, partition fractions, zone of inhibition.

INTRODUCTION

In the recent time, high percentage of resistance of bacteria to antibiotics has further strengthened the demand for medicinal plants (Ibekwe, 2000). Plant extracts have been used to treat many diseases and ailments. However, the practitioners do not have implicit knowledge of the principles of actions of the herbal preparations nor the aetiological agents of the diseases they treat (Gill, 1992; Burkill, 1985).

Alstonia (devil tree) is a native to tropical and subtropical Africa, Central America and Malaysian Region. It belongs to the family *Apocynaceae* and tribe *plumerriae*. The genus consists of about 60 authenticated species (Burkill, 1985). The leathery sessile, simple leaves, are elliptical, ovate, linear or lanceolate and wedge-shaped at the base (Dalziel, 1968; Irvine, 1961).

Alstonia boonei (De Wild) has been identified as one of the most medically useful plants in the Tropics (Uaeze and Pricilla 1986). *A. boonei* has potential therapeutic value to cure certain diseases like malaria, typhoid fever, gastroenteritis, urinary tract infections, dental carries, rheumatism, mouth odour and snake bite and its latex is used to treat cough, sore throat and fever (Personal communication).

This study has been undertaken to establish the antibacterial activity of partitioned methanolic leaf extracts of *Alstonia boonei* on some medically significant pathogens.

MATERIALS AND METHODS

Plant collection and Identification

Fresh leaves of *A. boonei* were obtained from the campus of the University of Ado-Ekiti,

Nigeria. The sample was identified and confirmed in the Herbarium Unit of the Department of Plant Science and Forestry, University of Ado-Ekiti, Nigeria. The leaves were air-dried and pulverized to fine particles with an electronic blender (SBG -320).

The finely pulverized sample was soaked in methanol at ratio 1:10 in a conical flask. This was covered and shaken at interval of 30min for a period of 6h. The suspension was allowed to stand for 48h. The solution was shaken and filtered using Whatman filter No 1. The filtrate was evaporated to dryness in the water bath. The extract was stored below the ambient temperature.

Partitioning of crude methanolic extract

The dried crude methanolic extract was mixed with distilled water and the slurry was partitioned successively between n-hexane, chloroform and ethylacetate to yield four fractions in all. These were concentrated and stored at 4°C until used.

Reconstitution of the partitioned extracts

The final extracts (partitioned) was reconstituted with 30% Dimethylsulphoxide (DMSO) to obtain a stock concentration of 100 mg/ml. lower concentrations were further obtained by doubling dilutions.

Source of test organisms and inocula standardization

The test organisms were obtained from the Department of Microbiology, University of Ado-Ekiti, Nigeria. The organisms used were *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus* sp, *Proteus vulgaris*, *Klebsella pneumoniae* and *Klebsiella* sp. They were grown (in separate tubes) at 37°C in Mueller-Hilton broth (Oxoid) for 16-18h with shaking and diluted to an optical density of 0.1 (0.5 McFarland Standard) at optical activity of 625nm and stored at 4°C to arrest further bacterial multiplication.

Determination of antibacterial activity

Agar diffusion method was used to assess the antimicrobial effect of the extracts. 0.2 ml of the standardized culture was seeded on freshly prepared Hilton-Mueller (Oxoid) agar. A sterile cork borer with 8.0 mm diameter was used to make a well in the agar medium. Each of the wells was filled with a particular concentration of the extracts. The plate was refrigerated at 4°C for 30 min before being incubated at 37°C. Zones of inhibition were measured after an incubation period of 24h.

The method of Osadebe and Ukwueze, (2000) was used to get the minimum inhibitory

Table 1: Minimum Inhibitory Concentration (MIC) (mg/ml) of the fractions on the test organisms

Test organism	Fraction			
	Hexane	Aqueous	Chloroform	Ethylacetate
<i>Salmonella typhi</i>	3.35	25.00	3.35	2.66
<i>Shigella dysenteriae</i>	3.35	2.51	3.16	2.99
<i>Enterococcus faecalis</i>	0	15.85	5.62	11.22
<i>Escherichia coli</i>	5.62	5.96	2.82	25.00
<i>Pseudomonas aeruginosa</i>	4.22	1.33	1.33	14.96
<i>Streptococcus</i> sp	12.59	1.41	2.51	25.00
<i>Klebsiella pneumoniae</i>	1.33	7.94	4.22	10.00
<i>Staphylococcus aureus</i>	2.82	25.00	25.00	25.00
<i>Proteus mirabilis</i>	14.13	5.96	14.96	25.00
<i>Klebsiella</i> sp	6.68	3.98	15.84	6.31

concentrations (MICs). The MIC was calculated plotting the logarithm of the concentration of the extract against the square of the zone of inhibition. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC value.

RESULTS AND DISCUSSION

Similar trends were observed in the Fig 1-4. The antibacterial activity decreases with decreases in concentration. In Fig 1, *E. faecalis* was

resistant to methanolic extract at 25mg/ml. Ethyl acetate fraction did not possess any antibacterial property at 25mg/ml against *Streptococcus* sp, *Staphylococcus aureus*, *Proteus mirabilis* and *Escherichia coli*. Chloroform, followed by aqueous extract performed better than the other extracts. This report negate the observations of Akujobi *et al.*, (2006) and Ibekwe *et al.*, (2001) that reported water extract to have low bacterial activity. The results presented in this study showed that the tested extracts contained antibacterial properties.

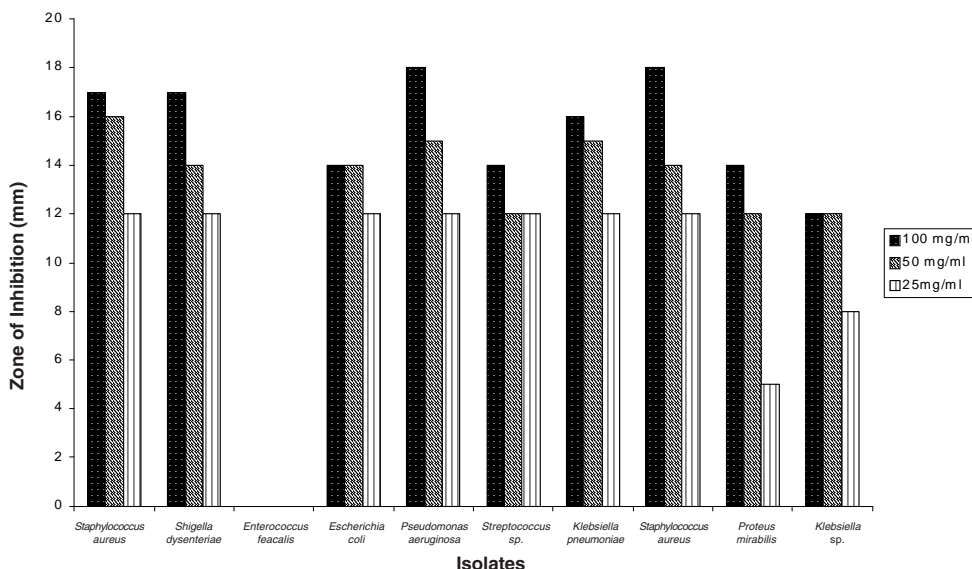


Fig. -1: Antibacterial activity of hexane fraction of partitioned leaf extract of *Alstonia boonei*

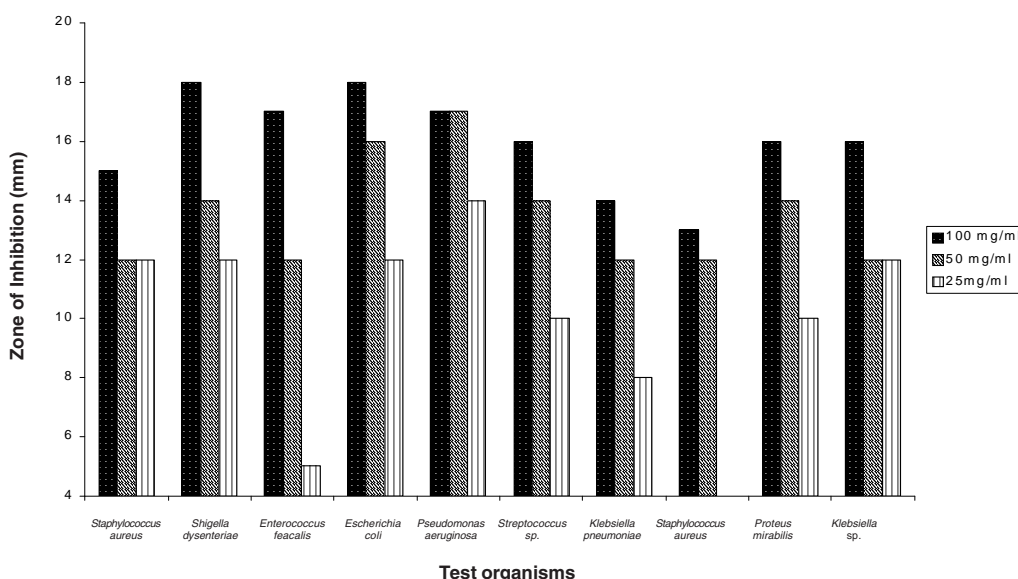


Fig. - 2: Antibacterial activity of aqueous fraction of partitioned leaf extract of *Alstonia boonei*

Though there was variation in their degree of antibacterial activity. Extracts are very potent against both Gram- positive and Gram-negative bacteria. This is in agreement with the reports of Ogueke *et al.*, (2006) and Oluma *et al.*, (2004). The exhibited antibacterial properties of these extracts

would be consequent upon their phytochemicals present in them (Obute, 2005; Wambebe, 1999; Wolinsky and Sote, 1984).

Methanol was identified by Cowan (1999) as one of the best solvent that extracts large quantity

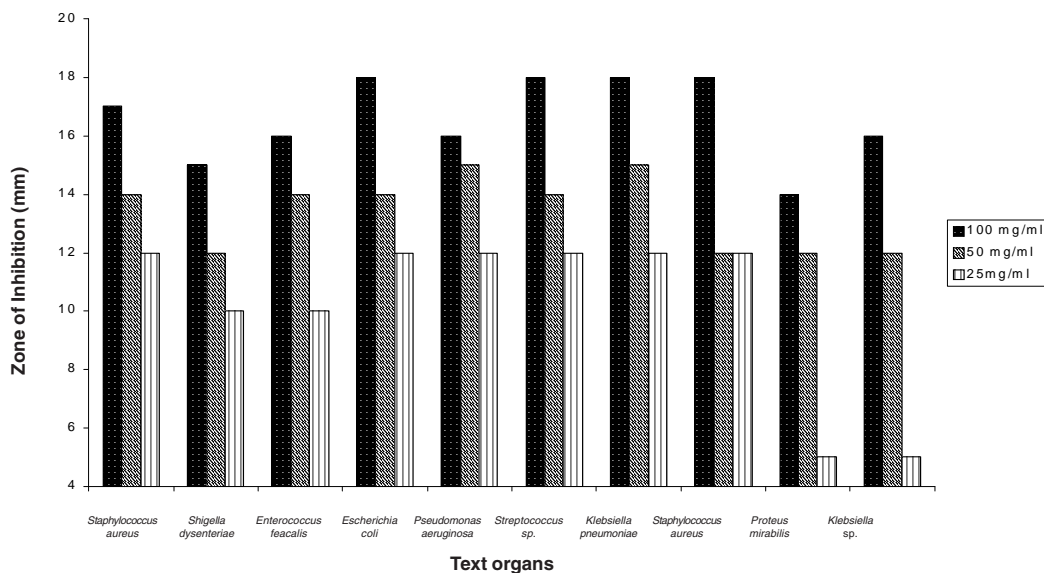


Fig. - 3: Antibacterial activity of chloroform fraction of partitioned leaf extract of *Alstonia boonei*.

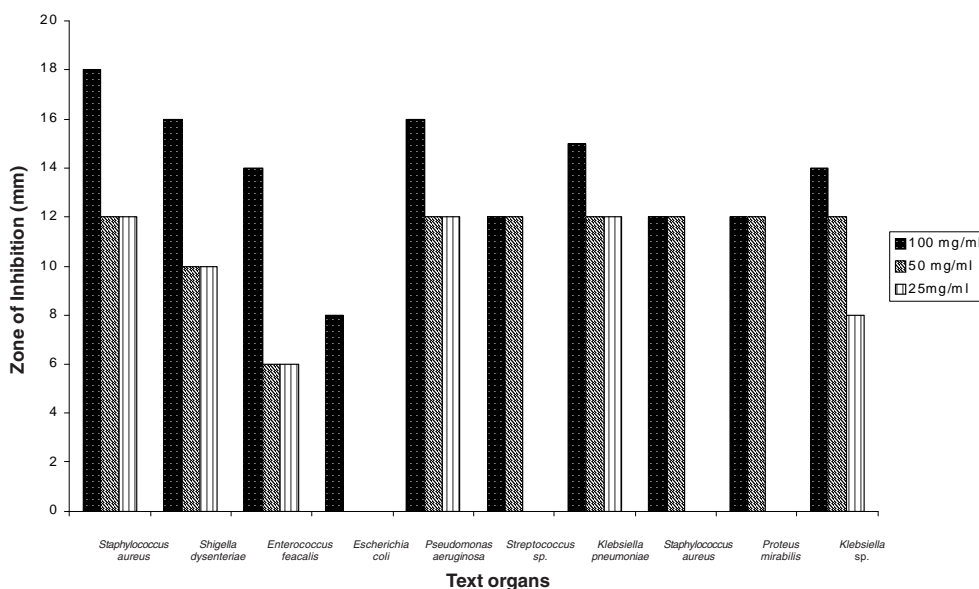


Fig. - 4: Antibacterial activity of ethylacetate fraction of partitioned leaf extract of *Alstonia boonei*.

and quite a number of phytochemical compounds. It is the only solvent without toxicity in bioassay. The adoption of different solvents for partitioning enables the solubility of the phytochemicals in their preferred solvents, thus enhancing their highest yield in such solvent. This could definitely prevent interaction of the active ingredients, hence improved the potency against targeted bacteria.

The potency of the extracts of *Alstonia boonei* justifies its uses in ethnomedical practices. The quantification and determination of the active ingredients on the test organisms should be the object of further investigation with the mechanism of action of their phytochemotherapy.

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