

Assessment of the cytotoxicity and genotoxicity of aqueous root extract of *Alstonia boonei* De wild using the *Allium* test

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Abstract

Herbal remedies are generally considered safe because they are natural. This study investigated the aqueous root extract of *Alstonia boonei* De Wild popularly utilized in herbal medicine, for its cytotoxicity and genotoxicity using the *Allium* test. Two hundred grams of the dried root was macerated in 1500 ml of tap water and left to stand for 24 hours. After filtration, the solution which formed the crude extract was diluted through four concentrations 25%, 50%, 75 % and 100% with a control for comparison. *Allium cepa* bulbs were planted in the extract for 48 hours, after which the root tips were observed for abnormalities using a microscope. The 25% extract concentration showed the highest range of abnormalities, including disturbed prophase and metaphase, precocious and lagging chromosomes in the anaphase and binucleate cells. The other concentrations showed a high level of prophase accumulation with bi-nucleate and multinucleate cells present, and inhibition of cell division to other phases. The mitotic index showed increasing values, indicative of low genotoxic effect, proving that for minimal amounts of root extract of *Alstonia boonei*, cytotoxicity can be observed. Therefore, its use as medicine should be viewed with caution despite the low genotoxic effect.

Keywords: aqueous root extract, *Alstonia boonei*, cytotoxicity, genotoxicity.

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Introduction

Alstonia boonei is a medicinal plant commonly found in Nigeria. It is known in Igbo as 'Egbu', in Yoruba as 'Awun', 'Ukhu' in Edo and 'Ukpukunu' in Urhobo. It is known in English as stool wood, cheesewood, pattern wood or alstonia. Its trade names include; stool wood, mujwa, pattern wood, cheesewood, alstonia or emien (Amole and Ilori, 2010). It belongs to the family Apocynaceae and the genus *Alstonia* has about 40 species occurring mostly in the moist lowland forests in Nigeria and sometimes spreads into drier areas such as the gentle, steep rocky hill sites in Liberia, it is also seen scattered or in small groups in wet or marshy places that are occasionally flooded. It is a large, deciduous tree growing up to 45 m tall and 1.2 m in

diameter with its bole often deeply fluted or grooved to 7 m with small buttresses present (Palla, 2005).

Akinmoladun et al. (2007) reported the presence of alkaloids, tannins, saponins, steroids, flavonoids and cardiac glycosides as phytochemicals present in the methanolic and aqueous stem bark extracts of *Alstonia boonei*, mineral elements such as iron, calcium, magnesium, potassium, a very high amount of phosphorus and the antioxidant, vitamin C were also reported present. The presence of two compounds, confirmed to be triterpenes were reported by Obiagwu et al. (2014) in a study carried out on the medicinal properties of the methanolic root-bark extract of *Alstonia boonei*.

Ethno-medicinally, the bark of the

Alstonia tree is one of the most potent analgesic herbs available in nature, it is widely used across Africa for treatment of various ailments. Reports have been made of its antimicrobial activity; ethanolic and aqueous extracts from its roots showed activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* (Opoku and Akoto, 2015).

A report on the toxicity of a polyherbal mixture of *A. boonei* stem bark and *Fadara zanthoyloids* stem bark showed nephrotoxic and hepatotoxic effects especially at high doses after long term use inferring that it may not be safe in the treatment of arthritis, rheumatism and other chronic diseases (Oghenesuvwe et al., 2015). The aqueous ethanolic extract of *A. boonei* stem bark has been reported to be nephrotoxic at high doses in guinea pigs (Oze et al., 2007).

In an oral acute toxicity (LD₅₀) study of the methanolic extract of *Alstonia boonei* root bark in mice, it was concluded that the administration of its extract in mice is safe at a dose less than or equal to 5000 mg/kg. However, doses that are extremely high may not be advised (Onwusonye et al., 2014). Akintonwa et al. (2009) carried out the *Allium cepa* assay using *Alstonia boonei* bark, the results obtained showed decreasing mitotic index and chromosomal aberrations.

The *Allium cepa* root meristem assay is a very convenient test system for evaluating harmful effects of chemical substances on biological materials, it is inexpensive and assures very clear chromosomes (Fikesjo, 1993). To the best of our knowledge, there are no reports on the use of this assay method to assess the cytotoxicity and genotoxicity of the aqueous root extract of *Alstonia boonei*.

Despite the fact that the numerous medicinal properties of *A. boonei* are well established in literature, information is lacking on the cytotoxic and genotoxic effects of its roots, which this study aims at evaluating using *Allium cepa* root tips.

Materials and Methods

Fresh roots of *A. boonei* De Wild were collected from within the environment of University of Nigeria, Nsukka campus. The following procedure is a modification of Akaneme and Amaefule (2012). The roots were dried at room temperature and ground into powder using a grinder. About 200 gm of the powder was added into 1500 ml of water, macerated and allowed to stand for 24 hours and

stored at 4 °C.

A filtrate was obtained by passing the powder/water mixture through a cheese cloth. This filtrate served as the root extract stock solution. The filtrate was reconstituted in tap water in appropriate concentration before administration. The dilution of the 300 ml stock of root extract, to its lower concentration required for treatments was made by measuring the required millimeters of stock solution into a container and making it up to 300 ml with tap water. For example, for 25 % concentration, 75 ml of the stock was added to 225 ml of distilled water, while for 50 % and 75 % concentrations, 150 ml and 225 ml of the stock were added to 150 ml and 75 ml of tap water respectively, while 300 ml of the stock solution formed the 100 % concentration.

Fresh bulbs of *Allium cepa* were purchased from the Nsukka main market. These were grown in disposable cups containing tap water for about 6 days to ensure proper root formation. The bulbs of *A. cepa* were then transferred into other disposable cups containing different concentrations of root extract (25 %, 50 %, 75 % and 100 %). A bulb of *A. cepa* was left in the tap water serving as the control.

After 48 hours, 4 to 6 roots were chopped off from each treated bulb including the control. They were washed three times in tap water and fixed in Carnoy's solution (a mixture of acetic acid and absolute ethanol) in the ratio 1:3. The fixed materials were stored in the refrigerator for 24 hours. Furthermore, the roots were rinsed in tap water three times before hydrolysing in 0.1 N hydrochloric acid for about 7 minutes at 60 °C. The milky portion of the root tips were subsequently, cut, squashed and stained with lacto-propionic orcein. The prepared slides were examined under the light microscope.

The 5 treatments (including control) were laid out in a completely randomized design (CRD) with three replications. A total number of 500 cells were counted for each treatment and various cells undergoing different stages of cell division- Prophase, metaphase, anaphase and telophase were observed and recorded for each treatment including the control. Phase indices of mitotic stages were recorded; Aberrations induced by each treatment at various stages were also recorded. Data pertaining to all dependable variables studied were analyzed using one-way ANOVA. Means were separated using the Least Square Difference at $\alpha=0.05$

Results

Table 1: Number of dividing and abnormal dividing cells, showing the mitotic index of *Allium cepa* roots treated with root extract of *Alstonia boonei*.

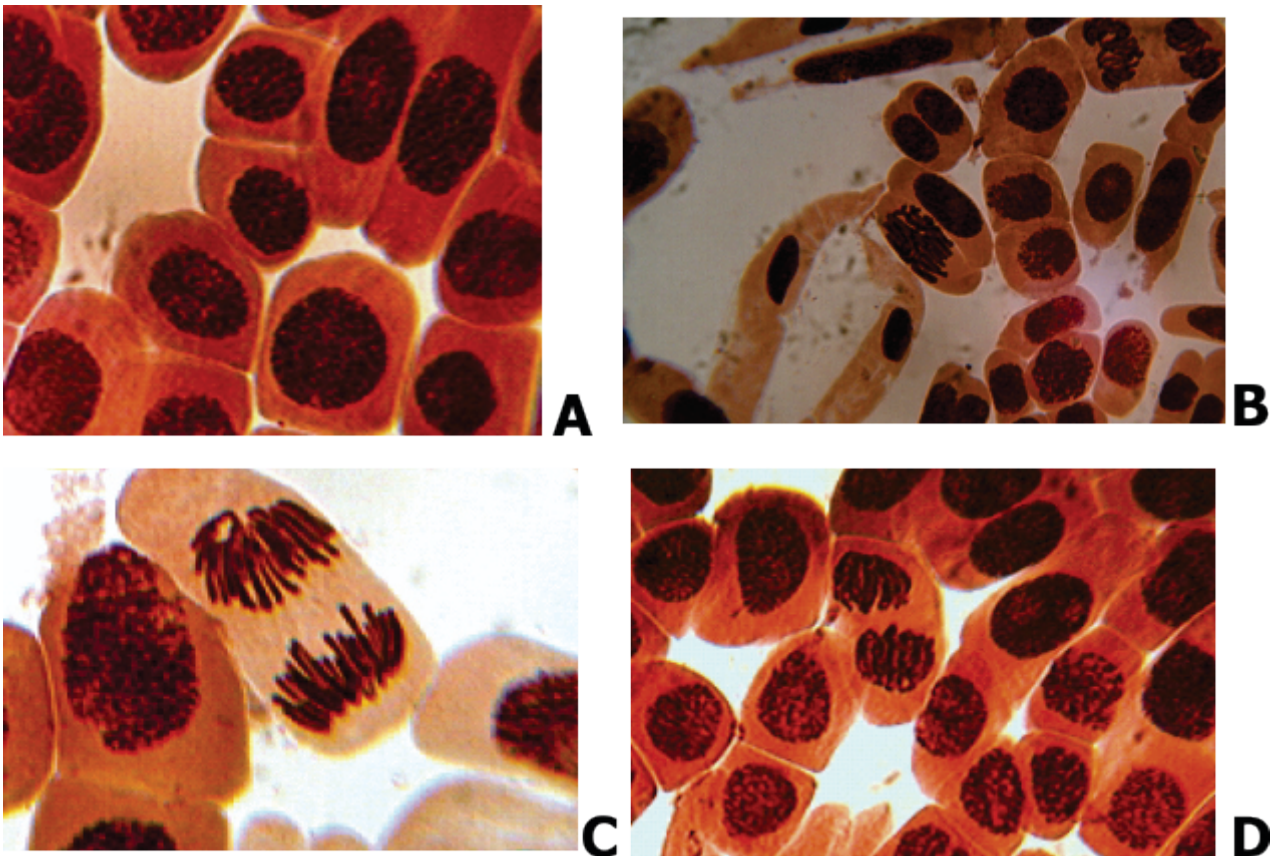
Conc./Treatment	Total no. of cells	No. of dividing cells	No. of abnormal dividing cells	Mitotic index	
				No.	%
Control	500	131±11.06	2±0.58	1.49	26.20
25 %	500	324±9.49	21±1.53	6.50	64.87
50 %	500	233±2.08	42±3.06	18.01	46.80
75 %	500	329±6.66	32±8.14	9.64	65.80
100 %	500	367±2.08	19±2.65	5.85	73.40
LSD (0.05)		10.64	6.03		2.14

As shown in Table 1, the total number of dividing cells ranged from 131.0 in the control, to 367.0 in the 100 % extract concentration out of 500 cells. The total number of dividing cells in all the treatments were much more than in the control, indicating that *Alstonia boonei* root extract increased the division of cells of *Allium cepa* root tips, and also increased abnormalities as the same table also shows that the number of abnormal cells in the treatments were also much more than that in the control. There exist variations in the number of dividing cells and abnormal cells observed in each treatment. The 100 % extract concentration had the highest number of dividing cells, while 50 % had the least, however, the number of dividing cells between the 25 % and 75 % extract concentrations were not significantly different. The number of abnormalities observed for each concentration showed marked variation with 50 % having the highest number of abnormalities but not significantly different from 75 %; but 100 % and 25% were significantly different from the 50 % extract concentration.

Moreover, Table 2 shows the phase indices for each mitotic stage treated with the root extract. Prophase stage had remarkably the highest number of cells for all treatments, a strong indication of prophase accumulation. The number of prophase cells in the 100 % concentration was the highest, followed by 75 %, 25 %, 50 % and the least being the control. For metaphase, anaphase and telophase stages of 50 %, 75 % and 100 % cells were not observed, but in each case showed a decrease from the control when compared with the 25 % concentration except in the telophase. Considering the phase indices, the prophase cells of the 100 % extract concentration was not significantly different at P (0.05) from the 50 % and 75 % extract concentrations. The metaphase, anaphase and telophase stages were absent, but the 25 % extract differed significantly at P (0.05) from the control, in these phases.

Table 2: Phase Indices

Conc./Treatment	Prophase		Metaphase		Anaphase		Telophase	
	No	%	No	%	No	%	No	%
Control	99±5.51	76.02	14±2.65	10.5	13±2.89	9.73	5.00±1.53	3.6
25 %	301±8.25	92.92	11±0.58	3.40	7±1.00	2.17	5.00±2.08	1.5
50 %	233±2.08	100.0	0±0.00	0.00	0±0.00	0.00	0±0.00	0.0
75 %	329±6.66	100.0	0±0.00	0.00	0±0.00	0.00	0±0.00	0.0
100 %	367±2.08	100.0	0±0.00	0.00	0±0.00	0.00	0±0.00	0.0
LSD (0.05)		8.04		1.77		1.99		1.69

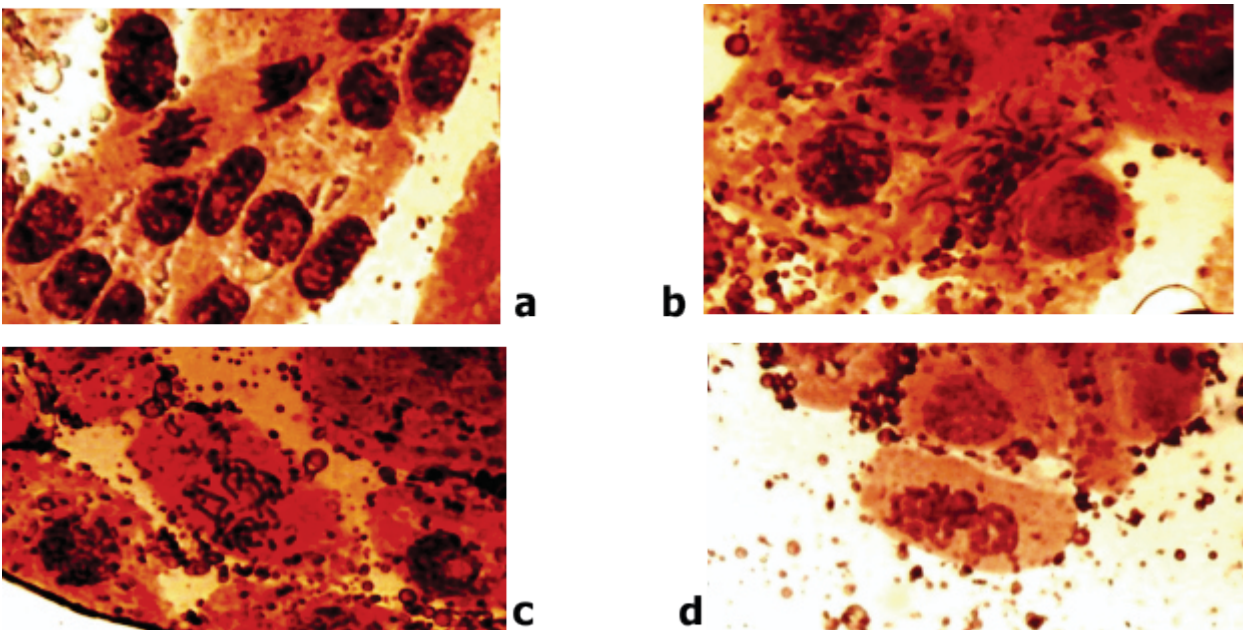


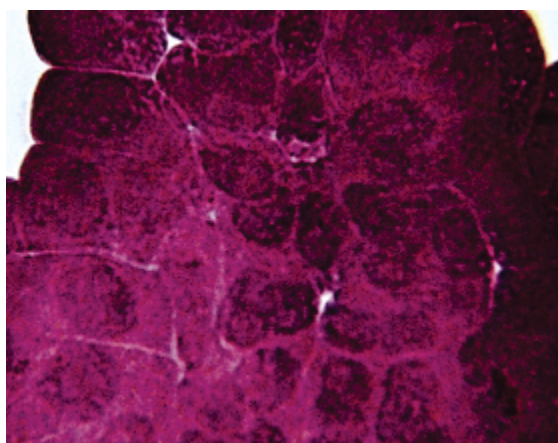
Plates I (A-D). Normal mitotic stages in *Allium cepa*. (A) Prophase (B) Metaphase (C) Anaphase and (D) Telophase

Plates I (A-D) shows the normal mitotic stages in *A. cepa*. The root extract of *Alstonia boonei* De Wild, showed a range of abnormalities as seen in plates II (a-f) which were of much diversity in the 25 % treatment concentration, since the 50 % - 100 % treatment concentrations showed no activity of division

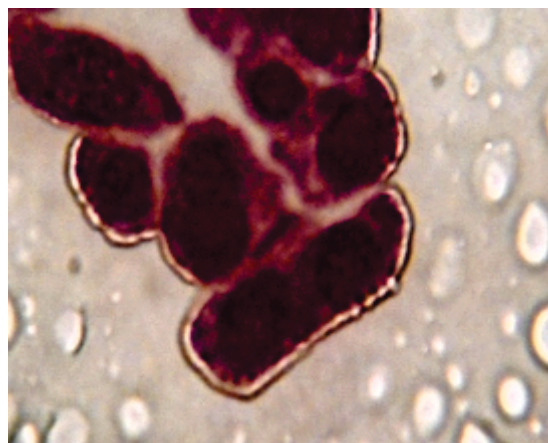
beyond the prophase stage. Precocious and lagging chromosome was observed in the anaphase stage of the 25 % treatment concentration. The formation of binucleated cells was observed in all extract concentration treatments.

Discussion





e



f

Plates II (a-f). Abnormal dividing cells: (a) Precocious Chromosome and lagging chromosome (b) disturbed metaphase(c) disturbed prophase (d) disturbed metaphase (e) multinucleated cell (f) binucleated cell.

The mitotic index of *Alstonia boonei* root extract increased with increasing treatment. Mitotic index is used as an indicator of cell proliferation biomarkers which measures the proportion of cells in the mitotic phase of the cell cycle (Rudolph et al., 1998). Hence, the increase in the mitotic index of *Allium cepa* meristematic cells could be interpreted as having low genotoxic effect. The presence of lagging chromosomes is attributed to the hindrance of pro-metaphase movement of chromosome, accompanied by adhesion of centromeres to the adjacent inner surface of the plasma membrane (Barthelmess, 1957; Soliman, 2010; Al-Ahmadi, 2013). Disturbed metaphase is attributed to lack of spindle fibre formation as observed by Sobieh et al. (2014), in the treatment of *Allium cepa* roots with *Rubus sanctus* Schreber extract. The formation of binucleated cells observed in all treatments is attributed to the inhibition of cytokinesis following telophase (Majewska et al., 2003). In *Allium cepa*, such inhibition arrests cell plate formation and this has been attributed to phlammogram inhibition at the early stage of telophase (Majewska et al., 2003; Fiskesjo, 1997; Rank et al., 2002).

The accumulation of prophase stages as observed in the extract treatment may be due to lack of spindle fibres formation that would have introduced the cells to another stage. The results agree with the work of El-Ghamery et al. (2000) and also that of Kumar and Rai (2007) on evaluation of cytological effects of Zn⁺ in relation to germination and root tip abnormalities. The high prophase accumulation could also be due to the blockage of the dividing cells at Chfr point which prevents prophase-metaphase transition as proved by Scolnick and Halazonetis (2000) who explained that Chfr protein delays chromosome condensation and nuclear envelope breakdown in response to drug such as

taxol and nocodazole that disrupt microtubule structure.

Conclusion

The observance of chromosomal aberrations in this study has proved that for minimal amounts of *Alstonia boonei* root extract, cytotoxicity can be observed, but the high mitotic index proves that there is no much genotoxic effect with the use of *A. boonei* root extract, therefore the need to standardize the dosages of medicinal plant extract for treatment of ailments and diseases.

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