

Full Length Research Paper

Phytochemical analysis and *in vitro* antibacterial evaluation of leaf and bark extracts of *Alstonia boonei*

D. J. Ajose¹, O. F. Onifade^{2*} and I. I. Wemambu¹

¹Department of Biological Sciences, College of Natural and Applied Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.

²Department of Food and Chemical Sciences, College of Natural and Applied Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.

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The antibacterial effect of the leaf, bark and leaf and bark combined of *Alstonia boonei* on bacterial pathogens, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Shigella dysenteriae* and *Salmonella typhimurium* was determined using agar diffusion technique to investigate their efficacy as anti-bacterial agent. The phytochemical components of the leaf and bark were studied. The phytochemical screening of the ethanolic leaf extract indicated the presence of tannins, phlobatannins, alkaloids, and cardiac glycosides, reducing sugar, saponins, anthraquinones and steroids, while the bark contains alkaloids, flavonoids, saponins, phlobatannins, anthraquinones, steroids and reducing sugars. The extracts showed varying inhibitory effect against *P. aeruginosa* and *K. pneumonia*. *S. typhimurium* (13±0.27 mm at 100 mg/ml for the leaf extract) being the most susceptible at all concentrations, 12±0.21 and 12±0.62 mm at concentrations 100 and 50 mg/ml respectively for bark extract and similarly for the leaf + bark extract. The result of the study suggests that the extracts of the different component parts of the plant can be used for the treatment of infections caused by the test organisms.

Key words: *In vitro*, antibacterial, *Alstonia boonei*, extracts, agar diffusion.

INTRODUCTION

Medicinal plants are natural products which provide numerous essential services in the ecosystem (Firenzuoli and Gori, 2007). It is also taken internally or used to bath as a remedy for dizziness, and given after childbirth to aid the delivery of the placenta (Orwa et al., 2009). The leaves, pulped to a mash, are applied topically to reduce

oedema, and leaf sap is used to cleanse sores (Orwa et al., 2009).

Various ethnopharmacological studies that have been carried out on this plant products which showed that the extracts possess antimalarial, antipyretic, analgesic and anti-inflammatory properties (Ojewole, 1984, Olajide et

*Corresponding author. E-mail: onifadeolayinka@gmail.com, Tel.: +234(0)8036641466.

al., 2000; Bello et al., 2009; Onifade and Maganda, 2015) anthelmintic (Weshche et al., 1990), diuretic, spasmolytic and hypotensive properties (Kucera et al., 1972), immunostimulant property (Taiwo et al., 1998), antipsychotic and anxiolytic effect (Elisabetsky and Costa-Campos, 2006), reversible antifertility effect (Raji et al., 2005).

The stem bark is anti-venom for snake bites and also used in traditional medicine to treat painful urination, insomnia and chronic diarrhea (Asuzu and Anaga, 1991). Infusion of the root and stem bark is used as a remedy for asthma, while that from the stem bark and leaves is used to treat impotence (Opoku and Akoto, 2015). Therapeutically, the bark has been found to possess antimicrobial and antibiotic properties (Kam et al., 1997; Faka, 2000; Hadi and Bremner, 2001). A decoction could be sweetened with pure honey and be taken up to 4 times daily as an effective painkiller for the following conditions: Painful menstruation (dysmenorrhoea), when associated with uterine fibroid or ovarian cysts in women; lower abdominal and pelvic congestion associated with gynaecological problems such as pelvic inflammatory diseases; and to relieve the painful urethritis common with gonococcus or other microbial infections in men (Adotey et al., 2012).

The cold infusion is also administered orally for the purpose of expelling round worms, threadworms (Abbiw, 1990), and other intestinal parasites in children. The bark decoction of *Alstonia boonei* is used with other preparations in the treatment of fractures or dislocation (Abbiw, 1990), jaundice for inducing breast milk and its latex is taken as a purgative (Adotey et al., 2012). *A. boonei* De Wild is regarded as one of few herbs with potential anti-HIV indicators (Adotey et al., 2012).

An array of chemical compounds has been isolated from *A. boonei*, which include alkaloids, tannins, iridoids, and triterpenoids (Akinmoladun et al., 2007). The alkaloids isolated from the plant include echitamine and other alkaloids, and the triterpenes b-amyrin, lupenol, and ursolic acid have all been isolated from leaves and stem bark (Adotey et al., 2012). Echitamine has anticancer activities (Adotey et al., 2012; Ashok et al., 2015), (Z)-9-Octadecenoic acid was found to be the most abundant volatile oil in the leaf and stem bark, while methyl (7 E)-7-octadecenoate was the most abundant in the root (Moronkola and Kunle, 2012).

In this study, the antibacterial activity of ethanol extracts of leaf, bark and equal combination of leaf and bark of *A. boonei* were analyzed against eight clinically pathogenic organisms using agar well diffusion method.

METHODOLOGY

Collection of samples

Fresh barks and leaves of *A. boonei* were obtained from the

botanical garden of the University of Lagos, Akoka (Lagos, Nigeria). Identification and authentication (LUH 6309) was done at the Herbarium, Faculty of Science, and Department of Botany of the University of Lagos. The leaves and stem were dried under shade for 2 weeks and ground into fine powder using a local grinding machine (the machine was pre-washed and dried before grinding to avoid contamination) at Oja market, Ogun State. The powder was stored in an air-tight vessel at room temperature.

Preparation of methanol extract

Extracts were prepared using the modified method of Opoku and Akoto (2015). 100 g of pulverized leaf and bark materials were soaked in 1000 ml of 70% ethanol separately and together (fifty grams each of leaf and bark) and left overnight. After 48 h, the mixtures were filtered with Whatman No. 1 filter paper and evaporated to dryness *in vacuo*. The crude extract was then stored at 4°C until further use.

Phytochemicals analysis

The phytochemical analysis was carried out according to Trease and Evans (2002). Chemical tests were carried out on the ethanolic extracts for the qualitative determination of phytochemical constituents.

Preparation of microorganism

The organisms used in this study were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, and *Salmonella typhimurium*. The strains were maintained at 37°C. Each bacterium was reactivated by inoculating onto sterile nutrient agar (Oxoid, CM003) plates and incubated appropriately. Mueller Hinton Agar (Oxoid, CM0337) medium was used as bacterial culture medium in the antibacterial assay.

Testing for antimicrobial activity

Antibacterial activity of ethanolic extracts of leaf and bark separately and leaf with bark together was tested using agar well diffusion method as described by Wemambu et al. (2018) with modification. 200 µl of bacteria suspension at 0.5 McFarland standards were aseptically introduced and spread using cotton swabs on surface of gelled sterile Muller Hilton agar plates. A well of about 8.0 mm diameter with sterile cork borer was aseptically punched on each agar plate. 100 µl of the reconstituted extracts at 100 mg/ml were introduced into the wells in the plates. A control well was made with 100 µl of the extracting solvent dimethylsulphoxide (DMSO) (undiluted). Plates were kept in laminar flow for 30 min for pre diffusion of extract to occur and then incubated at 37°C for 24 h. The diameter of the zone of inhibition around each well was measured.

RESULTS AND DISCUSSION

A. boonei has high quantities of alkaloids, tannins, saponins, steroids and flavonoids (which are the core antiparasitic agents), these could be responsible for the antimalarial efficacy (Ogiangbe et al., 2010). From

Table 1. Phytochemical constituents of the extracts.

Constituent	Observation	
	Stem	Leave
Flavonoid	+	-
Tannin	+	+
Phlobatannin	-	+
Alkaloid	+	+
Cardiac glycosides	+	-
Steroid	+	+
Reducing sugar	+	+
Saponin	+	+
Anthraquinone	+	+

Table showing the presence/absence of phytochemicals in *A. boonei* stem bark and leaves; + = presence of compound; - = absence of compound.

Table 2. Antibacterial susceptibility pattern of test organisms to the crude extracts.

Isolate	Extract and concentration (mg/ml)												
	Leaf				Bark				Leaf + bark				
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. aeru.</i>	12±0.43	12±0.87	-	-	-	-	-	-	-	-	-	-	-
<i>Strep. pneu.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>K. pneu.</i>	13±0.17	11±0.56	-	-	-	-	-	-	-	-	-	-	-
<i>S. dysenteriae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. typhi</i>	13±0.27	12±0.41	12±0.82	12±0.32	12±0.21	12±0.62	-	-	12±0.13	12±0.45	-	-	-

phytochemical screening, it was found that ethanolic extract of *A. boonei* stem bark contains alkaloids, flavonoids, saponins, phlobatannins, anthraquinones, steroids and reducing sugars. Tannins and cardiac glycoside were absent in the stem extract. Ethanolic extract of *A. boonei* leaves contains tannins, phlobatannins, alkaloids, cardiac glycosides, reducing sugar, saponins, anthraquinones and steroids. Flavonoids were absent (Onifade and Maganda, 2015), while steroidal compounds are known to behave like hormones, owing to their structural semblance. Tannins have also been found to be potentially anti-viral, anti-bacterial and anti-parasitic agents (Ene et al., 2008; Onifade and Maganda 2015). Tannins also hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Salah et al., 1995; Del-Rio et al., 1997; Okwu, 2004). Flavonoids also lower the risk

of heart diseases. Saponins are capable of neutralizing some enzymes in the intestine that can become harmful, building the immune system and promoting wound healing. Steroids and saponins have been found to be detrimental to several infectious protozoans (Delmas et al., 2000).

Three extracts (leaf, bark and leaf + bark) from the plant species, were screened for their potential antibacterial properties against *E. coli*, *S. aureus*, *P. aeruginosa*, *Streptococcus pneumoniae*, *P. mirabilis*, *K. pneumoniae*, *S. dysenteriae* and *S. typhimurium*. The extracts were prepared by sequentially extracting the plant material. The susceptibility pattern of the test organisms and phytochemical constituents of the extracts are represented in Tables 1 and 2. *E. coli*, *S. aureus*, *S. pneumoniae*, *P. mirabilis*, *S. dysenteriae* were all resistant to all extracts.

In this study, the antibacterial activity of ethanol extracts of leaf, bark and leaf + bark of *A. boonei* were analyzed against eight clinical isolates using agar well



Figure 1. *A. boonei*; leaves (left) and stem (right).

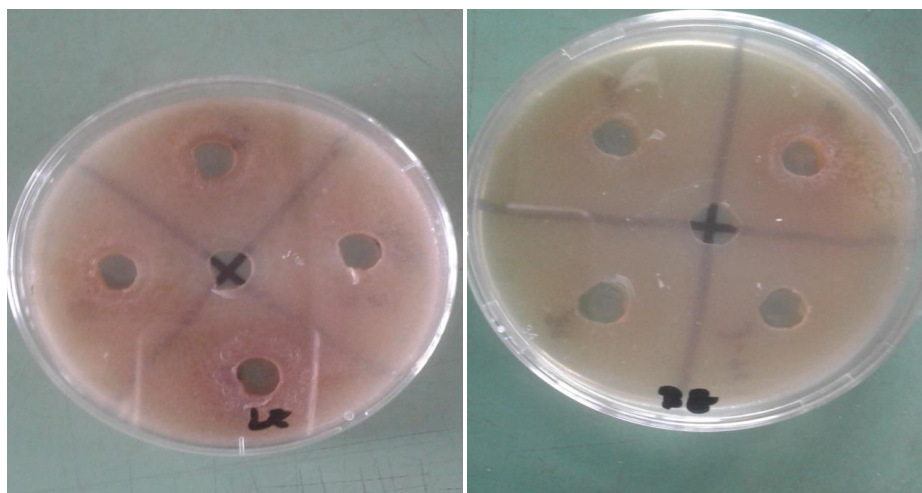


Figure 2. Plate showing the activity of crude leaf extract against *P. aeruginosa* (left) and bark extract (right) against *S. typhimurium* at concentrations 100, 50, 25 and 12.5 mg/ml. Negative control: DMSO (centre well).

diffusion method Figure 1. All test extracts showed varying degree of antibacterial activities at all tested concentrations which were compared with negative control DMSO that showed no activity with any of the extracts. *P. aeruginosa* was susceptible only to the leaf extract at 50 and 25 mg/ml and resistant to other extracts while *K. pneumoniae* was susceptible to leaf extract at concentrations 25 and 12.5 mg/ml and also resistant to other extracts. *S. typhimurium* was the most susceptible of all tested organisms Figure 2. It had the highest zone of inhibition (13 ± 0.27) for leaf extract at 100 mg/ml and lowest for other concentrations of the leaf extract and also at 100 and 50 mg/ml for both the bark extract and leaf + bark extract as presented in Table 2. The poor activity of the extracts could be as a result of the seventy per cent ethanol used for extraction as other researchers (Portillo et al., 2001; Alphonse et al., 2003; Koduru et al.,

2006; Aiyegoro et al., 2008; Ashafa et al., 2008; Igbiosa et al., 2009) have reported no activity with aqueous extracts against most bacterial strains. This could be due to the insolubility of the active compounds in water. Also, ethanol has a lower polarity as compared to methanol, and thus, may have not extracted all secondary metabolites from the samples used. It is also worthy to note that the resistant organisms could be highly resistant or multidrug resistant organisms and hence showing insensitivity to the extracts.

Conclusion

The result of this study shows that the tested organisms were more susceptible to the leaf extract than the bark the combined extracts. The combined effect of the leaf

and bark extract of the tested plant was additive.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Abbiw D (1990). *Useful Plants of Ghana: West African Uses of Wild and Cultivated Plants*. Intermediate Technology Publications, Royal Botanical Garden, Kew, London.
- Adotey JP, Adukpo GE, Boahen YO, Armah FA (2012). A Review of the Ethnobotany and Pharmacological Importance of *Alstonia boonei* DeWild (Apocynaceae). *International Scholarly Research* 1:1-9.
- Aiyegoro OA, Akinpelu DA, Afolayan AJ, Okoh AI (2008). Antibacterial activities of crude stem bark extracts of *Distemonanthus benthamianus* Baill. *Journal of Biological Sciences* 8(2): 356-361.
- Akinmoladun AC, Ibukun EO, Afor E (2007) Chemical constituents and antioxidant activity of *Alstonia boonei*. *African Journal of Biotechnology* 6(10):1197-201.
- Alphonse WW, Sumesh CC, Gerald M, Wilson N, Paul KT (2003): Molluscicidal and antimicrobial activity of *Solanum aculeastrum*. *Fitoterapia* 74:298-301.
- Ashafa AOT, Grierson DS, Afolayan AJ (2008). Antimicrobial activity of extract from *Felicia muricata* Thunb. *Journal of Biological Sciences* 8(6):1062-1066.
- Ashok R, Lokesh SJ, Hari Kiran (2015). Studies on the anticataract activity of echitamine, an alkaloid from the stem bark of *Alstonia scholaris*. *Der Pharmacia Lettre* 7(5):197-199
- Asuzu IU, Anaga AO (1991) Pharmacological screening of the aqueous extract of *Alstonia boonei* stems bark. *Fitoter* 63:411-417.
- Bello IS, Oduola T, Adeosun OG, Omisore NOA, Raheem GO and Ademosun AA. (2009). Evaluation of antimalarial activity of various fractions of *Morinda lucida* and *Alstonia boonei* stem bark. *Global Journal of Pharmacology* 3(3):163-165.
- Delmas F, Di Giorgio C, Elias R, Gasquet M, Azas N, Mshvildadze V, Dekanosidze G, Kemertelidze E, Timon-David P (2000). Antileishmanial activity of three saponins isolated from ivy, α -hederin, β -hederin and hederacolchiside A (1), as compared to their action on mammalian cells cultured in vitro. *Planta Medica* 66:343-347.
- Elisabetsky E, Costa-Campos L (2006). The alkaloid alstonine: a review of its pharmacological properties. *Evidence-Based Complementary and Alternative Medicine* 3:39-48.
- Ene AC, Ameh DA, Kwanashie HO, Agomuo PU, Atawodi SE (2008). Preliminary in vivo antimalarial screening of petroleum ether, chloroform and methanol extracts of fifteen plants grown in Nigeria. *Journal of Pharmacology and Toxicology* 32:254-260.
- Firenzuoli F, Gori L. (2007). Herbal medicine today: clinical and research issues. *Evidence-based Complementary and Alternative Medicine* 4(1):37-40.
- Hadi S, Bremner JB (2001). Initial studies on alkaloids from Lombok medicinal plants. *Molecules* 6(2):117-129.
- Igbinosa OO, Igbinosa EO, Aiyegoro OA (2009) Antimicrobial activity and phytochemical gel: A review update. *Journal of Ethno pharmacology* 68:3-37.
- Kam TS, Nyeoh KT, Sim KM, Yoganathan K (1997). Alkaloids from *Alstonia scholaris*. *Phytochemistry* 45(6):1303-1305.
- Koduru S, Grierson DS, Afolayan AJ (2006). Antimicrobial activity of *Solanum aculeastrum* (Solanaceae). *Pharmaceutical Biology* 44:284-286.
- Kucera M, Marquis VO, Kuceroval N (1972). Contribution to the knowledge of Nigerian medicinal plants; JLC separation and qualitative evaluation of *Alstonia boonei* alkaloids. *Plantamedical* 21:343-346.
- Moronkola DO, Kunle OF (2012). Essential oil compositions of leaf, stem bark and root of *Alstonia boonei* de Wild (Apocynaceae). *International Journal of Biological and Pharmaceutical Research* 3(1):51-60.
- Ogiangbe ON, Igbinosa IB, Tamo M (2010). Insecticidal properties of an alkaloid from *Alstonia boonei* De Wild. *Journal of Biopesticides* 3(1):265-270
- Ojewole JAO (1984) Studies on pharmacology of echitamine, an alkaloid from the stem bark of *Alstonia boonei* L. (Apocynaceae). *International Journal of Crude Drug Research* 22:121-143.
- Okwu DE (2004). Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. *Journal of Sustainable Agriculture and the Environment* 6:30-34
- Okwu DE, Okwu ME (2004). Chemical composition of *Spondias mombia* Linn plant parts. *Journal of Sustainable Agriculture and the Environment* 6:140-147.
- Olajide AO, Awe SO, Makinde JM, Ekhehar AI, Olusola A (2000) Studies on the anti-inflammatory, antipyretic and analgesic properties of *Alstonia boonei* stem bark. *Journal of Ethnopharmacology* 71:179-186.
- Onifade OF, Maganda Vera (2015). *In Vivo* Activity of Ethanolic Extract of *Alstonia Boonei* Leaves against *Plasmodium berghei* in Mice. *Journal for Worldwide Holistic Sustainable Development* 1(4):60-68.
- Opoku F, Akoto O. (2015). Antimicrobial and Phytochemical Properties of *Alstonia Boonei* Extracts. *Organic Chemistry Current Research* 1:137.
- Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). *Agroforestry Database: a tree reference and selection guide version 4.0* (http://www.worldagroforestry.org/sites/treedbs/tree_databases.asp). Diakses tanggal 10(09), 2016.
- Portillo A, Vila R, Freixa B, Adzet T, Canigüeral S (2001): Antifungal activity of Paraguayan plants used in traditional medicine. *Journal of Ethnopharmacology* 76:93-98.
- Raji YI, Salmon TM, Akinsomsoye OS (2005). Reproductive functions in male rats treated with methanolic extract of *Alstonia boonei* stem bark. *African Journal of Biomedical Research* 8:105-111.
- Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry and Biophysics* 322(2):339-346
- Taiwo OB, Kroes BH, Beukelman CJ, Horsten STAJ, Makinde JM (1998). Activity of stem bark of *Alstonia boonei* de wild on human complement and polymorph nuclear leucocytes. *Indian Journal of Pharmacology* 30(3):169.
- Trease GE, Evans WC (2002). *Pharmacognosy*. 15th Edn. Saunders, pp. 214-393.
- Wemambu II, Ajose DJ, Eni CC (2018). Antibacterial Effect of *Carica papaya* Root Extract on Some Selected Pathogens from Clinical Isolates. *Acta Scientific Microbiology* 1(7):6-10.
- Weshche D, Black D, Millious WK (1990). Book of Abstract, 39th Annual meeting of the American Society of tropical medicine and hygiene, New Orleans. Poster No 138.