

Synergistic Efficacy of Tissue Extracts of *Alstonia boonei* and *Vitex grandifolia* against Some Common Bacteria Pathogens

Justice Oyindeinyefa Epidi*, Esther Benafegha Enaregha

Department of Biology, Isaac Jasper Boro College of Education, Sagbama, Bayelsa State, Nigeria

*Corresponding Author: Justice Oyindeinyefa Epidi, Department of Biology, Isaac Jasper Boro College of Education, Sagbama, Bayelsa State, Nigeria

Abstract: This study evaluated the Synergistic efficacy of tissue extracts of *Alstonia boonei* and *Vitex grandifolia* against some common bacteria pathogens. Both plants were obtained from freshwater swamp in Wilberforce Island, Nigeria. Water, ethanol, methanol and hexane were used to extract the dried plant materials in a ratio of 1:1. Agar well diffusion technique was employed for the sensitivity testing. The results revealed that ethanolic extracts had superior zone of inhibition on the overall. The highest zone of inhibition for synergistic potency of the ethanolic extracts were 13.00mm (*Bacillus* sp) in root + leaf, 15.00 mm (*S. aureus*) in root + leaf, 13.00 mm (*P. aeruginosa*) in stem + leaf, 14.00 mm (*Proteus* sp) in stem + leaf, and 15.00mm (*Salmonella* sp) in root + leaf. There was significant variations ($P < 0.05$) in the zone of inhibition that was achieved for each of the bacterium under study and plant parts. The synergistic results of two or more parts of the plant combinations were superior as compared to the individual tissue extracts. The antimicrobial activities of the synergy of *Alstonia boonei* and *Vitex grandifolia* tissues suggests that it can be used as broad spectrum antibiotics.

Keywords: Antibacterial, *Alstonia boonei*, Medicinal plants, Microorganisms, *Vitex grandifolia*

1. INTRODUCTION

Despite the advancement made in the field of pharmaceutical microbiology and chemistry there is still a problem of antibiotics resistance. This is a major source of concern globally probably due to the resistance to emerging and re-emerging microbial infection. To this effect, studies have been carried out on potential alternative to this antibiotics and plant extracts have emerged as credible candidate. Plants with medicinal purpose has been widely used in the treatment of various types of diseases.

Authors have variously reported that medicinal plant are plants that one or more parts (including leaves, flower, fruit, latex, stem-bark, root etc) have medicinal potentials (Izah et al., 2018a-d; Izah and Aseibai, 2018; Izah, 2018; Kigigha et al., 2018, 2016, 2015). Medicinal plant is nature's gift to humanity and have been widely used. Authors have variously reported that approximately 75 – 80% of world population still rely on the use of herbs for the treatment and management of some ailments (Gahlaut and Chhillar, 2013; Fatima et al., 2011; Minochecherhomji and Vyas, 2014; Epidi et al., 2016a,b; Kigigha et al., 2015, 2016). Most of the individuals that rely on traditional medicine practitioners for the management of diseases reside in the rural area in many regions of developing nations. Amole and Ilori (2010) attributed this to economic factors and unavailability of modern drugs in the rural areas. A significant number of world population reside in rural areas in many developing nations. As such medicinal plants form the basis of primary health care services for many people in the region.

Many medicinal plant is a rich source of antimicrobial agents. This is probably due to the bioactive ingredients they contain. As such traditional medicine are source of potentially useful compounds for the development of phytotherapeutic agents with antimicrobial potentials. Plants with antimicrobial potentials have enormous therapeutic likelihood for the treatment of diseases caused by microbes. Several plants have been reported to have antimicrobial potentials in literature. But information about the synergy of one or more plant parts or species is often scanty in literature.

For instance, plant such as *Vitex grandifolia* and *Alstonia boonei* have been reported to contain antibacterial activity (Epidi et al., 2016a,b). *Alstonia boonei* which is popularly known as *Alstonia*,

stoolwood etc belongs to *Apocynaceae* family, and are used by traditional health practitioners in rural areas to manage several disease conditions (Epidi et al., 2016a). *Alstonia boonei* is a facultative plant having estimated occurrence probability of 33 – 67% in both wetland and non-wetland areas (Epidi et al., 2016a). Furthermore, *Vitex grandifolia* is also known as Black plum, Chocolate and berry tree (Azokou et al., 2013). The plant is also found in the tropical rain forest of the Niger Delta but predominantly found in the non-wetland region of Nigeria. Thus, it is a facultative upland plant found in both wetland and non-wetland areas. Typically the genus *Vitex* are known to have pharmacological potentials (Owolabi et al., 2010; Rani and Sharma, 2013) probably due to the presence of essential oils, flavonoids, iridoids, glycosides, diterpenoids and ligands they contain (Rani and Sharma, 2013). Even in the traditional setting they have been applied for the treatment of several diseases (Ganapaty and Vidyadhar, 2005) and controlling pests such as *Tribolium castaneum* (Epidi and Odili, 2009).

The synergy of the parts of *Vitex grandifolia* has been reported in literature using different solvents for extraction (Epidi et al., 2016b). Furthermore, Epidi et al. (2018a) also reported the antibacterial activities of different parts of *Alstonia boonei*. Hence the present study aimed at assessing the synergistic effectiveness of the different parts of both *Vitex grandifolia* and *Alstonia boonei*.

2. MATERIALS AND METHODS

2.1. Source of Plant Material

The *Vitex grandifolia* and *Alstonia boonei* were obtained from the freshwater swamp in Wilberforce Island. For each of the plants, the different tissues (leaf, root and stem) were obtained. The *Alstonia boonei* was identified based on the morphological characteristics presented by Adomi and Umukoro (2010), Nyananyo (2006), Onwusoye and Uwakwe (2014), Akinloye et al. (2013), Momoh et al. (2014). While the *Vitex grandifolia* was identified based on the information presented by Owolabi et al. (2010), Lemmens (2008) and Azokou et al. (2013).

2.2. Samples Collection, Preparation and Extraction

The plant tissues were allowed to air dry in the laboratory. The different tissues of both plants were macerated using electronic blender. The various parts of *Vitex grandifolia* were combined with that of *Alstonia boonei* in a 1:1 ratio. For instance, the root of *Vitex grandifolia* were combined with the root of *Alstonia boonei*, the stem-bark of *Vitex grandifolia* were combined with the stem-bark of *Alstonia boonei* and the leaves of *Vitex grandifolia* were combined with the leaves of *Alstonia boonei*. A total of 40g of the powdered plant (viz 20g from *Vitex grandifolia* part and 20g from *Alstonia boonei* parts) were soaked in 100ml of each solvent i.e water, hexane, ethanol and methanol for 48 hours. Thereafter, the samples were filtered using muslin cloth. The extracts were further filtered using Whateman #1 filter paper. The filtrates were then concentrated using rotary evaporator. This process was carried out in triplicate.

2.3. Source and Preparation of Organisms

The bacteria isolates used for the study including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus*, *Salmonella* and *Proteus* species were obtained from the stock culture in the Medical Microbiology and Parasitology Department, College of Health Sciences, Niger Delta University, Nigeria. The purity of the isolates were determined following standard biochemical procedure including gram reaction, motility, catalase, coagulase, oxidase, citrate, urease and indole on the organisms using the scheme of Benson (2002), Cheesbrough (2004). Furthermore, the *Staphylococcus aureus* was streaked in Mannitol salt Agar which showed yellow pigmentation. Klingon Iron Agar was also used for the confirmatory test of *Pseudomonas aeruginosa*, *Salmonella* and *Proteus* species.

2.4. Antimicrobial Screening of the Extract

Agar diffusion technique previously described by Opoku and Akoto (2015), Ere et al. (2014) and has been applied by Izah and Aseibai (2018), Izah et al. (2018b-d), Kigigha et al. (2018, 2016, 2015) were employed. Approximately 2ml of 24 hours culture were spread on the prepared Mueller Hilton agar. Then after, four wells of 6.0 mm diameter were made on the agar plates using sterile cork borer. 2ml of the concentrated aqueous, ethanolic, methanolic and hexane extract were dispensed into each of the wells using micropipette. The plates were properly labeled and then incubated. The plates were

incubated at 37°C for 24 hours under aerobic conditions. Thereafter, the zone of inhibition was measured using a meter rule.

2.5. Statistical Analysis

SPSS software version 16 was used to carry out the statistical analysis. The data were expressed as Mean \pm standard error. Significant difference was determined at $\alpha = 0.05$ using one-way analysis of variance. Where significant variations exist, Duncan Multiple Range Test was used to compare the means.

3. RESULTS AND DISCUSSION

The Zone of inhibition for the various extracts (i.e. ethanol, methanol, hexane and water) of *V. grandifolia* + *A. boonei* is presented in Table 1. The zone of inhibition of the ethanolic extracts ranged from 11.00 – 13.00 mm (root), 9.67 – 13.00 mm (stem), 11.00 – 13.00 mm (leaf), 10.67 – 13.00 (root + stem), 12.00 – 15.00 mm (root + leaf), 10.00 – 14.00 mm (stem + leaf) and 10.00 – 13.00 mm (stem + leaf + root). Basically, there were significant differences ($P < 0.05$) among the different microorganisms across the various plant tissues.

The synergistic potency of the ethanol extracts was highest at root + leaf with zone of inhibition of 13.00mm (*Bacillus* sp), root + leaf with zone of inhibition of 15 mm (*S. aureus*), stem + leaf with zone of inhibition of 13 mm (*P. aeruginosa*), stem + leaf with zone of inhibition of 14 mm (*Proteus* sp) and root + leaf with zone of inhibition of 15mm (*Salmonella* sp). There were significant variations ($P < 0.05$) in the highest zone of inhibition that was achieved for each of the bacterium under study.

The zone of inhibition of the methanolic extracts ranged from 11.00 – 13.67 mm (root), 10.00 – 13.00 mm (stem), 9.00 – 13.33 mm (leaf), 10.00 – 12.33 (root + stem), 11.00 – 14.00 mm (root + leaf), 11.00 – 13.00 mm (stem + leaf) and 9.00 – 12.33 mm (stem + leaf + root). Fundamentally, there were significant differences ($P < 0.05$) among the different microorganisms across the various plant tissues.

The synergistic result of the methanolic extracts was highest at root + leaf with zone of inhibition of 14.00mm (*Bacillus* sp), root + leaf with zone of inhibition of 13 mm (*S. aureus*), stem + leaf with zone of inhibition of 13.33 mm (*P. aeruginosa*), root + leaf and root with zone of inhibition of 13 mm (*Proteus* sp) and stem with zone of inhibition of 13mm (*Salmonella* sp). Statistically, there were significant variations ($P < 0.05$) in the highest zone of inhibition that was achieved in each of the bacterium under study.

The zone of inhibition of the different bacteria from the hexane extracts of the different plant tissues ranged from 10.67 – 13.00 mm (root), 10.00 – 13.00mm (stem), 10.00 – 12.33 mm (leaf), 11.67 – 13.00mm apart from *Bacillus* sp that was resistant (root + stem), 12.33 – 14.33 mm (root + leaf), 11 – 13.00 mm (stem + leaf) and 10.00 – 13.67 mm apart *P. aeruginosa* which was resistant (stem+ root+ leaf). However, there were significant differences ($P < 0.05$) among most of the bacteria in each of the plant tissues and its combinations.

The synergistic potency results of the different plant tissues showed that the highest zones of inhibition of 13.67 mm (*Salmonella* sp), 12.33mm (*Bacillus* sp), 14.33mm (*P. aeruginosa*), 13.00 mm (*Proteus* sp) and 14.00mm (*S. aureus*) were obtained from root + leaf+ stem, root + leaf and root, leaf + root, root+ stem and stem + leaf + root and leaf + root respectively. Statistically, significant variations ($P < 0.05$) exist in the highest zone of inhibition that was achieved in each of the bacterium under study.

The zone of inhibition of the different bacteria from the aqueous water extracts of the different plant tissues ranged from 9.67 – 11.67 mm (root), 9.33 – 11.00mm (stem), 8.00 – 10.33 mm (leaf), 9.00 – 11.00mm (root + stem), 10.00 – 12.33 mm (root + leaf), 10.67 – 14.00 mm (stem and leaf) and 9.33 – 11.67 mm (stem+ root+ leaf). However, zone of inhibition of extracts of root for *S. aureus*, leaf for *Proteus* sp, root + stem for *Bacillus* sp, leaf + stem for *Proteus* and *Bacillus* sp, stem+ root+ leaf for *P. aeruginosa* were 0.00mm. There were significant differences ($P < 0.05$) among most of the bacteria in each of the plant tissues and its combinations.

Table1. Zone of inhibition for the various extracts of *V. grandifolia* + *A. boonei*

Plant tissues	Microorganisms	Ethanol	Methanol	Hexane	Water
Root	<i>Bacillus</i> sp	11.67±0.33abcd	11.33±0.33bcde	12.33±0.33defgh	9.67±0.33cde
	<i>S. aureus</i>	12.33±1.20cde	10.33±0.33abc	10.67±0.33bcd	0.00±0.00a
	<i>P. aeruginosa</i>	12.33±0.33cde	13.67±0.33gh	11.00±0.58bcde	9.67±0.33cde
	<i>Proteus</i> sp	13.00±0.58def	13.00±0.58efgh	13.00±0.58fghi	11.33±0.33fgh
	<i>Salmonella</i> sp	11.00±0.58abcd	11.00±0.58bcd	12.67±0.33efghi	11.67±0.33gh
Stem	<i>Bacillus</i> sp	11.00±0.58abcd	11.00±0.58bcd	11.00±0.58bcde	11.00±0.58efgh
	<i>S. aureus</i>	10.00±0.58ab	10.00±0.58ab	10.00±0.58b	10.00±0.58cde
	<i>P. aeruginosa</i>	9.67±0.33a	10.33±0.33abc	10.33±0.33bc	10.33±0.33cdefg
	<i>Proteus</i> sp	11.00±0.58abcd	11.00±0.58bcd	11.00±0.58bcde	9.33±0.33bcd
	<i>Salmonella</i> sp	13.00±0.58def	13.00±0.58efgh	13.00±0.58fghi	10.33±0.33cdefg
Leaf	<i>Bacillus</i> sp	11.00±0.58abcd	11.00±0.58bcd	10.00±0.58b	8.00±0.58b
	<i>S. aureus</i>	12.67±0.88cde	11.33±0.33bcde	11.33±0.33bcdef	10.00±0.00cdef
	<i>P. aeruginosa</i>	11.00±0.58abcd	13.00±0.88efgh	12.33±0.33defgh	10.33±0.33cdefg
	<i>Proteus</i> sp	11.00±0.58abcd	11.00±0.58bcd	11.00±0.58bcde	0.00±0.00a
	<i>Salmonella</i> sp	13.00±0.58def	9.00±0.58a	10.00±0.58b	9.00±0.58bc
Root + stem	<i>Bacillus</i> sp	12.00±0.58bcde	10.00±0.58ab	0.00±0.00a	0.00±0.00a
	<i>S. aureus</i>	13.00±0.58def	10.67±0.33abcd	13.00±0.58fghi	10.67±0.33defg
	<i>P. aeruginosa</i>	10.67±0.33abc	11.67±0.88bcde	11.67±0.88bcdef	9.00±0.58bc
	<i>Proteus</i> sp	11.67±0.88abcd	10.67±0.88abcd	13.00±0.58fghi	11.00±0.58efgh
	<i>Salmonella</i> sp	12.67±0.33cde	12.33±0.33defgh	11.67±0.33bcdef	10.67±0.33defg
Root + leaf	<i>Bacillus</i> sp	13.00±0.58def	14.00±0.58h	12.33±0.58defgh	12.33±0.33h
	<i>S. aureus</i>	15.00±0.58f	13.00±0.58efgh	14.00±0.58hi	10.00±0.58cdef
	<i>P. aeruginosa</i>	12.00±0.58bcde	11.00±0.58bcd	14.33±0.33i	10.67±0.33defg
	<i>Proteus</i> sp	13.00±0.58def	13.00±0.58efgh	11.67±0.33bcdef	11.67±0.33gh
	<i>Salmonella</i> sp	15.00±0.58f	12.33±0.33defgh	12.33±0.33defgh	11.33±0.33fgh
Stem +leaf	<i>Bacillus</i> sp	12.67±1.45cde	12.33±0.33defgh	12.00±0.58cdefg	11.67±1.20gh
	<i>S. aureus</i>	10.00±0.00ab	12.00±0.00cdefg	12.33±0.67defgh	11.67±0.67gh
	<i>P. aeruginosa</i>	13.00±1.54def	13.33±0.58fgh	13.00±0.58fghi	11.00±0.58efgh
	<i>Proteus</i> sp	14.00±0.58ef	11.00±0.58bcd	11.00±0.58bcde	10.67±0.33defg
	<i>Salmonella</i> sp	11.00±0.58abcd	12.00±0.58cdefg	13.00±0.58fghi	14.00±0.58i
Stem+ leaf+ root	<i>Bacillus</i> sp	12.00±0.58bcde	12.00±0.58cdefg	10.00±0.58b	10.00±0.58cdef
	<i>S. aureus</i>	13.00±0.58def	12.00±0.58cdefg	12.00±0.58cdefg	11.67±0.33gh
	<i>P. aeruginosa</i>	10.00±0.58ab	9.00±0.58a	0.00 ±0.00a	0.00±0.00a
	<i>Proteus</i> sp	13.00±0.58def	12.00±0.58cdefg	13.00±0.58fghi	10.00±0.58cdef
	<i>Salmonella</i> sp	13.00±0.58def	12.33±0.58defgh	13.67±0.67ghi	9.33±0.33bcd

Each value is expressed as mean ± standard error (n = 3). Different letters (a, b, c, d, e, f, g, h, i) in each column indicate significant differences at P< 0.05 according to the Duncan Statistics

The synergistic potency results of the different plant tissues showed that the highest zones of inhibition of 14.00 mm (*Salmonella* sp), 12.33mm (*Bacillus* sp), 11.00mm (*P. aeruginosa*), 11.67 mm (*Proteus* sp) and 11.67 mm (*S. aureus*) were obtained from leaf + stem, root + leaf, stem + leaf, root + leaf and stem +leaf+ root and stem + leaf respectively. There were significant variations (P<0.05) in the highest zone of inhibition that was achieved on each of the bacteria under study

The antibacterial potency of the extracts suggests the presence of bioactive ingredients. Epiidi et al. (2016a) reported that *A.boonei* contain phytochemicals such as tannins, saponins, flavonoids, cardiac glycosides and alkaloids. Epiidi et al. (2016b) reported that *Vitex grandifolia* contain phytochemicals such as tannins, saponins, flavonoids, cardiac glycosides and alkaloids. Some of these phytochemicals have the tendency to wade off pests including microorganisms. The variations in the antimicrobial potentials of the different tissues of the plants could also be due to differences in the bioactive constituents in the various tissues (Epiidi et al., 2016a,b).

The zone of inhibition were in the order of ethanol > methanol > hexane > aqueous. This trend has been reported for individual plants i.e *A. boonei* (Epiidi et al., 2016a) and *V. grandifolia* (Epiidi et al., 2016b). This could be due to differences in polarity of the solvents (Izah, 2018) and their extraction

potentials. Generally, the values reported in this study appears to be superior than the result of the individual plants as reported by Epidi et al. (2016a,b). This is contrary to the findings of Karmegam *et al.* (2008) that reported an improved result of the synergistic effects of *Balantes aegyptiaca*, *Hyptis suaveolens*, *Lawsonia inermis*, *Leucasaspera*, *Lobelia nicotianaefolia* and *Phyllanthus madraspatana* using ethanol and water as extraction solvent. This trend could be due to the metabolism and physiology of the test organisms. Generally studies have reported that pH of the medium, temperature, water activity, oxygen and nutrient availability, choice of solvent, source of the organisms, biochemistry, physiology, metabolism and adaptation strategies of the microbes, plant species, biochemistry, age and parts, concentration of the plant extract and period of extraction could influence the sensitivity of plant extracts (Izah et al., 2018a-d; Izah and Aseibai, 2018; Izah, 2018; Kigigha et al., 2018, 2016, 2015).

Some of the bacteria were resistant to some of the extracts. The trend in some extracts i.e. microbes being resistant to some solvents is in agreement with the report of Gahlaut and Chillar (2013), Epidi et al. (2016a,b). Generally, the ability of both plants to inhibit the growth of the microbes used in this study suggest that they can be used as broad spectrum antibiotics (Izah et al., 2018a-d; Izah and Aseibai, 2018; Izah, 2018; Kigigha et al., 2018, 2016, 2015; Epidi et al., 2016a,b).

4. CONCLUSION

This study found that the combination of *V. grandifolia* and *A. boonei* extracts gives zone of inhibition in the order of ethanol > methanol > hexane > aqueous. The combination of two or more tissues also gave superior results. On the overall, both plants have antibacterial activities against the various bacteria under study. Hence they can be used as broad spectrum antibiotics.

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