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RESEARCH ARTICLE

SYNERGISTIC EFFECTS OF ALUM AND GUAVA *(PSIDIUM GUAJAVA)* LEAF EXTRACTS ON SOME PATHOGENS FROM CLINICAL SAMPLES

***Amadi, L. O., Wanabia, D. and Amadi, V.**

Department of Science Laboratory Technology, Ken Saro-Wiwa Polytechnic, P.M.B. 20, Bori, Rivers State, Nigeria

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ABSTRACT

The study investigates the synergistic effects of Guava (*Psidium guajava*) aqueous and methanolic leaf extracts in combination with potassium aluminum sulphate (Alum) respectively. The various concentrations (0.5, 1.0, 1.5 and 2.0%) of these combinations; methanolic leaf extract plus Alum (MLE+Alum) and aqueous leaf extract plus Alum (ALE+Alum) were tested respectively for antibacterial activity on some bacterial pathogens isolated from clinical samples. These were compared with an antibiotic standard, Chloramphenicol as positive control. Antibacterial activity was assessed using both disc diffusion method (DDM) and agar well diffusion method (AWDM) respectively. Of these combinations, ALE+Alum concentrations exhibited the largest mean diameter of inhibitory zone (DIZ) values of 12.0-21.0mm and 8.5-15.0mm on the test bacteria using AWDM and DDM respectively. In contrast, there was no inhibition with MLE+Alum concentrations by AWDM whereas DIZ values ranged from 8.0-13.5mm by DDM. The susceptibility of these pathogens to the combinations were much more pronounced against the Gram-negative bacteria, *Pseudomonas aeruginosa* (21.0mm) and *Escherichia coli* (20.0mm) than Gram-positive, *Staphylococcus aureus* and *Bacillus subtilis* (19.0mm) respectively, thus, indicative of broad spectrum activity particularly using the AWDM. This result is almost comparable with Chloramphenicol which exhibited the overall largest mean DIZ values. However, the activity of Chloramphenicol was marginally influenced by the methodology of antibacterial assessment which reinforces the fact that purified and tested antibiotics should be used as the drug of choice for treatments. The synergistic effects of the *P. guajava* leaf extracts plus Alum against test bacteria reveals that they can be used as a novel antibacterial agent against infections and/or diseases caused by these pathogens.

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INTRODUCTION

Guava (*Psidium guajava*) is a tropical plant which belongs to the family Myrtaceae. It is considered to have originated in tropical South America but grown in other tropical and subtropical regions of the world with long history of medicinal uses (Nwinyi *et al.,* 2008; Biswas *et al.,* 2013). Guava is a phytotherapic plant used in folk medicine with known bioactive components that help to treat and manage various diseases (Fagbohun *et al.,* 2013; Biswas *et al.,* 2013). Various parts of the plant extracts of root, bark and leaves have been used to treat a variety of diseases such as gastroenteritis, diarrhoea, dysentery, ulcers, sore throat, diabetes, hypertension, obesity, etc (Abdelrahim *et al.,* 2002; Begum *et al.,* 2004; Sunagawa *et al.,* 2004; Ismail *et al.,* 2012). Other species are used for regulation of vigor, fruit quality improvement and resistance to pest and disease (Mani *et al.,* 2011). The bioactive components consisting of flavonoids, phenols, terpenoids, tannins and glycosides in guava leaf have been reported to fight against foodborne pathogens and spoilage bacteria, regulate blood glucose levels and weight loss (Hoque *et al.,* 2007; Ismail *et al.,* 2012; Biswas *et al.,* 2013). Recently, there is widespread interest in evaluating drugs derived from plant sources and those used in prehistoric times in treating human diseases because they are economically safer, readily available and cost effective (Osuala *et al.,* 2009). These has prompted several investigators to evaluate the antimicrobial effects of various extracts of *P. guajava* leaves

****Corresponding author: Amadi, L. O.,***
Department of Science Laboratory Technology, Ken Saro-Wiwa Polytechnic, P.M.B. 20, Bori, Rivers State, Nigeria.

and roots (Gutierrez *et al.,* 2008; Dhiman *et al.,* 2011; Fagbohun *et al.,* 2013). Potassium Aluminum Sulphate (Potash alum/Alum) is used as an astringent and antisepsis in various food preservation and preparation processes such as pickling, fermentation as well as a decontaminant and flocculant for water purification (Oo *et al.,* 1993; Narayanan, 2009; Bestoon, 2012; Efiuvwevwere and Amadi, 2015). Alum has also been recommended by the U.S Food and Drug Administration (FDA) as a category 1 active ingredient in mouthwashes (Olmez *et al.,* 1998), used for the treatment of burns and ulcers in the oral cavity and has anticariogenic effect (Mourughan and Suryakanth, 2004). The antibacterial property of alum has also been documented (Ahmed, 2011; Bnyan *et al.,* 2014) as well as its use as deodorant lotion, after shaving astringent cream and gel (Alzomor *et al.,* 2014), medicinally as an adjuvant to enhance the body's response to immunogens; such vaccines include hepatitis A and B (Doherty, 2005) and used in Nigeria for the treatment of pediatric cough (Bestoon, 2012).

Sometimes the use of single drug/antimicrobial agent does not produce the desired effects, so a combination of drugs often exhibit synergism which surpasses individual performance. Synergistic effects of plant extracts in combination with antimicrobial agents/standard antibiotics against some microbial pathogens or reduction of microbial load have been reported (Dalen *et al.,* 2009; Adham, 2015; Mahmoud *et al.,* 2016). However, there is no study on the synergistic effects of a combination of *P. guajava* leaf extracts and alum. Since these agents have been reported to possess antimicrobial activity it becomes imperative to potentiate their effects by combining them. Therefore, the present study was to investigate the synergism of *P. guajava* leaf extracts and alum on some bacterial pathogens from clinical samples using disc diffusion and agar well diffusion methods.

MATERIALS AND METHODS

Collection of Guava (*P. guajava*) leaves

Foliage of *P. guajava* leaves were collected in a sterile polyethylene bag from the School of Management campus of the Polytechnic during the rainy season between the months of July and August, 2015. The leaves were taken to the Department of Science Laboratory Technology for identification and extraction.

Preparation of crude plant extract

Prior to extraction, the leaves were washed in clean tap water to remove extraneous debris and kept in the oven at 40^{o}C for 3-4days before pulverization using electric homogenizer. One (1) gram of the pulverized leaf sample was dissolved in 10mL of 99% Methanol solvent in 50mL sterile beaker (to obtain a ratio of 1:10 concentration).

The mixture was wrapped with aluminum foil and kept in the dark (to avoid evaporation and exposure to solar energy) for 3days at ambient temperature. The mixture was filtered into another 50mL beaker using standard Whatman No.1 and the filtrate kept in a water bath at 37^{o}C to ensure complete evaporation of methanol before storage in the refrigerator at 4 ^{o}C.

Test bacterial pathogens

The test bacterial pathogens; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* isolated from wound and stool samples were obtained from the stock cultures of Mainland clinic laboratory, Okrika Local Government Area, Rivers State, Nigeria.

Preparation of combinations of Guava leaf extracts (GLE) and Alum

Different combinations of GLE and Alum (Vickers Laboratories, Ltd, England.) were made to obtain concentrations of 0.5, 1.0, 1.5 and 2.0% (w/v) at a ratio of 1:1 respectively. The potency of these combinations was compared with chloramphenicol as control.

Susceptibility test for combinations of *P. guajava* leaf extracts with Alum

The antibacterial susceptibility test was performed by the disc diffusion method (DDM) and agar well diffusion method (AWDM) (Bauer *et al.,* 1966; NCCLS, 1997; CLSI, 2011). About 10µL of each of the bacterial suspensions from the overnight culture, following adjustment to 0.5 McFarland turbidity standards were spread-plated on Mueller Hinton agar (MHA (Titan Biotech Ltd. Bhiwadi-301019, Rajasthan, India.) and allowed to dry for 2 to 5 minutes (Selvamohan *et al.,* 2012). Filter paper discs, made as described by Ochei and Kolhatkar (2008) were impregnated with aqueous leaf extracts (ALE) + Alum and methanolic leaf extract (MLE) + Alum concentrations respectively.

These combinations and discs of commercially supplied Chloramphenicol (CH = 30µg, positive control) (Abtek Biologicals Ltd., Uk.) and blank filter paper discs (as negative control) were placed on the surface-dried inoculated MHA with sterile forceps. For agar well diffusion method, equal volume of the bacterial suspensions was spread-plated onto surface-dried MHA. Thereafter, four (4) wells of 6mm diameter were made on the agar plates using sterile cork borer. Equal volumes of aqueous or methanol extracts with Alum concentrations were dispensed into the wells including 30µg/mL chloramphenicol as positive control respectively. Duplicate plates were incubated at 37^{o}C for 24hours. The diameter of inhibition zones (DIZ) were measured with a transparent ruler and expressed in millimeters (mm). The mean and standard deviation values of DIZ were calculated and compared with Chloramphenicol. Interpretation of results was based on the zones of inhibition, susceptible or resistant (Smith, 2004; Cheesbrough, 2006; Forbes *et al.,* 2007).

Statistical analysis

All data were obtained from at least two replicated experiments and the mean values estimated.

RESULTS AND DISCUSSION

DIZ values of the various MLE+Alum concentrations compared with a standard antibiotic, Chloramphenicol are presented in Table 1.

Table 1. Antibacterial activity of *P. guajava* MLE+Alum

Bacterial	Diameter of inhibitory zone (DIZ (mm)				
	Concentrations of MLE + Alum (%)				CH
Pathogens	0.5	1.0	1.5	2.0	30µg
S. aureus	8.0	9.0	10.5	11.5	23.0
B. subtilis	8.5	9.5	10.0	12.0	27.0
E. coli	9.5	10.0	11.0	13.0	29.0
P. aeruginosa	8.5	9.5	12.0	13.5	27.0

Legend: MLE = Methanolic leaf extract; CH= Chloramphenicol.

The DIZ values increased with increasing concentrations of MLE+Alum, with the highest mean inhibition zone of 13.5mm against *P. aeruginosa* and least mean zone of 11.5mm against *S. aureus* at 2.0% concentration respectively. The least mean zones of inhibition against test bacteria occurred at 0.5% concentration. Similarly, the inhibition of *S. aureus, E. coli* and *P. aeruginosa* by MLE using DDM has been earlier reported (Dhiman *et al.,* 2011). Chloramphenicol showed the highest mean DIZ values against all the test bacteria (Table 1). This confirms the fact that standard antibiotics are purified compounds with active antibacterial agents whereas the relatively weak activity of *P. guajava* leaf extracts may be attributed to the fact that they are crude. Generally, the antibacterial activity was slightly higher on Gram-negative bacteria than Gram-positive contrary to earlier reports of MLE without Alum (Stefanello *et al.,* 2008; Tajkarimi *et al.,* 2010; Biswas *et al.,* 2013). This phenomenon may not be unconnected with the presence of Alum which potentiated antibacterial activity by the hydrolysis of Alum in solution to form sulphuric acid which raises the acidity of the microenvironment, thus, resulting in increased inhibition (Bulon *et al.,* 1984; Dutta *et al.,* 1996; Ahmed, 2011; Efiuvwevwere and Amadi, 2015). Similar trends in antibacterial activity were observed with increasing concentrations of ALE+Alum but with much higher inhibitory effects (Table 2).

Table 2. Antibacterial activity of *P. guajava* ALE +Alum

Bacterial	Diameter of inhibitory zones (mm)				
	Concentrations of ALE + Alum (%) CH				
Pathogens	0.5	1.0	1.5	2.0	30µg
S. aureus	8.5	9.0	11.0	13.0	23.0
B. subtilis	9.0	10.0	11.0	13.0	27.0
E. coli	9.0	11.0	12.0	14.0	29.0
P. aeruginosa	9.0	11.0	13.0	15.0	27.0

Legend: ALE = Aqueous leaf extract; CH = Chloramphenicol

Indicating ALE+Alum combinations to be more beneficial than MLE+Alum in terms of antibacterial efficacy/potency. The ability of these combinations to inhibit both the Gram positive and Gram negative bacteria demonstrates broad spectrum antibacterial activity. Consequently, the inhibitory effects of *P. guajava* ALE on the genus *Staphylococcus* corroborates earlier findings reported by several workers (Vieira *et al.,* 2001; Gnan and Demello, 1999; Fagbohun *et al.,* 2013) and such antibacterial activity was linked to the presence of guajaverin and psidiolic acid (Caceres *et al.,* 1993) whereas Alum also, had been previously reported to inhibit *S. aureus*, *E. coli*, and *P. aeruginosa* (Bestoon, 2012). With the agar well diffusion method (AWDM), MLE+Alum concentrations surprisingly displayed no antibacterial activity except the antibiotic standard (Table 3).

Table 3. Antibacterial activity of P. guajava MLE+Alum

Bacterial	Diameter of inhibitory zones (mm)				
	Concentrations of MLE + Alum (%)				CH
Pathogens	0.5	1.0	1.5	2.0	30µg
S. aureus	–	–	–	–	22.0
B. subtilis	–	–	–	–	33.0
E. coli	–	–	–	–	22.0
P. aeruginosa	–	–	–	–	26.0

Legend: MLE = Methanolic leaf extract; CH = Chloramphenicol

Table 4. Antibacterial activity of P. guajava ALE +Alum

Bacterial	Diameter of inhibitory zones (mm)				
	Concentrations of ALE + Alum (%)				CH
Pathogens	0.5	1.0	1.5	2.0	30µg
S. aureus	12.0	13.5	15.0	19.0	22.0
B. subtilis	13.0	14.0	17.0	19.0	23.0
E. coli	14.0	15.5	16.0	20.0	22.0
P. aeruginosa	15.0	17.0	19.0	21.0	24.0

Legend: ALE = Aqueous leaf extract; CH = Chloramphenicol

Perhaps, the method used (i.e., AWDM) or molecular interactions would have resulted in neutralization of the active ingredients such that there was no inhibitory zones or by factors which cannot be clearly elucidated here as there was activity using the DDM (Table 1). There were remarkable increases in DIZ with all the ALE+Alum concentrations against all test bacteria which suggests synergism in antibacterial activity, almost comparable to the commercial antibiotic standard (Table 4). Increase in DIZ with increasing concentrations of alum up to 2.0% using AWDM with some of the test bacteria had previously been reported (Gutierrez *et al.,* 2008; Bestoon, 2012; Alzomor *et al.,* 2014). Comparatively, the methodology invariably would have had some influence on the values of DIZ as shown in the respective Tables. The antibacterial activities of *P. guajava* ALE+Alum concentrations were demonstrated by increases in DIZ values using AWDM (Table 4). In contrast, the DIZ of the commercial antibiotic standard decreased (Tables 3 and 4) but increased (23-29mm) using DDM (Tables 1 and 2).

Conclusion

The present study demonstrated the antibacterial potentials of ALE+Alum and MLE+Alum concentrations respectively. The results indicate that ALE+Alum concentrations using AWDM, inhibited all the test bacteria with DIZ values almost comparable with the standard antibiotic control. Furthermore, the ALE+Alum concentrations showed more beneficial antibacterial activity than MLE+Alum concentrations.

The plant extract-Alum combinations exhibited broad spectrum antibacterial activity against test bacteria. The occurrence of largest DIZ with chloramphenicol reinforces the fact that commercially perfected and tested antibiotics should be used as the drug of choice in therapy. The results also revealed the synergistic effects of *P. guajava* leaf extracts plus alum concentrations against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* and can be used as a novel source of antibacterial agent against infections and/or diseases caused by these pathogenic microorganisms.

Conflict of Interest

There is no conflict of interest.

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