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Full Length Research Paper

Evaluation of antimicrobial synergy of scent plant (*Ocimum gratissimum*) and guava (*Psidium guajava*) extracts against some intestinal pathogens

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Antimicrobial drugs resistance by pathogenic microorganisms, prevalent in orthodox medicine in recent time, has brought about search for natural alternative phytochemical; otherwise called medicinal plants, for the treatment of diseases and infections caused by these microbes. Therefore, this study was designed to evaluate the efficacy of scent plant (*Ocimum gratissimum*) and guava (*Psidium guajava*) extracts synergistically against intestinal pathogens; *Escherichia coli* O157:H7, *Salmonella typhi*, *Pseudomonad aureginosa* and *Staphylococcus aureus*. Moreover, the sensitivity of the pathogens to different concentrations of the plants extracts was carried out using agar diffusion technique, and the zone of inhibition was measured in millimeter. Concerning the study data, One-way ANOVA was done using statistical software package SPSS version 20. The percentage inhibition synergistically mediated by *O. gratissimum* and *P. guajava* against the pathogens is arranged in ascending order as follows; 5% ethanolic extraction inhibited 114% *E. coli* O15:H7, 140% *S. typhi*, 152% *P. aureginosa*, and 100% *S. aureus*. For 5% methanol extraction, 100% *E. coli* O157:H7 was inhibited, followed by 133% *S. typhi*, 140% *P. aureginosa*, while *S. aureus* had the lowest inhibition of 127%. Moreover, the aqueous extraction of the plants extract had the following inhibition; *S. aureus* 50%, *E. coli* O157:H7 43%, *P. aureginosa* 40% while the lowest inhibition was found in *S. typhi* 33%. In conclusion, this study has been able to show that the synergy of *O. gratissimum* and *Psidium guajava* mediated excellent inhibitory capacity against the enteric pathogens assayed in this study. Therefore, these plants extracts could be adopted for the control of infections caused by these pathogens.

Key words: *Ocimum gratissimum*, *Psidium guajava*, extracts, synergy, enteric pathogens.

INTRODUCTION

Several plants and fruits extracts have been found to exhibit antimicrobial activity against different organisms such as pathogenic *Escherichia coli*, *Shigella* species (Abraham et al., 2011; Ademokoya and Adebolu, 2012; Aguiyi et al., 2000). The guava plant (*Psidium guajava*) is a phytotherapeutic plant used in herbal medicine that is believed to have active components that manage various diseases (Abraham et al., 2011; Betty et al., 2007). According to the report of Karawya et al. (1999), many parts of the plant have been used in traditional medicine to treat diseases like malaria, diarrhoea, dysentery, toothache, coughs, sore throat, inflamed gums and a number of other conditions. Moreover, this plant has also been used for the prophylaxis against life-threatening conditions such as diabetes, hypertension, and obesity (Begum et al., 2004). The research work published by Cheesbrough (2000) revealed that guava (*P. guajava*) leaves extracted with or without ethanol as the solvent effectively suppressed pathogens like *Bacillus cereus* and *staphylococcus aureus*.

The plant guava (*P. guajava*) belongs to the family Myrtaceae, which is considered to have originated in tropic South America (Goncalves et al., 2008). Guava crops are grown in tropical and subtropical area of the

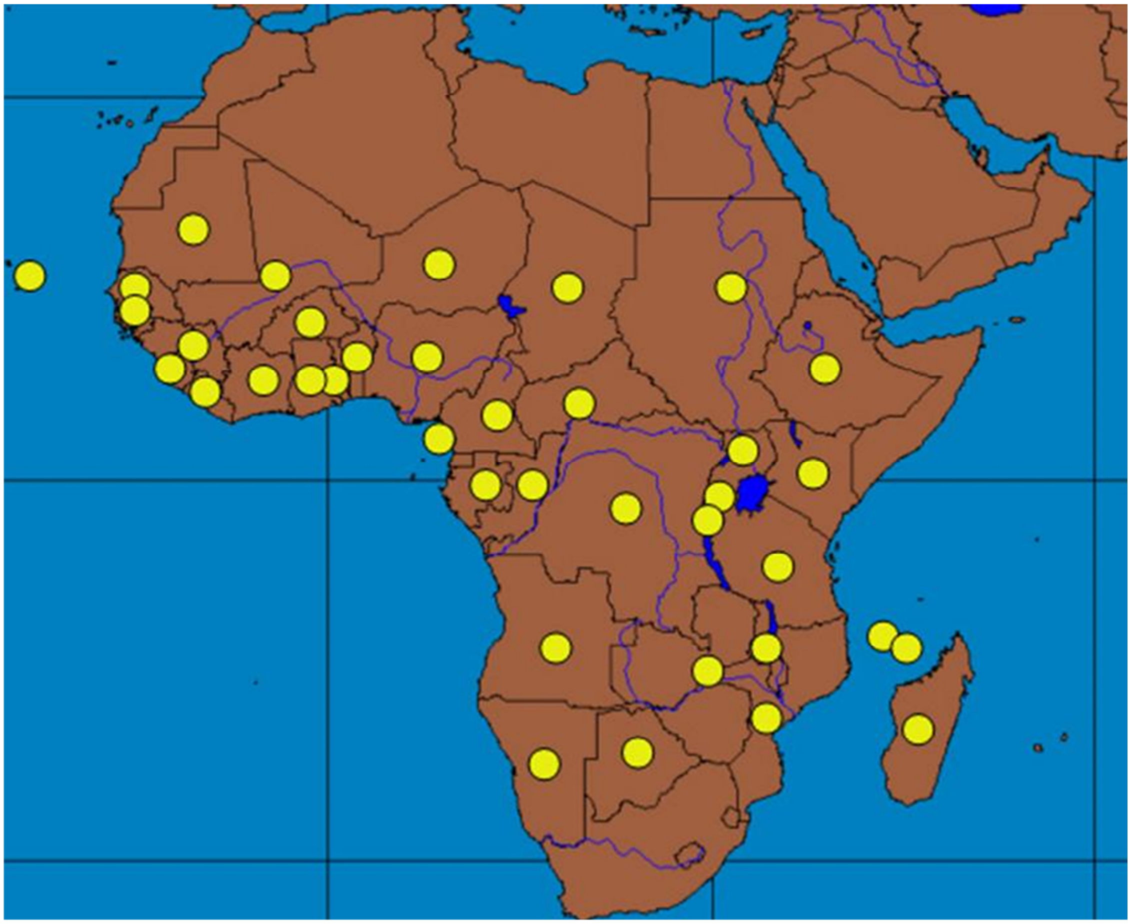
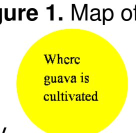


Figure 1. Map of Africa showing area where guava (*Psidium guajava*) crop is cultivated.

Key



world like Asia, Egypt, Hawaii, Florida, Africa, Nigeria, Ghana (Figure 1), Palestine, and others (Bipul et al., 2013). However, *O. gratissimum* on the other hand, is a native of Africa, Madagascar, Asia, Mexico, Panama, Brazil and Bolivia (Eugene et al., 2004). Guava is an evergreen small tree with 2 to 6 inches long and 1 to 2 inches wide leaves characterized with aromatic flavor when crushed (Nweze and Eze, 2009). This later authors recorded the bioactive component in guava leaf that are capable of inhibiting pathogens, regulate blood glucose levels, and can even aid weight lost. The leaves of guava contain an essential oil rich in cineol tannins, triterpene, flavonoides, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a number of other fixed substances (Begum et al., 2004; Betty et al., 2007). Ademokoya and Adebolu (2012) conducted a study where they screened the antimicrobial effect of essential oils and methanol, hexane, and ethyl acetate extracted from guava leaves. The extracts were screened against bacteria strains isolated from seabed shrimp and laboratory culture strains. They found that the methanol extract showed greater bacterial inhibition. No statistical significant differences were observed between the tested extract concentration and the effect. The essential oil extracts showed inhibitory activity against *S. aureus* and *Salmonella typhi*. The authors concluded that guava leaf extract and essential oil are very active against *S. aureus*, thus serving as important potential sources of new antimicrobial compounds. In the report of Aguiyi et al. (2000), mechanism of action of guava essential oil and extract in the inhibition of microorganism was discussed. In the report, the oils and extracts penetrate the lipid bi-layer of the cell membrane, rendering it more permeable, leading to the leakage of vital cell content (Nweze and Eze, 2009; Shittu et al., 2016). However, Begum et al. (2004) evaluated the antibacterial activity of guava against gram-positive and gram-negative bacteria testing ethanol. Hence, water extract of *P. guajava* leaves, stem,

Table 1. Comparison of percentage inhibition of *Ocimum gratissimum* on some enteric pathogens.

Extract	*E. coli* O157:H7	*Salmonella typhi*	*Pseudomonad aureginosa*	S. *aureus*
Ethanol	86	67	100	33
Methanol	71	50	60	27
Aqueous	43	17	20	13
Tetracycline	35	30	25	30

Table 2. Comparison of percentage inhibition of *Psidium guajava* on some intestinal pathogens.

Extract	*E. coli* O157:H7	*Salmonella typhi*	*Pseudomonad aureginosa*	*S. aureus*
Ethanol	100	83	60	83
Methanol	86	66	40	100
Aqueous	43	17	0	0

bark and root, as well as aqueous extract against *S. aureus* were found to be more active by using ethanol and water extract than with just aqueous extract.

Scent and guava plant extracts are one of the phytochemical nonnutritive components produced by plants for their own protection, but they have been found to protect humans against diseases through recent research (Oboh et al., 2009). Therefore, this study was conducted to evaluate the antimicrobial synergy of scent plant and guava against five different enteric pathogens.

METHODOLOGY

The solvents used in this study were 30% ethanol, 30% methanol and water (aqueous solvent). The solvents excluding water were purchased from laboratory chemical supplier in Adekunle Ajasin University, Akungba Akoko. The plants: *O. gratissimum* and *P. guajava* leaves were harvested in the vegetable garden of the Institution and brought to Microbiology Departmental Laboratory. The plants leaves were cleaned and sun-dried for 10 days before grinding using Excella grinder (Model: QTY: 1PC). 25 g of each plant leave powder was dispensed into 100 ml of each solvent, thoroughly mixed and kept for 3 days before the assay.

The intestinal pathogens which include *E. coli* O157:H7, *S. typhi, P. aureginosa* and *S. aureus* on the other hand were obtained from Culture Collection Center, Adekunle Ajasin University Centre Laboratory and kept in refrigerator at 4°C until used.

The antimicrobial assay of the plants leaves extracts was done using the method of Ademokoya and Adebolu (2012) and Begum et al. (2004). Overnight broth culture of the pathogens was prepared and seeded on Muller Hinton agar. Sterile cork borer was used to make well inside the agar and 3 drops of each extract was dispensed in the well. Zones of inhibition were measured in mm ruler after 24 h of incubation. Control was setup using commercially available antibiotic- tetracycline and the percentage inhibition was calculated using the formula:

C –T/C X 100,

where C = control, T = test pathogen.

RESULTS

The result of the percentage inhibition of *O. gratissimum* on some enteric pathogens can be seen in Table 1. The highest percentage inhibition of the extract against *E. coli* O157:H7 was found in ethanol extraction (86%), followed by methanol extraction (71%) while the lowest inhibition was found in aqueous extraction (43%). The highest percentage inhibition of *S. typhi* was found in ethanol extraction (67%), followed by methanol extraction (50%) while the lowest was found in aqueous extraction. The same trend of inhibition was found in *P. aureginosa* and *S. aureus*. On the other hand, *P. guajava* inhibition of the pathogens also followed similar trend whereby ethanol extraction had the highest inhibition capacity followed by methanol extraction and the lowest was found in aqueous extraction except in *S. aureus* in which methanol extraction had the highest inhibition of 100%, followed by ethanol extraction (83%) and the aqueous extraction had nothing (Table 2).

Figure 2 shows the antimicrobial synergy of the plants extracts. The ethanol extraction of both plants had the highest percentage inhibition of *P. aureginosa* (160%), followed by *S. typhi* (140%), *E. coli* O157:H7 (114%) while the lowest was found in *S. aureus* (100%). Concerning methanol extraction, the highest percentage of inhibition was found in *P. aureginosa* (140%) followed by *S. typhi* (133%), *S. aureus* (127%) while *E. coli*

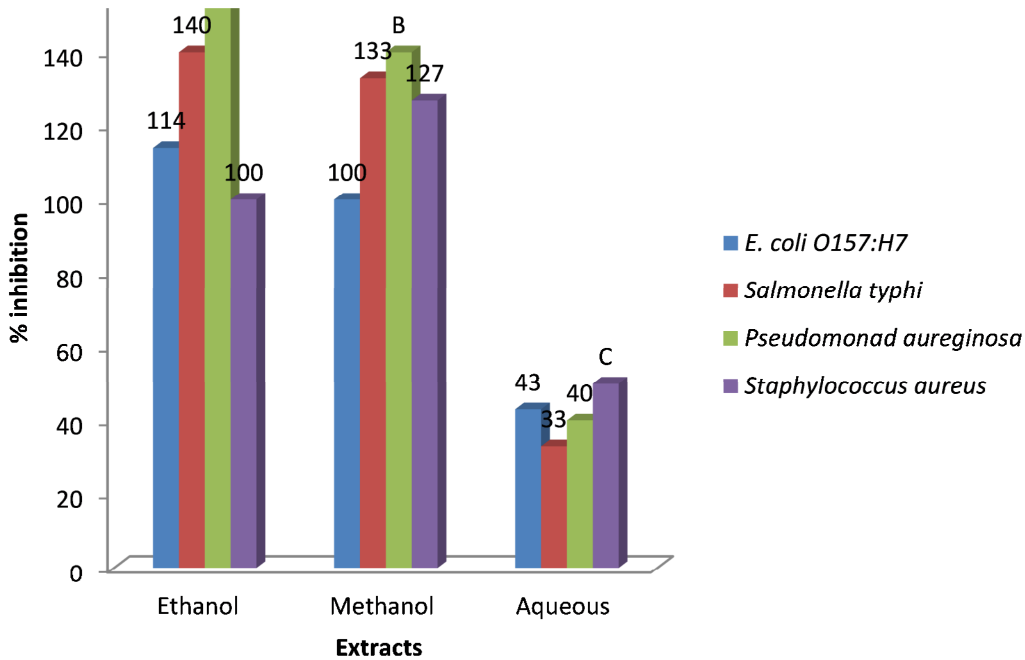


Figure 2. Percentage inhibition of some enteric pathogens mediated by synergetic action of *Ocimum gratissimum and Psidium guajava* extracts. Note: Mean with different alphabets outside end of the bars are significantly different at P<0.5.

O15:H7 had the lowest percentage inhibition (100%).

Moreover, the aqueous extraction which had the lowest antimicrobial efficacy against the pathogens had the following percentages; *S. aureus* (50%), followed by *E. coli* O157:H7 (43%), *P. aureginosa* (40%) while the lowest was found in *S. typhi* (33%).

DISCUSION

The evaluation of antimicrobial synergy of *O. gratissimum* and *P. guajava* against some enteric pathogens was carried out in this study. The percentage inhibition of *E. coli* O15:H7, *S. aureus*, *P. aureginosa*, and *S. typhi* found in ethanolic extraction in this study for both plants 160 and 140%, respectively is higher than the values obtained in USA by Bipul 8.27 and 12.3 mm reported by Betty et al. (2007). The discrepancy could be due to the test pathogens used by the second authors which were; *B. cereus* and *S. aureus*.

In this study, the percentage inhibition observed in each plant was lower than the combine mixture of the extracts. Thus the combination of the two plant extracts produced a positive effect than the individual plant extract. This increase in inhibitory effect of *O. gratissimum* and guava plant extracts combination is similar in action to some commercially sold antibiotics-orthodox medicines. For instance, the drugs used to treat tuberculosis – Isoniazid is used in combination with streptomycin antibiotics to produce synergetic inhibition of the disease pathogen *Mycobacterium tuberculosis* (Betty et al., 2007). It was also observed that there was a sharp difference in the inhibition capacity of the plant extracts based on the solvents used for the extraction. For example, ethanolic extraction of both plants produced the highest inhibition than methanol and aqueous extractions. These discrepancies could be due to the hydrophobic and hydrophobic property of the solvents (Bipul et al., 2013). Moreover, the cellular permeability of the pathogens, the efflux capacity of porin proteins of the pathogens could have played a significant role in this biochemical process (Eugene et al., 2004). The highest percentage of inhibition produced by ethanolic extraction of *O. gratissimum* and *P. guajava* on some intestinal pathogens (160%) in this study authenticates the importance of using ethanolic extract of these plants to treat infections caused by these organisms.

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