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Antimicrobial and Phytochemical Potential of *Psidium guajava* (Linneaus) Ethanolic and Aqueous Leaf Extracts

Setufe, S.B.,[1]* Ajani, E.K.,[2] Emikpe, B.O.[3], and Ogunbanwo, S.T.[4]
[1]School of Natural Resources, University of Energy and Natural Resources, Sunyani, Ghana
[2]Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria
[3]Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria

*Corresponding Author E-mail:
scyramsarah@yahoo.com

Abstract

This study investigated the antimicrobial and phytochemical potentials of ethanolic and aqueous leaf extracts of *Psidium guajava*. Extraction was done using ethanol and aqueous solvents. Phytochemical screening followed standard phytochemical procedure. Agar-well diffusion technique was used to test the antimicrobial potential against eight pathogens, namely, *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Streptococcus agalacticeae*. The quantitative analysis revealed flavonoids (0.60±0.40) %, saponins (1.30±0.10) % and steroids (0.20±0.10) % in aqueous leaf extract while ethanolic leaf extract revealed saponins (0.60±0.10) and steroids (0.50±0.10). Alkanoids were not detected in both extracts. Antimicrobial potential of ethanolic extract against eight pathogens was highest in Escherichia *coli* (19.33±3.06) mm compared with aqueous extract which was highest in *Pseudomonas aeriginosa* (11.33±1.15) mm and *Klebsiella pneumoniae* (11.33±1.15) mm. The analyses have provided baseline for use of *Psidium guajava* leaf as a phytobiotic agent in human and veterinary medicine.

Keywords:
Psidium guajava, Ethanolic leaf extract, Aqueous leaf extract, Antimicrobial, Phytoconstituents

Introduction

Medicinal plants are plants which have built-in active compounds used in the treatment of diseases in humans and animals (Okigho *et al*., 2008). Many developing countries have been involved in the use of medicinal plants as remedial agents in the maintenance of health (Adesokan *et al*., 2008). This development is necessitated due to the therapeutic properties of plants in the treatment of diseases. These include their use as anti-oxidative, anti-microbial and phytochemical constituents (Adesokan *et al*., 2008).

Due to the negative side effects of conventional medicine, alternate use of natural products in the treatment of diseases is currently being investigated by researchers (Kumari *et al*., 2011). In addition, measures are being taken in the production of safer phytomedicines and biologically active compounds, isolated from plants species in relation to efficacious therapeutic index for novel drugs development (Kumari *et al*., 2011).

Guava (*Psidium guajava* Linnaeus) is a tropical evergreen shrub, belonging to the family myrtaceae (Joseph and Priya, 2011). It is indigenous to and widely distributed in Mexico, Central America and Africa (Gupta *et al*., 2009). Guava leaves, bark and stem have been proven over years to have antiviral, antiscorbutic, antibacterial, antifungal and molluscidal activities in man (Gupta *et al*., 2009). There is however a dearth of information on its potential as phytochemical and antimicrobe in disease treatment

in animals. Therefore, this study evaluated the phytochemical and antimicrobial properties of ethanolic and aqueous leaf extracts of guava (*Psidium guajava* Linnaeus) in disease treatment of animals.

Materials and Methods

Plant collection, identification and extraction

Guava leaves were collected from a reputable botanical garden in Akure, Ondo State. They were validated in Forestry Herbarium of Forestry Research Institute of Nigeria (FRIN) with the validation number 110937 (Senthilkumar, 2013).

For ethanol extraction, 200g of guava leaves was macerated at room temperature (30°C) and soaked in 100mL 98% ethanol for 72 hours. Also 200g of the leaves macerated at room temperature (30°C) and soaked in 100mL of distilled water for 72 hours (Arvind *et al*., 2010). The resultant extracts were vigorously shaken for thorough extraction in clean sterile glass containers. Thereafter, clean muslin cloths were used in filtering the extracts to obtain the resultant aqueous and ethanol extracts which were, air-dried and stored at 40°C until used (Arvind *et al*., 2010).

Quantitative phytochemical analysis

Ethanolic and aqueous extracts of Guava leaves were subjected to further analytical tests for the quantification of phytochemical compounds. The quantities of phytochemicals which were found in the extracts were quantitatively determined by standard procedures. These were done in triplicates for proper statistical analysis (Arvind *et al*., 2010).

Antimicrobial screening of guava leaf extracts

The antimicrobial activities of the ethanolic and aqueous extracts of guava leaves were determined, using eight pathogenic organisms that were biochemically characterised in the Department of Microbiology of the University of Ibadan. They are *Aeromonas hydrophila, Klebsiella pneumonia, Bacillus subtilis, Staphylococus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Streptococcus agalacticeae*. These pathogens were obtained from the Department of Microbiology of the University of Ibadan. All the microbes were kept on nutrient slants (Fagbemi *et al*., 2009).

Afterwards these pathogenic organisms were sub-cultured into freshly prepared nutrient broth. One gram of each of the plant extracts was dissolved in 10mL of their solvents of extraction to obtain a concentration of 1000mg/mL ((Richard *et al.,* 2007). This concentration was used as the basal concentration to investigate the antimicrobial activities of the plant extracts against the tested microbes. Nutrient agar was used all through as the medium for growth for the pathogens. This was prepared according to the manufacturer's manual.

The medium was weighed and dissolved in measured distilled water in a conical flask, capped tightly and then sterilized at 121°C for 15 minutes by autoclaving. It was then allowed to cool to 40°C, and 20mL of it was poured to sterile petri dishes and allowed to solidify. Well diffusion technique was used to screen the extracts for antimicrobial activity (Richard *et al.,* 2007). The medium was seeded with the test microbes by employing sterile swab sticks in spreading the inoculums of each microbe evenly over the surface of the prepared plates. After the seeded plates were dried, standard cork borer of 5 mm in diameter was used to make holes on the surface of prepared plates (Richard *et al.,* 2007). The extracts were introduced into the holes using sterile syringes of 5mL. The plates were all incubated aerobically at 37°C for 24 hours thereafter, the zones of inhibition of the growths were observed and measurements of the zones were taken using a transparent ruler (Richard *et al*., 2007).

Statistical Analyses

Data were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 20.0). Duncan multiple range test (DMRT, 1955) was used to compare differences among individual means at (P> 0.05).

Results

The quantitative analysis of aqueous and ethanolic extracts of guava (*Psidium guajava*) leaves is presented in Table 1. It showed that flavonoids (0.60±0.40) % were present in the aqueous extract and absent in the ethanolic extract, saponins (1.30±0.10%) were significantly present in the aqueous and ethanolic extracts (0.60±0.10%) . The quantity of steroids (0.20±0.0.10%) present in aqueous extract was significantly lower (P<0.05) than that present in the ethanolic extract (0.50±0.10%) . There was no presence of alkanoids in both extracts.

Table 1: Quantitative analysis of aqueous and ethanolic extracts of guava (*Psidium guajava*) leaves

Phytochemical Constituents (%)	Aqueous Extract	Ethanolic Extract
Alkanoids	N.A.	N.A.
Flavonoids	0.60 ± 0.40^{a}	N.A.
Saponins	1.30 ± 0.10^{a}	0.60 ± 0.10^{b}
Steroids	$0.20\pm0.0.10^{b}$	0.50 ± 0.10^{a}

Means with the same letter in a row showed no significant difference (P>0.05)

Antibacterial activities of ethanolic and aqueous extracts of guava (*Psidium guajava*) leaves

The result of the antimicrobial activities showed that ethanolic leaf extract (18.00±3.46mm) had the highest zone of inhibition against *Aeromonas hydrophila,* and this was followed by streptomycin, the positive control (11.33±1.10mm) and then aqueous leaf extract. There was significant difference (P<0.05) in the anitimicrobial activities (Table 2).

This same trend was observed with *Klebsiella pneumoniae*, *Bacillus subtilis*, *Proteus vulgaris,* and *Staphylococcus aureus*. There was no significant difference (P>0.05) in the inhibition zones of EE

Table 2: Antibacterial activities (diameter of inhibition zone mm) of ethanolic and aqueous extracts of guava (*Psidium guajava*) leaves

Pathogens	Diameter of zone of inhibition (mm)			
	Ethanolic	Aqueous	-ve	+ve
Aeromonas hydrophila	18.00 ± 3.46^{a}	10.00 ± 0.00^{c}	-	11.33 ± 1.10^{b}
Klebsiella pneumonia	17.67 ± 3.22^{a}	11.33 ± 1.15^{c}	-	13.67 ± 0.58^{b}
Bacillus subtilis	18.00 ± 2.00^{a}	10.00 ± 2.00^{c}	-	11.00 ± 1.41^{b}
Staphylococcus aureus	18.67 ± 4.16^{a}	10.00 ± 2.00^{c}	-	17.33 ± 6.43^{b}
Escherichia coli	19.33 ± 3.06^{a}	8.67 ± 1.15^{b}	-	19.00 ± 1.73^{a}
Proteus vulga ris	18.00 ± 4.00^{a}	10.67 ± 1.15^{c}	-	15.67 ± 2.08^{b}
Pseudomonas aeruginosa	19.00 ± 1.00^{a}	11.33 ± 1.15^{b}	-	19.00 ± 4.36^{a}
Streptococcus agalactiae	18.67 ± 3.06^{b}	10.67 ± 1.16^{c}	-	21.00 ± 1.00^{a}

Means with the same letter in a row showed no significant difference (P>0.05)
-ve, Negative control (sterile water); +ve, Positive control (streptomycin)

(19.33±3.06mm) and the positive control (19.00 ±1.73mm) against *Escherichia coli* while a lower zone of inhibition with significant difference (P<0.05) was observed in AE (8.67±1.15mm) . This was the trend observed with the antagonistic test against *Pseudomonas aeruginosa*. The result of the antagonistic test against *Streptococcus agalacticeae* showed the positive control (21.00±1.00mm) with the highest zone of inhibition, this was followed by ethanolic leaf extract (18.67±3.06mm) and aqueous leaf extract (10.67± 1.16mm) and varied significantly (P<0.05).

Discussion

The presence of phytochemicals in the extracts of guava leaves *(Psidium guajava*) suggests its possible medicinal uses. Saponins are known to induce adverse physiological reactions in animals whereby generating cytoxic effect and causing growth inhibitions against various cells (Elumalai and Chinna, 2011). This prevents inflammations and tumorous growth in animals. Steroidal compounds found in both extracts of guava leaves (*Psidium guajava*) suggests that it is good for pharmaceutical purposes due to its relationship with sex hormones, promotion of immune functions of the skin of animals and man and in the reduction of inflammation (Iniaghe *et al*., 2009). Flavonoids which are free radical scavengers, help in the prevention of oxidative damage of cells, inhibit tumorous growth and induce anti-cancer mechanisms (Ugwu *et al*., 2013). Anthraquinones have anti-microbial properties. Thus, they help in adhesin binding by forming complex with cell wall, and in the inactivation of enzymes. Terpenoids are also antimicrobial in nature. Hence, they are engaged in membrane distruption (Mbosso *et al*., 2010).

The result of the screening of this experiment in Table 2 revealed that guava leaves could be used as a phytotherapeutic agent in replacing antibiotics in the treatment of fish bacterial diseases. This agreed with the result of Sridevi *et al. (*2010), who reported the sensitivity of bacteria isolated from Champavathi estuary on some medicinal plants in India. This agreed with the results obtained in this study as it was observed that guava (*Psidium guajava*) leaves aqueous extract inhibited the gram negative bacteria. Conversely, Mahfuzul-Haque *et al*. (2007) reported that guava sprouts produced negative result against gram negative bacteria. In addition to these results, aqueous extract of Asiatic pennywort did not inhibit *A. hydrophila* whereas, its ether extract did.

These reports suggest that the susceptibility of any bacteria to a given anti-bacterial agent may depend on the species of the organism, extraction process, or mode of action (Mahfuzul-Haque *et al*., 2007). This corresponds with this current study which reported the antibacterial activity of *P. guajava* against gram negative and gram positive bacteria.

Conclusion

From the results of these analyses, it could be deduced that guava (*Psidium guajava* Linnaeus) leaf extracts have antibacterial properties. Hence, this study has provided a baseline for the use of *Psidium guajava* leaf as a phytobiotic agent in fish medicine.

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