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Abstract: Various parts of guava plant are used in the tropic as herbs in curing dysentery, gastro intestinal disorder, typhoid fever, diarrhoea and other bacterial infections due to their antibacterial activities. Antibacterial sensitivities of ethanol, hot and cold water extracts of guava leaf, bark and fruit were tested against ten human pathogenic bacteria using cup plate agar diffusion method (plate diffusion method). The result showed that ethanol fruit extracts of guava exhibited the highest phytotoxicity against *Bacillus subtilis* (1.80cm,) this was closely followed by *Klebsiella pneumonia* and *Streptococcus faecalis* (1.70cm) while *Clostridium sporogenes* and *Staphylococcus aureus* showed resistance to both leaf and fruits extracts, except the bark extracts. *Staphylococcus aureus* was most inhibited by both cold and hot water fruit extract of guava by 1.40cm and 7.00cm respectively. Moreover, *Staphylococcus aureus* (2.10cm) was most inhibited by leaf extract of guava, followed by *Bacillus cereus* (1.90cm). However, *Bacillus cereus* (1.70cm) was most inhibited by cold water extract of guava leaf. Hot water leaf extract of guava was most inhibitive by 1.80cm against *Bacillus cereus*, *Clostridium sporogenes*, and *Bacillus subtilis*. Ethanol bark extract of guava most inhibited *Pseudomonas aeruginosa* (2.00cm). Hot water leaf extract of guava was most antibacterial on *Klebsiella pneumonia* (1.40cm) while *Escherichia coli* (1.50cm) was most retarded by cold water of leaf extract of guava. The activity of guava extracts is bactericidal on the test pathogenic microbes.

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Keyword: guava plant, human pathogenic microbes, plant extracts

Introduction

Medicinal plants are used in African communities to treat bacterial infections (Sofowora, 1993). Guava plants belong to the family Myrtaceae. *P. guajava* is a tropical and semi tropical plant, a native of tropical America. It is a low evergreen shrub of 6-25ft high, with wide spread branches and square downy twig common names to guava in various localities in Nigeria are gwaaba (Hausa), woba (Efik), ugbowoba (Igbo), guafa (Yoruba) Iwu (1993), while guava is called goyave and goieba in French and Portuguese respectively. Decoctions of leaf, root or stem of guava plant have been used in anti-diarrhoea therapy in many systems of traditional medicines in the tropical countries (Luterroft *et al.*, 1999). Ibrahim (2004) reported the presence of phenol, essential oils and flavonoids, tannins, saponins, carotenoids, lectins, vitamins, fibre and fatty acids. Most importantly, guava plant extracts have shown antimicrobial effects on pathogenic microbes responsible for enteric infections such as diarrhoea, respiratory infection, urinogenital infections, skin infections, wound, ulcer and typhoid fever (Ngemenya, *et al.*, 2006). Cowan (1999) reported that a large number of phytochemicals have been shown to have phytotoxic capacity on all types of microbes *in vitro*. Gistene *et al.*, (2000) reported that phytochemicals in guava plants have antimicrobial effects on some pathogenic organisms

which belong to both gram-ve and +ve bacteria. Therapeutic capacity of guava has been associated with its flavonoid. In Guatemala, guava is used for the treatment of gastro-intestinal disorders (Caceres *et al.*, 1993). The Indians use guava for sore throat, vomiting, stomach upsets and vertigo. Bark of guava plant is astringent, febrifuge and antiseptic while its fruit is laxative (Nadkarni *et al.*, 1991). The aim of this research is to evaluate the effects of *P. guajava* on some human pathogenic bacteria.

Methodology

Plant materials *Psidium guajava* fresh leaves, bark and fruits were collected from the vicinity of the campus of Adekunle Ajasin University, Akungba Akoko.

Preparation of extracts

Fresh leaves, bark and fruit used for this experiment were washed in water, air dried at room temperature of 25⁰C, oven dried at 60⁰C for 2 days and machine pulverised. Ethanol, hot and cold water extraction were carried out on each plant material. Twenty five gram each of leaf and bark, and 15g of the fruit were extracted with 150ml of 60% ethanol, hot and cold water for a total of 48 hours. Absolute ethanol (99%) was diluted in the ratio of 60:40 with water to get 60% ethanol while running tap water was heated till 100% and allowed to cool down to 60⁰C and cold running tap water. The measured plant

materials (25kg of leaf, bark and 15g of the fruit) were then soaked with different solvents in air-tight sterile containers.

PLANT PARTS	SOLVENTS		
	Ethanol	Hot water	Cold water
Leaf	Pgle	Pglh	Pglc
Bark	Pgbe	Pgbh	Pgbc
fruit	pgfe	pgfh	pgfc

Key: Pgfe-*P. guava* leaf, Pgbe-*P. guava* bark, pgfe- *P. guava* fruit in ethanol
Pglh-*P. guava* leaf, Pgbh-*P. guava* bark, Pglh- *P. guava* fruit in hot water
Pgfc-*P. guava* leaf, Pgbc-*P. guava* bark, Pgfc- *P. guava* fruit cold water

Test micro-organisms

Ten pathogenic microbes were used for these experiments. They were all typed organisms from stock cultures obtained from varied culture collection centres. *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538, *Escherichia coli* ATCC 25922, and *Bacillus subtilis* ATCC 6633 were all obtained from the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos State while *Bacillus cereus* NCIB 6349, *Pseudomonas aeruginosa* NCIB 950 and *Clostridium sporogenes* NCIB 533 were all obtained from the Department of Microbiology, Obafemi Awolowo University (OAU), Ile Ife, Osun State. These pathogenic micro-organisms were maintained on nutrient agar slants in the refrigerator and sub-cultured into nutrient broth and incubated at 37°C for 18-24 hours before use. Only 24 hours old cultures were used for this experiment.

Culture media

Nutrient agar was used for the preparations of the plates for the sub-culturing of the microbes. Nutrient broth prepared from nutrient agar by separating out the agar-agar with the aid of sterile filter paper before sterilisation was used for the sensitivity test. Nutrient agar was likewise used for the antibacterial sensitivity test.

Antibacterial sensitivity test

Plates of nutrient agar were prepared according to the manufacturer's specification and seeded with each of the test microbes using spread plate method 0.5ml of the freshly prepared (24 hours old) cultures was introduced unto the surface of the prepared media with the aid of pipette and aseptically spread on the surface of the plate. Holes were made on each plate (specifically 4 holes per plate) and each with a diameter of 0.5cm. These holes were made with the aid of cork borer. Three (3) out of those holes were filled with the extracts from the guava plant parts

(leaf, bark and fruit extract) while the remaining hole was filled with a control which is chloramphenicol antibiotic (250mg dissolved in 50ml of sterile water). Incubation was made at 37°C for 24 hours. Zones of inhibition were observed and measured with metre rule. The diameters were recorded against each extract on each microbe. Plates were left in the incubator for another 24 hours to reflect the kind of action the extracts have on the pathogenic microbes (bactericidal or bacteriostatic).

Results

The results reveal sensitivity and resistant of these pathogenic microbes to the plant extracts. The efficacies of extractive properties of various solvents (ethanol, hot and cold water) were established. Ethanol extract of the *P. guajava* proved most effective in inhibiting the growth of the microbes, followed by cold water and hot water extract. *Bacillus subtilis* was the most sensitive ethanol extract, followed by *Klebsiella pneumonia* while *Proteus vulgaris* and *Bacillus cereus* showed the same sensitivity to the extract. *Streptococcus faecalis* and *Escherichia coli* were the least sensitive to the extracts. *Staphylococcus aureus* was resistant to the fruit ethanol extract. All the microbes showed greater susceptibility or sensitivity to the control (chloramphenicol-250mg) than all the extracts which proved potent with the exception on *Pseudomonas aeruginosa* which was resistant (Table 1). Ethanol extract of *P. guajava* leaf was more effective, followed by hot water and lastly cold water. *S. aureus* showed the highest sensitivity to the ethanol extract of *Psidium guajava* leaf; this was followed by *Bacillus cereus* and *Pseudomonas aeruginosa* and *Streptococcus faecalis*. *Bacillus subtilis* and *Escherichia coli* showed the extent of sensitivity while *Proteus vulgaris* and *Clostridium sporogenes* showed the least sensitivity (Table 2). Ethanol extract of the *P. guajava* bark showed more activity with hot and cold water extract showing close range of activity. *Pseudomonas aeruginosa* showed the highest sensitivity to the extract with *Bacillus subtilis*, *Streptococcus faecalis*, *Proteus vulgaris* and *Bacillus subtilis* following the extent of sensitivity. *Klebsiella pneumonia* and *Clostridium sporogenes* were however resistant to the extract. Those zones of inhibition with asterisk (*) were not so clear compared with the standard (chloramphenicol- a broad spectrum antibiotic). The result of the experiment showed that there were no significant increases or decrease in the zone of inhibition after 48 hours of incubation, implying that the activity of guava plant extracts were bactericidal and not bacteriostatic (Table 3).

Table 1: Antimicrobial effects of extract of extracts of *Psidium guajava* fruit

Microorganisms	Zones of Inhibition (cm)			
	Ethanol	Hot water	Cold water	Control
<i>Escherichia coli</i>	0.70	-	-	2.80
<i>Staphylococcus aureus</i>	-	1.4	7.00	3.00
<i>Bacillus subtilis</i>	1.80	-	0.90	3.10
<i>Klebsiella pneumonia</i>	1.7	-	1.00	3.00
<i>Pseudomonas aeruginosa</i>	1.40	-	-	-
<i>Proteus vulgaris</i>	1.5	0.8	1.00	2.80
<i>Clostridium sporogenes</i>	-	-	-	2.00
<i>Bacillus cereus</i>	1.50	0.80	1.20	3.00
<i>Streptococcus faecalis</i>	1.70	1.30	1.00	3.00

Table 2: Antimicrobial effects of extracts of *Psidium guajava* leaf

Micro-organisms	Zones of Inhibition (cm)			
	Ethanol	Hot water	Cold water	Control
<i>Escherichia coli</i>	1.50	?	1.30	2.80
<i>Staphylococcus aureus</i>	2.10	1.50	1.60	3.00
<i>Bacillus subtilis</i>	1.50	1.80	0.80	3.10
<i>Klebsiella pneumonia</i>	-	0.80	1.50	3.00
<i>Pseudomonas aeruginosa</i>	*1.80	*1.40	*1.40	-
<i>Proteus vulgaris</i>	1.20	0.50	0.70	2.50
<i>Clostridium sporogenes</i>	1.30	1.80	1.50	2.00
<i>Bacillus cereus</i>	1.90	1.80	1.70	3.00
<i>Streptococcus faecalis</i>	1.70	1.30	1.00	3.00

Table 3: Antimicrobial effects of extracts of *Psidium guajava* bark

Microorganisms	Zones of Inhibition (cm)			
	Ethanol	Hot water	Cold water	Control
<i>Escherichia coli</i>	0.70	1.30	1.50	2.80
<i>Staphylococcus aureus</i>	0.50	*0.30	*0.30	3.00
<i>Bacillus subtilis</i>	1.20	1.11	1.80	3.10
<i>Klebsiella pneumonia</i>	-	1.40	1.10	2.00
<i>Pseudomonas aeruginosa</i>	2.60	1.30	1.40	-
<i>Proteus vulgaris</i>	1.50	*1.20	1.00	2.80
<i>Clostridium sporogenes</i>	?	-	-	2.00
<i>Bacillus cereus</i>	1.70	1.20	1.40	3.00
<i>Streptococcus faecalis</i>	1.50	1.20	0.80	3.20

Discussion, Conclusion And Recommendation

P. guajava is a potent antimicrobial against many pathogenic micro-organisms including gram positive and gram negative bacteria. *Pseudomonas aeruginosa* was found resistant to the control (chloramphenicol, 250gm) but susceptible to ethanol, hot and cold water extract of *P. guajava* fruit, leaf and bark. However, *C. sporogenes* which was susceptible to chloramphenicol was resistant to extract of *P. guajava* fruit and bark extract but sensitive to the leaf extract of guava. Varied extracts of guava parts exhibited high bactericidal activities against the tested organisms. This agreed with the reports of Dweck data that guava plant contains flavonoids especially quercetin and much of guava plant therapeutic

properties are attributed to the compound. Guava leaf extract alone evoked bio-active effect against *C. sporogene* but resistant to other extracts. Guava leaf extract had the highest activity compared to guava bark and fruit extracts. Ethanol proved the most potent in extraction of phytochemicals in guava plants. Hot water was also effective for extracting phytochemical principles from guava plant. This corroborated the report of Doughari (2006) that bioactive compound are heat stable and this explained the ethno-botanical application process of plants where boiling at very high temperature for extended period of time are often practiced without the concoction losing their efficacy. The results of antibacterial effect of *P. guajava* plant extracts against pathogenic microbes have really

supported the traditional uses of guava plant for the treatment of ailments. Therefore, guava plant extracts possess bioactive compounds that can be used as antimicrobial agents in curing bacterial infections, diarrheal, respiratory infections, urino-genital infections, skin infections, and typhoid fever alongside with therapeutic benefits of relieving pain. As part of the solutions outlined by centre for disease control (CDC), the development of new antimicrobials could be a possible solution to a number of infections (Fuci, 1998). Hence, guava plant provides great succour to tackle some pathogenic bacteria of health importance. However, further pharmacological evaluations, toxicological studies and possible identification and standardization of the therapeutic antibacterial agent from *P. guajava* need to be carried out.

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