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***In vitro* Anti-Microbial Activity of *Psidium guajava* Extracts**

Rasha S. Ahmed and Sanaa O. Yagoub

Department of Microbiology and Molecular Biology, Faculty of Science and Technology,
El Neelain University, P.O. Box 12702, Khartoum, Sudan

Abstract: Petroleum ether, methanol and water extracts of *Psidium guajava* leaves were tested to determine their possible antimicrobial activity against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli* and *Salmonella typhi*) and fungi (*Aspergillus niger* and *Candida albicans*). Each extract was used in concentration of 100, 50, 25 and 10%. Gram-positive and Gram-negative bacteria showed susceptibility toward all extracts, the zones of their inhibition ranged between (13-28 mm). *Aspergillus niger* showed complete resistance toward all extracts at all concentrations while *Candida albicans* was inhibited by water extract, all concentrations of petroleum ether extract and only concentration at 10% of methanol extract, the zones of inhibition ranged between (13-20 mm).

Key words: *Psidium guajava*, antimicrobial activity, gram-positive bacteria, gram-negative bacteria, fungi

INTRODUCTION

Psidium guajava L. also known as (Guava), belongs to the family Myrtaceae which is widely distributed in tropical and warm temperature regions of the world (Wilson *et al.*, 2001). *Psidium guajava* is rich in tannins, triterpens, flavonoids, essential oil, saponins, lactins, vitamins, fiber, fatty acids, alanine, oxalic acid, palmitic acid, quercetin, glutamic acid, D-galactose and histidine (Li *et al.*, 1999; Jaiarj, 1999; Arima and Danno, 2002; Begum *et al.*, 2002; Gilani, 2002; Michael *et al.*, 2002; Jordan *et al.*, 2003).

Psidium guajava is used in folk medicine of many countries including Sudan. The leaves and infusion or decoction prepared from them are used for the treatment of dysentery, vomiting, stomach upsets, vertigo, regulation of menstrual periods, bleeding gums, prevention of hangovers, intestinal worms, edema, cough and as a douche for vaginal discharge and tightening of vaginal walls after childbirth (Abdelrahim *et al.*, 2002).

A decoction of the bark and/or leaves or flower infusion is used topically for the treatment of wounds, ulcers, skin sore and to painful eye conditions such as strain, conjunctivitis and injuries (Holetz *et al.*, 2002; Lozoya *et al.*, 2002). Conde Garcia *et al.* (2003) reported that in guinea pigs, *Psidium guajava* leaf extracts have antioxidant effect beneficial to the heart and improve myocardial function.

Antimicrobial activity of *Psidium guajava* leaf extract and fruit juice was examined against infantile Rota viral enteritis and results showed that recovery rate occurred within 3 days (Wei *et al.*, 2000; Concealves *et al.*, 2005). In several studies, *Psidium guajava* bark and leaf extracts showed significant antibacterial activity against diarrhea-causing bacteria including food poisoning

Corresponding Author: Rasha S. Ahmed, Department of Microbiology and Molecular Biology,
Faculty of Science and Technology, El Neelain University, P.O. Box 12702,
Khartoum, Sudan Fax: 00249-83-776338

Staphylococcus aureus, *Shigella* spp., *Salmonella typhi*, *Salmonella paratyphi* A, B, C, cholera causative agent, *Bacillus* spp., *E. coli*, *Clostridium* spp. and *Pseudomonas* spp. as well as antifungal, anti-yeast, antiamebic and antimalarial action (Lutterodt *et al.*, 1999; Arima and Danno, 2002; Holetz *et al.*, 2002).

The present study was planned to investigate the possible inhibitory effect of *Psidium guajava* leaves extracts (petroleum ether, methanol and water) against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli* and *Salmonella typhi*) and fungi (*Aspergillus niger* and *Candida albicans*).

MATERIALS AND METHODS

Area of the Study

This study was conducted in El Neelain University Faculty of Science and Technology, Khartoum, Sudan during 2006.

Plant Material

The leaves of *Psidium guajava* were collected, identified by our Taxonomist Dr. Alawia A. El-Awad, Department of Biology, School of Life Sciences, El-Neelain University. The plant was washed in water, air-dried at room temperature and ground by mortar and pestle.

Preparation of the Crude Extracts

Hundred grams of each of the air-dried and coarsely powdered plant material were exhaustively extracted for 2 h with petroleum ether (60-80°C) in soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure using Rota-vapor (Heidolph, Heizbad, Laborota 4001, Germany, 2000). The extracted plant material was then air-dried, repacked in the soxhlet apparatus and exhaustively extracted with methanol (98.8%) for 2 h. The methanol extract was filtered and evaporated under reduced pressure using Rota-vapor.

The extracts were dissolved in dimethyl sulphoxide to make the final concentrations and kept in refrigerator till used.

Simultaneously, water extract was prepared by adding (10 mL) of boiled distilled water to 5 g of coarsely powdered leaves in a beaker on water bath with occasional stirring for 4 h. The aqueous extract was then filtered, rewashed with small volume of boiled distilled water and added to the filtrate, which was then adjusted to (5 mL) volume and used immediately.

Preparation of the Tested Organisms

A) Preparation of Bacterial Suspensions

The average number of viable *B. subtilis*, *E. coli*, *Staph. aureus* and *S. typhi* organisms per mL of the stock suspensions was determined by means of the surface viable counting technique (Miles and Misra, 1938). About (10^8 - 10^9) colony-forming units per mL was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

B) Preparation of Standard Fungal Suspensions

The fungal cultures (*Aspergillus niger*, *Candida albicans*) were maintained on Saboraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 mL of sterile normal saline and the suspension was stored in refrigerator till used.

In vitro Testing of Extracts for Antimicrobial Activity**Testing for Antibacterial Activity**

The cup-plate agar diffusion method was adopted according to Kavanagh (1972) to assess the antibacterial activity of the prepared extracts. 0.6 mL of standardized bacterial stock suspensions (10^8 - 10^9) colony-forming units per mL was thoroughly mixed with 60 mL of sterile nutrient agar. Twenty milliliter of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 10 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1 mL of each extracts using microtiter-pipette and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37°C for 18 h. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated.

Testing for Anti-Fungal Activity

The same method as for bacteria was adopted. Instead of nutrient agar, yeast and mould extract agar was used. The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for *Aspergillus niger*.

RESULTS

All *Psidium guajava* extracts showed antibacterial effect on both Gram-positive and Gram-negative bacteria. The methanol extract was more effective than petroleum ether or water extract. As shown in Table 1, the concentration of the plant leaf extracts showed un-rhythmic pattern. The highest inhibition zone (21 mm) was observed against *Staph. aureus* at 100% methanol extract, other concentrations gave inhibition zones that ranged between 19-20 mm. The petroleum ether extract showed inhibition zones against *Staphylococcus aureus* that ranged between 14-17 mm while the water extract showed inhibition zone at 16 mm. On the other hand, the highest inhibition zone (25 mm) was observed against *Bacillus subtilis* at concentration of 50% of the methanol extract while the other methanol extract concentrations gave inhibition zones that ranged between 19-23 mm. The petroleum ether extract showed inhibition zones, which ranged between 15-16 mm at different concentrations while the water extract showed inhibition zone at 14 mm.

S. typhi was the most susceptible organism to methanol extracts used in 100-10% concentrations with inhibition zones that ranged between 25-28 mm. *E. coli* was more sensitive than *S. typhi* to petroleum ether extract used at 25% concentration. *S. typhi* showed similar inhibition zone compared

Table 1: Antimicrobial activity of *Psidium guajava* leaf extracts

Microorganisms	Mean inhibition zones (mm)								
	Methanol extract (%)				Petroleum ether extract (%)				Water extract
	100	50	25	10	100	50	25	10	
<i>Staph. aureus</i>	21	19	20	20	16	17	15	14	16
<i>B. subtilis</i>	19	25	23	20	15	16	15	15	14
<i>E. coli</i>	23	25	20	17	16	17	25	10	15
<i>S. typhi</i>	25	26	28	25	19	17	15	25	20
<i>C. albicans</i>	-	-	-	17	16	20	19	13	18
<i>Asper. niger</i>	-	-	-	-	-	-	-	-	-

-: No inhibitory activity observed

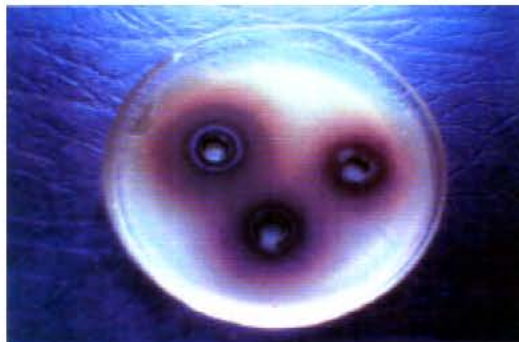


Fig. 1a: Antimicrobial activity of methanol extract of *Psidium guajava* leaves against *Salmonella typhi*



Fig. 1b: Antimicrobial activity of methanol extract of *Psidium guajava* leaves against *Staphylococcus aureus*

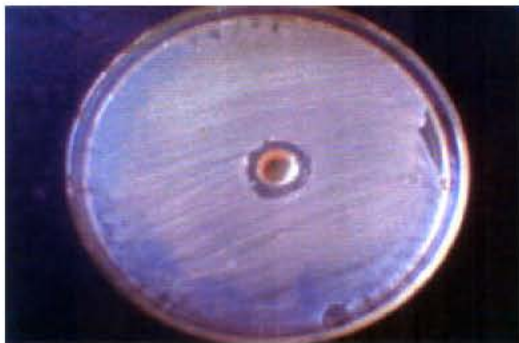


Fig. 1c: Antimicrobial activity of *Psidium guajava* leaf water extract against *Candida albicans*

with Gram-positive bacteria (Fig. 1a and b). The largest inhibition zone (28 mm) was shown at 25% methanol extract and the other concentrations of the same extract showed inhibition zones that ranged between 25-26 mm. The petroleum ether extract showed inhibition zones, which ranged between 15-25 mm, while water extract showed inhibition zone as 20 mm. The *E. coli* showed largest inhibition zone (25 mm) with both 50% methanol extract and 25% petroleum ether extracts. The other concentrations of methanol extract gave inhibition zones that ranged between 17-20 mm, the petroleum ether extracts showed inhibition zones that ranged between 10-17 mm while water extract showed inhibition zone of 15 mm.

Aspergillus niger showed clear resistance to all concentrations of the plant leaf extracts used. *Candida albicans* showed zone of inhibition at 18 mm by water extract (Fig. 1c), 17 mm at 10% methanol extract while at other concentrations, 25, 50 and 100%, resistance was developed. Petroleum ether extracts gave the highest zone of inhibition at concentration of 50% at 20 mm followed by 19, 16 and 13 mm at concentrations of 25, 100 and 10%, respectively.

DISCUSSION

All concentrations of *Psidium guajava* extracts showed inhibitory effect on *Staph. aureus*, *B. subtilis*, *E. coli* and *S. typhi*. The effect was perceived through the presence of zones of inhibition (15-28 mm) by methanol extract, (14-20 mm) by petroleum ether and (14-20 mm) by aqueous extract. The antibacterial activity of the *Psidium guajava* leaf extracts might be due to the presence of flavonoids, polyphenols and tannins which are well known as antimicrobial substance as reported by Jaiarj (1999), Viera *et al.* (2001), Lozoya *et al.* (2002) and Wei (2002). These findings support the use of the plant in traditional medicine as antidiarrheic.

In this study, Gram-negative bacteria were more inhibited by the plant extracts compared with gram positive bacteria, these might be due to the different in the cell wall constituents. *S. typhi* showed the highest sensitivity towards all plant extracts with different dilutions. It is interesting to note that the plant leaf extracts can be used against *S. typhi*, the causative agent of typhoid. The results also showed that growth of *Staph. aureus*, a common cause of food poisoning and pus-causing wounds, was markedly inhibited by the plant leaf extracts that could be used as an alternative in the treatment of wound infection caused by this bacterium as well as in the treatment of multiple-resistant *S. aureus* (MRSA). Present results support the findings of Jimenez (2001), Viera *et al.* (2001), Abdelrahim (2002), Lozoya *et al.* (2002) and Conde Garcia (2003).

Aspergillus niger showed clear resistance to all concentrations of the plant leaf extracts used, these might support that *Psidium guajava* extracts affect prokaryotes but not Eukaryotes. *Candida albicans* showed good sensitivity towards both water and petroleum ether extracts but it was surprising that with methanol extract, *Candida albicans* was inhibited by only the lowest used concentration (10%) and showed clear resistance with higher methanol extract concentrations.

CONCLUSION

We conclude that the *Psidium guajava* leaf extracts have a significant antimicrobial activity against Gram-positive and Gram-negative bacteria as well as *Candida albicans*. *Aspergillus niger* showed clear resistance towards the *Psidium guajava* leaf extracts. The demonstration of antimicrobial activity of *Psidium guajava* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

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