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ORIGINAL ARTICLE

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF PSIDIUM GUAJAVA LINN ('ZEYTUNA') USED IN ETHIOPIAN FOLK MEDICINE

Hirut Lemma, MSc, Daniel Melaku, Mulugeta Guta, Kelbesa Urga, MSc, Midist Yirsaw, Negero Gemeda

ABSTRACT

BACKGRAOUND: *Medicinal plants are natural resources, yielding valuable herbal products, which are often used in the treatment of various ailments. The bark of Psidium guajava Linn is used for treatment of intestinal diseases such as diarrhea, dysentery, stomach ache, cramps and abdominal distention as well as skin diseases. The present investigation was made to evaluate the antibacterial and antifungal activity of leaves of Psidium guajava*

METHODS: *Eighty percent ethanolic leaf extract of Psidium guajava was screened for antimicrobial activity against different strains of bacteria and fungi. The Microorganisms that were included in the test were Staphylococcus aureus ATCC 6538, Klebsiella pneumoniae ATCC 13883, Escherichia coli ATCC 25922, Pseudomonas aeroginosa ATCC 27853, Trichophlyton mentagraphytes ATCC 18748, Aspergillus flavus ATCC 13697, Aspergillus niger ATCC 10535, and clinical isolates of Staphylococcus aureus, Klebsiella pneumoniae, Esherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae, Streptococcus pyogenes, Shigella dysentrieae, Salmonella typhi, Candida albicans, Cryptococcus neoformans, Aspergillus flavus, Aspergillus niger, Tricophyton violaceum and Microsporum canis*

The tests were carried out using agar dilution method at different concentration levels of crude extract (0.25, 0.5, 0.75, 1.0, 1.5 mg/ml for antibacterial and 1.0, 1.5, 2.0 2.5, 3.0, 3.5,4.0mg/ml for antifungal) and the Minimum Inhibitory Concentration of the crude extract of P. guajava. Ampicillin and Ketoconazol were employed as a positive control.

RESULTS: *The minimum inhibitory concentration of Ethanol leaf extract of Psidium guajuva ranged from 0.5 to 1.5mg/ml.The lowest values observed against standard strain of S. aureus, K. pneumoniae , and medically important clinical isolates of S. aureus and S. Pyogenes. Whereas, low activity was seen against clinical isolates of S. typhi and P. aeruginosa. Its inhibitory activity against yeasts ranged from 1.5 to 2.0 mg/ml. But the inhibitory activity against other fungi was between 3.0 and 3.5mg/ml. Candida albicans and Cryptococcus neoformans were more susceptible to the ethanol extract compared to other fungal strains.*

CONCLUSTION: *The results showed that Psidium guajava has antibacterial and antifungal effect on common bacterial and fungal species. Relatively high concentrations were required to inhibit all fungi than bacteria.*

KEY WORDS: *Antibacterial activity, Antifungal activity, Psidium guagava*

INTRODUCTION

Medicinal plants have been used as sources of medicine in virtually all cultures (1). Traditional medicine has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (2). According to WHO, herbal medicines serve the health needs of about 80% of the world's population especially for millions of people in the vast rural areas of developing countries (3).

In Ethiopia, traditional medicine represents an integral component of the cultural beliefs and attitudes (4). In one community it is found that more than 95% of traditional medical preparations are of plant origin (5). Antimicrobial and wound healing plants are among some of the major medicinal plants that are commonly available in markets (6).

Higher plants with activity against human pathogenic fungi and bacteria are of interest because present antibiotic therapeutics is often toxic, induce problematic drug-drug interactions and become non-effective when resistance develops (7). Given these problems, the development of natural antifungal and antibacterial agents is an attractive objective.

Previous studies showed that water, methanol and chloroform extract of *Psidium guajava* dry leaves and, bark were found to possess antimicrobial activities (8, 9, 10).The study also indicated that *Psidium guajava* contains tannin, triterpenes, saponin sterols, flovonoids

antimicrobial activity of *Psidium guajava* well documented from few countries, there has been no report on the antimicrobial activity of the plant from Ethiopia. In this study, P. *guajuva* Linn. was investigated for its antimicrobial activities on commonly known pathogenic bacterial and fungal strains.

MATERIALS AND METHODS

Psidium guajava L. leaves were collected in June 2004 from town of Miesso, Oromiya Region, Ethiopia. The voucher specimens (Voucher no. AD 37) authenticated and have been deposited at Herbarium of the Department of Drug Research, Ethiopian Health and Nutrition Research Institute

The plant material was dried at ambient temperature (25-30°C), protected from light and grounded to fine powder using a centrifugal mill (Retsh Muhle, Germany). Eighty percent ethanol (Sigma Chemical Co. St. Loulis, USA) (3 x 500ml) extract was prepared by macerating the dried plant powder (100gm) for 3 days. The macerated product was then filtered, evaporated in a Rotavapour (Buchi, Switzerland) and the remaining water is removed by freeze dryer (LABCONCO). The dry extract (7.5 gm) was kept in airtight bottle. Yield of the ethanol extract was 7.5% on dry weight.

The following bacterial stains were used in the bioassays: *Staphylococcus aureus* ATCC (American Type Culture Collection) 6538, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Pseudomonas aeroginosa* ATCC 27853, *Trichophlyton mentagraphytes* ATCC 18748, *Aspergillus flavus* ATCC 13697, *Aspergillus niger* ATCC 10535, all American Type Culture Collections were obtained from the Department of Drug Research, Ethiopian Health and Nutrition Research Institute. One from each clinical isolates of *Staphylococcus aureus, Klebsiella pneumoniae, Esherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae, Streptococcus pyogenes, Shigella dysentrieae, Salmonella typhi, Candida albicans, Cryptococcus neoformans, Aspergillus flavus, Aspergillus niger, Tricophyton violaceum and Microsporum canis* were obtained from the Clinical Bacteriology Laboratory, Infectious and Other Diseases Research Department, Ethiopian Health .and Nutrition Research Institute. The clinical isolates were collected from patients' urine, stool, blood, different body discharge, skin lesion, skin scrap and throat swab depending on the infection site of each microorganism. They were identified by using different methods (Microscopy, Morphology, biochemical and serological tests) in the same department where referral diagnostic service is given.

The inoculum for the test was prepared following the methods of Leven et al. and Matsen (13, 14). In brief the inoculum for bacterial tests was prepared by suspending colonies from overnight growth in nutrient broth (Difco Laboratories, USA). Test inocula of the yeast were sub-cultured in liquid Sabouraud Dextrose broth (OXID Ltd. UK) medium for 48h at 25°C; moulds were prepared by harvesting mature sporulating cultures with the same broth media. The turbidity of each cell suspension of all test microorganisms was measured to match that of 0.5 McFareland turbidity standards (10^8CFU/ml). The turbidity standard was monitored using a spectrophotometer at 625nm to give optical density in the range of 0.08-0.1. By using calibrated loop, 0.001–0.002ml of bacterial suspension (corresponding to $1x10^4$) was delivered to the agar surface. For inoculation with dermatophytes, very small portions (2mm) of three weeks old mycelia cultures were used for the tests.

The antibacterial activity of the extract was determined by the agar dilution streak method (15). The plant extract was dissolved in 80% ethanol and diluted with water to give 20% ethanol for 5.0, 7.5, 10.0 and 15.0mg/ml concentration for antibacterial assays and 15.0,20.0,30.0,35.0,40.0mg/ml stoke concentration for antifungal assays. Two ml from each concentration of the extract was added to 18ml of sterile cooled (45-50°C) agar media (Muller Hinton Agar, BDH Chemicals Ltd. UK for antibacterial and Saburaud dextrose agar from OXID Ltd. UK for antifungal assay) in culture tubes and mixed with a vortex mixer (Whirl mixer, Fisher, UK). Subsequently, poured into sterile Petri dishes and allowed to solidify.This gave the final plant extract concentration of 0.25,0,5,0.75,1.0,1.5mg/ml in the media for antibacterial and 1.5, 2.0, 3.0, 3.5, 4.0mg/ml for antifungal test. Ampicillin (IPCA Lab. Bombay, India) was used as positive control for antibacterial assay and its concentration was prepared with sterile distilled water. Whereas, Ketoconazole, (USP Batch No KT-4061) was used for antifungal assay and its concentration was prepared with ethanol. Preparation of the standard drugs in the agar media was similar with preparation of the plant extract assay. Then by using a calibrated loop the adjusted inoculum suspension of the bacteria, fungi and the small portion of the dermatophytes were delivered to inoculate the dried agar surface of all the prepared plates. To check for purity and viability of each test organisms, plates without plant extract or standard drugs were included as negative control. Finally, the plates inoculated with bacterial were incubated at 37°C for 24h while the plates inoculated with fungi were incubated at 25°C for 3-7 days. All the test organisms were incubated in aerobic environment. The minimum inhibitory concentration (MIC) for each test was determined visually. The lowest concentration of sample at which the subcultures from the test dilution yielded no viable organisms was recorded as the Minimum Inhibitory Concentration. The test was carried out by triplicate.

RESULTS

The antibacterial activity of the plant extract exhibited strong inhibitory activity on the growth of both Gram positive and Gram negatives bacteria. The MIC ranged from 0.5 to 1.5mg/ml with lowest values observed against standard strain of *S. aureus*, *K. pneumoniae* , and medically important clinical isolates of *S. aureus* and *S. Pyogenes* whereas low activity against clinical isolates of *S. typhi* and *P. aeruginosa* (Table 1).

The plant extract showed highest activity against yeasts and the result is 1.5 and 2.0 mg/ml. The standard strain *A. flavus* was inhibited at the concentration of 3.0mg/ml whereas standard organisms of *T. mentagraphytes*, *A. niger* and the clinical isolates of *T. violacium* and *M. canis* were inhibited at the concentration of 3.5mg/ml. The clinical isolates of moulds were inhibited by ethanolic *P. guajuva* leaf extract at the concentration of 4 mg/ml which is the highest concentration tested in the study. The standard antifungal drug Ketoconazole tested revealed that *C. neoformans,* and *C. albicanus*, were sensitive at the concentration of 2µg/ml and 4µg/ml, respectively. The standard organisms of *T mentagraphytes*, and both the standard and clinical isolates of *A. flavus* were inhibited at the concentration of 32µg/ml.Whereas both the standard and clinical isolates of *A. niger* were inhibited at the concentration 64µg/ml.The clinical isolates of *M. canis* and *T. violaceum* were inhibited at 128 µg/ml concentrations(table-2).

Table 1: Anti-bacterial activity of *p. guajuva* and standard drugs against the reference strains and clinical isolates

Plant extract and standard drugs	Conc. Mg/ml	Standard strains				Clinical isolates							
		S.aureus	*K. Pneum.*	*E.coli*	*P.Aureg*	*S.aureus*	*K.pneum.*	*E. coli*	*P.aureug.*	S.*neum.*	S. *Pyogen.*	S. dysent.	S. typhi
Psidium guajuva													-
	0.25	-	-	-	-	-	-	-	-	-	-	-	
	0.5	+	+	-	-	+	-	-	-	-	+	-	-
	0.75	+	+	-	+	+	-	-	-	+	+	-	-
	1.0	+	+	+	+	+	+	+	-	+	+	+	-
	1.5	+	+	+	+	+	+	+	+	+	+	+	+
Ampicillin													
	0.001	-	-	-	-	-	-	-	-	-	-	-	-
	0.002	-	+	-	+	-	-	-	-	-	-	-	-
	0.004	+	+	+	+	+	-	+	+	+	-	+	+
	0.008	+	+	+	+	+	+	+	+	+	+	+	+

+ showed activities
- No activities

Table 2: Antifungal activity of *Psidium guajuva* and standard drugs against the standard strains and clinical isolates

Plant extract and standard drug	Conc. mg/ml	Standard strains	Clinical isolates							
		T. Menta.	*A. flavus*	*A. niger*	*C. albicans*	*C. neoformance*	*T.violacium Viola*	*A. flavus*	*A. niger*	*Micro. canis*
P.guajuva	1.0	-	-	-	-	-	-	-	-	-
	1.5	-	-	-	+	-	-	-	-	-
	2.0	-	-	-	+	+	-	-	-	-
	2.5	-	-	-	+	+	-	-	-	-
	3.0	-	+	-	+	+	-	-	-	-
	3.5	+	+	+	+	+	+	-	-	+
	4.0	+	+	+	+	+	+	+	+	+
Ketoconazol	0.002	-	-	-	-	+	-	-	-	-
	0.004	-	-	-	+	+	-	-	-	-
	0.008	-	-	-	+	+	-	-	-	-
	0.016	-	-	-	+	+	-	-	-	-
	0.032	+	+	-	+	+	-	+	-	-
	0.064	+	+	+	+	+	-	+	+	-
	0.128	+	+	+	+	+	+	+	+	+
	0.256	+	+	+	+	+	+	+	+	+

+ showed activities
- No activities

DISCUSSION

Different parts of *P. guajava* Linn is used for treatment of different ailments (16,17).

Our results compared favorably with others findings showing an inhibition of *S. aureus* by different extracts (18,19). Different medicinal plant extracts were also found to inhibit *S. aureus, E.colia* and *Aspergillus niger* (20,21). In a similar study carried using extracts of *Lippia adoensis,* growth of *S. aureus and P. aeroginosa* at a concentration of 1.25mg/ml whereas *E. coli* was inhibited at concentration of 10mg/ml. On the other hand the growth of these organisms was inhibited by extracts of *Olinia rochetiana* at concentration of 5mg/ml and 10mg/ml, respectively (22). Ethanol leaf extract of *P. guajava* is thus more active than extracts of *Olinia rochetiana* and *Lippia adoensis* against Gram positive and Gram negative bacteria.

Comparing ethanol *P. giajava* leaf extract with known antibiotic used in the treatment of some diseases, the finding was similar with Ampicillin at a concentration less than 1.0mg/ml against test organisms except for clinical isolates of *P. aeroginosa* and *Salmonella typhi.* The complete inhibition of all microorganisms tested with the exception of clinical isolates of *Pseudomonas aeroginosa* and *Salmonella typhi* by ethanolic *P. guajava* leaf extract, at a concentration in the range of 0.5 to 1. 0mg/ml is very important since these microorganisms are causative agents of diseases.

The antibacterial activity of ethanolic *P. guajava* leaf extract may be attributed to the presence tannin, triterpenes, saponin sterols, flovonoids and an essential oil rich in cineol compounds. The contributions to antibacterial activity of essential oils, flvonoids and terpenes have been previous reported elsewhere (23, 24). Similar to other studies our result showed more susceptible of Gram positive bacteria such as *S. aureus* than Gram negative bacteria such as *S. typhi, K. Peneumoniae*, and *E. col* (25). This difference may be ascribed to the morphological difference between these microorganisms. Gram negative bacteria has an outer phospholipidic membrane carrying the structural lipopolysaccharide compounds which makes the cell wall impermeable to lipohilic solutes, while purines not an effective permeability barrier (26, 27)

Most tested bacteria (83.3%) were totally inhibited at concentration of $\leq$1.0 mg/ml and the rest were inhibited at the concentration of 1.5mg/ml, which is the highest concentration included in study for the anti bacterial test.

Regarding the antifungal activity, the ethanol leaf extract of *P.guajava* also significantly inhibited all fungi included in the study; however, relatively high concentrations were required to inhibit all fungi than bacteria. This supported the observations made by other investigators that pathogenic fungi are less susceptible to plant extracts than pathogenic bacteria (28). Majority (77.7%) of the moulds and the dermatophytes were inhibited at concentrations $\leq$ 3.5mg/ml. The remaining inhibited at concentration of 4mg/ml. Antifungal activity of *Allaca neisinni* and *Albizia gumifera* against *C. albicans* indicated the MIC of the extracts to be 3.125 mg/ml and 6.25, respectively (29). The ethanolic *P. guajava* leaf extract thus could be an important source of new antimicrobial compounds.

In conclusion, the present investigation showed the scientific evidence for antifungal and antibacterial activities of *P. guajava* leaf extract against pathogenic bacteria, yeast, dermatophytes and moulds. The utilization of this plant by communities in Ethiopian traditional medicine for the treatment of infections diseases might therefore be justified.

As *P. guajava* leaves showed interesting antimicrobial effects, further study should be conducted including the identification of bioactive principle and toxicological effects.

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ORIGNAL ARTICL

CLIENTS' SATISFACTION ON VOLUNTARY COUNSELING AND TESTING FOR HIV IN JIMMA TOWN, SOUTHWEST ETHIOPIA

Misra Abdulahi, BSc.,PH, Abebe G/mariam, BSc,PH,MPH, Sofonias Getachew, MD.,MPH

ABSTRACT

BACKGROUND: *Client satisfaction of voluntary counseling and testing for Human Immuno deficiency virus is a useful measure that provides a direct indication of quality of care. However, studies on satisfaction of clients on Human Immuno deficiency virus Voluntary Counseling and Testing services are scarce both locally and nationally. The objective of this study was to assess clients' satisfaction on Voluntary Counseling and Testing services rendered at governmental and non-governmental clinics sites in Jimma Town*

METHOD: *A cross sectional study was conducted from February13-March 11, 2006 on voluntary counseling and testing services rendered at four facilities in Jimma Town. A sample of clients aged fifteen years and above who visited the Voluntary counseling and testing for Human Immuno deficiency virus centers during the study period were included in the study.Data on socio demographic factors, reason for testing, clients' satisfaction on the overall services, information given to clients, preference of age and gender of the counselor and their suggestions for improvement of Voluntary Counseling and Testing services were collected. The data was analyzed using SPSS version 12.0.1.*

RESULTS: *data were collected from 365 clients making a response rate of 95.0%. Two hundred eighty six (78.4%) of the respondents reported that they were satisfied with the overall services. The main reasons mentioned for satisfaction were: comfort of the waiting area 323 (88.5%); sufficiency of the time spent with the counselor for discussion 323 (88.5%); comfort with the way the counselor treated them 237 (65.0%); length of the time to see the counselor 153 (41.9%); discussed issues or information given 317 (86.8%); maintenance of privacy 339 (92.9%); convenience of location of the clinic site 279 (76.4%) and reasonable distance from home 316 (86.5%). Three hundred twenty eight(89.9%)] and 347(95.1%) of the studied subjects preferred to receive care from counselors of same sex and age,respectively It was observed that study subjects who have monthly income of Birr 101 – 300; 301 – 500 and more than 500 and above are 1.7, 1.4, and 4.1 times more likely to be satisfied with the Voluntary Counseling and Testing service provided [OR(95%Cl: 1.68(0.34,16.28); 1.38(0.24,14.43); and 4.05(0.85,38.25)] respectively, but was not statistically significant (p>0.05).*

CONCLUSION: *Most of the respondents were satisfied with the services in terms of comfort with the waiting area, sufficiency of the time spent with the counselor for discussion and convenience of the location of the clinic site. Only about less than half of them were satisfied with the length of the time to see the counselor. They also preferred to receive care from counselors of same sex and age. Therefore, Waiting time to see the counselor should be minimized by increasing number of counselors. Counselors of both gender/sex and different age group should be availed and give service in each VCT center in order to make sure that the needs of the clients are fulfilled.*

KEY WORDS: *Voluntary counseling and testing, satisfaction, Client, Jimma.*

INTRODUCTION

HIV/AIDS is the worst pandemic in human history, never before did a single disease have such global crisis and significance in terms of its threatening impact on social, economical, political, security and life expectancy of individuals and the community (1, 2).

According to 2006 UNAIDS report, an estimated 38.6 million people worldwide were living with HIV in 2005. An estimated 4.1 million became newly infected and 2.8 million lost their lives to AIDS. Despite progress made in many countries, the pandemic continue to outstrip global efforts to contain it (2). The Sub-Saharan Africa (SSA) is the highly affected region by HIV/AIDS epidemic with an estimated 2.7 million newly infected people (2, 3).

Until recently, many HIV infected people in Ethiopia do not know their status, as there was poor access to Voluntary counseling and testing (VCT) services (4, 5, 6). Findings of a situational assessment on VCT practices in Ethiopia conducted in 2000 revealed that the demand for HIV testing was growing but service provision by government facilities was limited due to shortage of physical facilities, test kits and trained personnel (7, 8). HIV counseling and testing began in the late 1980s with services expanding throughout the 1990s. In the early 1990s, several national level training programs were conducted for nurses and social workers from all regional hospitals and those in Addis Ababa. In addition to setting up counseling services in health institution, the Ministry of Health collaborated with other