INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 10, p. 188-192, 2014

SHORT COMMUNICATION OPEN ACCESS

In vitro antimicrobial activity of guava leaves extract against important bacterial and fungal strain

Muhammad Mushtaq[4], Basreen Akhtar[2], Muhammad Daud[1*], Saadullah[1], Samrana[2], Fakhar Mohsin[2], Ghazi Rehman[3], Sajid Iqbal[3], Zaffar Iqbal[1], Malik Zeb Khan[5], Umar Saddique[4]

[1]*Department of Microbiology, Hazara University, Manshera, Khyber Pakhtoonkhwa, Pakistan.*

[2]*Department of Botany, Kohat University of Science and Technology, Kohat, Khyber Pakhtoonkhwa, Pakistan*

[3]*Department of Microbiology, Kohat University of Science and Technology Kohat, Khyber Pakhtoonkhwa, Pakistan.*

[4]*Faculty of Animal Husbandry and Veterinary Sciences, Agriculture University Khyber Pakhtoonkhwa, Pakistan*

[5]*Institute of Kidney Diseases Department of Pathology Hayatabad, Peshawar, Khyber Pakhtoonkhwa, Pakistan*

Key words: Antibacterial, antifungal, *Psidium guajava* extracts.

http://dx.doi.org/10.12692/ijb/4.10.188-192 Article published on May 23, 2014

Abstract

The leaves of Guava is used in the treatment of the Acute diarrheal diseases, Bronchitis, asthma attacks, cough, pulmonary diseases could be also treated with guava teas and could also be useful as anti-inflammatory and haemostatic agent. Aim of the Research work was to find out the antibacterial and antifungal activity of guava leaves extract. This work was carried out in the Laboratory of Microbiology Department, Hazara University Manshera, Khyber Pakhtoonkhwa, Pakistan, during the month of January 2013.The methanol, acetone and N, N-dimethyl formamide (DMF) fractions of leaves of *Psidium guajava L.* were evaluated for antibacterial and antifungal activity. Piperacillin and gentamicin were used as standards for antibacterial assay, while nystatin and flucanazole were used as standards for antifungal assay. Twenty three (23) important microorganism was used were used for the study which were unidentified isolates as well as identified strains. The antibacterial activity was more pronounced against fungal strains. Moderate activity was shown against the gram-negative bacterial strains studied.

* **Corresponding Author:** Muhammad Daud ✉ Khattaks2010@gmail.com

Introduction

Guava is a small tree it is present mostly in tropical region, it grows up to 35 feet. It belongs to Myrtaceae family, 133 genra and 3800 species .The leaves of P.guajava have long history of medicinal uses that are still employed today. (Nwinyi *et al.*, 2008).

The leaves of Guava contains tannins, Pentacyclic, uvaol, pentacyclic triterpenoid guajanoic acid, oleanolic acid, maslinic acid, ursolic acid, volatile oils, triterpenoids, and flavonoids (Meckes *et al.*, 1996.)

The Presence of flavanoids in the leaves of guava has medicinal and antibacterial activity. A decoction of leaves is used tropically for wounds, ulcers and skin sores. In "Ducth-Pharmacopoea " the leaves of guava is used for the treatment of Diarrhea.In the present work an assessment of the antibacterial potential of leaves of Guava has been carried out. (M Heinrich *et al.*, 1998).

The leaves of Guava are used in the treatment of wounds, diarrhea, toothache, ulcer, stomach ache and in diabetes. (M Heinrich *et al.*, 1998). The decoctions of leaves are used in the leucorrhoea like condition (P Conway., 2001). In the Latin America, and South East Asia the decoction of the leaves are used as gargles or the sore throats, laryngitis,swelling of mouth, external ulcer on the skin and in vaginal irriation.(JA Ojewole 2005., XL Yang 2007). Due to anti-inflammatory property of leaves it is used in various lung problems. In addition to this, leaves are used in various bacterial infection, diarrhoea, blood cleansing (XL Yang *et al* 2007) . Leaf extract of the Psidium guajava has been reported for the strong antimicrobial properties (Oliver-Bever 1986).
The plant of guava is frost sensitive and it grow mostly in tropical and subtropical regions.The temperature required for its growth is 23 to 28°C. It is grown in different types of soil ranging from heavy clays to sands with pH values varying from acidic to alkaline (Qureshi and Barrett., 1998). The guava tree best grows in the month of june- September when the annual rainfall is below 100cm.

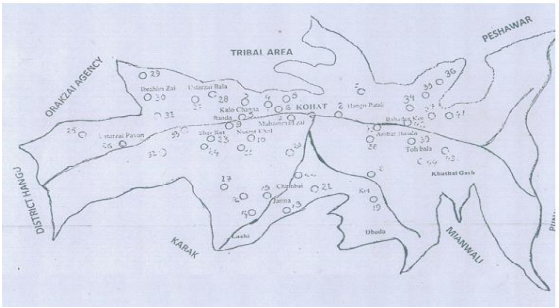
Guava is grown best in those areas of Punjab where climate and soil is suitable for its production. In Larkana, Dadu and in Shikarpur Pear shaped guava with smaller seed core is grown.In Khyber Pakhtoonkhwa (Kohat, Bannu, Haripur, D.I.khan and Malakand is famous for good quality of guava production.In KhyberPakhtoonkhwa during 2012-2013 the total area under cultivation occupied by guava's was 1557 hectars,which produce 18750 tones of guava fruit.Of which Kohat alone produce 33%. (Agricultural Statistics of Pakistan 2004 -2005).

Guava fruit is produce twice a year, that's why guava fruit is available in the market throughout the year.In valley of Kohat different kinds of guava are grown, it includes (White Allahabadi, Red Allahabadi, and Local/Desi). Among these kinds White Allahabadi is commercially accepted guava fruit due to its good quality, colour, shape (rounded in shape and white in colour) and large shelf life. While Red Allahabadi is also good quality, rounded shape and red colour but its shelf life is not up to the mark. Local/Desi variety, which has got two different shapes i.e., rounded rough surface and oval. It is also commercially not accepted due to its low shell life.The aim of this research study was to evaluate the antimicrobial activity of Guava leaves extract against important Bacteria and Fungal strains.

Materials and methods

Leaves Collection/ Sample Collection

The leaves of Guava plant was collected from the local farm land of Kohat KhyberPakhtoonkhwa, Including areas are (Hangu Patak, Jarma, Dodha, Toghbala, Tanda Daim and many local areas of District Kohat) and brought it to the laboratory of Microbiology, Hazara University Manshera for further processing.



Figu. 1. Showing the Major areas of Kohat.

Washing, Drying and Grinding of the leaves

In Laboratory leaves of guava are washed with distill water or tap water to remove the soil or dust particles from it; later on it is washed with disinfectant (Ethanol) for further cleaning of leaves. The leaves are cut in to small pieces with the help of scissors and shades dry it for 5 days. After Drying grind the leaves with help of pistol and mortar or with the help of electric grinder. The powder form of the leaves is stored in a closed sterile container for further use. (Daud *et al.,* 2014)**.**

Preparation of samples

Methanol, acetone and DMF extracts were dissolved in DMSO at a concentration of 25 mg/ml and 12.5 mg/ml respectively and used as working stocks. Sterile discs (Hi-media Labs) were impregnated with 20 µl of the stock solution. Gentamicin and piperacillin for bacteria; nystatin and fluconazole (Himedia Labs) for fungus were used as standards for comparative studies.

Antimicrobial study

Antimicrobial activity was performed by disc diffusion method. (Daud *et al* 2014) The bacterial strains were grown in nutrient broth while fungal strains were grown in MGYP (Malt glucose yeast peptone) broth. Mueller Hinton agar was the media used to study the antibacterial susceptibility while Sabroaud agar was used to study the antifungal susceptibility test. The cultures were grown for 24 hours, and the turbidity of the culture was maintained according to the 0.5 MacFarland standards. The inoculum's size was 1 X 10^8 cells.

Statistical analysis

All the treatment of experiment were performed in triplicates. Statistical analysis of the data was conducted by using graph pad prism. The data was arranged and mean value was compared with Standard deviation.

Results

In the present study *guava* leaf extracts extracted in methanol (PME), acetone (PAE) and N, N-dimethylformamide (DMF) (PDE) were investigated at two different concentrations for their antimicrobial potentiality against seven bacterial strains and 16 fungal strains. All the three extracts showed similar activity profiles against gram-negative bacterial strains studied. They were active against 76.36% of the total gram-negative bacteria studied which included 73.68% *Pseudomonas spps*., 93.75% *E. coli*, 83.33% *Klebsiella spps* and 66.66% of *Proteus spps*. All of the extracts were inactive against one of the three *Citrobacter spps* and *Alcaligenes fecalis*, while they were active against *Salmonella typhimurium*. The three extracts showed varying results against the fungal strains. PME-500 was active against 37.5%, PAE-500 was active against 56.25% and PDE-500 was active against 31.25% of the total fungal strains studied. All the extracts were inactive against the three *Aspergillus spps* studied. Details of the result are shown in Table 1 and Table 2.

Table 1. Antimicrobial activity of guava leaves extract against seven important bacterial strains (inhibition zone in mm).

S.No	Strains	Control	Extracts						Antibiotics			
		DMSO	PME-500	PAE-500	PDE-500	PME-250	PAE-250	PDE-250	G	Pc	Fu	Ns
1	Pseudomonas spp	-	10±1.73	14.6±0.3	19±1.15	14.6±0.33	18.6±0.3	13±3.4	-	-	NT	NT
2	E. coli spp	-	14.5 ±0.28	16 ±0.5	16.5±0.5	14±0.58	12.5±0.2	13.5±0.2	17.83±0.16	14.5±0.5	NT	NT
3	Klebsiella spp	-	16±0.58	14.3±1.6	14.3±1.2	14±0.58	12.6±0.8	12.3±1.2	22±0.58	-	NT	NT
4	Proteus spp	-	16±1.001	17±1.001	16±2.002	15±0.58	14.6±0.3	15±0.58	-	14±0.58	NT	NT
5	Salmonella	-	11.5±0.28	11.3±0.33	12.3±0.3	12±0.58	13±0.58	12±0.58	18.5±0.28	-	NT	NT
6	Citrobacter spp	-	-	-	-	-	-	-	12.33±0.33	-	NT	NT
7	*Alcaligenes fecalis*	-	-	-	-	-	-	-	18.33±0.66	-	NT	NT

Table 2. Antimicrobial activity of guava leaves extract against 16 important Fungal strains (inhibition zone in mm).

S.No	Strains	Control	Extracts						Antibiotics			
		DMSO	PME-500	PAE-500	PDE-500	PME-250	PAE-250	PDE-250	G	Pc	Fu	Ns
1	*Candida albicans [1]*	-	-	-	-	-	-	-	0.3±11.3	-	NT	NT
2	*Candida albicans [2]*	-	8 ±0.58	9±0.58	9 ±1.15	-	-	-	18 ±0.58	-	NT	NT
3	*Candida spps. [3]*	-	8 ±0.58	8±0.58	-	-	-	-	14 ±0.58	-	NT	NT
4	*Candida spps. [4]*	-	8.5±0.8	9±1.15	-	-	9 ±1.15	-	14 ±0.58	-	NT	NT
5	*Candida spps. [5]*	-	-	7.5 ±0.2	-	-	-	-	10 ±0.58	-	NT	NT
6	*Candida albicans ATCC 2091*	-	-	-	-	-	-	-	13±0.5	17.6±0.3	NT	NT
7	*Candida albicans ATCC 18804*	-	10 ±1.15	8.3±0.8	8.5±0.2	8±0.5	-	-	0.3±14.3	-	NT	NT
8	*Candida glabrata NCIM 3448*	-	-	-	-	-	-	-	22 ±0.5	39.6±0.8	NT	NT
9	*Candida tropicalis ATCC 4563*	-	8.5 ±0.29	8 ±0.58	9±0.5	8.5 ±0.29	8.5 ±0.29	8 ±0.58	-	8.3±0.3	NT	NT
10	*Candida apicola NCIM 3367*	-	18.3±0.3	18.6±0.8	-	-	-	-	-	21.3±0.8	NT	NT
11	*Cryptococcus neoformans ATCC 34664*	-	-	9 ±1.15	7.5±0.29	-	-	-	17±0.5	21.3±0.3	NT	NT
12	*Cryptococcus luteolus ATCC 32044*	-	-	-	-	-	-	-	17.6±0.8	23.6±0.8	NT	NT
13	*Trichosporan beigelii NCIM 3404*	-	-	8.5±0.88	7.5±0.29	-	-	-	-	-	NT	NT
14	*Aspergillus flavus NCIM 538*	-	-	-	-	-	-	-	-	-	NT	NT
15	*Aspergillus candidus NCIM 883*	-	-	-	-	-	-	-	-	-	NT	NT
16	*Aspergillus niger ATCC 6275*	-	-	-	-	-	-	-	-	-	NT	NT

Conclusions

All the three extracts of *P. guajava* showed dose dependent activity. Acetone extract was highly active against fungal strains while all of the extracts were equally active against gram-negative strains. From the results, it is concluded that acetone extract of *Psidium guajava* is highly active against 74.72% of the total 23 microbial strains studied. The acetone extract of *P.guajava* should further be studied for its phytochemical constituents in order to elucidate the active principle within the extract which can turn out to be a novel antimicrobial agent of the future.

Acknowledgment

We are thankful to the Department of Microbiology Hazara University, Manshera, Pakistan for providing the research facility. We are thankful to Dr. Zenat Fatima Khattak for arranging and reviewing the data.

References:

Agricultural Statistic of Pakistan. 2004-2005. Governoment. of Pakistan, Ministry of Food, Agriculture and Livestock (Economic Wing) Islamabad.

Ojewole JA. 2005. Hypoglycemic and hypotensive effects of Psidium guajava L, (Myrtaceae) leaf aqueous extracts. Methods Find in Experimental Clinical Pharmacology **27,** 689-695.

Heinrich M, Ankli A, Frei B, Weimann C, Sticher O. 1998. Medicinal plants in Mexico. healers consensus and cultural importance Social Science and Medicine **47,** 1859-1871.

Meckes M, Calzada F, Tortoriello J, Gonzalez JL, Martinez M. 1996. Terpenoids isolated from Psidium guajava hexane extract with depressant activity on central nervous system. Phytotherapy Research **10,** 600–603.

Daud M, Hidayatullah, Saadullah, Haris K, Faheema Umer F, Abdul S, Noor Z, Mukhtiar A, Mujadad UR, Tauseef A. 2014. Antibacterial Activity of Leaf Extract of Euphorbia cf. serrata against Known Human Pathogenic Bacteria American-Eurasian Journal of Agriculture and Environmental Science **14(1),** 07-10- 2014.

Daud Sohail M, Saadullah Hasnain.N, Hidayatullah Muhammad M, Basreen A , Zenat FK. 2014.Comparative Invitro Study of Euphorbia cf Serrata Stem Extract against Different Pathogenic Bacteria, International Journal of Biosciences.**4(5),** 182-186 p, http://dx.doi.org/10.12692/ijb/4.5.182-186

Nwinyi OC, Chinedu NS, Ajani OO. .2008.Evaluation of antibacterial activity of Psidium guajava and Gongronema Latifolium, Journal of Medicinal. Plants Research. **2(8),** 189-192.

Oliver-Bever Bep. 1986. Medicinal Plants in tropical West Africa. Cambridge University Press, Cambridge.

P Conway, 2001. Tree Medicine. A Comprehensive Guide to the Healing Power of Over 170 Trees. Judy Piatkus (Publishers) Ltd **2002,** 2173-2177.

Qureshi RH, Barrett-lennard EG. 1998. Saline Agriculture for Irrigated Land in Pakistan-A handbook. ACIAR, Australia, 90-91 p.

Yang XL, Hsieh KL, Liu JK, Guajadial. 2007. An unusual monoterpenoid from guava leaves. Organic letter. **24,** 5135-5138.