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A STUDY OF ANTIBACTERIAL ACTIVITY OF *PSIDIUM GUAJAVA* LINN. FRUIT EXTRACTS AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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ABSTRACT

Plants have been a valuable source of natural products for maintaining human health. so, today, the world is gradually turning to herbal formulations, which are known to be effective against a large repertoire of diseases and ailments. More importantly, they are not known to cause any notable derogatory effects and are readily available at affordable prices. The present study was designed to evaluate the antibacterial activity of crude fruit extracts with different solvents (methanol, ethanol and aqueous) of *Psidium guajava* Linn. was evaluated against two gram-positive (*Micrococcus luteus* and *Bacillius subtilis*) as well as one gram-negative (*Escherichia coli*) bacterial strains. For this, agar well diffusion assay (AWDA) method was applied to determine the antibacterial activity of the extracts. The crude fruit extracts with different solvents showed remarkable antibacterial activity against the tested both types of bacterial strains. Among the tested bacterial strains, *M. luteus* was found more sensitive to the fruit extract as compared to the *B. subtilis* and *E. coli.* The methanolic extract showed higher antibacterial potential as compared to other two solvent (ethanol and aqueous) extracts as well as commercially available standard antibiotic streptomycin.

1. INTRODUCTION

Medicinal plants have important contributions in the health care system of local communities as the main source of medicine for the majority of the rural population[1]. Out of the total 422,000 flowering plants reported from the world, more than 50,000 are used for medicinal purposes[2]. About 60% of the world population and 80% of the population of developing countries rely on traditional medicine. Use of herbal medicines in Asia represents a long history of human interactions with the environment. India has a wealth of medicinal plants, most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generations. In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants[3].

Plants used in traditional medicine to contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance[4]. Medicines that are used today are definitely not the same as those that were used in ancient times or even in the recent past. Several modifications, improvement, sophistication and newer discoveries contribute continuously to the type, quality, presentation and concept of medicinal preparation. The therapeutic use of development of human knowledge, scientists endeavored to isolate different chemical constituents of plants, put them to biological and pharmacological tests and thus have been used to prepare modern medicines[3]. Plants with possible antimicrobial activity have been identified by a number of researchers in different parts of the world[5-9]. Much work has been done on ethnomedicinal plants in India[10-12]. It has been suggested that aqueous and ethanolic extracts of plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents[8, 13].

Recently, the natural products have been evaluated as sources of antimicrobial agents with efficacy against a variety of microorganisms. Plants are rich in a wide variety of secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes etc., which have been found *in vitro* to have antimicrobial properties[14-17]. The selection of crude plant extracts of screening programs has the potential of being more successful in the initial steps than the screening of pure compounds isolated from natural products[9].

Psidium guajava Linn. (Myrtaceae) is an evergreen shrub native to tropical America that has naturalized in south-east Asia. It is a medium sized tree with evergreen, opposite, aromatic,

short-petiolated leaves. It is a phytotherapic plant used in folk medicine that has been used for the management of various disease conditions and is believed to act. Various parts of the plant has been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions[18,19]. Thus its uses in traditional medicine are well established against enteric human bacteria. This plant has also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity[19-21]. Leaves and bark of *P. guajava* plant has a long history of medicinal uses that are still employed today[22]. Hence, in the present study, an attempt has been made to the study of antibacterial activity of *P. guajava* fruit extract against two gram-positive and one gram-negative bacterial strains.

2. MATERIALS AND METHODS

2.1 Collection of plant material

A fresh fruit of *Psidium guajava* Linn. (Figure 1) was collected from the campus of Pt. N. R. S. Govt. College, Rohtak, Haryana, India, and brought to the laboratory in polythene bags. Then the fruit was rinsed twice with distilled water and made into small pieces using sharp, sterile knife, air dried on a clean sheet for one week at room temperature. After drying, the pieces were ground into powder using sterile mortar and pestle.

Figure 1: *Psidium guajava* Linn., Studied plant

2.2 Preparation of plant extracts

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant. In the present study the extraction was done at room temperature

by the simple extraction method. For this, 20 g dried fruit powder was immersed in 20 ml of different solvent (methanol, ethanol and aqueous) contained in 100 ml sterile conical flasks and covered with cotton wool separately. It was placed aside with intermittent shaking for 2 days. They were first filtered with double layered muslin cloth and then through Whatman No. 1 filter paper, and the march was discarded. The filtrate was subjected to evaporation by treating at 40°C in an oven to obtain a dried extract. The dried extract was dissolved in 10% Dimethyl sulphoxide (DMSO) solvent in a ratio of 200 mg/ml to determine the antibacterial activity by agar well diffusion assay (AWDA) method.

2.3 Source of Bacterial Strains

Three bacterial strains, including two gram-positive as well as one gram-negative were obtained from: *Micococcus luteus* (MTCC106) (Dr. S. K. Tiwari, Department of Genetics), *Bacillus subtilis* (Dr. Bijender Singh, Department of Microbiology), and *Escherichia coli* strain DH5α (Dr. Krishan Kant, Department of Microbiology) M. D. University, Rohtak, Haryana, India. These bacterial cultures were grown in nutrient broth medium, pH 7.0. Stock cultures were maintained on a nutrient agar slant pH 7.0 at 4°C until needed. The media components were purchased from Hi-media, Mumbai, India.

2.4 Antibacterial Activity by agar well diffusion assay (AWDA) method

Antibacterial susceptibility testing of the crude fruit extracts with different solvents (methanolic, ethanolic and aqueous) of *Psidium guajava* Linn. against gram-positive as well as gram-negative bacterial strains were evaluated by agar well diffusion assay method[23]. For this, a well (6 mm diameter) was made with the help of a borer in cooled nutrient agar plate, overlaid with soft agar (5 ml), seeded with a target strain (~10^6 cfu/ml). Aliquots (100µl) of the test compound were introduced into the well and the plates were incubated overnight at 37º C. The diameters of the inhibition zones were measured in millimeters (mm). For each bacterial strain, the dissolving solvent 10% DMSO was used as negative control and streptomycin (50µg/ml) was used as positive control

2.5 STATISTICAL ANALYSIS

The experiment was carried out in three independent sets, each consisting of 3 replicates. Values shown here represent mean ± standard error of the mean (SEM).

3. RESULTS AND DISCUSSION

Medicinal plants continue to be an important therapeutic aid in alleviating the ailments of humankind. The search for eternal health and longevity and for remedies to relieve pain and

discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal products, and minerals, etc. and the development of a variety of therapeutic agents. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs are mainly due to the current widespread belief that "green medicine" is safer and more dependable than the costly synthetic drugs, many of which have adverse side effects[24].

There are many literatures reporting the ethno-medicinal values of *P. guajava* Linn., but there is little scientific proof for further using this plant commercially or in a more effective form[25-28]. Therefore, in order to evaluate the antibacterial activity of *P. guajava* Linn. fruit extracts against gram-positive and gram-negative bacterial strains was assessed by the AWDA method. In the present study, the crude fruit extracts exhibited excellent antibacterial activity against the tested two gram-positive and one gram-negative bacterial strains as shown in Figure 2. The resulted data shows, *M. luteus* was the most sensitive among the tested bacterial strains with the zone of inhibition (mm) 30 (methanol), 25 (ethanol), 21 (aqueous). However the gram-negative bacteria *E. coli* exhibited less sensitivity as compared to the other two gram-positive bacterial strains (*M. Luteus and B. subtilis*) with the zone of inhibition (mm) 25 (methanol), 20 (ethanol), 18 (aqueous). *B. subtilis* bacterial strain shows intermediate sensitivity among the tested bacterial strains with the inhibition zone (mm) 26 (methanol), 22 (ethanol), 19 (aqueous). The present study revealed that the remarkable antibacterial activity was shown against both gram-positive as well as gram-negative bacterial strains. However, methanolic extract showed the higher zone of inhibition as compared to the other two solvent (ethanol and aqueous) extracts as well as commercially available standard antibiotic streptomycin with the inhibition zone (mm) *M. luteus* (27), *B. subtilis* (23) and *E. coli* (24). In contrast, no inhibition zones were observed against 10% DMSO (result not shown in the Figure 2). The results indicate that the different solvent extracts showed antibacterial activity against tested bacterial strains with to the various degrees.

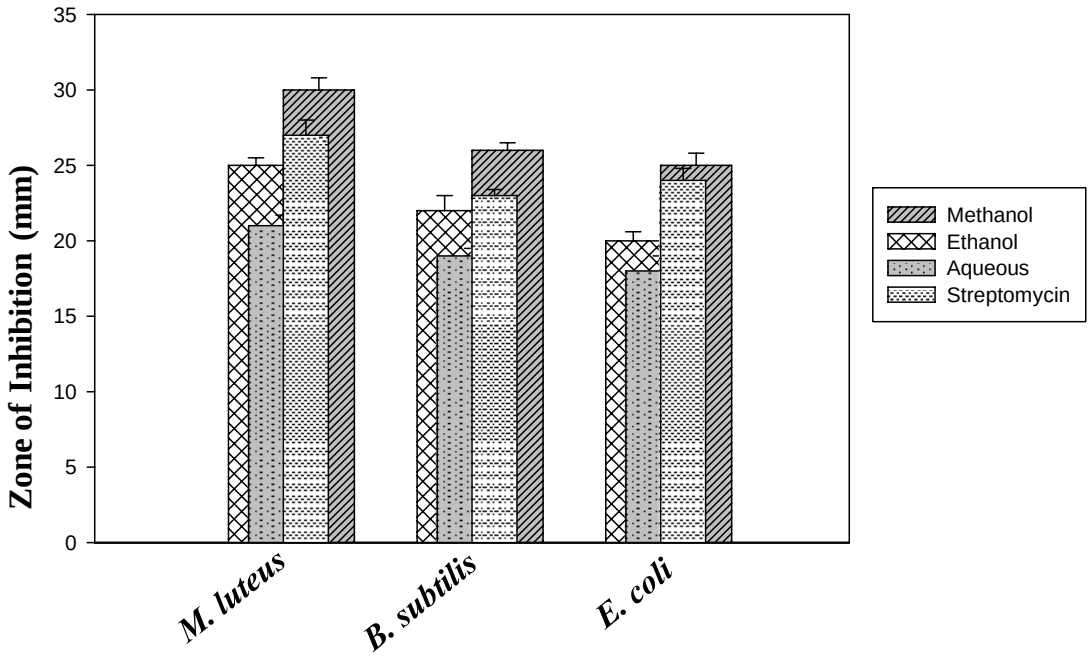

Figure 2: Antibacterial activity of fruit extracts of *Psidium guajava* Linn.

It is known that the successful prediction of extracting compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional practitioners make use of water as a primer solvent, but from the results obtained from the present study it was apparent that the methanolic extract of *P. guajava* Linn. was the most effective as the widest inhibitory zone was observed compared to the ethanolic as well as aqueous extract used. This may be due to better solubility of the active compounds in organic solvents. Though similar response has been reported that methanol plant extracts inhibited the growth of testing bacterial strains more than the other solvent extracts[9, 23, 29-32]. The present study also supports the above observations. In the present investigation, antibacterial activity of the *P. guajava* crude fruit extracts against tested gram-positive as well as gram-negative bacterial strains are an indication that there is a possibility of discovering an alternative antibiotic substance in these plants for the development of newer antibacterial agents and carry out further pharmacological evaluation.

4. CONCLUSION

The crude fruit extracts of *P. guajava* Linn. showed remarkable antibacterial activity against tested two gram-positive (*M. luteus* and *B. subtilis*) as well as one gram-negative (*E. coli*) bacterial strains. The results indicate that the methanolic extract of the fruit exhibited better antibacterial activity as compared to the other two solvent (ethanol and aqueous) extracts as

well as commercially available standard antibiotic streptomycin. It is inferred from the present investigation the remarkable antibacterial activity of the studied plant material extracts against both gram-positive as well as gram-negative bacterial strains may be indicative of the presence of broad-spectrum antibacterial compounds, suggests that there is a scientific basis for its utilization as antibacterial agents in designing and developing new drugs. Further studies are needed to identify the antibacterial compounds and to evaluate the efficiency against human pathogenic bacterial strains.

5. COMPETING INTERESTS

The author declares that they have no competing interests.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. Ahmad, M., Khan,M. A., Rashid, U., Zafar, M., Arshad, M.. Sultana, S., "Quality assurance of herbal drug valerian by chemotaxonomic markers". *African Journal of Biotechnology*, 2009; 8 (6): 1148–1154.
2. Hamilton, C., "Medicinal plants, conservation and livelihoods". *Biodiversity and Conservation*, 2004; 13 (8): 1477– 1517.
3. Mishra, G., Jawla, S., Srivastava, V., *Melia azedarach*: A Review. *International Journal of Medicinal Chemistry & Analysis*, 2013; 3(2): 53-56.
4. Diallo, D., Hveem, B., Mahmoud, M. A., Betge, G., Paulsen, B.S., Maiga, A., An ethnobotanical survey of herbal drugs of Gourma district, Mali. *Pharmaceutical Biology*, 1999; 37: 80-91.
5. Samy, R.P., Ignacimuthu, S., Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. *Journal of Ethnopharmacology*, 2000; 69: 63-71.
6. Erdogul, O.T., Antibacterial activities of some plant extracts used in folk medicine. *Pharmaceutical Biology*, 2002; 40: 269-273.
7. Farombi, E.O.,African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *African Journal of Biotechnology*, 2003; 2: 662-671.
8. Kiruthika, K.A., Jaisheeba, A.A., Sornaraj, R., Evaluation of antibacterial activity of some selected Angiosperm flower extract. *International Journal of ChemTech Research*, 2011; 3: 1945-1951.
9. Kumar, M., Gitika, Sharma, A., *In vitro* antibacterial activity of flower extracts of *Quisqualis indica* Linn. against gram-positive and gram-negative bacteria. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 2014; 3 (3): 781-785.
10. Nair, R., Kalariya, T., Chanda, S. Antimicrobial activity of some selected Indian medicinal flora. *Turkish Journal of Biology*, 2005; 29: 41-47.

11. Sharma ,M., Pandey, G. Ethnomedicinal plants for prevention and treatment of tumours. *International Journal of Green Pharmacy*, 2009; 3: 2 - 5.
12. Rout, S.D., Panda, T., Mishra, N., Ethno-medicinal plants used to cure different diseases by Tribals of Mayurbhanj district of north Orissa. *Ethno-Medicine*, 2009; 3: 27-32.
13. Reddy, P.S., Jamil. K., Madhusudhan, P., Antibacterial activity of isolates from *Piper longnum* and *Taxus baccata*. *Pharmaceutical Biology*, 2001; 39: 236-238.
14. Edeoga, H.O., Okwu, D.E., Mbaebie, B.O., Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 2005; 4: 685-688.
15. Sher, A., Antimicrobial activity of natural products from medicinal plants. *Gomal Journal of Medical Sciences*, 2009; 7: 72-78.
16. Lalitha, P., Arathi, K.A., Shubashini, K., Sripathi., Hemalatha. S., Jayanthi, P., Antimicrobial activity and phytochemical screening of an ornamental foliage plant, *Pothos aurea* (Linden ex Andre). *International Journal of Chemistry*, 2010; 1: 63-71.
17. Hussain, H., Badawy, A., Elshazly, A., Elsayed, A., Krohn, K., Riaz, M., Schulz, B., Chemical constituents and antimicrobial activity of *Salix subserrata*. *Records of Natural Products*, 2011; 5: 133-137.
18. Jaiarj, P., Khoohaswan, P., Saraya, M.L.S., Ruangsomboom, O., Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. *Journal of Ethnopharmacology*, 1999; 67: 203.
19. Abdelrahim, S.I., Almagboul, A.Z., Omer, M.E.A., Elegami, A., Antimicrobial activity of *Psidium guajava* L. *Fitoterapia*, 2002; 73: 713–715.
20. Begum, S., Hassan, S.I., Ali, S.N., Siddiqui, B.S., Chemical constituents from the leaves of *Psidium guajava*. *Natural Product Research*, 2004; 18: 135–140.
21. Sunagawa, M., Shimada, S., Zhang, Z., Oonishi, A., Nakamura, M., Kosugi, T., Plasma insulin concentration was increased by long-term ingestion of guava juice in spontaneous non-insulin dependent diabetes mellitus (NIDDM) rats. *Journal of Health Science*, 2004; 50: 674–678.
22. Kumar, A., Importance for Life '*Psidium guava*'. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2012; 3: 137- 143.
23. Kumar, M., Gitika, Evaluation of antibacterial activity of *Nelumbo nucifera* (Gaertn.) flower extracts against gram-positive bacteria. *World Journal of Pharmaceutical Research*, 2014; 4: 1155-1161.
24. Nair, R., Chanda, S.V., Antibacterial Activities of Some Medicinal Plants of the Western Region of India. *Turkish Journal of Biology*, 2007; 31: 231-236.
25. Phatthalung, P.N., Chusri, S., Thai ethnomedicinal plants as resistant modifying agents for combating *Acinetobacter baumannii* infections. *BMC Complementary and Alternative Medicine*, 2012; 12: 56.
26. Sahu, J., Patel, P.K., Dubey, B., *Quisqualis indica* Linn: A Review of its Medicinal Properties. *International Journal of Pharmaceutical and Phytopharmacological Research*, 2012; 1: 313-321.
27. Limsuwan, S., Anti-*Streptococcus pyogenes* Activity of Selected Medicinal Plant Extracts Used in Thai Traditional Medicine. *Tropical Journal of Pharmaceutical Research*, 2013; 12: 535-540.
28. Kambar, Y., Asha, M.M., Chaithra, M., Prashith Kekuda, T.R., Antibacterial activity of leaf and flower extract of *Quisqualis indica* Linn. against clinical isolates of *Staphylococcus aureus*. *Research Journal of Science and Technology*, 2014; 6: 23-24.

29. Selvamohan, T., Ramadas, V., Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Advances in Applied Science Research*, 2012; 3: 3374-3381.
30. Sanguri, S., Kapil, S., Gopinathan, P., Pandey, F.K., Bhatnagar, T., Comparative screening of antibacterial and antifungal activities of some weeds and medicinal plants leaf extracts: An *in vitro* study. *Elixir Applied Botany*, 2012; 47: 8903-8905.
31. Jani, M., Shah, S., Prajapati, S., Antibacterial screening and qualitative phytochemical estimation of selected aquatic plants. *Advances in Biological Research*, 2012; 6: 19-23.
32. Abu-Shanab, B., Adwan, G., Abu-Safiya, D., Adwan, K., Abu-Shanab, M., Antibacterial activity of *Rhus coriaria* L. extracts growing in Palestine. *Journal of Islamic University of Gaza*, 2005; 13: 147-153.