Research Article [Kumar, 6(3): March, 2015:4363-4368]
CODEN (USA): IJPLCP ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

In vitro evaluation of antibacterial activity of *Petunia axillaris* leaves extracts against human pathogenic bacteria

Manoj Kumar*
Department of Botany, Pt. N. R. S. Govt. College, Rohtak, (Haryana) - India

Abstract

Medicinal plants have been very intensively screened for their bioactivity in order to treat various disease and disorders in human. In the present study, crude leaves extracts with different solvents (methanol, ethanol and aqueous) of *Petunia axillaris* were evaluated for antibacterial activity against medically important human pathogenic bacterial strains including two gram-positive (*Staphylococcus aureus* and *Streptococcus* sp.) and two gram-negative (*Pseudomonas aeroginosa* and *Salmonella typhimurium*). The different extracts were tested for the presence of antibacterial activity by agar well diffusion assay (AWDA) method. The leaves extracts with different solvents showed significant antibacterial activity against all the tested four bacterial strains. Among the tested bacterial strains, *Staphylococcus aureus* was highly susceptible to the extract as compared with other three bacterial strains. The results also showed that the methanolic extract of *Petunia axillaris* was the most effective as the widest inhibitory zone was observed as compared to the ethanolic as well as aqueous extract. The use of leaves extracts of the *Petunia axillaris* with known antibacterial properties can be of great significance in therapeutic treatments.

Key-Words: Agar well diffusion assay, Antibacterial activity, Human pathogens, Petunia axillaris, Solvents

Introduction

Use of plants as a source of medicine has been inherited and is an important component of the health care system. Medicinal plants are valuable natural sources effective against various infectious agents and are rich in bioactive compounds which can resist health hazards. Plant extracts has been used traditionally to treat a number of infectious diseases including those caused by bacteria and fungi (Yoshida, et al., 2005; Nejad and Deokule, 2009; Gao and Zhang, 2010). New antimicrobial agents are needed to treat diseases in humans and animals caused by drug resistant microorganisms. Antimicrobial substances are substances that inhibit the growth and existence of microorganisms (Thenmozhi and Sivaraj, 2011). These microorganisms could be pathogenic or non pathogenic, hence, antimicrobial substances are used in the treatment of various ailments. Quite a number of antimicrobial substances exist and they are gotten from diverse sources such as microbial, plant, animal and chemical sources (Ganellin and Roberts, 1999). Medicinal uses of these plants range from the administration of the plant's roots, bark, stem, leaves, fruits and seeds, to the use of extracts from the whole plant (Akujobi, et al., 2004).

*** Corresponding Author**
E.mail: manojgenetics@yahoo.com
Ph: - + 91-1262-274965
Fax- + 91-1262-274190

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants (Jigna and Chanda, 2006). This search for new antimicrobial properties of natural products cannot be ignored because this can be found in the most remote parts of the world where medical doctors are not present (Olukemi and Kandakai-Olukemi, 2004). Among the diseases that have been managed successfully by traditional (herbal) medicine include malaria, epilepsy, infertility, convulsion, diarrhoea, dysentery, gonorrhoea, flatulence, tonsillitis, bacterial and fungal infections, mental illness and worm infections (Sofowora, 1996).

Inspite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care (Ravikumar, et al., 2010). It is estimated that about 35,000 to 70,000 plants species are used as medicinal plants among 4, 22,127 species of plants (Kaur, et al., 2015). According to World Health Organization (WHO), the medicinal plants would be the best source to obtain a variety of drugs (Santos, et al., 1995). The use of plant extracts, with known antimicrobial properties, can be of great significance in the treatment of various microbial infections. Recently, a wide range of these plants have been screened for antimicrobial property (Martin and Ernst, 2003; Upadhyay, et al., 2010). The WHO

estimated that more than 80% population of the world for some aspect of primary health care use herbal medicines (Ahmad, et al., 2003). In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases, but also induce adverse side effects. Therefore, there is the need to search for useful herbs (Jain and Varshney, 2011)

Petunia axillaris is an annual herbaceous plant in the family Solanaceae. Several species of *Petunia* are ornamentals grown in gardens for their large, showy; multicoloured flowers (Thenmozhi and Sivaraj, 2011). Hence, in the present study, an attempt has been made to the evaluation of antibacterial activity of *Petunia axillaris* crude leaves extracts with different solvents (methanol, ethanol and aqueous) against two gram-positive (*Staphylococcus aureus* and *Streptococcus* sp.) and two gram-negative (*Pseudomonas aeroginosa* and *Salmonella typhimurium*) human pathogenic bacteria.

Material and Methods

Collection of plant material

A fresh leaves of *Petunia axillaris* (Figure 1) were collected from the campus of Pt. N. R. S. Govt. College, Rohtak, Haryana, India, and brought to the laboratory in polythene bags. Then the leaves were rinsed twice with distilled water, dried in the shade for 15 days and then ground into coarse powder using sterile mortar and pestle.

Preparation of plant extracts

An extract is a mixture of phytochemical 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 leaves 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 method.

Source of Microorganisms

In the present study four human pathogenic bacterial strains, including two gram-positive *Staphylococcus aureus* MTCC 6908 and *Streptococcus* sp. MTCC 9724 as well as two gram-negative *Pseudomonas aeroginosa* MTCC 4673 and *Salmonella typhimurium* MTCC 3224, were obtained from the microbial type culture collection (MTCC), Chandigarh, 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.

Antibacterial susceptibility assays

Antibacterial susceptibility of *Petunia axillaris* crude leaves extracts with different solvents (methanol, ethanol and aqueous) against gram-positive as well as gram-negative bacterial strains was determined by agar well diffusion assay method (Kumar and Gitika, 2014). 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

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).

Results and Discussion

Medicinal plants play a central role not only as traditional medicines, but also as commercial commodities meeting the demand of distant markets. To compete with the growing market, there is a need to expeditiously utilize and scientifically validate more medicinally useful plants. Because of the appearance of drug resistance to antimicrobial agents, more effort is being made to find alternative antimicrobial components. It had been suggested that natural products are a preferable option than synthetic ones. Literature indicates that medicinal plants are the backbone of traditional medicine, and the antimicrobial activity of plant extract is due to different chemical agent in the extract with antimicrobial compounds (Rojas, et al., 1992; Farnsworth, 1994).

The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Girish and Satish, .2008). Many studies have been undertaken with the aim of determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic drugs.

Increase of antibiotic resistance as well as undesirable side effects of synthetic drugs have triggered immense interest in the search for new antimicrobial agents of plant origin (Akinpelu and Onakoya, 2006; Chopra, 2007; Ogu, et al., 2010; Ali, et al., 2011).

Therefore, in order to evaluate the antibacterial activity of crude leaves extracts of *Petunia axillaris* against gram-positive as well as gram-negative human pathogenic bacterial strains by the AWDA method. In the present study, the crude leaves extract showed significant antibacterial activity against all tested gram-positive as well as gram-negative human pathogenic bacterial strains as shown in Figure 2.

The results shows *Staphylococcus aureus* was the most sensitive gram-positive bacterial strain with the inhibition zone (mm) 20 (methanol), 17 (ethanol), 15 (aqueous) as compared with the *Streptococcus* sp. with the inhibition zone (mm) 18 (methanol), 16 (ethanol), 14 (aqueous). However, among the tested two gram-negative bacterial strains *Pseudomonas aeroginosa* exhibited less sensitivity with the inhibition zone (mm) 14 (methanol), 12 (ethanol), 10 (aqueous) as compared to the *Salmonella typhimurium* with the inhibition zone (mm) 16 (methanol), 13 (ethanol), 11 (aqueous). However used commercially available, standard antibiotic streptomycin showed the greater zone of inhibition (mm) *Staphylococcus aureus* (22), *Streptococcus* sp. (20), *Pseudomonas aeroginosa* (16) and *Salmonella typhimurium* (18) as compared with the used different solvents (methanol, ethanol and aqueous) extract. In contrast, no inhibition zones were observed against 10% DMSO (result of DMSO not shown in the figure 2). The results indicate that the different solvent extracts showed inhibition of growth against tested bacterial strains with to the various degrees. From the results obtained it was apparent that the methanolic extract of *Petunia axillaris* was the most effective as the widest inhibitory zone was observed as compared to the ethanolic as well as aqueous extract used.

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 *Petunia axillaris* 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 (Abu-Shanab, et al., 2005; Jani, et al., 2012; Sanguri, et al., 2012; Selvamohan, et al., 2012; Kumar and Gitika, 2014; Kaur et al., 2015). Significant antibacterial activity of the *Petunia axillaris* crude leaves extracts against tested gram-positive as well as gram-negative human pathogenic 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.

The activity of plant extracts against both gram-positive and gram-negative bacteria may be an indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. The implication of the broad spectrum action of crude leaves extracts is that they can be useful in antiseptic and disinfectant formulation as well as in chemotherapy if the active principle can be isolated (Olukoya, et al., 1993). This study does not only show the scientific basis for some of the therapeutic uses of this plant in traditional medicine, but also confirms the fact that the ethnobotanical approach should be considered when investigating antimicrobial properties of plants (Adesanya, et al., 2005).

Conclusion

The crude leaves extracts of *Petunia axillaris* showed significant antibacterial activity against tested human pathogenic bacterial strains, including both gram-positive (*Staphylococcus aureus* and *Streptococcus* sp.) as well as gram-negative (*Pseudomonas aeroginosa* and *Salmonella typhimurium*). The results indicate that the methanolic extract of the leaves showed better antibacterial activity as compared to the other two solvent (ethanol and aqueous) while lesser than commercially available standards antibiotic streptomycin. Therefore, they could be further subjected for screening and identification of active ingredients which are responsible for the antibacterial activity against more pathogenic microorganisms associated with various human diseases. Hence, it may be recommended that the leaves extracts of this plant possess biologically active compounds with high antibacterial properties that can be used as antibacterial agents in designing and developing new drugs. Further studies should be need to isolation of bioactive compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

Acknowledgement

The author expresses their sincere thanks to the Principal, Pt. N.R.S Govt. College, Rohtak for cooperation and encouragement. The author also gratefully acknowledges Professor S. Srivastava, Department of Genetics, University of Delhi South

Campus, New Delhi, India for suggestion and guidance.

References

1. Abu-Shanab, B., Adwan, G., Abu-Safiya, D., Adwan, K. and Abu-Shanab, M. (2005). Antibacterial activity of *Rhus coriaria* L. extracts growing in Palestine. *Journal of Islamic University of Gaza*, 13: 147-153.
2. Adesanya, S. A. (2005). In: Theories and realities. Inaugural lecture series 181, Obafemi Awolowo University Press Ltd Ile-Ife, Osun State Nigeria.
3. Ahmad, M., Qureshi, M., khan, A., Saqib, M. (2003). Ethnobotanical studies of some cultivated plants of Chhahh region (District Attock). *Pakistan Scient Khyb*, 16: 109-121.
4. Akinpelu, D.A. and Onakoya, T.M. (2006). Antimicrobial activities of medicinal plants used in folklore remedies in south-western Africa. *African Journal of Traditional, Complementary and Alternative medicines*, 3:112-115.
5. Akujobi, C.O., Ogbulie, J.N. and Okorondu, T. (2004). Antibacterial and nutrient potentials of *Gongronema latitolium* and *Piper guineenses* used in herbal remedies and as species. *Nigerian Journal of Microbiology*, 18: 241-246.
6. Ali, S., Ahmad, G., Ahmad, M. N. and Hassan, R. (2011). Antimicrobial activity of aqueous and methanolic extracts of pomegranate fruit skin. *Avicenna Journal of Phytomedicine*, Vol. 1 (2): 67-73.
7. Chopra, I. (2007). The increasing use of silver based products as microbial agents: A useful development or a concern. *Journal of Antimicrobial Chemotherapy*, 59: 587-590.
8. Farnsworth, N.R. (1994). Ethnopharmacology and Drug Discovery. In: Ciba Foundation Symposium 185. Wiley, Chichester, pp. 42-59.
9. Ganellin, C.R. and Roberts, S. (1999). "Medicinal Chemistry" The Role of Organic Chemistry in Drug Research (2nd Ed.). Academic Press Limited, pp. 122-123.
10. Gao, D. and Zhang, Y. (2010). Comparative antibacterial activities of extracts of dried ginger and processed ginger. *Pharmacognosy Journal*, 2(15): 41-44.
11. Girish, H.V. and Satish, S. (2008). Antibacterial activity of important medicinal plants on human pathogenic bacteria-a comparative analysis. *World Applied Sciences Journal*, 5 (3): 267-271.
12. Jani, M., Shah, S. and Prajapati, S. (2012). Antibacterial screening and qualitative phytochemical estimation of selected aquatic plants. *Advances in Biological Research*, 6: 19-23.
13. Jigna, P. and Chanda, S. (2006). *In vitro* antimicrobial activities of extracts of *Launaea procumbeus* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). *African Journal of Biomedical Research*, 9(2): 89 – 93.
14. Kaur, S., Kaur, H.P. and Aggarwal, S. (2015). Evaluation of antibacterial activity, antioxidant potential and phytochemicals of *Withania somnifera* (Ashwagandha). *World Journal of Pharmacy and Pharmaceutical Sciences*, 4: 1032--1042.
15. Kumar, M. and Gitika (2014). Evaluation of antibacterial activity of *Nelumbo nucifera* (Gaertn.) flower extracts against gram-positive and gram-negative bacteria. *World Journal of Pharmaceutical Research*, 4: 1155-1161.
16. Martin, K.W. and Ernst, E. (2003). Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. *Journal of Antimicrobial Chemotherapy*, 51:241-246.
17. Nejad, B.S. and Deokule, S.S. (2009). Anti-dermatophytic activity of *Drynaria quercifolia* (L.) J. Smith. *Journal of Microbiology*, 2: 25-30.
18. Ogu, G.I., Ekeanyanwu, R.C. and Igborgbor, C.J. (2010). Phytochemical screening and *In vitro* Antibacterial activity of stem bark extract of *Caesalpinia pulcherrima* against some Clinical isolates. *International Journal of Natural and Applied Sciences*, 6(3):329-337.
19. Olukemi, M.A. and Kandakai-Olukemi, Y.T. (2004). Antibacterial activity of the ethanolic extracts of *Daniella oliveri*, *Annona senegallensis* and *Mitragyna sipulosa*; *Nigerian Journal of Microbiology*, 18: 35-239.
20. Olukoya, D.K., Ndika, N. and Odugbemi, T.O. (1993). Antibacterial activity of some medicinal plants in Nigeria. *Journal of Ethnopharmacology*, 39: 69-72.
21. Ravikumar, S., Selvan, G.P. and Gracelin, A.A. (2010). Antimicrobial activity of medicinal plants along Kanyakumari coast, Tamil Nadu, India. *African Journal of Basic and Applied Sciences*, 2: 153-157.
22. Rojas, A., Hernandez, L., Pereda-Miranda, R. and Mata, R. (1992). Screening for antimicrobial activity of crude drug extract and

natural products from Mexican medicinal plants. *Journal of Ethnopharmacology*, 35:111-115.

23. Sanguri, S., Kapil, S., Gopinathan, P., Pandey, F.K. and Bhatnagar, T. (2012). Comparative screening of antibacterial and antifungal activities of some weeds and medicinal plants leaf extracts: An *in vitro* study. *Elixir Applied Botany*, 47: 8903-8905.
24. Santos, P.R.V., Oliveira, A.C.X. and Tomassini, T.C.B. (1995). Controle microbiógico de produtos fitoterápicos. *Rev Farm Bioquím*, 31: 35-38.
25. Selvamohan, T., Ramadas, V. and Shibila, S.K.S. (2012). Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Advances in Applied Science Research*, 3: 3374-3381.
26. Jain, P. and Varshney, R. (2011). Antimicrobial activity of aqueous and ethanolic extracts of *Withania somnifera* (Ashwagandha). Journal of Chemical and Pharmaceutical Research, 3(3):260-263.
27. Soforowa, E.A. (1996). Research on medicinal plants and traditional medicine in Africa. *Journal of Alternative and Complementary Medicine*, 2(3): 365-372.
28. Thenmozhi, M. and Sivaraj, R. (2011). *In Vitro* evaluation of the antibacterial activity of *Petunia* leaf and callus extracts. *Journal of Agricultural Technology*, 7(2): 321-330.
29. Upadhyay, R.K., Dwivedi, P. and Ahmad, S. (2010). Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. *Asian Journal of Medical Sciences*, 2: 152-158.
30. Yoshida, M., Fuchigami, M., Nagao, T., Okabe, H., Matsunaga, K. and Takata, J. (2005). Antiproliferative constituents from Umbelliferae plants VII. Active triterpenes and rosmarinic acid from Centella asiatica. *Biological and Pharmaceutical Bulletin*, 28: 173-5.

Fig. 1: *Petunia axillaris,* studied plant

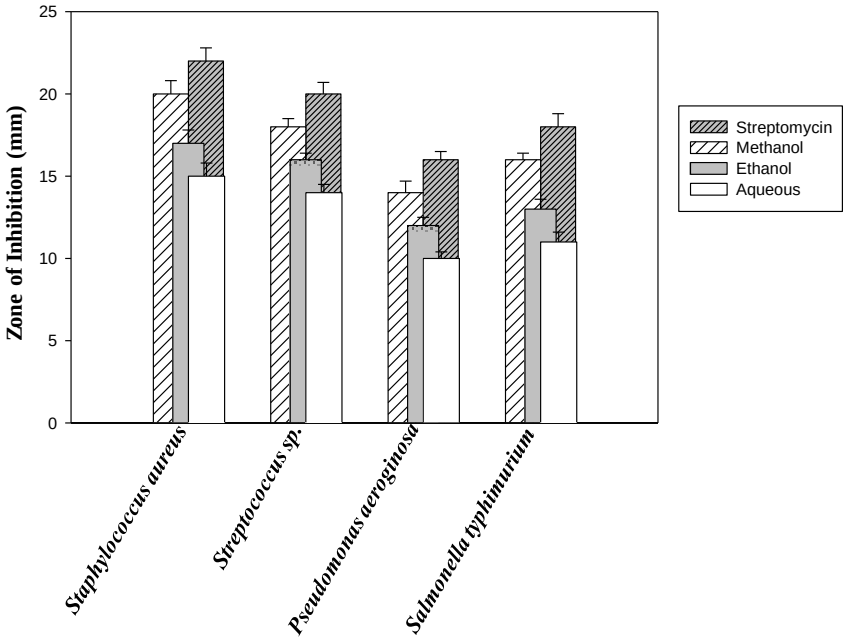

Fig. 2: Antibacterial activity of crude leaves extracts of *Petunia axillaris*

How to cite this article

Kumar M. (2015). *In vitro* evaluation of antibacterial activity of *Petunia axillaris* leaves extracts against human pathogenic bacteria**.** *Int. J. Pharm. Life Sci.*, 6(3):4363-4368.

Source of Support: Nil; Conflict of Interest: None declared

Received: 13.02.15; Revised: 20.03.15; Accepted: 15.03.15