



***In-vitro* analysis of potential antibacterial activity of three medicinal plants**

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Abstract: The present study was aimed to examine and compare the antibacterial activity of hot methanolic extract of medicinal plants viz. *Portulaca oleracea* (purslane), *Syzygium cumini* (L.) (jamun), *Psidium guajava* (L.) (guava). Antibacterial activity was carried by using agar well diffusion method, against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*). Results indicated that all the three plant extracts possess antibacterial property against Gram-positive bacteria and no activity was found against Gram-negative bacteria. Moderate zone of inhibition against *Staphylococcus aureus* and *Bacillus subtilis* was exhibited by *S. cumini* (L.) (11mm and 12mm) and *P. guajava* (L.) (10mm and 11mm) and weak zone of inhibition was exhibited by *P. oleracea* (5 mm and 6mm). In conclusion, *S. cumini* (L.) and *P. guajava* (L.) possess better capabilities of being a good candidate in search for natural antibacterial agent against infections and diseases causing Gram-positive bacteria as compared to *P. oleracea*.

Keywords: Agar well diffusion method, Antibacterial activity, Gram-negative bacteria, Gram-positive, Medicinal plants

INTRODUCTION

In recent years, there have been renaissance of interest in natural or herbal remedies worldwide; partly because of the realization that modern medicine is not capable of proving a “cure-all” solution against human diseases as well as the presence of side effects is almost unavoidable (Hostettmann *et al.*, 1995 and Handa, 2014). Medicinal plants and derived medicine are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals (Dixit *et al.*, 2013). Medicinal plants represent a rich source from which antimicrobial agents may be obtained due to the presence of active compounds such as tannins, flavonoids, terpenoids, saponins (Mujeeb *et al.*, 2014). These active compounds can inhibit the growth of microbes in many ways such as by inhibiting protein synthesis, interfering with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting metabolic pathways, interfering with cell wall synthesis or by preventing utilization of available nutrients by the microorganisms (Sumitra and Kaneria, 2011). Now-a-days, herbs are selected as antibacterial sources due to the development of drug resistance in human pathogens against commonly used antibiotics (Dixit *et al.*, 2013). Unlike modern drugs that invariably comprise of a single active species, the plant extract contain multiple active compounds which render them with medicinal characteristics (Donehower and Rowinsky, 1993). In 2009

WHO called antibiotic resistance one of the three greatest threats to human health, and in 2011 the focus of World Health Day was ‘Combating Antibiotic Resistance’ (World Health Organisation 2011). Numerous studies have been conducted with the extracts of various plants and revealed the health benefits of medicinal plants such as *Portulaca oleracea*, *Syzygium cumini* (L.), *Psidium guajava* (L.). But no comparative analysis of their antibacterial properties is being made till date. *P. oleracea* belongs to Potulacaceae family and is also known as Purslane. Leaves and stem of *P. oleracea* is reported to be used as antidiarrhoeal, anti-helminthic, antiphlogistic and bactericide in bacillary dysentery, hemorrhoids, enterorrhagia, antidiabetic (Bakkiyaraj and Pandiyaraj, 2011). Whereas, leaves of *S. cumini* (L.) (jamun) belonging to Myrtaceae family, used in diabetes, antiallergic, antioxidant, antiviral, antibacterial, anti inflammation and helps in preventing DNA damage. (Chaudhary and Mukhopadhyay, 2012). Leaves of *P. guajava* (L.) (guava) belonging to Myrtaceae family, are used in diarrhoea, dysentery, gastroenteritis, anti cough, ulcers, bowels, cholera, hypoglycaemic, anti-inflammatory, analgesic, antipyretic (Dey *et al.*, 2010). The present study was aimed to determine the antibacterial potential of three medicinal plants viz. *P. oleracea*, *S. cumini* (L.), *P. guajava* (L.) along with their comparative analysis. The selection was guided by lack of information in literature on comparative study of antibacterial activity of hot methanolic extract of the selected plants.

MATERIALS AND METHODS

Collection of plant material: Mature leaves and stem of *P. oleracea*, leaves of *S. cumini* (L.) and *P. guajava* (L.) were collected from Haryana Agricultural University, Hisar, Haryana during the month of April 2014. The collected plant material was thoroughly washed under tap water, rinsed in distilled water and shade dried.

Preparation of methanol extract: The shade dried plant material was grounded into powder. About 40 gm powder was extracted with 250 ml of methanol at 55-60°C for 4 hours. The extracts obtained were filtered, concentrated after dryness in rota vapor. The dried extracts were stored in air tight containers at 4°C for further study. (Choudhary *et al.*, 2012)

Test organisms: The pure cultures of common human pathogenic Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*) were obtained from the Department of Veterinary Microbiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar and sub-cultured in nutrient agar plates and slants. Three to five well-isolated colonies of micro-organism were aseptically transferred into the tube containing 5ml of Brain Heart Infusion Broth. The inoculated broth tubes were incubated at 37°C for 24 hours. The growth was observed in the form of turbidity and the tubes were stored at 4°C for further experimental work.

Screening of antibacterial activity: The modified agar well diffusion method of Dey *et al.*, (2010) was employed to study the antibacterial activity of the plant extracts. The Nutrient agar medium (28 g nutrient agar in 1000 ml distilled water) was autoclaved at 15 lbs pressure (121°C) for 15 minutes. After autoclaving, the media was poured into sterilized petri plates with a uniform thickness of approximately 4 mm. The agar plates were allowed to solidify at ambient temperature and were used after 24 hours. Three uniform wells were prepared with the help of sterile cork borer of 5 mm diameter in agar plates. The entire agar surface was then inoculated by sterile cotton swab dipped in bacterial culture and left for 3 to 4 minutes. Different hot methanolic plant extracts were added to the grooves and one control (methanol) of each was added to groove on separate agar plate. The plates were incubated for 24 hours at 37°C. Plates were examined and zone of inhibition of the bacteria growth was measured in mm by using diameter measurement scale. All the assays were performed in triplicate and expressed as average values. Antibacterial activity of plant extracts and control was interpreted as no activity (- mm), weak (<6mm), moderate (7-12mm) and strong activity (>12mm).

RESULTS AND DISCUSSION

The main objective of this study was to screen antibacterial activity of three medicinal plants and compare them to identify the potential antibacterial plant extract

for the therapeutic use. Hot methanolic extracts of the selected parts of the plants were prepared by soxhlet extraction process. Agar well diffusion method was employed to test the antibacterial efficiency of all the three plant extracts. The bacterial resistance was tested against two Gram-positive bacteria (*S. aureus* and *B. subtilis*) and one Gram-negative bacteria (*E. coli*). The results of the present study revealed that the three hot methanolic plant extracts under study possess antibacterial activity against Gram-positive bacteria (*S. aureus* and *B. subtilis*), which are very much in conformation with the results of the studies conducted by Mohamed *et al.*, 2013, Bakkiyaraj and Pandiyaraj 2011, and Egga *et al.*, 2014. All hot methanolic plant extracts did not display any antibacterial activity against *Escherichia coli* as presented in table 1. Zero antibacterial activity was exhibited by control (methanol) against tested bacteria under study. The resistance of the Gram-negative bacteria to several plant extracts was previously highlighted by Biswas *et al.*, 2003 and suggested that such activity could be attributed to the cell wall structure of *E. coli*, as it has an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which restricts the penetration of the extruding plant extract. Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts. Among the tested plant extracts, moderate zone of inhibition against *S. aureus* was exhibited by *S. cumini* (L.) (11mm) and *P. guajava* (L.) (10mm) and weak zone of inhibition against *S. aureus* was exhibited by *P. oleracea* (5 mm). In case of *B. subtilis* moderate zone of inhibition was exhibited by *S. cumini* (L.) (12mm) and *P. guajava* (L.) (11mm) and weak zone of inhibition was exhibited by *P. oleracea* (6 mm) as shown in figure 1. Effective antibacterial activity of leaf extract of *S. cumini* (L.) especially against Gram-positive bacteria (*S. aureus*) has been reported by Shafi *et al.*, 2002 and Shyamala and Vasantha, 2010, rendered the antibacterial activity to the presence of phenol and tannin constituents in the leaf extract in their study. Goncalves *et al.*, 2008, Sanches *et al.*, 2005, Vieira *et al.*, 2001 reported high activity of guava leaves extract and essential oil against

Table 1: Antibacterial efficacy of hot methanolic leaves extract of selected plants against bacteria.

Plants	Zone of inhibition (mm)		
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>
<i>P. oleracea</i>	5mm	6mm	-
<i>S. cumini</i> (L.)	11mm	12mm	-
<i>P. guajava</i> (L.)	10mm	11mm	-
Control (methanol)	-	-	-

Bacteria activity: - no activity; <6mm : weak; 7-12: Moderate; > 12mm: Strong

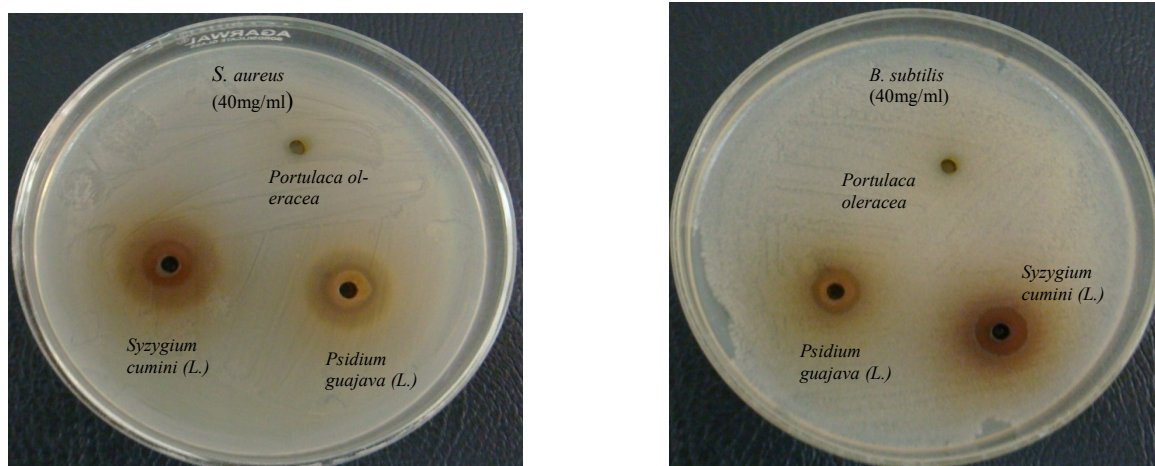


Fig 1. Zone of inhibition of plants under study against Gram-positive bacteria.

S. aureus, which could be attributed to the presence of active compounds such as tannin, flavonoids, eugenol, mallic acid present in the leaves (Ncube *et al.*, 2008). Although Methanolic extract of *P. oleracea* have been reported to possess good antibacterial property against two Gram-positive bacteria (*S. aureus* and *B. subtilis*) and such activity was rendered to the presence of chemical compounds of this plant (Bakkiyraj and Pandiyaraj, 2011). Whereas in the present study the antibacterial activity of hot methanolic extract of *P. oleracea* was weak as compared to *S. cumini* (L.) and *P. guajava* (L.). This might be due to the difference in number and type of chemical compounds present in the extract, which are responsible for antibacterial property. The results suggest that among the three plants screened hot methanolic extract of *S. cumini* (L.) and *P. guajava* (L.) belonging to the family Myrtaceae exhibited almost similar antibacterial activity and can be used as potential antibacterial agent against Gram-positive bacteria.

Conclusion

The present work demonstrates the antibacterial potential of hot methanolic extracts of *P. oleracea*, *S. cumini* (L.) and *P. guajava* (L.). The observed inhibition of Gram-positive bacteria (*S. aureus* and *B. subtilis*) suggests that plants under study possess compounds containing antibacterial properties that can effectively suppress the growth when extracted using methanol as solvent. On the basis of the present finding, it can be concluded that *S. cumini* (L.) and *P. guajava* (L.) possess better capabilities of being a good candidate in search for natural antibacterial agent against infections and diseases causing Gram-positive bacteria as compared to *P. oleracea*. Also, detailed investigation is required for the quantitative and qualitative analysis of chemical compounds also known as phytochemicals present in the plants under study, which are responsible for their antibacterial activity.

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