Advanced Research Journal of Microbiology Vol. 1 (4), pp. 056-059, December, 2013. Available online at www.advancedscholarsjournals.org © Advanced Scholars Journals

Full Length Research Paper

Antibacterial activity of *Lallemantia royleana* (Benth.) indigenous to Pakistan

Seemab Mahmood, Muhammad Qasim Hayat, Alia Sadiq*, Shabina Ishtiaq, Sadia Malik and Muhammad Ashraf

Medicinal Plants Research Laboratory, Department of Plant Biotechnology, Atta-Ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), H-12 Islamabad, Pakistan.

Accepted 8 November, 2013

***Lallemantia royleana* (Benth.) is an important folk medicine in Pakistan. This natural herb is used as folk remedy for number of ailments. This study was conducted to assess the antibacterial activity of four different organic extracts of *L. royleana* seeds against four bacterial strains (*Escherichia coli*, *Enterobacter cloaceae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) for the first time by disc diffusion method. Except aqueous extracts, all organic extracts of *L. royleana* seeds displayed significant anti-bacterial activity against all the tested bacteria. The chloroform extract exhibited highest anti-bacterial activity for all bacterial strains. Results shows that *L. royleana* seeds possess significant antibacterial potential against *S. aureus*, *E. coli* and *E. cloaceae*, therefore, it can be a good remedy for skin disease and gastro-intestinal problems caused by human pathogenic bacterial strains. Further screening for phytochemicals should be carried out in search of novel therapeutic compounds.**

Key words: Antibacterial activity, zone of inhibition, phytochemical, aqueous extracts, antibiotic.

INTRODUCTION

Lallemantia royleana (Benth.) is an annual herb, belonging to the family *Lamiaceae* and is commonly known as “Lady’s mantle” (English) and Tukhum-malanga (Urdu). Geographically, *Lallemantia* is represented by five species which are distributed in Afghanistan, China, India, Kazakhstan, Kyrgyzstan, Pakistan, Russia, Tajikistan, Turkmenistan, Uzbekistan, SW Asia and Europe. This medicinally important plant is originally native to tropical Asia, throughout Afghanistan, Turkestan, India and Pakistan. *L. royleana* seeds with vernacular name of Balangu is widely grown in different regions of Pakistan namely, Attock, Layyah, Bhakkar, Bahawalpur, Hasilpur and Chishtian etc (Morton, 1990; Ghannadi and Zolfaghari, 2003; Abbas et al., 2012; Hayat et al., 2008). Traditionally, it is a very common practice that local people use indigenous plants to cure infectious diseases. These indigenous plants or plant products, or those are the part of food as dietary components are termed as ethnomedicine. Although, there are very few reports on mechanism of action and phytochemistry of these plant based phytomedicines. But traditional knowledge report that these plants possess potential to cure infectious diseases. Nowadays, these ethnomdicines have been receiving considerable attention by scientist and pharmaceutical research industries with the aim to investigate for more effective substitute (Dogruoz et al., 2008; Karsha and Lakshmi, 2010; Samee et al., 2009). *L. royleana* seeds are ethno-botanically well-established worldwide for the treatment of abscesses, inflammation and respiratory problems, particularly Muslim used its seeds in drinks due to sedative effects (Abbas et al., 2012; Abdulrasool et al.,

*Corresponding author. E-mail: aliasadiq55@hotmail.com, alia.11.dirphd.56@asab.nust.edu.pk.

2011; Khare, 2007). Seeds of this plant are more common part of folk medicine and but there is very limited information about its nutritive components. It contains carbohydrates, fiber, oil, protein and tannins (Naghibi et al., 2005; Razavi and Karazhiyan, 2009; Razavi and Moghaddam, 2011). Recent trend toward natural remedies support the fact that plant and food medicine are good source of natural and safe healing therapies (Dogruoz et al., 2008; Karsha and Lakshmi, 2010).

Previous studies were focused on its ethno-botany, while medicinal properties of *L. royleana* are not much evaluated on scientific merits. So far, not a single study has been reported with reference to its antibacterial potential (Razavi and Moghaddam, 2011; Amiri et al., 2012; Akber et al., 2011). Therefore, the present study has been carried out to evaluate the antibacterial potential of organic and aqueous extracts of *L. royleana* seeds against human pathogenic bacterial strains.

MATERIALS AND METHODS

Collection and processing of seeds

Seeds sample was purchased from the local market of Gulrez and Bahria town, Rawalpindi. Identification was done by Plant Taxonomist Dr. Muhammad Qasim Hayat, and voucher specimen was then deposited to Medicinal Plant laboratory at Atta-ur-Rehman School of Applied Biosciences, NUST Islamabad, Pakistan. Seeds were then grinded to very fine powder, followed by preservation at room temperature in air tight bottles and wrapped up in aluminum foil to avoid contact with light.

Seeds extract preparation

The powdered seeds of *L. royleana* were used to prepare aqueous and organic extracts (organic solvents are ethanol, methanol and chloroform). Primarily, crude extracts of *L. royleana* were prepared by using dried powdered seeds samples (10 g) immersed in 100 ml methanol and kept on rotary shaker at 109 to 220 rpm for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min and the supernatant was collected. The residue were transferred to a conical flask and washed with 80% ethanol. The procedure was repeated twice to obtain the supernatants. The combined supernatant was filtered and then concentrated to about 50 ml under vacuum using a rotary evaporator at 40°C. The concentrated sample was successively partitioned with chloroform and water. Each fraction was evaporated *in vacuo* to yield the concentrated residues and stored at 4°C in air tight bottles (Samee et al., 2009).

Bacterial strains

Tested bacterial strains including gram-positive, *Staphylococcus aureus* and gram-negative strains *Enterobacter cloacae* (IARS 7), *Pseudomonas aeruginosa* (IARS 9) and *Escherichia coli* (IARS 3) were obtained from Pakistan Institute of Medical Sciences (PIMS) Islamabad, Pakistan.

Preparation of inoculums

Suspensions of bacterial culture were prepared as per McFarland's standard for all bacterial strains (Gulfraz et al., 2011). Bacterial cultures (24 h old) were used for the preparation of bacterial inoculums and maintained approximately at 1.5×10^8 cells/ml.

Antibacterial assay

The antibacterial activity of *L. royleana* seeds extracts was evaluated by using disc diffusion method (Karcioglu et al., 2011) at three different concentrations (100, 50 and 10 mg/ml) against all bacterial strains. Standard antibiotics Kanamycine and Ampiciline were used as positive control whereas dimethyl sulfoxide (DMSO) has been used as negative control against test bacterial cultures. Bacterial culture plates were prepared by spreading 100 µl of liquid bacterial culture on plates and then tested against effect of each extract. All aqueous and organic extract were applied (10 µl) onto a 6 mm sterile filter paper disc separately and plates were inverted and incubated at 37°C for 24 h. All tests were carried out in triplicate. After incubation, antibacterial effect was observed and measured as zone of inhibition and recorded in a tabulated form.

Statistical analysis

Statistical analysis was carried out using the student's t-test, for the estimation of results as mean ± SD (standard deviation) and percentage values (Gulfraz et al., 2011).

RESULTS

Results for antibacterial activity of all extracts of *L. royleana* seeds (Table 1) show that organic extracts possess a significant antibacterial potential against all the studied bacterial strains while aqueous extracts did not show antibacterial activity at any concentration. Results were also compared with standard antibiotics Ampicillin and Kanamycine. Chloroform extracts of *L. royleana* seeds exhibited highest zone of inhibition (14.67 mm) at 100 mg/ml against *S. aureus*, which revealed that chloroform extract is most effective against *S. aureus*.

Furthermore, *E. coli* and *E. cloaceae* were also found more sensitive to these organic extracts (zone of inhibition, 12.83 and 13.83 mm, respectively) while *P. aeruginosa* showed less sensitivity. *S. aureus* was found to be the most susceptible organism showing the maximum inhibition zone as compared to other bacterial strains. Hence, the results have shown that *L. royleana* seeds extracts have significant antibiotic potential against *S. aureus*; while standard antibiotic testing revealed that *P. aeruginosa* and *E. coli* were resistant to ampicillin.

DISCUSSION

In the present investigation, we have demonstrated the antibacterial activity of various extracts of *L. royleana* seeds against wide range of gram positive and gram negative bacteria with the highest antibacterial activity being demonstrated against *S. aureus.* It has been observed that antibacterial efficacy of different extracts proceed in a dose-dependent manner for different bac-

Table 1. Antibacterial activity of different organic extracts of *L. royleana* seeds by disc diffusion method.

Plant extract	Extract concentration (mg/ml)	Bacterial strains diameter of zone of inhibition (mm ± SD)			
		S. aureus	***E. cloacae***	***E. coli***	***P. aeruginosa***
Methanol	100	13.17 ± 2.0	12.00 ± 1.3	09.33 ± 1.0	11.83 ± 0.5
	50	13.00 ± 2.7	10.17 ± 2.0	10.17 ± 0.7	09.00 ± 0.5
	10	06.17 ± 5.3	12.33 ± 1.2	11.67 ± 2.3	07.00 ± 6.08
Ethanol	100	13.83 ± 4.48	11.17 ± 1.04	11.17 ± 1.04	10.33 ± 1.26
	50	12.67 ± 2.84	12.33 ± 3.82	12.83 ± 0.76	10.83 ± 1.44
	10	12.33 ± 3.75	13.83 ± 1.26	10.00 ± 1.5	03.67 ± 6.35
Chloroform	100	14.67 ± 0.58	14.00 ± 1.5	11.83 ± 3.79	10.67 ± 1.44
	50	13.33 ± 2.93	11.83 ± 1.89	11.00 ± 1.32	13.67 ± 3.75
	10	06.67 ± 6.29	11.67 ± 0.76	12.16 ± 0.76	10.50 ± 3.5
Aqueous	100	-	-	-	-
	50	-	-	-	-
	10	-	-	-	-
Standard antibiotics Amp	10 ug/ml	28± 0.5	12± 0.8	-	-
Kana	10 ug/ml	22.5± 0.4	35± 0.1	25± 0.11	19± 0.7
DMSO (50%)	5 ul	-	-	-	-

DMSO, Dimethyl sulphoxide; Amp, ampiciline; Kana, kanamycine; -, no inhibition. Values are in terms of mean ±SD after triplicate analysis.

terial strains. Similar results were observed in another research study in different system (Abraham and Thomas, 2012). Various extracts of *L. royleana* seeds have shown variability in antibacterial activity. It was noted that methanol, ethanol and chloroform extracts of the seeds possess greater antibacterial potential for all bacterial strains. However, the aqueous extract showed no antibacterial activity at any concentration against any bacteria. In contrast to our study, it has been observed that local traditional healers use water as a solvent for their preparations (Hayat et al., 2008). Gram-positive *S. aureus* was found to be the most sensitive bacterial strain, showing the maximum inhibition zone as compared to other micro-organisms in case of all the extracts. Therefore, it is very obvious from results that *L. royleana* seeds extracts have significant antibiotic potential against gram positive and negative bacterial strains which is an indicative of presence of broad spectrum antibiotic phytoconstituents (Doughari, 2006) which impart respective bioactivity to these plant extracts (Gulfraz et al., 2011).

Furthermore, still, there is a requirement to determine the active compounds present in the seeds, to classify the compounds that might be more effective against these human pathogenic bacterial strains and to use a specific formulation of only those compounds in the drug synthesis (Abraham and Thomas, 2012).

Conclusions

The present study suggests that seeds extracts of *L. royleana* exhibit significant antibacterial activity. The degree of concordance between traditional use and observed antibacterial properties validate ethno-pharmacological use of this folk medicine. Therefore, *L. royleana* seeds extracts as potent antibiotic can be good candidate for further studies leading toward comprehensive investigation for its mechanism of action.

ACKNOWLEDGMENTS

The authors are grateful to Medicinal Plant laboratory, Department of Plant Biotechnology, ASAB, NUST Pakistan for providing facilities and financial support.

REFERENCES

Abbas M, Mehmood T, Bashir A, Zafar M, Afzal A (2012). Economics of *Lallemantia royleana* (tukham-ebalangoo) production in the low intensity cropping zone of the Punjab, Pakistan. Pak. J. Agric. Res . 25:2.

Abdulrasool AA, Naseer AA, Rahi FA (2011). Application of seed mucilage extracted from *Lallemantia royleana* as a suspending agent. Iraqi. J. Pharm. Sci. 20.

Abraham J, Thomas TD (2012). Antibacterial activity of medicinal plant

Cyclea peltata (Lam) Hooks & Thoms. Asian Pac. J. Trop. Dis. S280-S284.

Akber M, Seeraj S, Islam F, Ferdausi D, Ahmed R, Nasrin D, Nahar N, Ahsan S, Jamal F, Rahmatullah M (2011). A Survey of Medicinal Plants Used by the Traditional Medicinal Practitioners of Khulna City, Bangladesh. Am. Eurasian J. Sustain. Agric. 5 (2): 177-195.

Amiri MS, Parham J, Mahdi A (2012). An ethnobotanical survey of medicinal plants used by indigenous people in Zangelanlo district, Northeast Iran. J. Med. Plants Res. 6 (5):749-753.

Dogruoz N, Zeybek Z, Karagoz A (2008). Antibacterial Activity of Some Plant Extracts. IUFS J. Biol. 67(1):17-21.

Doughari JH (2006). Antimicrobial activity of Tamarindus indica Linn. Trop. J. Pharm. Res. 5:597.

Ghannadi AR, Zolfaghari B (2003). Compositional Analysis of the Essential Oil of *Lallemantia royleana* Benth. J. Flavour Fragr.18: 237-239.

Gulfraz M, Sadiq A, Tariq H, Imran M, Qurashi R (2011). Phytochemical analysis and antibacterial activity of *Eruca sativa* seeds. Pak. J. Bot. 43(2):1351-1359.

Hayat MQ, Khan MA, Ahmad M, Shaheen N, Yasmin G, Akhter S (2008). Ethnotaxonomical Approach in the Identification of Useful Medicinal Flora of Tehsil Pindigheb (District Attock) Pakistan. Ethnobot. Res. Appl. 6:035-062.

Karcioglu L, Tanis H, Comlekcioglu N, Diraz E, Kirecci E, Aygan A (2011). Antimicrobial Activity of *Salvia trichoclada* in Southern Turkey. Int. J. Agric. Biol. 13:134-136.

Karsha PV, Lakshmi OB (2010). Antibacterial activity of black pepper (*Piper nigrum* Linn.) with special reference to its mode of action on bacteria. Indian J. Nat. Prod. Resour. 1(2):213-215.

Khare CP (2007). Indian Medicinal Plants. An Illustrated Dictionary Springer-Verlag Heidelberg.

Morton JF (1990). Mucilaginous plants and their uses in medicine. J. Ethnopharmacol. 29 (3):245-66.

Naghibi F, Mosaddegh M, Motamed SM, Ghorbani A (2005). Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iran J. Pharm. Res. 2:63-79.

Razavi SMA, Karazhiyan H (2009). Flow properties and thixotropy of selected hydrocolloids, Experimental and modeling studies. Food Hydrocoll. 23 (3):908-912.

Razavi SMA, Moghaddam TM (2011). Influence of different substitution levels of *Lallemantia royleana* seed gum on textural characteristics of selected hydrocolloids. Electron. J. Environ. Agric. Food Chem. 10:2826-2837.

Samee H, Li ZX, Lin H, Khalid J, Guo YC (2009). Anti-allergic effects of ethanol extracts from brown seaweeds. J. Zhejiang Univ. Sci. *B*. 10(2):147-153.