Pak. J. Bot., 45(1): 275-278, 2013.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *LAWSONIA INERMIS*, *LANTANA CAMARA* AND *SWERTIA ANGUSTIFOLIA*

ZARRIN FATIMA RIZVI[1], RABIA MUKHTAR[2], M. FAYYAZ CHAUDHARY[3] AND MUHAMMAD ZIA[*2]

[1]*Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan*
[2]*Department of Biotechnology, Quaid-i-Azam University Islamabad, Pakistan*
[3]*Department of Nanotechnology, Preston University Islamabad, Pakistan*
[*]*Corresponding author e-mail: ziachaudhary@gmail.com*

Abstract

Three medicinally important plants *Lawsonia inermis*, *Lantana camara* and *Swertia angustifolia* were evaluated for their antibacterial and antifungal activities. *L. inermis* extract showed maximum activity against *staphylococcus aureus* and *Klebsiella pneumoniae*. *L. camara* extract was not much active against any bacterial strain. A range of zone of inhibition 9-12.3 mm was observed against all bacterial strains tested. *S. angustifolia* did not show activity against any bacterial strain. In case of fungal growth inhibition *L. inermis* showed good inhibition of 3 fungal strains *Fusarium solani*, *Alternaria* and *Mucor*. The percent inhibitions were 78.8, 65.3 and 71.1, respectively. These findings demonstrate that *L. inermis* is a good candidate for further studies that carries both antibacterial and antifungal active constituents.

Introduction

The role of natural products as medicine in treatment of ailments has always been very important all over the world. Sources of natural products are both terrestrial and aquatic that includes plants, microorganisms, vertebrates and invertebrates (Newman *et al*., 2000). Plants produce a variety of toxic compounds that can act as drugs against pathogenic microorganisms. There is a long history related to the plants used in treatment of human diseases. For example licorice (*Glycyrrhiza glabra*), myrrh (*Commiphora* species) and poppy capsule latex (*Papaver somniferum*), have written historic record to be used in 2600 B.C. and these plants are still used in treatments either as a part of drug or as herbal preparations in traditional medicine (Newman *et al*., 2000). Traditional use of plants as therapeutic tools especially those with ethnopharmacological uses serve as basis for their use in modern medicines. According to a recent analysis 80% of 122 plant-derived drugs are related to their original traditional uses (Fabricant & Farnsworth, 2001).

About 50% of all deaths occur due to infectious diseases in tropical areas of world (Iwu *et al*., 1999). As far as causes of deaths are concerned, recent surveys proves it to be the second major cause of death worldwide and third major reason in developed countries (Nathan, 2004). Although antibiotics are major means of treating such infectious diseases but they are not effective in all cases and some microorganisms sometimes cannot be treated by them.

One major limitation of efficacy of antibiotics is development of resistance in microbes and this resistance is spreading all over the world (Livemore, 2003; Walsh & Amyes, 2004). As a result cases of therapy failure are increasing. Likewise increasing costs is another limitation to the use of antibiotics (Solomon *et al*., 2003; Alder, 2005). Although more than 99% known organic compounds are synthetic but still natural products are the basis of more than a third of all drugs sales (Newmann *et al*., 2003).

Keeping in view importance of natural sources especially plants, this study was conducted to evaluate antibacterial and antifungal potential of 3 important medicinal plants; *Lawsonia inermis*, *Lantana camara* and *Swertia angustifolia*. For antibacterial analysis, agar well diffusion method was done while antifungal assay was performed through tube dilution method.

Materials and Methods

Collection and identification of plant material: Fresh plant material (*Lawsonia inermis* leaves, *Lantana camara* fruit and *Swertia angustifolia* whole plant) was collected from different areas of Pakistan. Plants were identified by a Taxonomist, Department of Plant Sciences, Quaid-i-Azam University Islamabad. The plants specimens were deposited in the Herbarium Department of Plant Sciences Quaid-i-Azam University.

Drying and extraction: Plant materials were thoroughly washed and dried under shade. Dried material (*L. inermis* 1 Kg, *L. camara* 0.8 Kg and *S. angustifolia* 1.5 Kg) and ground to fine powder. Cold maceration technique was used for extraction. Powdered plant material was dipped in methanol and kept at room temperature. After 7 days, the extract was filtered through Whatman filter paper No. 1 under vacuum. The residue was again dipped in methanol for 7 days and filtered thereafter. The filtrate was combined and the methanol was evaporated under vacuum using rotary evaporator at 45°C. The dried extracts were stored at 4°C until further analysis.

Antibacterial activity: The agar well diffusion method was performed to exploit antibacterial potential of extracts (Bibi *et al*., 2010). Each extract (200mg) was dissolved in 10 ml of dimethyl sulfoxide (DMSO) to get 20mg/ml concentration. Cefotaxime (2mg/ml) in DMSO was prepared as positive control. Pure DMSO was used as negative control (Table 1).

Table 1. Antibacterial potential (mean zone of inhibition (mm) of three medicinal plants against some bacteria.

Plant	*S. setubal*	*E. aerogenase*	*M. luteus*	*E. coli*	*K. pneumoniae*	*S. aureus*
L. inermis	10.5	11	14	14.1	15.6	16
L. camara	10	9	12	12.1	12.3	10
S. angustifolia	-	-	-	-	-	-
Cefotaxime	47.3	36	38	45	29	31
DMSO	-	-	-	-	-	-

Test microorganisms: Six bacterial strains were used for assay. These were *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240), *Salmonella setubal* (ATCC 19196), *Entarobacter aerogenase* (ATCC13048), *Klebsiella* pneumoniae (ATCC 1705) and *Escherichia coli* (ATCC5224). The nutrient broth medium (8 g/l) was prepared by dissolving nutrient broth (Merck) in distilled water. The pH of media was adjusted at 7.0 and 50 ml was dispensed in 100 ml flask and then autoclaved. Bacterial cultures were inoculated individually and kept at 37°C overnight in shaker incubator at 150 rpm.

Turbidity Standard and preparation of inocula: The turbidity standard was prepared by adding 0.5 ml of 0.048 M $BaCl_2$ to 99.5 ml 0.3 6N $H_2SO_4.BaSO_4$. The standard was taken in screw cap test tube and used to compare the turbidity. Overnight grown bacterial culture of selected strains was mixed with physiological saline. Turbidity was corrected by adding sterile saline until a McFarland 0.5 $BaSO_4$ turbidity standard 10^8 Colony Forming Unit (CFU) per ml was achieved. These inocula were used for seeding the nutrient agar.

Procedure: Nutrient agar medium was prepared by suspending nutrient agar (Merck) 20 g/l in distilled water. pH of media was adjusted at 7.0, autoclaved, and allowed to cool upto 45°C. The media was seeded with 10 ml prepared inocula. Seeded medium (75-80 ml) was poured in pre-labeled Petri plates (diameter=14 cm) and allowed to solidify. Required numbers of wells per plate (six wells for extracts, one for positive and negative control each) were made with 8 mm sterile cork borer. These wells were sealed by pouring 20 µl of liquid nutrient agar medium in each well.

Using micropipette, 100 µl of test solution was poured in respective well. Sample of extracts, one positive control (Cefotaxime), and one negative control (DMSO) were applied to each Petri plate. Plates were incubated at 37°C. After 24 hr of incubation, diameter of clear zones, showing no bacterial growth around each well was measured. Triplicate plates were prepared for each extract and bacterial strain. Mean zone of inhibition with standard deviation was calculated. The percentage growth inhibition was calculated by,

$$\text{Percentage inhibition} = (TS-SC)/PC \times 100$$

TS: test sample; SC: Solvent control; PC positive control

Antifungal activity: The agar tube dilution method was performed to determine antifungal activity of plant extract (Fatima *et al*., 2009). Three fungal strains were used which were *Mucor* specie, *Aspergillus fumigatus* and *Fusarium moliniforme*. Fungal cultures were maintained on Sabouraud dextrose agar media in tubes in slanting position at 27°C. To perform assay, media was prepared by mixing 32.5 g Sabouraud dextrose agar (Merck) in 500 ml distilled water. It was dissolved on heating and 5 ml was poured in each screw cap tube. Tubes were labeled and autoclaved at 121°C for 20 min. Tubes were allowed to cool and 100 µl of plant extract (20 mg/ml in DMSO) was added just before solidification to obtain a concentration of 400 µg/ml. For positive control, 83 µl of fluconazole (12 mg/ml in DMSO) was added in each tube to get concentration of 200 µg/ml. Pure DMSO (100 µl/tube) was used as negative control. Tubes were shaken well and allowed to solidify at slanting position at room temperature. A piece of fungal inoculum (4 mm diameter) from seven days old culture was inoculated in each tube. The tubes were incubated at 27°C for seven days. Growth was determined by measuring linear growth (mm). Test was performed in triplicate and growth inhibition was calculated with reference to negative control with the help of following formula:

$$\% \text{ inhibition of fungal growth} = 100 - \frac{\text{Linear growth in test}}{\text{Linear growth in control}} \times 100$$

Results and Discussion

Three medicinally important plants were evaluated for their antibacterial and antifungal activities. It was observed that *Lawsonia inermis* and *Lantana camara* extracts were active against all bacterial and fungal strains tested. *Swertia angustifolia* did not show activity against any bacterial strain while active against only two fungal strains.

L. inermis extract showed maximum activity (16 mm) against *staphylococcus aureus* (16 mm) and *Klebsiella pneumoniae* (15.6 mm). The same extract was active against *Micrococcus luteus* and *E. coli* representing near about equal zone of inhibition (14 & 14.1 mm,

respectively). Minute activity was observed by *L. inermis* extract against *Salmonella setubal* (10.5 mm) and *Entarobacter aerogenase* (11 mm). Habbal *et al*., 2005 demonstrated that fresh and dry leaves and seeds of the *L. inermis* contained antibacterial activity against standard strains as well as patients' isolated strains.

Lantana camara extract was not much active against any bacterial strain. A range of zone of inhibition 12-12.3 mm was observed against *Micrococcus luteus, E.coli* and *Klebsiella pneumoniae*. A mean zone of inhibition 10 mm was observed against *Salmonella setubal* and *staphylococcus aureus*. *Entarobacter aerogenase* showed little zone of inhibition (9 mm). Basu *et al*., (2005) reported presence of Emodin and Physcion in *L. camara* extract that showed mild activities against different bacterial strains. An antibacterial activity of different Lantana species has been evaluated (Barreto *et al*., 2010; Ganjewala *et al*., 2009) and it is found that Lantana species have good antibacterial agents. *S. angustifolia* did not show activity against any bacterial strain tested.

Percentage inhibition of bacterial growth by these plant's extracts is presented in Fig 1. The figure shows that *L. inermis* extract inhibited bacterial growth more actively as compared with Lantana extract. Maximum percent inhibition by *L. inermis* extract was observed against *Klebsiella pneumoniae* (53.7%) leading to *Stephylococcus aureus* (51.6). All other three bacterial strains were inhibited in a range of 22.1-38.8%). *L. camara* extract also showed maximum percent inhibition of *Klebsiella pneumonia* (42.2%). *Micrococcus luteus*, *E. coli* and *Staphylococcus aureus* were percent inhibited in a range of 31.8 to 33.3. Percent inhibition 21.1 and 25 were observed against *Salmonella setubal* and *Entarobacter aerogenase*, respectively.

L. inermis showed good inhibition of three fungal strains; *Fusarium solani*, *Alternaria* and *Mucor*. The percent inhibitions were 78.8, 65.3 and 71.1, respectively. Sharma & Sharma (2011) also demonstrated that *L. inermis* has fungicidal potential against a number of isolated i.e. *Alterneria solani*, *Drechslera halodes*, *Rhizoctonia solani*, *Fusarium solani*, *Curvularia lunata*, *Dreschlera graminae*, *Fusarium moniliformae*, *Aspergillus flavus*, *A. parasiticus* var. globosus, *Trichophyton rubrum*, *Aspergillus fumigatus*. *Lantana camara* fruit extract was mildly active against *Mucor* and *Fusarium solani* representing 41.2 and 46.1 percent inhibition, respectively. *Swertia angustifolia* extract showed mild activity against *Alternaria* (41.6% inhibition) and low activity against *Fusarium solani* (23.7%). Deena & Thoppil (2000) and Sharma & Kumar (2009) also demonstrated that Lantana extract was moderately active against different fungal strains. *S. angustifolia* extract was inactive against *Mucor* specie and did not inhibit its growth (Fig. 2).

These findings demonstrate that *Lawsonia inermis* is a good candidate carrying both antibacterial and antifungal active constituents. While, *Lantana camara* fruit extract is moderately active against tested bacterial and fungal strains. Therefore, the extracts of these plants were considered as suitable candidates for antibacterial and/or antifungal drug discovery. Based on our findings, we visualize that the finding of novel antibacterial agent from natural springs (plants) will help to minimize the bad effects of mock drugs.

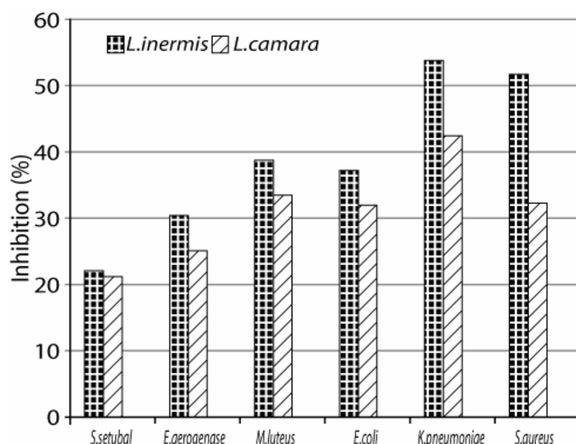

Fig. 1. Percent bacterial growth inhibition by *L. inermis* and *L. camara* extracts

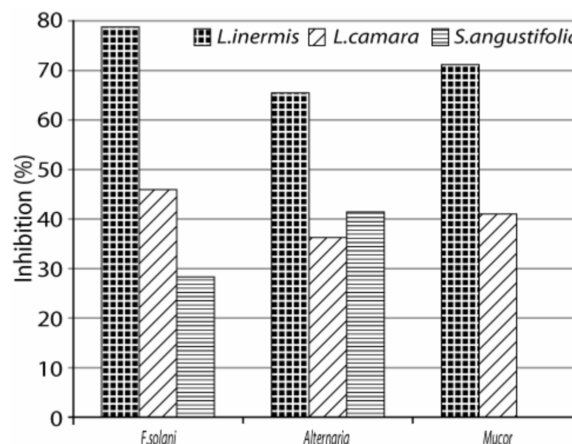

Fig. 2. Percent fungal growth inhibition by *L. inermis, L. camara and S. angustifolia* extracts

References

Alder, J.D. 2005. Daptomycin, a new drug class for the treatment of Gram-positive infections. *Drugs Today*, 41(2): 81.

Barreto, F.S., E.O. Sousa, A.R. Campos, J.G.M. Costa and F.F.G. Rodrigues. 2010. Antibacterial activity of *Lantana camara* Linn and *Lantana montevidensis* brig extracts from Cariri-Ceará, *Brazil J. Young Pharm*., 2(1): 42-44.

Basu, S., A. Ghosh and B. Hazra. 2005. Evaluation of the antibacterial activity of *Ventilago madraspatana* Gaertn., *Rubia cordifolia* Linn. and *Lantana camara* Linn.: isolation of emodin and physcion as active antibacterial agents *Phytother. Res.* 19, 888-894.

Bibi, Y., S. Nisa, A. Waheed, M. Zia, S. Sarwar, S. Ahmed and M.F. Chaudhary. 2010. Evaluation of *Vibernum foetens* for anticancer and antibacterial potential and phytochemical analysis. *African J. Biotechnol*., 9(34): 566-5615.

Deena, M.J. and J.E. Thoppil. 2000. Antimicrobial activity of the essential oil of *Lantana camara*. *Fitoterapia*, 71: 453-455.

Fabricant, D.S. and N.R. Farnsworth. 2001. The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect*., 109: 69-75.

Fatima, N., M. Zia, Riaz-ur-Rehman, Z.F. Rizvi, S. Ahmed, B. Mirza and M.F. Chaudhary. 2009. Biological activities of *Rumex dentatus* L: Evaluation of methanol and hexane extracts. *African J. Biotech*., 8(24): 6945-6951.

Ganjewala, D., S. Sam and K.H. Khan. 2009. Biochemical compositions and antibacterial activities of *Lantana camara* plants with yellow, lavender, red and white flowers *EurAsia J. BioSci*., 3: 69-77.

Habbal, O.A., A.A. Al-Jabri, A.H. El-Hag, Z.H. Al-Mahrooqi and N.A. Al-Hashmi. 2005. In-vitro antimicrobial activity of *Lawsonia inermis* Linn (henna). A pilot study on the *Omani henna*. *J. Saudi Med*., 26(1): 69-72.

Iwu, M.W., A.R. Duncan and C.O. Okunji. 1999. New antimicrobials of plant origin In: Perspectives on new crops and new uses, edited by Janick J, ASHS Press, Alexandria, VA. pp. 457-462.

Livemore, D.M. 2003. Overstretching the mutant prevention concentration. *J. Antimicrob. Chemother*. 52: 732.

Nathan, C. 2004. Antibiotics at the crossroads. *Nature,* 431: 899-902.

Newman, D.J., G.M. Cragg and K.M. Snader. 2000. Influence of natural products upon drug discovery. *Nat. Prod. Rep*., 17: 215-234.

Newman, D.J., G.M. Cragg and K.M. Snader. 2003. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.,* 66: 1022-1037.

Sharma, A. and K. Sharma. 2011. Assay of antifungal activity of *Lawsonia inermis* Linn. and *Eucalyptus citriodora* Hook. *J. Pharmacy Res.,* 4(5): 1313-1314.

Sharma, B. and P. Kumar. 2009. Bioefficacy of *Lantana camara* L. against some human pathogens *Indian J. Pharm Sci*., 71(5): 589-593.

Solomon, S., T. Horan, M. Andrus, J. Edwards, S. Fridkin, J. Koganti, G. Peavy and J. Tolson. 2003. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992 through June 2003, issued August 2003. *Am. J. Infect. Control*., 31: 481-498.

Walsh, F.M. SGB and Amyes. 2004. Microbiology and drug resistance mechanisms of fully resistant pathogens. *Curr. Opi. Micro*., 7: 439-444.

(Received for publication 11 July 2011)