www.ajlifesciences.com / ISSN: 2663-1040 *AJ Life Sci. 2021, 4 (2): 59-65*

Research Article

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.4.2.7

Invitro antimicrobial efficiency of *Lawsonia inermis* L (Henna) extracts against Multidrug Resistant Microorganisms

Shehla Javaid*[1,2], Amer Jamil*[1], Hina Awais[2], Samiah Shahid[3], Saira Khan[4] and Hafiz Muhammad Rehman[2].

[1] *Molecular Biochemistry Lab, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan*
[2] *University Institute of Medical Lab Technology, University of Lahore, Lahore-Pakistan.*
[3] *Institute of Molecular Biology and Biochemistry, University of Lahore, Lahore-Pakistan*
[4] *Gulab Devi educational complex Lahore-Pakistan*

Abstract

Antibiotics are becoming less effective as drug resistance spreads throughout the world, making it ever more difficult to treat the disease. The effect of biotic stress on antimicrobial activity of *Lawsonia inermis* L. (Henna) was studied as an alternate to antimicrobial agents against multidrug resistant microorganisms. The study was carried out in Molecular Biochemistry Lab, Department of Biochemistry, University of Agriculture Faisalabad. The plant seedlings were given biotic stress with *Fusarium solani*. The induced extracts were harvested at different time intervals and the antimicrobial activity was investigated by disc diffusion method. The induced plant extracts revealed highest susceptibility against *S. aureus* (30±0.02) and *Pasteurella multocida* (30±0.01) at 12 hpi. The least activity was shown against all the strains at 0 hpi. The highest antifungal activity was found against *Ganoderma lucidum* (55±0.05) at 24 hpi. The results demonstrated a significant difference in the antimicrobial activities of all the strains with and without fungal stress (P<0.05). The fungal induced extracts of *Lawsonia inermis* L (Henna) with enhanced antimicrobial activity may have the potential of being alternative and cost- effective agents against antimicrobial resistance.

Key words: Antimicrobial; In-vitro; *Lawsonia inermis*; Drug resistance; Antibiotics.

Article Info:
Received:
August 31, 2021
Received Revised:
December 29, 2021
Accepted:
December 29, 2021
Available online:
December 31, 2021

**Corresponding Author:*
shehla.javaid@mlt.uol.edu.pk and
amerjamil@yahoo.com

1. INTRODUCTION

Medicinal plants are playing a vital role in providing the defence mechanism against viral and bacterial infections[1]. Being sources of many lives sustaining metabolites, the research is still on for plants to be used in the treatment of several diseases. This in part is due to the growing problem of worldwide antibacterial resistance. The massive use of antibiotics has tremendously increased the prevalence of drug resistant strains and that is the major cause of insufficient control to fight against microbial infections[2]**.**The measures used to combat this increasing resistance rate demands continuous investigation to search for non-toxic antimicrobials with improved efficacy as an alternate to fewer effective ones.

Lawsonia inermis L (also known as mehndiand/or henna) belongs to the *Lythraceae*family that contains tannic acid, gallic acid, mucilage and mannite, 2 – hydroxyl napthoquinone (lawsone), an organic compound containing protein binding affinity responsible for dyeing properties of henna[3]**.** Henna plant is known since with its healing attributes and is now the major subject of scientific research. It has been used in traditional medicines since old age to treat several diseases such as edema, menstrual disorder, rheumatism, haemorrhoids and bronchitis[4]**.** Its antifungal, antiamoebiasis, antibacterial, astringent, hypotensive, antihemorrhage and dermatological effects are attributed**.** It shows inhibitory action against Gram positive and negative both[5].

Recently, it has been reported that biotic stress affects plant growth and productivity. After biotic stress plants produce different biological compounds including PR (pathogenesis related) proteins and directly disease related proteins that are protective in nature. Biotic stress interacts with cell proteins to maintain their functional conformations, refold denatured proteins to gain functional conformation or prevent aggregation of non-native proteins[6]. *Fusarium Solani* is a plant pathogenic fungus that induces the compound production that is biologically active and concerned with the plant defence mechanism[7].

In the present study, *Lawsonia inermis* L. was given stress with *Fusarium solani* and then antimicrobial activity from different extracts of *Lawsonia inermis* L was analysed to check the effect of biotic stress on antimicrobial resistance. All the post-inoculated samples exhibited inhibitory activity against tested strains of bacteria and fungus.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

The seeds of the plant *Lawsonia inermis* L. were collected from botanical garden of the University of Agriculture Faisalabad, Pakistan. The seeds were soaked in distilled water for 5 minutes and then in 70% ethanol for 10 minutes under sterilized conditions. The ethanol was removed from the seeds, washed with distilled water and kept in 5% sodium hypochlorite (commercial bleach) for 15 min. The seeds were washed off with prior autoclaved distilled water for 3 times and incubated whole night for imbibition at 4 °C. The floated seeds were discarded to prevent contamination.

2.2 Microorganisms

The fungal strain *Fusarium Solani* was used for plant induction. Pure cultures from four fungal strains i.e. *Fusarium Solani*, *Alternaria alternata, Ganderma lucidum and Trichoderma Harizianum.* Four bacterial strains i.e., *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pasteurella multocida* were used. The selected strains were verified from the Institute of Microbiology, University of Agriculture Faisalabad, Pakistan.

2.3 Fungal induction

Lawsonia inermis L seeds 15-20 per plate were inoculated with *Fusarium solani* after the seed sprouting started within 8-9 days. The control plate had no fungal induction[9].

2.4 Sample extraction

The seeds were ground in a pre-chilled pestle and mortar under liquid nitrogen until powdered. One gram of the sample was extracted in 3 mL of extraction buffer (potassium phosphate buffer). 30 µL PMSF (as protease inhibitor) was added as protease inhibitor. The mixture was homogenized finely on homogenizer and centrifuged at 10,000 rpm, 4°C for 20 minutes. The supernatant was homogenized in 12 mL acetone, 60 µL β-mercaptoethanol and 3mg PVP (polyvinylpyrrolidone). Incubated overnight and centrifuged at 4 °C for 20 minutes. The pellet was dissolved in 1 mL dissolving buffer, transferred to a micro centrifuge tubes and stored at -40 °C till further analysis[10].

2.5 Protein content determination

The protein content of the sample was obtained by Bradford assay[11].

2.6 Determination of antimicrobial activity

Antimicrobial activity of *Lawsonia inermis L* extract collected at the time zero (0 hpi: without fungal stress) and then after fungal stress at different time intervals (2 hpi, 4 hpi, 8hpi, 12 hpi, 24 hpi, 48 hpi) was measured. Antibacterial and antifungal assays were performed by disc diffusion method. Chloramphenicol was used as the positive control and a blank disc without any antibiotic and extract was used as negative control[12]. The zones of inhibition measured by zone reader were expressed as mean along with standard deviation in Table 1& 2.

2.7 Statistical analysis

The statistical analyses were performed on SPSS software. The results were expressed as mean ± SD (standard deviation). All assays were performed in duplicate. The assays were compared by using one-way analysis of variance (ANOVA) and the *P* value (<0.05) indicates statistical significance.

3. RESULTS AND DISCUSSIONS

3.1Induction and harvesting

The pure culture of *Fusarium solani* was applied on the seedlings of the *Lawsonia inermis* L as biotic stress. First the seedlings were harvested before fungal stress (0 hour) that was used as control and then at 2, 4, 8, 12, 24, 48 hour post inoculation (hpi). Introduction of fungus i.e., *Fusarium solani* to the young seedlings (fungal induction) may result in the production of antimicrobial peptides that can defend the plants against pathogenic attack.

3.2 Determination of protein content

Peptides can inhibit pathogenic microorganism's growth by deactivating their virulence factors. These peptides encoding genes are expressed in different cells in the host and their expression is tightly controlled. They can be induced by pathogens and microorganisms appear to be less resistant against these antimicrobial peptides as compared to conventional antibiotics[13]. The protein contents of the samples were determined by Bradford assay[11]. The absorbance was noted at different hpi at 575 nm against blank (Fig.2). The protein content was (4 µg/mL) at 0 hpi in the sample without fungal induction. The protein content was increased with the time hours post inoculation after fungal stress representing highest concentration at 12 hpi. These results suggest that fungal stress might have induced the protein and/or peptides present in the *Lawsoniainermis* L having antimicrobial potential.

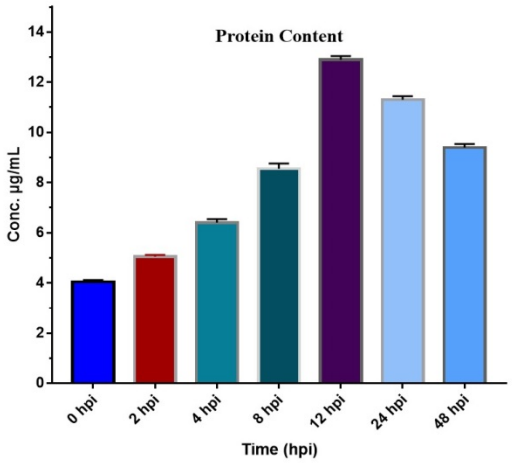

Figure 2. Concentration of protein in different extracts of *Lawsonia inermis* (henna) harvested at different time interval after biotic stress.

3.3 Antimicrobial activity

Antimicrobial activity of the protein extract of *Lawsoniainermis L* collected at the time zero (0 hpi: without fungal stress) and then after fungal stress at different time intervals was determined (Table 1& 2).

3.4 Antibacterial activities

Different hours protein extract of *inermis* L were tested against four bacterial strains including *S.aureus, E.coli, Bacillus subtilus*, *Pasteurella multocida* (Table 1). The fungal induced extracts revealed antibacterial activity against all strains but highest susceptibility was found against *S. aureus* (30±0.02) and *Pasteurella multocida* (30±0.01) at 12 hpi as measured by their zones of inhibition (Fig 3). Moreover the highest antibacterial activity of the plant extract against *B.subtilus* (22±0.02) *and E.coli* (21±0.02) was represented in 12 hpi sample.The least activity was shown against all the strains at 0 hpi. The zones formed against bacterial strains showed a statistically significant difference among antibacterial activity of the induced (12 hpi) and non-induced (0 hpi) extracts (P<0.05).

Table 1. Average antibacterial activity of fractions of *Lawsonia inermis L* harvested at different time intervals against selected bacterial strains.

Different fractions of *Lawsonia inermis*(hpi)	*Bacillus subtilus*	*Pasteurella multocida*	*Escherichea coli*	*Staphlococcus aureus*
0	16±0.01	20±0.02	15±0.01	24±0.02
2	18±0.02	20±0.02	17±0.01	25±0.02
4	19±0.01	24±0.02	17±0.01	27±0.02
8	20±0.02	24±0.02	20±0.004	29±0.02
12	22±0.02	30±0.01	21±0.02	30±0.02
24	20±0.02	26±0.02	21±0.02	29±0.02
48	17±0.02	24±0.02	13±0.01	22±0.02
Control (non-inoculated sample)	16±0.05	20±0.02	15±0.01	24±0.02
Positive control (chloramphenicol)	21±0.03	30±0.02	21±0.03	32±0.02

Zone size: zero/- (No activity); 1-6/+ (activity present); 6-9/++ (moderate activity); 9-12 or >12/+++ (strong activity)

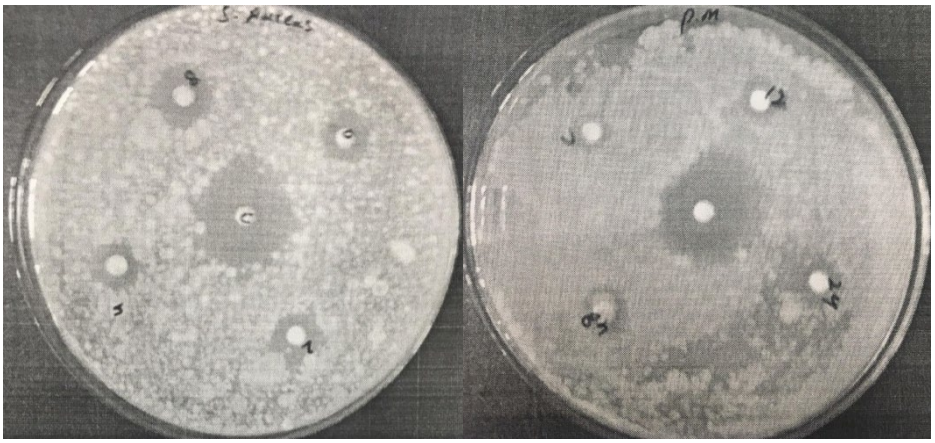

Figure 3. Antibacterial activity of *Lawsonia inermis* L gainst *S.aureus* and *P. multocida.* Chloramphenicol on central disc was used as +ve control.

3.5 Antifungal activity

The antifungal activity of *inermis* L extract was checked by disc diffusion assay at different hpi. The zone size against all the selected strains increased with increasing time of post hour inoculation (Table 2). The

highest activity was found against *Ganoderma lucidum* (55±0.05) at 24 hpias determined by inhibition zone diameter (Fig :4). Similarly, *Fusarium solani* showed highest inhibition zone of 25±0.02 at 24 hpi. *A. alternate* (9±1.009) and *T. harzianum* (21±0.09) showed their maximum inhibition at 24 hpi(Table 2). The results demonstrated a significant difference in the antifungal activities against all the strains with and without fungal stress (P<0.05).

Table 2. Average antifungal activity of fractions of *Lawsonia inermis* L harvested at different time interval against selected fungal strains

Fractions of *Lawsonia inermis* (hpi)	***Fusarium solani***	***Alternaria alternate***	***Ganoderma lucidum***	***Trichoderma harzianum***
0	19±0.02	5±0.008	43±0.05	12±0.02
2	19±0.01	5±0.005	44±0.05	13±0.01
4	20±0.01	6±0.006	45±0.04	14±0.02
8	21±0.02	8±0.009	48±0.004	18±0.01
12	23±0.01	9±0.005	50±0.04	20±0.02
24	25±0.02	9±1.009	55±0.05	21±0.09
48	23±0.01	8±1.009	53±0.05	19±0.01
Control(non-inoculated sample)	19±0.01	5±1.009	43±0.05	12±0.01

Zone size: zero/- (No activity); 1-6/+ (activity present); 6-9/++ (moderate activity); 9-12 or >12/+++ (strong activity)

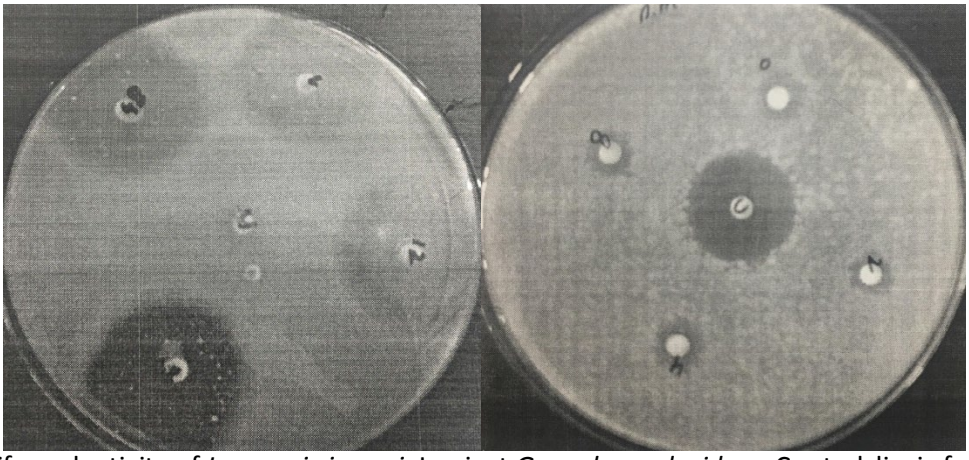

Figure 4. Antifungal activity of *Lawsonia inermis* L gainst *Ganoderma lucidum.* Central disc is for +ve control (Fluconazole).

In this study we have documented that *Lawsonia inermis* L (henna) plant species from Pakistan demonstrated antibacterial activity under biotic stress against multidrug resistant (MDR) bacterial strains including *E.coli, Staphylococcus aureus, Bascillus subtilis* and *P. multocida* with the highest antibacterial activity against *Staphylococcus aureus* and *P. multocida*. Antibiotic resistant microorganisms can increase mortality rates because they can survive and recover through their ability to obtain and transmit resistance after exposure to antibiotic drugs such as S. aureus, so that's way it was a strong indication to use these strains for the research purpose[14]. The different extracts of medicinal plants have well known antimicrobial properties but their efficiency against MDR bacteria under biotic stress have not been well studied in the local as well as international medical literature. Stress response in plants comprises repertoire of molecular, cellular cross-talk and signaling responses initiated through the detection of specific or combined biotic or abiotic stress effect that may result in the induction of secondary metabolites[15]. *Fusarium solani* is an opportunistic fungi that is intermittently able to penetrate into the host, to spread and to induce biotic stress[16]. After biotic stress in plants, *Fusarium solani* introduces some bioactive peptides in *Lawsonia inermis* that secrete reactive oxygen species and other detoxified enzymes which act on the cell membrane of bacteria. These bioactive peptides show antimicrobial activity by changing their structural modification[17]. So in the present study, fungal induced seedling extracts of *Lawsonia inermis* L were active against four different bacteria including *E.coli, S. aureus*, *B. subtilus* and *P. multocida* as they showed inhibition zone

against all the tested strains. The results were encouraging, as the *Lawsonia inermis* appeared to contain substances and peptides that had antimicrobial properties. As in the recent years, continuous increasing number of multi-drug resistant (MDR) bacteria is a challenge for the healthcare organizations. The least efficacy and increased toxicity of synthetic drugs further exaggerate this problem. So they believe that green medicines are safer and harmless as compared to synthetic ones directed the scientists to expand their interests towards natural or organic sources. Traditional medicines particularly medicinal plants have been used worldwide as therapeutic alternatives. Different studies have been carried out on medicinal plant extracts to check their potential as antimicrobial agent with good antimicrobial activities[18]. Jabeen and her co-workers reported that bioactive peptide plant extracts were more efficient as free radical scavengers after fungal stress[19].

The present work showed that antimicrobial activities against four bacterial and four fungal strains were exhibited by the *Lawsoniainermis* plant samples collected at different time intervals after fungal induction with *Fusarium solani*. Henna plant has long been used as a preservative, in cosmetics and in traditional herbal medicines in parts of Africa, Asia and in the Middle East. The plant extracts in different organic solvents exhibited antibacterial and antifungal activities[20].

During antifungal screening of higher plants, the leaves of *L. inermis* were found to exhibit strong fungitoxicity. The samples showed inhibitory activity against a wide range of the tested fungal strains. Wong and Ng[21] purified an antifungal peptide from the seeds of haricot beans (*Phaseolus vulgaris*) having antifungal activity against different fungal strains. *Lawsonia inermis* may produce compounds or bioactive peptides after fungal induction with a potential to fight against drug resistant pathogens mainly by causing membrane permeabilization[22]. The inducible peptides or compounds could be a source of new antimicrobials in traditional medicine to treat bacterial and fungal diseases.

4. CONCLUSIONS

The results obtained in the current study revealed that *Lawsonia inermis* L. (henna) under biotic stress showed enhanced antibacterial activity against *E.coli, S. aureus*, *B. subtilus* and *P.multocida*. The fungal induced extracts at different hpi also showed antifungal activity against *Fusarium Solani, Alternaria Alternata, Ganderma lucidum* and *T. Harizianum*. Further studies are required to isolate, purify and quantify these bioactive and inducible proteins/peptides from the selected plant. The positive outcomes from successive studies would certainly strengthen the potential of *Lawsonia inermis* L. (henna) under biotic as an alternative cost effective and novel agent against multi-drug resistant microorganisms.

ACKNOWLEDGMENT

The authors are thankful to Molecular Biochemistry Lab, Department of Biochemistry, University of Agriculture Faisalabad for providing research facilities and financial support.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

REFERENCES

1. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pacific journal of tropical biomedicine. 2012 Apr 1;2(4):320-30.
2. Babu PD, Subhasree RS. Antimicrobial activities of Lawsonia inermis-a review. Acad J Plant Sci. 2009;2(4):231-2.
3. Muhammad HS, Muhammad S. The use of Lawsonia inermis Linn.(henna) in the management of burn wound infections. African Journal of Biotechnology. 2005;4(9).
4. Cuong NX, Nhiem NX, Thao NP, Nam NH, Dat NT, Anh HL, Van Kiem P, Van Minh C, Won JH, Chung WY, Kim YH. Inhibitors of osteoclastogenesis from Lawsonia inermis leaves. Bioorganic & medicinal chemistry letters. 2010 Aug 15;20(16):4782-4.

5. Saadabi MA. Evaluation of Lawsonia inermis Linn.(Sudanese henna) leaf extracts as an antimicrobial agent. Research Journal of Biological Sciences. 2007;2(4):419-23.
6. Kamal AH, Kim KH, Shin KH, Kim DE, Oh MW, Choi JS, Hirano H, Heo HY, Woo SH. Proteomics-based dissection of biotic stress responsive proteins in bread wheat (Triticum aestivum L.). African Journal of Biotechnology. 2010;9(43):7239-55.
7. Lim HS, Kim YS, Kim SD. Pseudomonas stutzeri YPL-1 genetic transformation and antifungal mechanism against Fusarium solani, an agent of plant root rot. Applied and Environmental Microbiology. 1991 Feb;57(2):510-6.
8. JaEeen R, Jamil A, Shahid M, Ashraf M. Bioactive Potential of 1ormal and Fungal Stressed Extracts from Ricinus Communis L.(Castor) Whole Plant Relative Potential of Protein/PeStide. Oxid Commun. 2015 Jan 1;38(4):1612-21.
9. Falak S, Jamil A. Expression profiling of bioactive genes from a medicinal plant Nigella sativa L. Applied biochemistry and biotechnology. 2013 Jul;170(6):1472-81.
10. Bradford MM. A dye binding assay for protein. Anal Biochem. 1976;72(248):e54.
11. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and antifungal activities of spices. International journal of molecular sciences. 2017 Jun;18(6):1283.
12. Khameneh B, Iranshahy M, Soheili V, Bazzaz BS. Review on plant antimicrobials: A mechanistic viewpoint. Antimicrobial Resistance & Infection Control. 2019 Dec;8(1):1-28.
13. Isah T. Stress and defense responses in plant secondary metabolites production. Biological research. 2019;52.
14. Hof H. The Medical Relevance of Fusarium spp. Journal of Fungi. 2020 Sep;6(3):117.
15. Abushaala AR, Ben R1, Fahej MAS. *In vitro* antifungal activity of some plant extracts against seed-borne pathogens. *IOSR-JAVS*. 2017 Aprl 10 (4): 49-57.
16. JaEeen R, Jamil A, Shahid M, Ashraf M. Bioactive Potential of 1ormal and Fungal Stressed Extracts from Ricinus Communis L.(Castor) Whole Plant Relative Potential of Protein/PeStide. Oxid Commun. 2015 Jan 1;38(4):1612-21.
17. Kouadri F. In vitro antibacterial and antifungal activities of the saudi Lawsonia inermis extracts against some nosocomial infection pathogens. Journal of Pure and Applied Microbiology. 2018 Mar 1;12(1):281-6.
18. Nigussie D, Davey G, Tufa TB, Brewster M, Legesse BA, Fekadu A, Makonnen E. Antibacterial and antifungal activities of Ethiopian medicinal plants: a systematic review. Frontiers in pharmacology. 2021;12.
19. Chaudhary G, Goyal S, Poonia P. Lawsonia inermis Linnaeus: a phytopharmacological review. International journal of pharmaceutical sciences and drug research. 2010;2(2):91-8.
20. Wong JH, Ng TB. Vulgarinin, a broad-spectrum antifungal peptide from haricot beans (Phaseolus vulgaris). The international journal of biochemistry & cell biology. 2005 Aug 1;37(8):1626-32.
21. Huang X, Xie WJ, Gong ZZ. Characteristics and antifungal activity of a chitin binding protein from Ginkgo biloba. FEBS letters. 2000 Jul 28;478(1-2):123-6