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Antimicrobial, Phytochemical and Toxicological Evaluation of *Lawsonia inermis* Extracts against Clinical Isolates of Pathogenic Bacteria

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ABSTRACT

The search of new antimicrobial agents is a current demand due to emerging resistance of pathogen against the currently available antimicrobial agents. The use of medicinal plants as natural substitute is the paramount area of research to overwhelm the drug resistance of infectious agents. In the present study, antimicrobial activity of *Lawsonia inermis* was investigated against clinical isolates of eight bacteria including five Gram negative (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella sonnei*) and three Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) using disc diffusion method. Four types of *Lawsonia inermis* extracts were prepared using methanol, chloroform, acetone and water as extraction solvents while DMSO (Dimethyl sulfoxide) and water as dissolution solvents. The rate and extent of bacterial killing was determined by Time-kill kinetic assay at 1×MIC of each bacterial isolate. The overall safety of *Lawsonia inermis* extracts was assessed in mice. The minimum value of MIC for different bacterial isolates ranged from 3-9.50 mg mL⁻¹. At 1×MIC of each bacterial isolate, 3log₁₀ decrease in CFU was recorded after 6 h of drug exposure and no growth was observed in all tested bacteria after 24 h of exposure except *B. subtilis* and *S. epidermidis*. No sign of toxidrome were observed during *in vivo* toxicity evaluation in mice at 300 mg kg⁻¹ concentration. The present study provides the scientific rational for medicinal use of *Lawsonia inermis*. The use of *Lawsonia inermis* extracts is of great significance as substitute antimicrobial agent in therapeutics.

Key words: *Lawsonia inermis*, antimicrobial activity, time-kill kinetic assay

INTRODUCTION

Medicinal plants have been used for many years traditionally for herbal medicine (Ushimaru *et al.*, 2007). Therefore, plants have attained status of natural source of new and potent antimicrobial agents (Blanks *et al.*, 1998). Medicinal plants are used as ethnomedicine in different countries around the world (Srivastava *et al.*, 1996) and are source of natural products providing unlimited opportunity for new drugs because of readily available medicinal diversity (Cos *et al.*, 2006).

Lawsonia inermis (*L. inermis*) is a scientific name of a tall shrub plant commonly known as Henna or Mehndi (Muhammad and Muhammad, 2005) or mignonette tree (Bailey and Bailey, 1976) belongs to Kingdom: Plantae, Division: Angiospermae, Class: Dicotyledoneae, Order: Myrtales, Family: Lythraceae, Genus: *Lawsonia*, Species: *L. inermis* (Varghese *et al.*, 2010). Henna is a flowering plant, having a height of 5 m, natal to subtropical and tropical regions of world including South Asia, Africa, oases of Sahara dessert and even in northern regions of Australia. Leaves of henna plant are entire, opposite, sub-sessile, oval-shaped and smooth (Ashnagar and Shiri, 2011). Leaves have length of 2-3 cm with 1-2 cm width (Muhammad and Muhammad, 2005). Henna shrub is highly branched and has greyish-brown barks (Rahmoun *et al.*, 2010).

Lawsone (2-hydroxynaphthoquinone), mucilage, mannite, gallic acid and tannic acid are the main chemical constituents of henna (Al-Rubiay *et al.*, 2008). Henna is known to be used as a cosmetic agent for dyeing hair, nails and skin (Rostkowska *et al.*, 1998).

In traditional medicine, henna plant is used to treat many diseases like oedema, bronchitis, menstrual disorder, rheumatism, hemorrhoids and even in jaundice, leprosy, pain, spleen enlargement, dysentery and skin problems (Rahmoun *et al.*, 2010; Cuong *et al.*, 2009; Bhuvaneshwari *et al.*, 2002; Warriar *et al.*, 1995). Henna can also be used as an astringent and antihemorrhagic agent and is also known for its hypotensive, cardio inhibitory and sedative effects (Rahmoun *et al.*, 2010). In addition, henna is reported to show some other properties including hypoglycemic (Symsudin and Winarno, 2008), immunostimulant (Mikhaeil *et al.*, 2004), hepatoprotective (Chaudary *et al.*, 2012), anti-inflammatory (Singh *et al.*, 1982), tuberculostatic (Sharma, 1990), anti-cancer and antioxidant properties (Kamal and Jawaid, 2010).

MATERIALS AND METHODS

Bacterial cultures: Bacterial cultures used in the present study were clinical isolates collected from Ma-o-Shishu Hospital, Chittagong and Bnagldesh. The cultures comprise of five Gram negative bacterial isolates namely *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella sonnei* and three Gram positive bacterial isolates namely *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Maintenance of bacterial cultures: All the bacterial isolates were cultured and maintained in LB (Luria Bertani) medium (1% Tryptone, 1% sodium chloride, 0.5% yeast extract) during all the experiments of the study until mentioned. The bacterial cultures were refreshed fortnightly.

Plant material: The dried leaves of *Lawsonia inermis* (Henna) used in the present study were collected from the local market of Chittagong, Bangladesh.

Preparation of plant extract: Four different types of extracts including methanol extract, aqueous extract, chloroform extract and acetone extract were prepared to determine the *in vitro* antimicrobial activity of *Lawsonia inermis*.

For the preparation of extracts, dried leaves of Henna were ground to fine powder mechanically in electric grinder. Powdered leaves (10 g) were added in four flasks of 100 mL volume and 50 mL of each solvent was added to each flask separately. The flasks were kept in incubator at 37°C overnight with shaking at 180 rpm. The contents of flask were first filtered through four layers of muslin cloth and then through Whatman filter paper. The filtrate was evaporated in rotary evaporator at 50°C. The weight of residues was recorded. The DMSO (Dimethyl sulfoxide) was used

to dissolve the residues of methanol, acetone and chloroform extracts while aqueous extract residues were dissolved in distilled water at different concentrations. The resulting extracts were stored at 4°C for further use in experiments.

Inoculum preparation: Ten milliliter of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria of the experimental species were added into the tube and vortex was done. The turbidity of this tube is comparable with Mcfarland 0.5 standard solution (contain 99.5 mL of 1% sulfuric acid and 0.5 mL of 1.175% barium chloride) which provides a bacterial suspension containing 1.5×10^6 CFU mL^{-1} formerly NCCLS (1999).

Antimicrobial sensitivity test using disc diffusion method: The assay of antimicrobial activity of *Lawsonia inermis* extracts was performed by Kirby-Bauer's disc diffusion method (Kirby-Bauer, 1996). Disc impregnated with DMSO were used as control. The diameter of zones of inhibition formed was measured in millimeters. The test was performed in triplicate with each bacterial isolates and mean zone of inhibition was recorded.

Determination of Minimum Inhibitory Concentration (MIC): MIC of four different extracts of *Lawsonia inermis* was determined against the test bacterial cultures using the method described by Natta *et al.* (2008) with slight modifications. Briefly, starting from highest concentration of each extract of *Lawsonia inermis* serial dilution were prepared ranging from 15-500 mg mL^{-1} for methanol extract, 2.2-75 mg mL^{-1} for chloroform extract, 20.50-650 mg mL^{-1} for aqueous extract and 2.30-74.5 mg mL^{-1} for acetone extract. The DMSO was used as diluent for all extract except for aqueous extract where water was used instead of DMSO. Sterile discs were dipped in different dilutions for 1 min and placed on LB agar plates seeded with each bacterial culture separately. The whole experiment was performed under aseptic conditions. Plates were then incubated at 37°C for 16 h. The minimum concentration of each extract with clear zone of inhibition was considered as MIC. The zone of inhibition in each case was measured as the diameter of the clear zone and results were recorded. Each experiment was performed in triplicate.

Phytochemical analysis: The extracts of *Lawsonia inermis* prepared in the present study were screened for phytochemicals including carbohydrates, cardioglycosides, terpenoids, tannins, phenolic compounds, proteins and quinones by the following phytochemical analysis (Thenmozhi *et al.*, 2010).

- **Carbohydrates:** The 1 mL of each of four different extracts was taken in test tubes separately and treated with 5 mL of Fehling's solution (Solution A: 34.6 g of copper (II) sulfate pentahydrate dissolved in 500 mL distilled water, Solution B: 125 g of potassium hydroxide and 173 g of potassium sodium tartrate tetrahydrate dissolved in 500 mL of distilled water, combine solution A and solution B (1:1) just before use). The test tubes were placed in boiling water bath for 5 min. The tubes were observed for appearance of yellow or red color precipitates indicating the presence of reducing sugars
- **Terpenoids:** The 5 mL of each of the four extracts was taken in test tubes separately and mixed with 2 mL of chloroform. Concentrated sulphuric acid was added to form a layer. Test tubes were observed for the appearance of reddish brown colour at the interface

- **Proteins:** The 1 mL of each extract was taken in test tubes separately. The 2 drops of freshly prepared 0.2% ninhydrin reagent (2.5 g of ninhydrin dissolved in 50 mL n-butyl alcohol on mild heating and stirring and diluted 500 mL with n-butyl alcohol) were added. Test tubes were heated for few minutes. Test tubes were observed for the appearance of blue color
- **Cardioglycosides:** The 5 mL of each of the four extracts was taken in test tubes separately and treated with 2 mL of glacial acetic acid having a drop of ferric chloride solution. The 1 mL of the concentrated sulphuric acid was added to each test tube. Test tubes were observed for the appearance of brown coloured ring at the interface indicating the presence of cardioglycosides
- **Phenolic compounds:** The 1 mL of each extract of *Lawsonia inermis* was mixed with 4 drops of ethanol and 3 drops of 0.1% ferric chloride solution in test tube separately. Test tubes were observed for the appearance of red color
- **Tannins:** The 2 mL of each extract of *Lawsonia inermis* was mixed with few drops of 0.1% ferric chloride solution in test tubes separately. Test tubes were observed for the appearance of brownish green colour
- **Quinones:** Few drops of 1 N sodium hydroxide solution were mixed with 1 mL of each extract of *Lawsonia inermis* in test tubes separately. Test tubes were observed for the appearance of red colour indicating the presence of quinones

Time-kill kinetic analysis: The rate of bacterial killing was determined using *Lawsonia inermis* extracts with least MIC value for each bacterial isolate by time-kill kinetic assay as described by Miyasaki *et al.* (2013) with slight modifications. Briefly, overnight bacterial cultures were diluted to the 1.5×10^6 CFU mL⁻¹ with LB broth supplemented with 1×MIC of *Lawsonia inermis* extract for each bacterial isolate. The cultures were grown at 37°C with agitation at 160 rpm. The aliquots of cultures were collected at different time intervals (0, 1, 6, 12, 24, 36 and 48 h), serially diluted in LB broth and plated onto LB agar plates. After incubating the plates at 37°C for 16 h viable colonies were enumerated. The results were recorded in terms of log₁₀ CFU and plotted vs. time for each bacterial isolate.

Toxicological evaluation of *Lawsonia inermis* extract: The experiments of acute toxicity in animals were performed in Chittagong Vatanary and Animal Science University, Chittagong, Bangladesh. Each of the two groups comprised of 10 male albino mice (weighing 150-250 g) were used to evaluate the acute toxicity of *Lawsonia inermis* ethanol extract. The animals were housed in cages and served with proper diet according to the international standards. One group was administered with ethanol extract of *Lawsonia inermis* (300 mg kg⁻¹) and other group with equal volume of vehicle (DMSO) daily through subcutaneous route for 2 weeks by subcutaneous injection. The animals were continuously observed for signs of toxidromes such as aggression, sedation, rising fur, increased respiration, altered cardiac rate, excitation, convulsion, stupor, vomiting, etc. or death in first 2 h and then after 24 h.

Statistical analysis: Values are mean of Mean±Standard deviation standard deviation of three triplicates.

RESULTS

Dried leaves of *Lawsonia inermis* were used to prepare the extracts as it has been reported that dried preparation have more concentrated active phytochemical compounds than fresh plant material (Romero *et al.*, 2005). The results revealed that all extracts exhibited antimicrobial

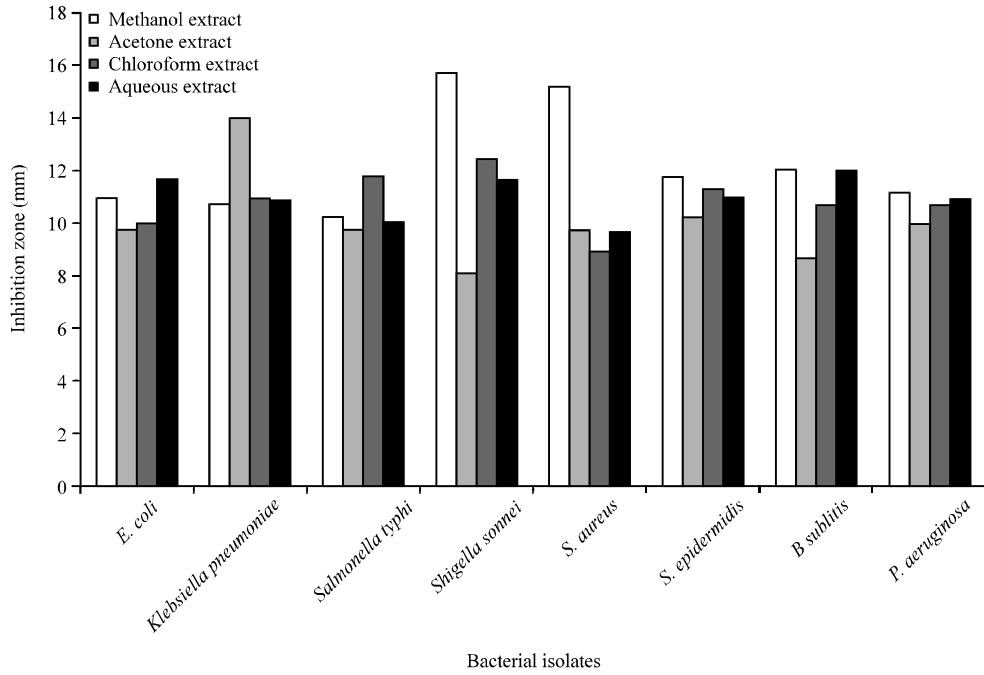


Fig. 1: Zone of inhibition (mm) of *Lawsonia inermis* extracts against tested bacterial isolates

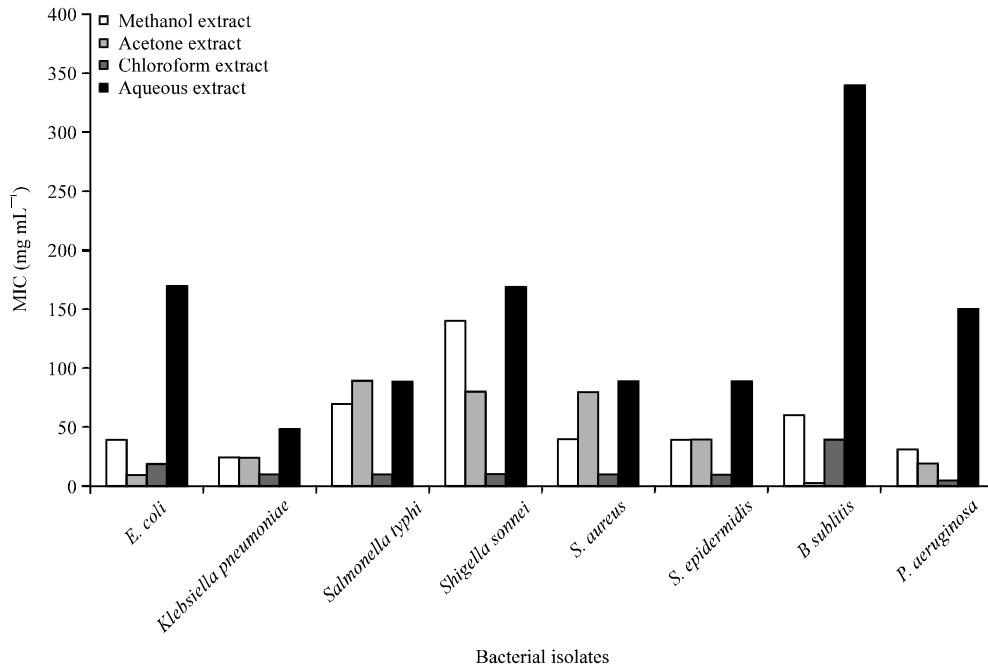


Fig. 2: Minimum Inhibitory Concentration (MIC) of different extracts of *Lawsonia inermis* against tested bacterial isolates

activity against all bacterial isolates. However, bacterial isolates showed differential sensitivity for each extract (Fig. 1). Antimicrobial activity was not observed with controls (DMSO and water).

The values of MIC for each tested bacterial strain had been shown in Fig. 2. From the data in Fig. 2, it was illustrated that all of the tested bacterial isolates showed minimum value

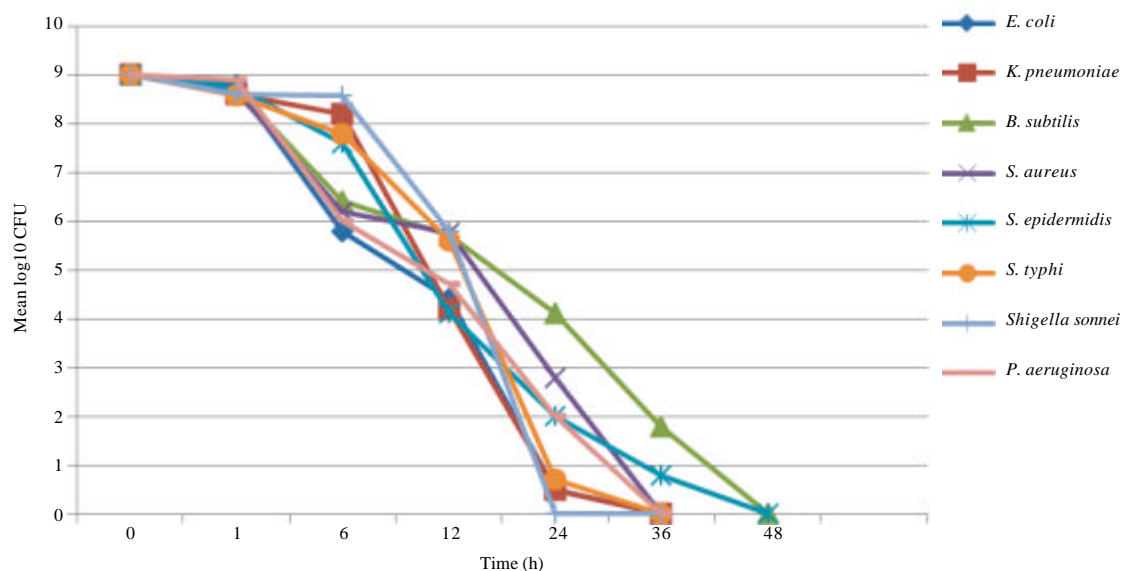


Fig. 3: Time-kill kinetic analysis of tested bacterial isolates

Table 1: Phytochemical analysis of different extracts of *Lawsonia inermis*

Phytochemicals	Different extracts of <i>Lawsonia inermis</i>			
	Methanol	Chloroform	Acetone	Aqueous
Cardioglycosides	+	+	+	+
Terpenoids	+	-	+	+
Carbohydrates	+	+	+	+
Proteins	-	-	-	-
Phenols	+	+	+	+
Quinones	+	+	+	+
Tannins	+	+	+	+

of MIC for chloroform extract except *E. coli* and *B. subtilis*. The minimum MIC value of *E. coli* (9.50 mg mL⁻¹) and *B. subtilis* (3 mg mL⁻¹) was observed using acetone extract.

Phytochemical analysis was performed to identify the metabolites present in different extracts of *Lawsonia inermis*. The data in Table 1 depicted that cardioglycosides, terpenoids, carbohydrates, phenols, quinones and tannins were present in methanol, acetone and aqueous extracts. While proteins were absent in all three of these extracts. The chloroform extract had cardioglycosides, carbohydrates, phenols, quinones and tannins while proteins and terpenoids were absent. These metabolites solubilized in solvent on the bases for polarity.

The rate of bacterial killing after exposure to the 1×MIC of respective extract of *Lawsonia inermis* for each isolate is summarized in Fig. 3. The time required to achieve 3log₁₀ decrease in CFU is an acceptable index of bactericidal activity from time-kill analysis (NCCLS, 1999). The results illustrated that not a single bacterial isolate showed significant bactericidal activity in first hour. Whereas, 3log₁₀ reduction in viability of all tested bacterial isolates was observed after 12 h of exposure. The log₁₀ CFU of all bacterial isolates was reduced to zero after 24 h of exposure except *S. epidermidis* (48 h) and *B. subtilis* (48 h).

DISCUSSION

According to the study of Papageorgiou *et al.* (1999), phytochemical constituents of *Lawsonia inermis* exhibit antimicrobial activity only against gram positive bacteria while ineffective for gram negative bacteria. In this study, we were interested to note that *Lawsonia inermis* had antimicrobial activity against both gram positive (*S. aureus*, *B. subtilis* and *S. epidermidis*) and gram negative (*E. coli*, *S. typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Shigella sonnei*) bacteria. The study of Bhuvaneshwari *et al.* (2002), Habbal *et al.* (2005) and Hussain *et al.* (2011) support our findings of present study.

The MIC values of our study were less than the MIC values reported by Al-kurashy *et al.* (2011). They found MIC values in the range of 8-64 mg mL⁻¹ for aqueous extract and 32-64 mg mL⁻¹ for alcoholic extract of *Lawsonia inermis* against *E. coli*, *S. aureus*, *P. aeruginosa* and *E. faecalis*. It was established that chloroform extract of *Lawsonia inermis* was more promising antimicrobial agent for *S. aureus*, *S. epidermidis*, *S. typhi*, *Klebsiella* spp. and *Shigella* while its acetone extract for *E. coli* and *B. subtilis* at least in *in vitro* antimicrobial assay.

The secondary metabolites mainly attribute the antimicrobial activity of plants (Gonzalez-Lamothe *et al.*, 2009). Among these secondary metabolites, the active constituents include phenolic compounds and tannins (Haslam, 1996). In most of the plant materials, water soluble components includes starches, tannins, saponins, polypeptides, terpenoids, lectins and different ions (Darout *et al.*, 2000) while alcoholic extract includes flavonol, alkaloids, tannins, sterols polyphenols etc. (Ivanovska *et al.*, 1996). Lawsone (2-hydroxynaphthoquinone), mucilage, mannite, gallic acid and tannic acid are main chemical constituents of henna (Al-Rubiay *et al.*, 2008). From plants, about 25% of all medicines available in the market have been derived directly or indirectly (De Smet, 1997; WHO, 2005). Herbal medicines are generally believed as safe. However, to avoid fatal consequences, it is important to evaluate their biological safety before use (Kunle *et al.*, 2012). There is no doubt in pharmacological properties of *Lawsonia inermis* but its toxicological assessment is also indispensable. *In vivo* acute toxicity of *Lawsonia inermis* extracts was checked in mice. No mortality was observed during the study. All the signs of toxidrome were negative.

CONCLUSION

In conclusion, the present study provides the scientific rationale for medicinal use of *Lawsonia inermis*. As substitute antimicrobial agent in therapeutics, the use of *Lawsonia inermis* extracts has great significance.

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REFERENCES

- Al-Kurashy, H.M.K., S.A. Al-windy and A.K. Al-Buhadilly, 2011. Evaluation of the antimicrobial activity of *Lawsonia inermis*: *In vitro* study. Iraqi J. Sci., 52: 16-19.
- Al-Rubiay, K.K., N.N. Jaber, B.H. Al-Mhaawe and L.K. Alrubaiy, 2008. Antimicrobial efficacy of henna extracts. Oman Med. J., 23: 253-256.

- Ashnagar, A. and A. Shiri, 2011. Isolation and characterization of 2-hydroxy-1, 4-naphthoquinone (lawsone) from the powdered leaves of henna plant marketed in Ahwaz city of Iran. *Int. J. Chemtech Res.*, 3: 1941-1944.
- Bailey, L.H. and E.Z. Bailey, 1976. *Hortus Third: A Concise Dictionary of Plants Cultivated in the United States and Canada*. Macmillan, New York, Pages: 1290.
- Bhuvaneshwari, K., S.G. Poongothai and A. Kuruvilla, 2002. Appala raju B: Inhibitory concentrations of *Lawsonia inermis* dry powder for urinary pathogens. *Indian J. Pharmacol.*, 34: 260-263.
- Blanks, T., S. Brown, B. Cosgrave, J. Woody, V. Bentley, N. O'Sullivan and N. Graydon, 1998. *The Body shop Book of Wellbeing Mind, Body and Soul*. Ebury Press, London, pp: 173-192.
- Chaudary, G.D., P. Poonia, P. Kamboj and A.N. Kalia, 2012. Hepatoprotective protection of *Lawsonia inermis* L. (seeds). *Int. J. Phytopharmacol.*, 3: 66-73.
- Cos, P., A.J. Vlietinck, D.V. Berghe and L. Maes, 2006. Anti-infective potential of natural products: How to develop a stronger *in vitro* proof-of-concept. *J. Ethnopharmacol.*, 19: 290-302.
- Cuong, N.X., P.T. Binh, M.T.T. Thuy, N.H. Nam and H.L.T. Anh *et al.*, 2009. Isolation of lawsone from lawsonia inermis leaves and synthesis of its dimer derivative by cyclic voltammetry. *J. Chem.*, 47: 228-232.
- Darout, I.A., A.A. Christy, N. Skaug and P.K. Egeberg, 2000. Identification and quantification of some potentially antimicrobial anionic components in miswak extract. *Indian J. Pharmacol.*, 32: 11-14.
- De Smet, P.A., 1997. The role of plant-derived drugs and herbal medicines in healthcare. *Drugs*, 54: 801-840.
- Gonzalez-Lamothe, R., G. Mitchell, M. Gattuso, M. S. Diarra, F. Malouin and K. Bouarab, 2009. Plant antimicrobial agents and their effects on plant and human pathogens. *Int. J. Mol. Sci.*, 10: 3400-3419.
- Habbal, O.A., A.A. AlJabri, A.H. El-Hug, Z.H. Al-Mahroopi and N.A. Al-Hashmi, 2005. In-vitro antimicrobial activity of *Lawsonia inermis* Linn henna a pilot study on the Omani henna. *Saudi Med. J.*, 26: 69-72.
- Haslam, E., 1996. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *J. Nat. Prod.*, 59: 205-215.
- Hussain, T., M. Arshad, S. Khan, H. Sattar and M.S. Qureshi, 2011. *In vitro* screening of methanol plant extracts for their antibacterial activity. *Pak. J. Bot.*, 43: 531-538.
- Ivanovska, N., S. Philipov, R. Istatkova and P. Georgieva, 1996. Antimicrobial and immunological activity of ethanol extracts and fractions from *Isopyrum thalictroides*. *J. Ethnopharmacol.*, 54: 14-15.
- Kamal, M. and T. Jawaid, 2010. Pharmacological activities of *Lawsonia inermis* Linn. A review. *Int. J. Biomed. Res.*, 2: 62-68.
- Kirby-Bauer, A., 1996. Antimicrobial sensitivity testing by agar diffusion method. *J. Clin. Pathol.*, 44: 493-493.
- Kunle, O.F., H.O. Egharevba and P.O. Ahmadu, 2012. Standardization of herbal medicines: A review. *Int. J. Biodivers. Conserv.*, 4: 101-112.
- Mikhaeil, B.R., F.A. Badria, G.T. Maatooq and M.M. Amer, 2004. Antioxidant and immunomodulatory constituents of henna leaves. *J. Biosci.*, 59: 468-476.

- Miyasaki, Y., J.D. Rabenstein, J. Rhea, M.L. Crouch and U.M. Mocek *et al.*, 2013. Isolation and characterization of antimicrobial compounds in plant extracts against Multidrug-resistant *Acinetobacter baumannii*. PloS one, Vol. 8. 10.1371/journal.pone.0061594
- Muhammad, H.S. and S. Muhammad, 2005. The use of *Lawsonia inermis* linn. (henna) in the management of burn wound infections. Afr. J. Biotechnol., 4: 934-937.
- NCCLS, 1999. Methods for determining bactericidal activity of antimicrobial agents; Approved guideline. Vol. 19, No. 18, Approved Guideline M26-A, Clinical and Laboratory Standards Institute, Wayne, PA., USA., September 1999. http://shopping.netsuite.com/c.1253739/site/Sample_pdf/M26A_sample.pdf
- Natta, L., K. Orapin, N. Krittika and B. Pantip, 2008. Essential oil from five *Zingiberaceae* for anti food-borne bacteria. Int. Food Res. J., 15: 337-346.
- Papageorgiou, V.P., A.N. Assimopoulou, E.A. Couladouros, D. Hepworth and K.C. Nicolaou, 1999. The chemistry and biology of alkannin, shikonin and related naphthazarin natural products. Angewandte Chemie Int. Edn., 38: 270-301.
- Rahmoun, M.N., M. Benabdallah, D. Villemin, K. Boucherit, B. Mostefa-Kara, C. Ziani-Cherif and N. Choukchou-Braham, 2010. Antimicrobial screening of the Algerian *Lawsonia inermis* (henna). Der Pharma. Chemica, 2: 320-326.
- Romero, C.D., S.F. Chopin, G. Buck, E. Martinez, M. Garcia and L. Bixby, 2005. Antibacterial properties of common herbal remedies of the Southwest. J. Ethnopharmacol., 99: 253-257.
- Rostkowska, H., M.J. Nowak, L. Lapinski and L. Adamowicz, 1998. Molecular structure and infrared spectra of 2-hydroxy-1,4-naphthoquinone; Experimental matrix isolation and theoretical Hartree-Fock and post Hartree-Fock study. Spectrochimica Acta Part A: Mol. Biomol. Spectrosc., 54: 1091-1103.
- Sharma, K., 1990. Tuberculostatic activity of henna (*Lawsonia inermis* Linn). Tubercle, 71: 293-295.
- Singh, S., N.M. Shrivastava, N.T. Modi and A.Q. Saifi, 1982. Anti-inflammatory activity of *Lawsonia inermis*. Curr. Sci., 51: 470-471.
- Srivastava, J., J. Lambert and N. Vietmeyer, 1996. Medicinal plants: An expanding role in development. World Bank Technical Paper No. WTP 320, April 1996, The International Bank for Reconstruction and Development/The World Bank, Washington, DC., USA., pp: 1-36.
- Symsudin, I. and H. Winarno, 2008. The effect of Inai (*Lawsonia inermis* L.) leaves extract on blood sugar level: An experimental study. Res. J. Phrmacol., 2: 20-23.
- Thenmozhi, M., R. Sivaraj and R. Yadav, 2010. A comparative phytochemical analysis of alstonia scholaris, lawsonia inermis, ervatamia divaricata and asparagus racemosus. Int. J. Pharm. Res. Dev., 2: 86-91.
- Ushimaru, P.I., M.T.N. Da Silva, L.C. di Stasi, L. Barbosa and A. Jr. Fernandes, 2007. Antibacterial activity of medicinal plant extracts. Braz. J. Microbiol., 38: 717-719.
- Varghese, K.J., K.S. Silvipriya, S. Resmi and C.I. Jolly, 2010. *Lawsonia inermis* (henna): A natural dye of various therapeutic uses: A review. Inventi Impact: Cosmeceuticals.
- WHO, 2005. WHO Global Atlas of Traditional, Complementary and Alternative Medicine. WHO Centre for Health Development, Kobe, Japan, ISBN-13: 9789241562867, Pages: 314.
- Warrier, P.K., V.P.K. Nambiar and C. Ramakutty, 1995. Indian Medicinal Plants: A Compendium of 500 Species. Vol. 4, Orient Longman Publication, Hyderabad, India.