

Identification of Phytoconstituents in *Lawsonia inermis* Linn. Leaves Extract by GC-MS and their Antibacterial Potential

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ABSTRACT

Background: Plant extracts contains multiple active constituents which leads to the production of new drugs from plants and chemicals derived from various parts of plants. The objective of present study was to investigate the GC-MS analysis and antibacterial activity of *L. inermis* leaves extracts. **Material and Methods:** Crude methanol extract and its fractions were tested for the presence of active phytochemicals and GC-MS analysis of hexane; ethyl acetate and aqueous methanol fractions was performed. Antimicrobial activity against six bacterial strain's *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella* and *Pseudomonas aeruginosa* was also tested. **Results:** Phytochemical screening of extract confirmed the presence of carbohydrates, glycosides, quinones, steroids and phenol. In GC-MS chromatograms, 56, 108 and 19 peaks were obtained and out of these, 13, 17 and 7 compounds were identified in hexane, ethyl acetate and aqueous methanol fractions, respectively. **Conclusion:** For best of our knowledge in *L. inermis* leaves extract, Celidoniol and Vitamin E has not been reported earlier in hexane fractions. While 2, 3 dihydrobenzo furan, 1-H indole -1, 3 (2H)-dione, 1 (3H)-Isobenzofuranone, 1H Isoindole-1, 3 (2H) Dione, Napthelene, 2 ethoxy, 2 (4H) Benzofuranone, Vitamin E, Benzene, 1 isocyano 4 methyl are also identified for the first time in ethyl acetate fraction. Also, in aq. Methanol fraction 1(3H)-isobenzofuranone, Squalene and Vit E were not previously identified. Highest antibacterial activity was confirmed in crude methanol extract which might be due to all the antibacterial compounds present in its fractions. The present study helped in identifying phytoconstituents present in the extract and its fractions which are responsible for various biological and antibacterial activities.

Key words: *Lawsonia inermis* Linn., Methanol extract, Lawsone, 1,4 Napthelenedione, Antimicrobial activity, Medicinal plants.

INTRODUCTION

Since the beginning of human civilization plants are used by mankind for their therapeutic values.¹ The books on the ayurvedic medicine such as 'Susruta samhita' and 'Charak samhita' refer to the use more than 700 herbs, which are now becoming an important part of daily life despite the progress in modern medicinal and pharmaceutical research.²⁻³ Plant extracts contains multiple active constituents, unlike modern medicine that invariables comprise single active ingredients, presenting herbal cocktail showing synergistic and less side effect. It leads to the production of new drugs from plants and chemicals derived from various parts of plants.⁴⁻⁵ A wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavanoids are present in plants, which show antimicrobial properties,⁶⁻¹⁰ resulting their use as ethno medicine in different countries around the world.¹¹⁻¹³

Indian continent is blessed with 120 families and 130000 species of plants. Many of these are known to have medicinal properties. From historical time, various parts of these plants have been used in treatment of

communicable as well as non-communicable diseases. However, the bioactive phytoconstituents contributing to antimicrobial properties are yet to be discovered.¹⁴ As from the above, it can be easily understood that some medicinally and pharmacologically important active ingredients play an important role in the biological activities like antibacterial activity of plants. Herbals are a rich source of active ingredients and can be safer and cost effective treatment for skin diseases ranging from rashes to dreadful skin cancer.¹⁵ To well document their role, several researchers are investigating the biological activities of several plants, which are leading to the development of many synthetic antibiotics.¹⁶

Genus *Lawsonia* have only one sps. *Lawsonia inermis* Linn. common name Henna/Mehndi.¹⁷⁻¹⁸ Prominent areas for the growth of *L. inermis* Linn. are tropical regions of Asia, America and Africa.⁸⁻¹⁹ This plant is mignonette tree²⁰ belong to Kingdom- Plantae, Division- Angiosperm, Class- Dicotyledon, Order- Myrtales, Family- Lythraceae, Genus- Lawsonia,

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Species- *L. inermis*.²¹⁻²² Microscopic evaluation of *L. inermis* powders showed the fragment of mesophyll, fragment of parenchyma, epidermis layer with stomata, and the rosette crystal of calcium oxalate.²³ There is no much information about the physicochemical proprieties of henna's aromatic oil, plant parts gives yellowish liquid aromatic oil, from seed and leaves, brown or dark brown color and strong fragrance from the flowers, with 0.01-0.02 % of yield from flowers.²⁴

Earlier, this plant was used as hair dye and in cosmetics.²⁵ But now days, it is used as a medicinal plant.^{8,26} The leaves of this plant showed anti-inflammatory, antipyretic, analgesic, antifungal and antibacterial activity.²⁷⁻³¹ Lawsone (2-hydroxy naphthaquinone), mucilage, mannite, gallic acid and tannic acid were found to be the main chemical constituents which might be having a role in its medicinal properties.³²

Previous studies have shown that *L. inermis* Linn. exhibits antibacterial effect only against gram positive bacteria³³ but later antibacterial effect was also observed against gram negative bacteria.³⁴ Considering this, an attempt has been made to investigate the phytochemical screening and antibacterial activity of crude methanol extract and different solvent fractions of *L. inermis* Linn. leaves.

MATERIALS AND METHODS

Collection of plant material and authentication

L. inermis Linn. plant leaves were collected from G.L.A. University campus, Mathura and were authenticated by Dr. (Mrs.) A. S. Upadhye (Voucher no. L-081), Botany group, Plant Science Division, Agharkar Research Institute, Pune. Leaves were washed thoroughly with tap water then rinsed with distilled water three times. After it leaves were shade dried, coarsely powered and packed in airtight bottle for the preparation of extract.

Preparation and Fractionation of crude methanol extract

Method as described by Muhi et al.³⁵ was used with slight modifications for the preparation and fractionation of crude methanol extract of *L. inermis* leaves. In brief, 15 g of leaves dry powder was soaked in 250mL of methanol for one week with occasional shaking. At the end of the week, the extract was filtered using a Whatmans No.1 filter paper and crude methanol extract was evaporated to dryness at 40°C in oven. This extract was fractionated by using solvents of increasing polarity. Extract in 90% methanol was taken in to a separating funnel, and equal volume of hexane was mixed to it. Upper hexane layer was separated from lower methanol layer. Now, upper hexane layer was dried in oven at 40°C and methanol layer was further separated into ethyl acetate and aqueous methanol layer by adding equal volume of ethyl acetate and distilled water. Both the layers were dried in hot air oven at 40°C.

Preliminary phytochemical screening of plant leaves extract and its fractions

Crude methanol extract and its fractions were tested for the presence of active phytochemicals such as alkaloids, carbohydrates, saponins glycoside, flavanoids, triterpenoids and proteins by standard procedures as described by Debela.³⁶ Mayer's test, Hager's test and Dragendorff's test were performed for alkaloids, Legal's test for identifying glycosides while ferric chloride test to determine the presence of tannins and polyphenolic compounds. Ninhydrin and Biuret test were used to detect proteins and flavonoids were tested through alkaline tests. Furthermore, steroids were identified through Salkowski test and the presence of carbohydrates was done through Biuret and fehling's test.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The GC-MS was performed by using Agilent 7683 series model and the software used is Chem. Station software updated with Mass productivity software. The fused silica column was packed with HP-5MS (5% Phenyl-methylsulphoxane) of 30m x 250 µm x 0.25µm dimensions. The oven temperature was started from 50°C with an increase of 3°C/min up to 240°C with holding time of 5 min. Temperature further enhanced gradually at 15°C/min up to 270°C with holding time of 40 min. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. An aliquot of 1µl of sample was injected into the column at injector temperature 270°C with split ratio of 20:80. The ionizing energy of 70 eV was used for electron ionization of molecules. The mass range is 30-550amu. Total GC running time was 90 min. The identification was done with the help of Wiley 2010 library.

Bioassay studies

Test microorganisms

Antimicrobial activity against six bacterial strain's *Escherichia coli* (MTCC No. 40), *Staphylococcus aureus*, *Bacillus subtilis* (MTCC No. 10619), *Salmonella typhi* (MTCC No. 3231), *Klebsiella* and *Pseudomonas aeruginosa* (MTCC No. 424) was tested. All the strains were biochemically tested, identified and authenticated.

Determination of bacterial cell count

Each strain of bacteria was inoculated in nutrient broth for 24 h. After incubation of 24 h, nutrient broth was removed by centrifugation and bacterial pellet was reconstituted in normal saline. Bacterial cell count was estimated by matching the turbidity of bacterial cell suspension with McFarland nephelometer.³⁷

Antibacterial assay

Antibacterial activity against six bacterial strains was determined by disc diffusion method as described by Parekh et al.³⁹ 5, 10 and 20 mg/disc of extract and its fractions were loaded on filter discs and were screened against six bacterial strains on nutrient agar plates. One negative control disc was also placed to nullify the effect of solvent on bacterial growth. Each bacterial strain was also screened for standard antibiotic disc which acted as positive control. After incubation of 24h at 37°C, the plates were observed for the presence of zones of inhibition as evidence of antibacterial activity. The degree of sensitivity was determined by measuring the diameter of visible zones of inhibition to the nearest millimetres with respect to each bacterial strain and extract concentration.

RESULTS

Extract preparation (percentage yield)

The physical characteristics of crude methanol extract of the *Lawsonia inermis* leaves and its different solvent fractions are shown in Table 1. The methanol extract was dark green in colour, oily in texture and percentage yield was 17%. Aqueous methanol fraction has 11.70% percentage yield which is higher as compare to hexane (0.66%) and ethyl acetate fraction (0.96%), indicating higher percentage of polar compound as compared to nonpolar.

The percentage recovery of the individual fraction was 4.0, 5.7 and 70.0% respectively. Approximately 20% of extract was lost during extraction and drying procedure. Hexane and aqueous methanol extract were oily whereas ethyl acetate extract was dry powder in texture. Hexane, ethyl acetate and aqueous methanol were greenish, brownish and reddish in colour respectively, the variation in colour was due to the difference in the solubility of different chemical constituents.

Preliminary Phytochemical screening of plant leaves extract and its fractions

The presence of different phytochemical constituents in the crude methanol extract and its fractions are shown in Table 2. Carbohydrates along with glycosides, quinones, steroids, phenol were present in the extract and its fractions while proteins were absent.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

GC-MS of Hexane fraction

The GC–MS chromatograms (Figure 1a, 1b and 1c) of the hexane fraction clearly showed peaks indicating the presence of phytochemical compounds

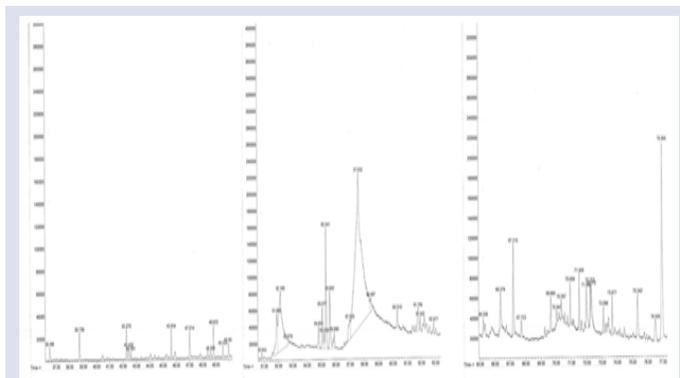


Figure 1: GC-MS Chromatograms of Hexane fraction of *L. inermis* leaves.

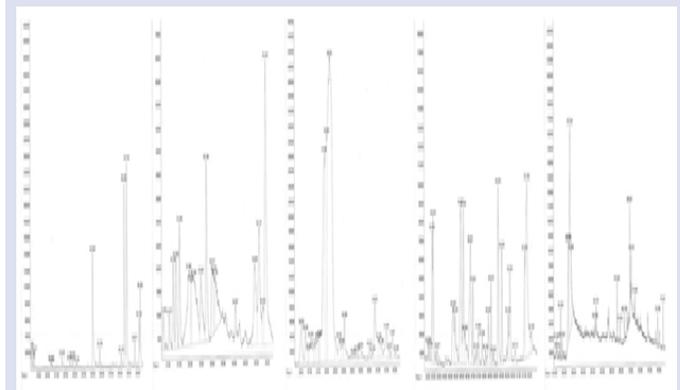


Figure 2: GC-MS Chromatograms of Ethyl acetate fraction of *L. inermis* leaves

and the chemical compounds identified are shown in Table 3. In GC-MS chromatogram 56 peaks were present. Out of these 13 peaks were identified and their biological properties were referenced from available literature (Table 3). In addition to these compounds, approximately 20 peaks were also obtained in the chromatogram. These peaks refer different compounds present in large quantities, like peak RT – 57.632 having area – 41.63, RT – 52.165 area- 9.47 but they were not identified by the library.

GC-MS of Ethyl acetate fraction

The GC–MS chromatograms of the ethyl acetate fraction clearly showed 108 peaks (Figure 2a, 2b, 2c, 2d and 2e) indicating the presence of large no. of phytochemical compounds. Their biological activities are indicated

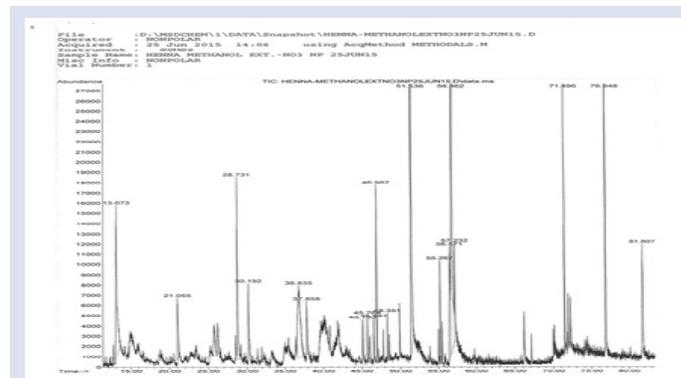


Figure 3: GC-MS chromatogram of aqueous methanol fraction of *L. inermis* leaves

Table 1: Percentage yield of *Lawsonia inermis* Linn. plant leaves extract.

| S.No. | Extract/ fraction of extract | Color | Texture | Percentage yield | Recovery yield |
|-------|------------------------------|----------|---------|------------------|----------------|
| 1. | Crude Methanol | Brownish | Oily | 17% | |
| 2. | Hexane | Greenish | Oily | 0.66% | 4% |
| 3. | Ethyl Acetate | Brownish | Dried | 0.96% | 5.7% |
| 4. | Aqueous methanol extract | Reddish | Oily | 11.7% | 70% |

Table 2: Qualitative analyses of Phytochemicals present in crude methanol extract and their different fractions of *Lawsonia inermis* Linn. plant leaves

| S. No. | Test used | (Crude methanol extract) | (Hexane fraction) | (Ethyl acetate fraction) | (Aqueous Methanol Fraction) |
|--------|----------------------|--------------------------|-------------------|--------------------------|-----------------------------|
| 1. | Alkaloids | +ve | +ve | +ve | +ve |
| 2. | Glycosides compounds | +ve | +ve | +ve | +ve |
| 3. | Flavonoids | -ve | +ve | +ve | +ve |
| 4. | Proteins | -ve | -ve | -ve | -ve |
| 5. | Carbohydrates | +ve | +ve | +ve | +ve |
| 6. | Steroids | +ve | +ve | +ve | +ve |
| 7. | Phenol | +ve | +ve | +ve | +ve |
| 8. | Quinones | +ve | +ve | +ve | +ve |

Key: - +ve = present, -ve = absent

Table 3: Chemical compounds identified in GC-MS of Hexane fraction with their biological properties

| S.No. | R.T. | Area | Compound Name | Biological activity |
|-------|--------|------|-------------------------------|---|
| 1. | 36.486 | 0.39 | Phenol | Antiseptic, Topical anaesthetic ³⁹ |
| 2. | 42.270 | 0.78 | Eicosane | Antitumor activity against human gastric SGC-7901 cell line ⁴⁰ |
| 3. | 45.634 | 0.67 | Nonadecane | No medicinal activity is known so far |
| 4. | 48.833 | 0.76 | Celidoniol | Anti inflammatory, antioxidant, wound healing activity ⁴¹ |
| 5. | 52.165 | 9.47 | Hexadecanoic acid | Antimicrobial activity ⁴² |
| 6. | 55.077 | 1.43 | Ethyl-9,12 octadecadienoate | Nematicide, Hepatoprotective, Anticoronary ⁴³ |
| 7. | 55.208 | 0.53 | 9-octadecanoic acid | Antioxidant, anti-inflammatory ⁴³ |
| 8. | 55.341 | 4.14 | 9,12,15-octadecatrienoic acid | Antioxidant, anti-inflammatory ⁴⁴ |
| 9. | 67.215 | 2.67 | 1,2 Benzenedicarboxylic acid | Antimicrobial activity ⁴² |

Table 4: Phytochemical compound identified in GC-MS of Ethyl acetate fraction of leaves of *L. inermis* and their biological activities

| S. No. | R.T. | Area | Compound Name | Biological activity |
|--------|--------------|-------|---------------------------------|---|
| 1. | 18.33 | 0.20 | Benzoic acid, methyl ester | No activity reported |
| 2. | 25.00 | 2.51 | 2,3 dihydrobenzo furan | Anti-inflammatory ⁵⁰ |
| 3. | 28.49 | 3.33 | 2-methoxy 4 vinyl phenol | Flavour and Perfumery ^{39,43} |
| 4. | 28.78 | 3.97 | Phthalic anhydride | Anticonvulsant activity ⁵¹ |
| 5. | 29.67 | 0.44 | 1-H indole -1,3 (2H)-dione | Antimicrobial and anticoagulant ⁵² |
| 6. | 30.15 | 0.71 | 1 (3H)-Isobenzofuranone | Antimicrobial, antifungal and anti-inflammatory ⁵³ |
| 7. | 37.83- 41.06 | 36.54 | 1,4 Naphthalenedione | Antimicrobial, antifungal, antiviral, insecticidal, anti-inflammatory, antipyretic ^{54,39} |
| 8. | 33.02-33.14 | 0.64 | 1,2,3-Benzenetriol | Antioxidant, antiseptic, antibacterial, antidermatic, fungicide, pesticide ^{39,43} |
| 9. | 34.05 | 0.07 | Phenol,2 methoxy-4 (2 Propenyl) | Antiseptic, topical anesthetic ³⁹ |
| 10. | 35.14 | 0.49 | 1H Isoindole-1, 3 (2H) Dione | Antibacterial, analgesic, anti-inflammatory, antipyretic ⁵⁵ |
| 11. | 36.77 | 0.86 | Naphthelene, 2 ethoxy | Antibacterial and antifungal ⁵⁶ |
| 12. | 46.19 | 1.28 | 2 (4H) Benzofuranone | Antimicrobial, antifungal and anti-inflammatory ⁵³ |
| 13. | 48.62 | 0.53 | 1,2 Benzene dicarboxylic acid | Antimicrobial activity ⁴² |
| 14. | 51.79 | 2.79 | Hexadecanoic acid | Antioxidant ^{57,47} |
| 15. | 55.31 | 0.39 | 9,12,15 octadecatrienoic acid | Antioxidant and anti-inflammatory ⁵⁸ |
| 16. | 76.90 | 0.44 | Vitamin E | Antioxidant ⁴⁹ |
| 17. | 28.13 | 0.125 | Benzene, 1 isocyno 4 methyl | Antibacterial and antifungal ⁵⁹ |

in Table 4. 1, 4 Naphthalenedione 2 hydroxy compound was present in highest concentration (36.54%).

GC-MS of Aqueous methanol fraction

In GC-MS (Figure 3) of aqueous methanol fraction, 19 peaks were obtained and out of which 7 compounds were identified. 9, 12-octadecadienoic acid ethyl ester was found in highest concentration. 1(3H)-isobenzofuranone, Squalene and Vit E were also identified in this fraction. Biological activities of identified phytochemical are shown in Table 5.

Bioassay studies

Table 6 showed antibacterial activity of methanol extract and their fractions at different concentrations against different bacterial strains.

The antibacterial activity was illustrated in terms of zone of inhibition (mm) formed against bacterial strains. Results are illustrated in Table 6. Hexane fraction showed least antibacterial response whereas ethyl acetate and aq. methanol fraction confirm more or less equal response against all bacterial strains. Extract and their fractions confirmed the effective response in dose dependent manner. Against all bacterial strain, positive control had highest antibacterial property as compared with extract and its fractions.

DISCUSSION

L. inermis is used in various cosmetic things such as dye from the earlier times but this plant also has good medicinal value due to the presence

Table 5: Biological activities of phytochemical compounds identified in GC-MS of aqueous methanol fraction of Leaves of *Lawsonia inermis*

| S. No. | Chemical compound | R.T. | Area | Biological Activity |
|--------|---------------------------------------|-------|-------|--|
| 1. | 4H-Pyran-4-one | 21.05 | 2.07 | Antimicrobial, ant proliferative and anti-inflammatory ⁶⁰ |
| 2. | 1,3-isobenzofurandione | 28.73 | 6.11 | Antimicrobial, antifungal and anti-inflammatory ⁵³ |
| 3. | 1(3H)-isobenzofuranone | 30.19 | 3.16 | Antimicrobial, antifungal and anti-inflammatory ⁵³ |
| 4. | n-Hexadecanoic acid | 51.53 | 15.03 | Antioxidant and Antimicrobial activity ^{42,47,57} |
| 5. | 9,12-octadecadienoic acid ethyl ester | 56.86 | 19.25 | Nematicide, Hepatoprotective, Anticoronary ^{39,43} |
| 6. | Squalene | 71.49 | 3.71 | Antioxidant, antitumor & immunostimulant ^{47,48} |
| 7. | Vit. E | 76.85 | 10.55 | Antioxidant activity ⁴⁹ |

of various phytochemicals which are responsible for various biological activities.²⁴ The present study was conducted to identify the phytochemical constituents responsible for their biological role and antibacterial activity in *L. inermis* leaves.

As shown in Table 1, the percentage yield of methanol extract of *L. inermis* was 17% while recovery yield of Hexane, ethyl acetate and methanol fractions were 4%, 5.7% and 70%, respectively. It indicates that approximately 20% was lost during the fractionation. Kawo and Kwa⁶¹ reported 1.8% ethyl acetate fraction yield which is lower than our report while recovery yield of aq. methanol fraction is higher (87.19%) than our reported value.

The result of phytochemical study confirmed the presence of various primary and secondary metabolites such as flavanoids, carbohydrates, quinones, steroids and phenols. Presence of these phytochemicals in *L. inermis* leaves extracts are responsible for various biological activities such as antibacterial, antioxidant, anti-inflammatory activities, etc.⁶² Most of the phytochemicals are present in extract and its fraction as shown in our study. Whereas protein was found to be absent in extract as well as in its fractions.

GC-MS analysis of different fractions of *L. inermis* revealed the presence of different phytochemical compounds. These phytochemical compounds have medicinal property as reported earlier. GC-MS analysis of hexane fraction showed 56 peaks based on retention time. Out of these, 13 phytochemical constituents were identified by GC-MS software library and most of them showed biological activity as evidences by literature (Table 3). For best of our knowledge, Celidoniol and Vitamin E has not been reported earlier in *L. inermis* leaves fractions. In the hexane extract, 5 chemical compounds were present in high concentration, out of which 2 were identified viz. Hexadecanoic acid (9.47%), Vit. E (7.41%) while 3 peaks which were not identified having % area i.e. 1.43%, 1.22% and 1.01% with RT- 55.07, 75.342 and 76.506 respectively.

In GC-MS of ethyl acetate fraction 108 peaks were observed and 17 phytochemical compounds were identified. Out of which 1, 4 Napthalenedione was identified as major constituent. Dama *et al.*⁶³ also reported this compound which is responsible for the antibacterial effect. This compound also has been reported in other literature and have antimicrobial, antifungal, antiviral, insecticidal, anti-inflammatory and antipyretic activities.^{39,64} Biological activities of other compounds are shown in Table 4. Benzoic acid, methyl ester was also identified in chromatogram, but no biological activity has been reported so far. Some phytoconstituents such as 2,3 dihydrobenzo furan, 1-H indole -1,3 (2H)-dione, 1 (3H)-Isobenzofuranone, 1H Isoindole-1, 3 (2H) Dione, Napthelene, 2 ethoxy, 2 (4H) Benzofuranone, Vitamin E, Benzene, 1 isocyano 4 methyl were also identified in this fraction for the first time and are not mentioned in earlier references. Ethyl acetate fraction had 5 peaks present in higher concentration. Out of these 4 peaks 1,4 Napthalenedione, 2 hydroxy

(36.54%), 2-methoxy 4 vinyl phenol (3.33%), Pthalic anhydride (3.97%), Hexadecanoic acid (2.79%) were identified and single peak present at RT- 57.31 was not identified having area 6.68% .

GC-MS analysis of aq. methanol fraction had 19 peaks and 7 compounds were identified. In this fraction 3 new compounds 1(3H)-isobenzofuranone, Squalene and Vit E were identified, and these compounds have biological properties. Methanol fraction had 5 major peaks based on % peak area viz {(9.44% and RT- 36.84), (8.53% and RT- 13.07), (5.06% and RT- 56.57), (3.25% and RT- 57.23) and (3.19% and RT- 46.98)} and all these major peaks were not identified.

Some common compounds such as Vitamin E and Hexadecanoic acid which were present in all fractions and has antioxidant activities.^{47,49,57} Phenol, 1, 2 Benzene carboxylic acid and 9,12,15 octadecatrienoic acid were present in hexane and ethyl acetate fractions and were absent in aq. methanol fraction. Furthermore, in aq. methanol and hexane fractions, Squalene and 9, 12-octadecadienoic acid ethyl ester were common but were not identified in ethyl acetate fraction. Common phytochemical compounds in methanol and ethyl acetate fractions were Pthalic anhydride and 1 (3H)-Isobenzofuranone.

As per Panchaksharam *et al.*⁶² *L. inermis* shows higher total phenolic content and possess a single peak for simple phenols as obtained from chromatog. Moreover, it exhibits maximum zone of inhibition on both g positive and g negative organisms. Since, the amount of phenolic compound was proportional to antimicrobial activity.

Different fractions of *L. inermis* were analyzed against 6 bacterial strains for antibacterial activity. These fractions showed antibacterial activity in dose dependent manner. Results are illustrated in Table 6. As shown in results, hexane fraction showed highest antibacterial activity against *E. coli* whereas moderate antibacterial activity was found against *Bacillus subtilis* and *Klebsella*. However, hexane fraction failed to show activity against *Staphylococcus citrus*, *S. typhi* and *Pseudomonas*. Hexane fraction showed least antimicrobial activity due to the presence of only 2-3 compounds responsible for antimicrobial activity such as Hexadecanoic acid. Sarojini *et al.*⁶⁶ also reported antibacterial activity of hexane fraction against *Staphylococcus*, *E. coli* and *Bacillus subtilis*.

Ethyl acetate fraction illustrated better results than hexane fraction. This fraction confirmed good activity against *Bacillus subtilis* and *E. coli* while moderate antibacterial activity was confirmed against other strains. This antibacterial activity might be due to the presence of 1, 4 Napthalenedione which was present in very high conc. Some researcher like Sarojini *et al.*⁶⁶ and Kawo and Kwa⁶³ demonstrated better antibacterial activity of ethyl acetate fraction as compare to hexane fraction which is similar to our results.

Aq. Methanol fraction demonstrated maximum zone of inhibition against *S. typhi* whereas this fraction had moderate antibacterial activity against other bacteria. However, it failed to show effective response

Table 6: Antibacterial activity of crude methanol extract and its fractions of *L. inermis*

| Bacterial Strain | Zone of inhibition (mm) | | | | | | | | | | | | | | +ve control | -ve control | | |
|------------------------------|--------------------------|------------|------------|-----------|------------|------------|------------|------------|---------------|------------|------------|------------|---------------------------------------|-----------|-------------|-------------|-----------|--|
| | Crude methanolic extract | | | | Hexane | | | | Ethyl acetate | | | | Fractions of crude methanolic extract | | | | | |
| | 5mg/disc | 10mg/disc | 20mg/disc | 5mg/disc | 10mg/disc | 20mg/disc | 5mg/disc | 10mg/disc | 20mg/disc | 5mg/disc | 10mg/disc | 20mg/disc | 5mg/disc | 10mg/disc | | | 20mg/disc | |
| <i>S. typhi</i> | 11.32±0.26 | 13.21±0.55 | 16.23±1.15 | 0 | 0 | 7.20±0.05 | 8.68±0.88 | 12.25±0.12 | 13.32±1.03 | 11.94±0.42 | 16.25±1.05 | 18.28±0.75 | 20.23±0.85 | 0 | 0 | | | |
| <i>E. coli</i> | 11.81±0.38 | 13.15±0.27 | 15.18±1.65 | 9.38±0.78 | 11.95±0.55 | 13.23±1.15 | 10.98±0.66 | 12.21±0.55 | 15.12±1.13 | 9.82±0.03 | 10.41±0.65 | 11.37±0.15 | 24.75±0.41 | 0 | 0 | | | |
| <i>Pseudomonas</i> | 10.26±0.54 | 13.51±0.14 | 14.51±0.45 | 7.95±0.28 | 7.77±0.65 | 8.11±0.75 | 10.00±0.01 | 11.20±0.05 | 13.23±1.15 | 7.18±1.65 | 8.51±0.45 | 11.00±1.98 | 24.95±0.76 | 0 | 0 | | | |
| <i>Klebsella</i> | 12.72±0.47 | 13.27±0.16 | 16.11±0.75 | 7.11±0.48 | 8.20±0.15 | 10.27±1.16 | 10.95±0.98 | 11.00±0.22 | 13.18±1.25 | 11.95±0.35 | 12.95±1.05 | 14.33±1.08 | 23.76±0.61 | 0 | 0 | | | |
| <i>Bacillus subtilis</i> | 10.63±0.63 | 13.11±0.75 | 15.27±1.98 | 8.00±0.11 | 80.17±0.22 | 11.11±0.75 | 11.37±1.44 | 14.44±0.52 | 16.00±1.05 | 10.75±0.85 | 12.65±1.00 | 14.73±0.08 | 22.22±0.41 | 0 | 0 | | | |
| <i>Staphylococcus citrus</i> | 7.27±0.23 | 8.95±0.05 | 10.00±1.75 | 0 | 8.75±0.41 | 8.95±0.98 | 6.95±0.76 | 8.38±0.78 | 10.95±0.28 | 6.11±0.48 | 7.00±0.11 | 9.89±0.77 | 23.00±0.01 | 0 | 0 | | | |

against *Staphylococcus citrus*. Antibacterial activity might be due to the presence of some phytoconstituents such as Hexadecanoic acid, 4H-Pyran-4-one, 1, 3-isobenzofurandione and 1(3H)-isobenzofuranone which are reported in our studies by GC-MS analysis.

Crude methanol extract showed highest antibacterial activity as compared to its fractions against most of the bacteria. It may be due to presence of all phytoconstituents which were present in its fractions. However, positive control was potent than all fractions.

Our study confirms the previous work on *L. inermis* leaves extracts and its fractions. According to our study, plant leaves extract and its fraction have various phytoconstituents exhibiting antimicrobial activity against gram +ve and gram -ve bacteria. It gives an idea that *L. inermis* contain various phytoconstituents which have effective antibacterial properties. In further studies *in-vivo* activity of this plant extract should be done, so that it can be used in human welfare as medicine for treating different diseases.

CONCLUSION

An attempt was made to identify the phytochemical constituents responsible for their biological role and antibacterial activity in *Lawsonia inermis* Linn. leaves extract. The result of phytochemical study confirmed the presence of various primary and secondary metabolites such as flavonoids, carbohydrates, quinones, steroids and phenols which are responsible for various biological activities. The present study also helped in identifying phytoconstituents present in the extract and its fractions which are responsible for various biological and antibacterial activities. Thus, the present study helped in identifying phytoconstituents present in the extract and its fractions which are responsible for various biological and antibacterial activities.

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CONFLICT OF INTEREST

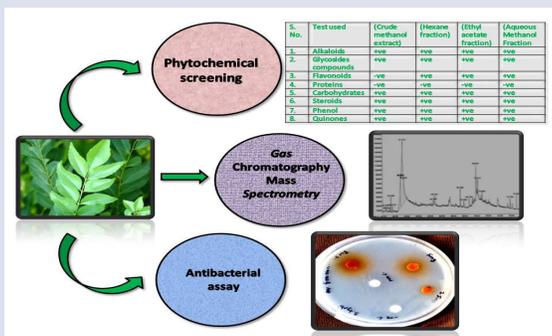
The Author(s) declare(s) that they have no conflicts of interest to disclose.

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GRAPHICAL ABSTRACT



SUMMARY

- *Lawsonia inermis* is a well-known ethno medicinal plant used cosmetically and medicinally for over 9,000 years. Its use in the Indian traditional folk medicines is well documented. It belongs to the Lythraceae family (a family of flowering plants that includes 620 species). It is the only plant species in the genus *Lawsonia*.
- Phytochemical screening of extract confirmed the presence of carbohydrates, glycosides, quinones, steroids and phenol. In GC-MS chromatograms, 56, 108 and 19 peaks were obtained and out of these, 13, 17 and 7 compounds were identified in hexane, ethyl acetate and aqueous methanol fractions, respectively.
- For best of our knowledge in *L inermis* leaves extract, Celidoniol and Vitamin E has not been reported earlier in hexane fractions. While 2, 3 dihydrobenzo furan, 1-H indole -1, 3 (2H)-dione, 1 (3H)-Isobenzofuranone, 1H Isoindole-1, 3 (2H) Dione, Napthelene, 2 ethoxy, 2 (4H) Benzofuranone, Vitamin E, Benzene, 1 isocyano 4 methyl are also identified for the first time in ethyl acetate fraction. Also, in aq. Methanol fraction 1(3H)-isobenzofuranone, Squalene and Vit E were not previously identified. Highest antibacterial activity was confirmed in crude methanol extract which might be due to all the antibacterial compounds present in its fractions. The present study helped in identifying phytoconstituents present in the extract and its fractions which are responsible for various biological and antibacterial activities.

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