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GC-MS analysis and antimicrobial activity of *Lawsonia inermis* L.

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Abstract
Lawsonia inermis L. also known as Henna occurs all over Indian Sub Continent. Traditional practitioners widely used the seeds, fruit and stem bark of this plant in treatment of Skin disease. The ethanol extract of fruit has been used to study activity against Fungal isolates *Candida albicans, Microsporum audouinii, Trichophyton rubrum, Trichophyton mentagrophytes* and the bacterial isolates of *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Bacillus pumilus* by disc diffusion method (Zone of Inhibition in mm at 100 µg / disc). It has been reported that fruit extract inhibited the growth of fungus *Trichophyton rubrum* and bacteria *Pseudomonas aeruginosa.* GC-MS analysis of ethanol extract of fruit of *Lawsonia inermis* revealed the presence of four chemical compounds i.e. N-Hexadecanoic acid, 9,12-Octadecanoic acid (Z, Z), 4, 4, 6a, 6b, 8a, 11, 11, 14b- Octamethyl 1, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a,14b- octadecahydro- 2Hpicen- 3- one, 12-Oleanen-3-yl acetate, (3a)-.

Keywords: ethnobotany, antimicrobial activity, zone of inhibition

Introduction
Ethno-botany deals with traditional and natural relationship between human societies and plants. The use of plant medicine has become the part of the art, traditions and culture of different communities which have developed from ancient civilization. The composition of different plant products, have been used since ages to cure diseases.

As time progressed it was proved that each plant has its own curative properties. The revival of interest in natural drugs, especially those derived from plants started in the last decades mainly because of the widespread belief that green medicines are healthier and safer than the synthetic ones.

To study the medical value of plants, the study of chemically active substances with a variety of structural arrangements and properties and the impact produced on a definite physiological action on the human body must be studied. Thus the present attempt was made for determination of chemical components and antimicrobial activity of ethanolic extract of *Lawsonia inermis* L. fruits.

Material and Methods
Solvent extraction of plant material
The material of study i.e., fruits were collected, washed and after complete shade drying the plant material was grinded. The extraction was done by using Soxhlet's extraction method with solvent ethanol.

1. Antimicrobial Activity
The extract was used for antimicrobial activity against pathogens e.g. Fungal isolates *Candida albicans, Microsporum audouinii, Trichophyton rubrum, Trichophyton mentagrophytes* and the bacterial isolates of *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Bacillus pumilus* by disc diffusion method (Zone of Inhibition in mm at 100 µg / disc).

2. GC-MS (Gas Chromatography and Mass Spectroscopy)
The samples were subjected to GC-MS analysis from Central Instrumentation Laboratory (CIL), Panjab University Chandigarh and results were obtained. GC-MS analysis of the samples were carried out using Perkin Elmerclarus 680 with mass spectrometer clarus 600 (EI) using TurboMass ver 5.4.2 Software with NIST – 2008 Library ver. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy 70 eV; a scan interval of 2 min and fragments from 50 to 600 Da. The chemical components form the different extracts of plants were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

Result and Discussion
1. Antimicrobial activity of Fruits extracts of *Lawsonia inermis* L.
Ethanol extracts showed positive microbial zone of inhibition of 9 mm against pathogen *Pseudomonas aeruginosa*. Also The zone of inhibition of 9 mm against pathogen *Trichophyton rubrum.* Ethanol extracts was found non reactive to other test organisms.

Table 1: Antimicrobial activity of fruits extracts of *Lawsonia inermis* L.

S. N.	Micro-organism	Ethanol
1	*Staphylococcus aureus*	00
2	*Streptococcus pyogenes*	00
3	*Pseudomonas aeruginosa*	9 mm
4	*Bacillus pumilus*	00
5	*Trichophyton rubrum*	9 mm
6	*Trichophyton mentagrophytes*	00
7	*Microsporangium audouinii*	00
8	*Candida albicans*	00

*Data represented in mean of three replicates.

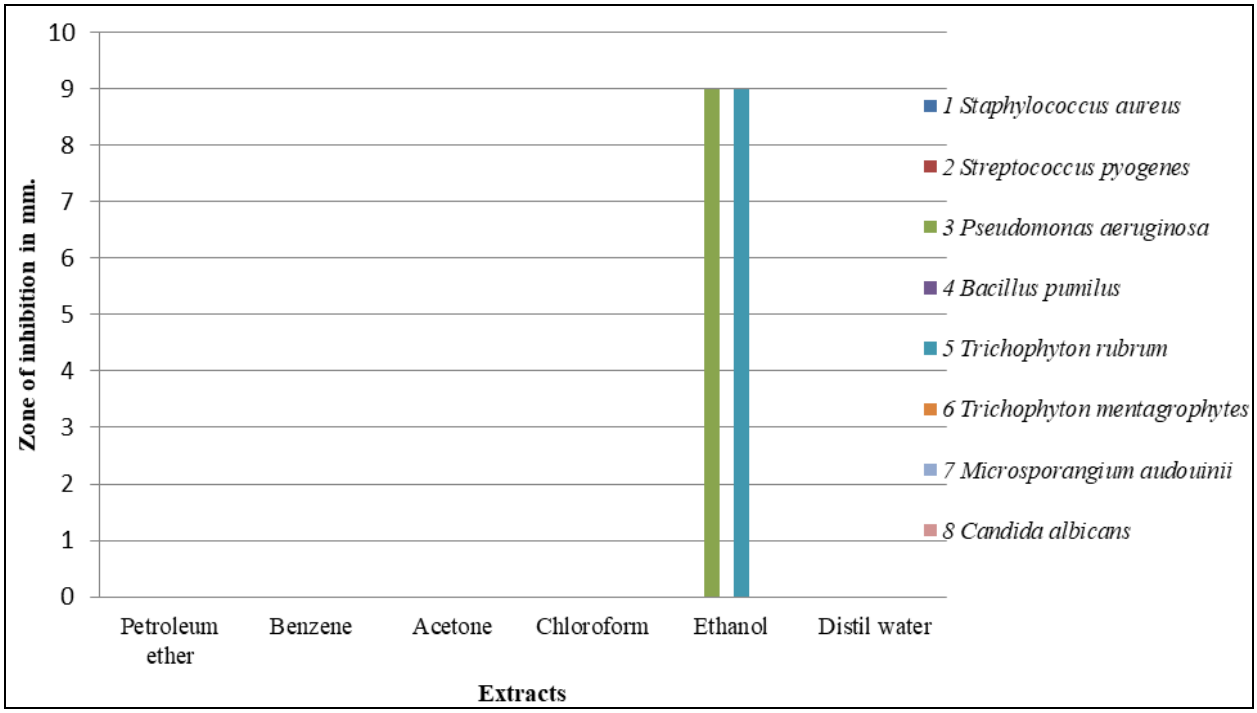


Fig 1: Analysis of antimicrobial sensitivity of fruits of *Lawsonia inermis* L.

2. GC-MS Analysis of *Lawsonia inermis* L.

The determination of the possible chemical components from fruits of *Lawsonia inermis* L. was carried out by GC-MS. On the basis of data obtained by GC-MS of Ethanol extract analysis revealed four peaks. These four peaks indicated the presence of four phytochemical compounds. The GC-MS chromatogram of the four peaks of the compound detected was shown in Fig.2.1. The four phytoconstituents were characterized and identified on comparison of the mass spectra of the constituents provided by NIST library. The Ethanol extract of *Lawsonia inermis* L. fruits analyzed by GC-MS shows the presence of compounds like N-Hexadecanoic acid, 9,12-Octadecanoic acid (Z, Z), 4, 4, 6a, 6b, 8a, 11, 11, 14b- Octamethy l 1, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b-octadecahydro- 2Hpicen- 3- one, 12- Oleanen- 3- yl acetate, (3a)-. The active principles with their retention time (RT), % peak area, Compound analyzed, molecular formula, probable structural formula and activity reported are presented in Table 2.1.

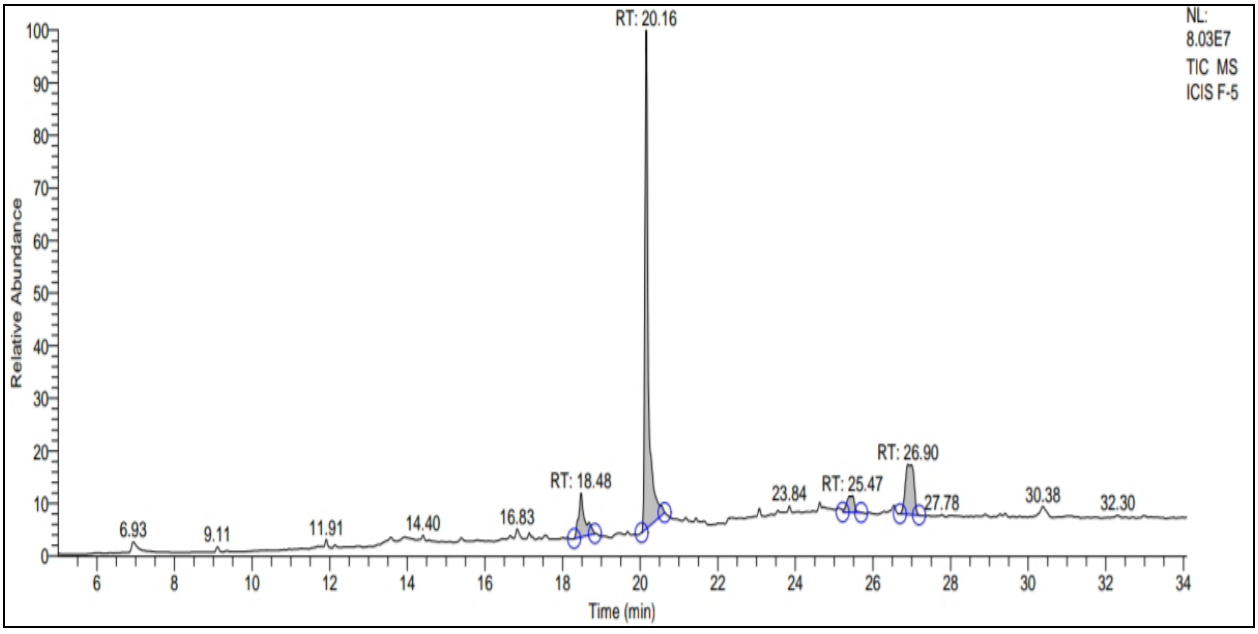
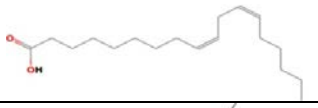
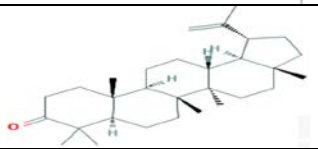
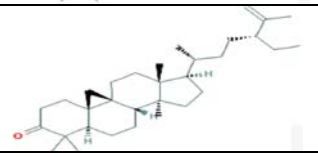


Fig 2: GC-MS chromatogram *Lawsonia inermis* L

The percentage of all the phyto-compounds present in the Ethanol extracts are listed in Table. The ethno-medicinal use of *Lawsonia inermis* L. fruits in the treatment of Skin disease may be attributed due to the presence of Phytocompounds and their activities shown in Ethanol extract (Table-2.1).

Table 2: GC-MS Analysis of *Lawsonia inermis* L.

Sr. No.	Retention Time	Peak area %	Compound Analyzed	Molecular formula	Probable Structural Formula	Activity reported
1	18.48	10.60	N-Hexadecanoic acid	$C_{16}H_{32}O_2$		Antiirritant, Antiinflammatory, Antiitching
2	20.16	67.83	9,12-Octadecanoic acid (Z,Z)	$C_{18}H_{32}O_2$		Antiinflammatory, Acnegenic,Skin lightening
3	25.47	4.87	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2Hpicen-3-one	$C_{30}H_{48}O$		Antifungal, Antiinflammatory
4	26.90	16.69	12-Oleanen-3-yl acetate, (3a)-	$C_{32}H_{52}O_2$		Antioxident

Conclusion

The present study incorporates antimicrobial activity and Gas chromatography and mass spectroscopic analysis of *Lawsonia inermis* L. The plant study was related to skin diseases. The plant species *Lawsonia inermis* L. (Fruits), ethanol extract was positive against pathogens *Trichophyton rubrum* and *Pseudomonas aeruginosa.* It is concluded that the pathogen *Pseudomonas aeruginosa* and *Trichophyton rubrum* were positive to plants which is used in skin diseases. N-Hexadecanoic acid, obtained from GC-MS analysis of plants used as anti-inflammatory, antiallergic, absorbent, demulcent, antiitch and antidermatic agents. This study would be precious and effective in cosmetology and treatment of skin diseases.

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