

## Antimicrobial activities and constituents of the leaf essential oil of *Lawsonia inermis* growing in Nepal

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### Abstract

The essential oil from the leaves of *Lawsonia inermis* L. (collected from Biratnagar, Nepal) was obtained by hydrodistillation and analyzed by GC-MS. A total of 40 compounds were identified in the oil, accounting for 100.0% of the oil. The majority of the essential oil was composed of (E)-phytol (27.5%), while the remainder of the essential oil was dominated by monoterpenoids including: limonene (20.0%), 1,8-cineole (6.9%), and linalool (7.0%). The oil was screened for antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Aspergillus niger* and showed marginal activity (MIC = 625 µg/mL).

Key words: *Lawsonia inermis*, essential oil composition, (E)-phytol, linalool, Nepal

## Introduction

*Lawsonia inermis* L. (Lythraceae), commonly known as “mehndi” or “henna”, is a perennial shrub (height 2-6 m) native to tropical and subtropical semi-arid climates in North Africa, South-West Asia, and northern Australasia. The leaves are small, sub-sessile and greenish brown to dull green in color, and have either a glabrous, obtuse or acute apex with a tapering base. Flowers are small of red or rose color (1-4).

Traditionally used in cosmetics, the leaf dye of *L. inermis* is used to stain hands, feet and nails with artistic patterns. However, *L. inermis* has also been used in ethnomedicine to treat various maladies including, but not limited to, arthritis, headaches, ulcers, diarrhea, leprosy, intestinal neoplasticity, jaundice, fever, leucorrhoea, diabetes, and smallpox (4-7).

Aside from traditional usage, extracts from various parts of *L. inermis* have shown antidiabetic activity (8-9), anti-oxidant activity (10), immunomodulatory effects (11), hepatoprotective activity (12), antimicrobial activity (13-14), cytotoxic activity (10), and protein glycation inhibitory activity (15). Secondary metabolites isolated from *L. inermis* are responsible for some of the activities listed above and include lawsone (2-hydroxy-1,4-naphthoquinone), hennatannic acid and olive green resin (16). The floral essential oil of *L. inermis* includes  $\alpha$ - and  $\beta$ -ionone as the major components (17-19).

To our knowledge, this is the first examination of the leaf essential oil of *L. inermis* from Nepal. The purpose of this investigation was to analyze the essential oil composition of *L. inermis* and evaluate its antimicrobial potential.

## Methods

### Plant material

The leaves of *Lawsonia inermis* were collected from the city of Biratnagar (26°28'N 87°16'E, 72 m above sea level) in the Morang district in the Koshi

Zone of Nepal on 18 May 2011. The plant was identified by Tilak Gautam, and a voucher specimen (1031) has been deposited in the herbarium of the Tribhuvan University Post-Graduate Campus Botany Department in Biratnagar. The fresh leaf sample (102 g) was crushed and hydrodistilled using a Clevenger type apparatus for 4 hours to give 0.02 g of a clear pale-yellow essential oil, which was stored at 4°C until analysis.

### Gas chromatographic – mass spectral analysis

The essential oil of *L. inermis* was analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25  $\mu$ m, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Injector temperature was 200°C and detector temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3°C/min to 200°C; increased 2°/min to 220°C. A 1% w/v solution of the sample in CH<sub>2</sub>Cl<sub>2</sub> was prepared and 1  $\mu$ L was injected using a splitless injection technique.

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature (20) and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080)]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The essential oil composition of *L. inermis* from Nepal is summarized in Table 1.

### Antimicrobial Screening

The essential oil was screened for antimicrobial activity against Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213); Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC No. 27853) and *Escherichia coli* (ATCC No. 10798). Minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique (21). Dilutions of the crude extracts were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50  $\mu$ L of 1% w/w solutions of crude extracts in DMSO plus 50  $\mu$ L CAMHB. The extract solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately  $1.5 \times 10^8$  colony-forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 hours; the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Geneticin was used as a positive antibiotic control; DMSO was used as a negative control. Antifungal activity against *Aspergillus niger* (ATCC No. 16888) was determined as above using YM broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

see Table 1.

### Results and Discussion

The leaf essential oil of *Lawsonia inermis* was obtained in 0.02% yield. A total of 40 compounds were identified, accounting for 100.0% of the oil composition. The majority of the essential oil was dominated by monoterpenoids (accounting for 49.9% of the oil) and was mostly composed of limonene (20.0%), 1,8-cineole (6.9%), and linalool (7.0%). The diterpene (*E*)-phytol (27.5%) was the major component of the essential oil, while eudesmol isomers comprised 7.5% of the oil. A previous report on a Nigerian sample of the leaf essential oil of *L. inermis*, dominated by monoterpenoids, showed a 1,8-cineole-rich chemotype with a notable

absence of (*E*)-phytol (22). A Malaysian sample, dominated by long-chain hydrocarbons, contained 10.3% (*E*)-phytol (10). In a different report on another Nigerian sample, a very different chemotype, contained ethyl hexadecanoate (24.4%) and (*E*)-methyl cinnamate (11.4%) (23).

The essential oil of *L. inermis* was screened for potential antimicrobial activity against *B. cereus*, *E. coli*, *P. aeruginosa*, *S. aureus*, and *A. niger*, but showed minimal activity against those microorganisms (MIC = 625  $\mu$ g/mL). Of the major components in the essential oil, neither limonene, 1,8-cineole, linalool,  $\alpha$ -thujone, camphor,  $\alpha$ -terpineol, nor eugenol are particularly antimicrobial (24,25,26). Phytol (27,28) and eudesmols (29), on the other hand, have demonstrated antibacterial activity. Ethanol and ethyl acetate extracts of *L. inermis* have shown antibacterial activity against Gram-positive and Gram-negative bacteria (30). In another report, quinonic compounds of *L. inermis* also showed antibacterial activity (31), while notable antifungal activities were reported from non-volatile fractions of *L. inermis* (32,33). In addition, lawsone has shown significant antifungal activity (34).

### Acknowledgments

PS and PP is grateful to Tribhuvan University for helping with the plant collection and access to laboratory equipment. WNS is grateful to an anonymous private donor for the gift of the GC-MS instrumentation. We would also like to express our gratitude to Tilak Gautam for identifying plants for our project.

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RI	Compound	%	RI	Compound	%
931	Tricyclene	tr	1143	Camphor	4.0
935	$\alpha$ -Thujene	tr	1172	Menthol	0.3
941	$\alpha$ -Pinene	1.9	1176	Terpinen-4-ol	0.3
953	Camphene	1.9	1189	$\alpha$ -Terpineol	1.3
976	Sabinene	1.5	1226	Nerol	0.2
978	$\beta$ -Pinene	1.1	1251	Geraniol	0.9
992	Myrcene	1.0	1270	Geranial	0.1
1010	$\delta$ -3-Carene	1.9	1271	<i>n</i> -Decanol	0.3
1016	$\alpha$ -Terpinene	tr	1311	<i>p</i> -Vinylguaiaicol	0.9
1024	<i>p</i> -Cymene	1.7	1356	Eugenol	1.0
1028	Limonene	20.0	1458	( <i>E</i> )- $\beta$ -Farnesene	1.0
1030	1,8-Cineole	6.9	1475	<i>n</i> -Dodecanol	0.9
1038	( <i>Z</i> )- $\beta$ -Ocimene	tr	1584	Globulol	0.9
1048	( <i>E</i> )- $\beta$ -Ocimene	0.7	1628	Eremoligenol	0.7
1058	$\gamma$ -Terpinene	0.3	1631	$\gamma$ -Eudesmol	0.8
1087	<i>p</i> -Mentha-2,4(8)-diene	0.3	1650	$\beta$ -Eudesmol	3.6
1100	Linalool	7.0	1653	$\alpha$ -Eudesmol	3.1
1105	$\alpha$ -Thujone	3.1	1844	Phytone	1.1
1116	$\beta$ -Thujone	0.5	1941	Isophytol	0.9
1124	Chrysanthenone	0.2	2108	( <i>E</i> )-Phytol	27.5
				Total Identified	100.0

Table 1: Chemical composition of Lawsonia inermis L. from Nepal