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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 sub-tropical 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 α- and β-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 Tribhuwan 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 µm, 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_2Cl_2 was prepared and 1 µL 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 µL of 1% w/w solutions of crude extracts in DMSO plus 50 µL CAMHB. The extract solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5 x 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 µg/mL). Of the major components in the essential oil, neither limonene, 1,8-cineole, linalool, α-thujone, camphor, α-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).

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

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