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Chemical composition and biological activity of *Centella asiatica* essential oil from Nepal

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Abstract
The essential oil from the aerial parts of *Centella asiatica*, collected from Kirtipur, Nepal, was obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry (GC-MS). The essential oil was dominated by sesquiterpene hydrocarbons, including (*E*)-β-farnesene (26.5%), α-humulene (20.9%), and (*E*)-caryophyllene (13.3%). The essential oil was screened for *in-vitro* cytotoxic activity against MCF-7 cells and antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Aspergillus niger*. The *C. asiatica* essential oil showed no antimicrobial activity and was only marginally cytotoxic to MCF-7 cells.

Keywords: Essential oil composition, *Centella asiatica*, (E)-β-farnesene, α-humulene, and (E)-caryophyllene, cytotoxicity, cluster analysis

1. Introduction
Centella asiatica (L.) Urb. (Apiaceae), commonly known as Asiatic pennywort or gotu kola, is a perennial creeper, native to tropical swampy areas of Asia [1]. This medicinal plant has a long history of use in Chinese traditional medicine as well as Indian Ayurvedic medicine [1]. Numerous medicinal benefits have been attributed to this herb including antineoplastic, antiviral, antibacterial, antifungal, anti-inflammatory, antioxidant, anticonvulsant, antipsoriatic, antiulcer, wound-healing, sedative, immunostimulant, cardioprotective, hepatoprotective, neuroprotective, antidiabetic, and insecticidal [1,2]. Extracts of *C. asiatica* contain large quantities of pentacyclic triterpenoids, which have been implicated in the biological activities of this plant [3]. In this work, we have investigated the essential oil of *C. asiatica* collected from Kirtipur, Nepal, and examined the cytotoxicity and antimicrobial activity of the essential oil.

2. Materials and Methods
2.1 Plant Material
The aerial parts of *C. asiatica* were collected from the city of Kirtipur (27.67° N, 85.28° E, 1360 m above sea level) in the Kathmandu district of the Bagmati Zone, Nepal, on 21 May 2011. The plant was identified by Nawal Shrestha, and a voucher specimen has been deposited in the herbarium of the Tribhuvan University Central Herbarium, Kirtipur, Nepal. The dry leaf sample (85 g) was crushed and hydrodistilled using a Clevenger type apparatus for 4 h to give 0.05% yield of a clear pale yellow essential oil, which was stored at 4°C until analysis.

2.2 Gas chromatography – mass spectrometry
The essential oil from the aerial parts of *C. asiatica* was subjected to gas chromatographic – mass spectral analysis using an Agilent 6890 GC with Agilent 5973 mass selective detector, fused silica capillary column [HP-5ms, (5% phenyl)-methyl polysiloxane stationary phase, film thickness 0.25 μm, 30 m length, 0.25 mm diameter], helium carrier gas, 1.0 mL/min flow rate; inj. temp. 200°C, oven temperature program: 40°C initial temperature, hold for 10 min; increased at 3°/min to 200°C; increased 2°/min to 220°C, and interface temperature 280°C; EIMS, electron energy, 70 eV. The sample was dissolved in CH_2Cl_2 to give a 1% w/v solutions; 1-mL injection using a splitless injection technique was used. Identification of oil components was achieved based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature [4] and stored on our own in-house reference library.

2.3 Hierarchical cluster analysis
A total of seven chemical compositions of *C. asiatica* essential oils, including the sample from this study in addition to those obtained from the published literature [5–10] were used to carry out the cluster analysis using the XLSTAT software, version 2015.4.01. The essential oil compositions were treated as operational taxonomic units (OTUs) and the percentages of 27 of the most abundant essential oil components (α-humulene, (*E*)-caryophyllene, (*E*)-β-farnesene, *p*-cymene, γ-cadinene, germacrene D, α-copaene, bicyclogermacrene, β-elemene, caryophyllene oxide, myrcene, falcarinone, alloaromadendrene, neophytadiene, germacrene B, α-pinene, γ-terpinene, (*E*)-nerolidol, δ-elemene, β-cubebene, limonene, humulene epoxide II, germacrene A, mintsulfide, caryophylla-4(12),8(13)-dien-5β-ol, linalool, and selin-11-en-4α-ol) were used to establish the chemical relationships of the *C. asiatica* essential oil samples using the agglomerative hierarchical cluster (AHC) method. Pearson correlation was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for definition of the clusters.

2.4 Antimicrobial Screening
The *C. asiatica* essential oil was screened for antibacterial activity against Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213), and Gram-negative bacteria, *Escherichia coli* (ATCC No. 254922) and *Pseudomonas aeruginosa* (ATCC No. 27853). Minimum inhibitory concentrations (MIC) were determined using the microbroth dilution technique. Dilutions of the chloroform extracts were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 μL of 1% w/w solutions of each crude extract in DMSO plus 50 μL CAMHB. The extracts were then serially diluted (1:1) in CAMHB in 96-well plates. Microorganisms at a concentration of approximately 1.5×10^8 colony forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 h; the lowest concentration without turbidity was defined as the final minimum inhibitory concentration (MIC). DMSO was used as a negative control and Gentamicin was used as a positive antibiotic control. Antifungal activity against *Aspergillus niger* (ATCC No. 16401) was determined as above using potato dextrose broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

2.5 Cytotoxicity Screening
Human breast adenocarcinoma (MCF-7, ATCC No. HTB-22) cells were grown in RPMI 1640 supplemented with 10% Fetal bovine serum (FBS), 30 mM HEPES, sodium bicarbonate, and 100,000 units penicillin/streptomycin (10 mg/L) at pH 7.35. MCF-7 cells were plated into 96-well cell culture plates at a concentration of 1.2×10^4 cells/well and a volume of 100 μL in each well and incubated at 37°C and 5% CO_2 for 48 hours. After 48 hours, the cells reached 70-80% confluent growth. The supernatant fluid was carefully aspirated and replaced with 100 μL growth medium containing 1 μL of essential oil (1% in DMSO), giving a final concentration of 100 μg/mL. The plate was then incubated at 37°C and 5% CO_2 for 48 hours, after which the supernatant liquid was gently aspirated from each well. Into each well, 100 μL of MTT solution was added and the pre-read absorbance was immediately measured spectrophotometrically at 570 nm (using a Molecular Devices SpectraMax Plus 384 microplate reader). The plate was incubated at 37°C and 5% CO_2 for 4 h, after which the supernatant liquid was removed and DMSO (100 μL) was used to dissolve the purple formazan crystals. The amount of formazan produced was determined spectrophotometrically at 570 nm. DMSO and tingenone [11] (100 μg/mL) served as negative and positive controls, respectively. Solutions were added to wells in eight replicates. Average absorbances, standard deviations, and percent kill ratios (% $\text{kill}_{\text{compound}}$ / % $\text{kill}_{\text{control}}$) were calculated.

3. Results and Discussion
The essential oil composition of *C. asiatica* is listed in Table 1. There were only trace amounts of monoterpenoids, but the oil was rich in sesquiterpene hydrocarbons (74.1%) and oxygenated sesquiterpenoids (13.0%). The most dominant compounds were the sesquiterpenes (*E*)-β-farnesene (26.5%), α-humulene (20.9%), (*E*)-caryophyllene (13.3%), and the diacetylene falcarinone (8.8%). Previous investigations on *C. asiatica* essential oils from Penang, Malaysia [5], Alice, South Africa [6], and Palampur, Himachal Pradesh, India [7], were also rich in α-humulene (33.7, 21.1, and 23.1%, respectively) and (*E*)-caryophyllene (26.8, 19.1, and 23.2%, respectively), but poor in (*E*)-β-farnesene (trace, not detected, and 6.3%, respectively). In contrast, the essential oil from Penang, Malaysia was rich in germacrene D (10.0%), while the sample from South Africa was rich in bicyclogermacrene (11.2%). An essential oil from Sabah, Malaysia was, however, also rich in (*E*)-β-farnesene, (*E*)-caryophyllene, and α-humulene (15.2, 10.0, and 9.2%, respectively) [8]. This oil, however, was also dominated by γ-cadinene (26.4%), which was only observed in the essential oil from Himachal Pradesh (0.2%). Interestingly, the essential oil of *C. asiatica* collected from Chettupuzha, Kerala, India [10], was dominated by *p*-cymene (44.0%), which was only a minor component or unobserved in the essential oils from the other geographical locations. Likewise, an essential oil sample from Istanbul, Turkey, was dominated by α-copaene (22.0%) [9], which was only a minor or unobserved component in *C. asiatica* oils from other sites. Headspace analysis of fresh *C. asiatica* leaf juice from Chiangmai, Thailand, showed high concentrations of α-humulene (1602 ng/L), (*E*)-caryophyllene (1344 ng/L), (*E*)-β-farnesene (552 ng/L), and α-copaene (538 ng/L), with small amounts of γ-cadinene (97 ng/L) [12].

These widely different chemical compositions suggest the possibility of multiple chemotypes for *C. asiatica*. A hierarchical cluster analysis (Figure 1) reveals five potential chemotypes: (1) an α-humulene/(*E*)-caryophyllene chemotype (Penang, South Africa, Himachal Pradesh); (2) a (*E*)-β-farnesene/α-humulene chemotype, represented by the sample from Nepal; (3) a γ-cadinene chemotype (Sabah); (4) an α-copaene chemotype (Istanbul); and (5) the *p*-cymene chemotype represented by the sample from Chettupuzha, Kerala, India.

The essential oil of *C. asiatica* from Nepal showed only weak cytotoxic activity to MCF-7 breast tumor cells (36.3 ± 5.4% kill at 100 μg/mL), but did show excellent brine shrimp (*Artemia salina*) lethality with IC_{50} = 2.24 ± 1.35 μg/mL. The essential oil from Sabah, Malaysia, showed no cytotoxic activity against P388 murine leukemia cells [8]. The methanol extract of *C. asiatica* did show cytotoxic activity to Ehrlich ascites carcinoma (EAC) and Dalton's lymphoma ascites (DLA) with IC_{50} of 75.0 and 62.0 μg/mL, respectively [13].

Table 1: Essential oil composition of *Centella asiatica* from Kirtipur, Nepal.

RI	Compound	%
1100	Linalool	tr
1189	α-Terpineol	tr
1251	Geraniol	tr
1337	δ-Elemene	tr
1392	β-Elemene	4.2
1412	Decyl acetate	1.4
1419	β-Caryophyllene	13.3
1453	α-Humulene	20.9
1459	(*E*)-β-Farnesene	26.5
1474	10-*epi*-β-Acoradiene	0.8
1481	Germacrene D	3.5
1485	Aristolochene	tr
1493	Viridiflorene	tr
1497	Bicyclogermacrene	tr
1505	Germacrene A	4.0
1557	Germacrene B	0.8
1566	(*E*)-Nerolidol	2.9
1578	Spathulenol	tr
1579	Caryophyllene oxide	2.3
1609	Humulene epoxide II	1.9
1628	(*Z*)-2,7-Bisaboladien-4-ol	1.3
1631	Caryophylla-4(12),8(13)-dien-5α-ol	0.7
1633	Caryophylla-4(12),8(13)-dien-5β-ol	1.2
1642	τ-Muurolol	tr
1645	α-Muurolol (= Torreyol)	tr
1654	Selin-11-en-4α-ol	1.9
1657	Neointermediol	tr
1685	Germacra-4(15),5,10(14)-trien-1α-ol	tr
1739	Mintsulfide	1.2
1838	Neophytadiene	0.9
2069	Falcarinone	8.8
	Sesquiterpene hydrocarbons	74.1
	Oxygenated sesquiterpenoids	13.0
	Total Identified	98.6

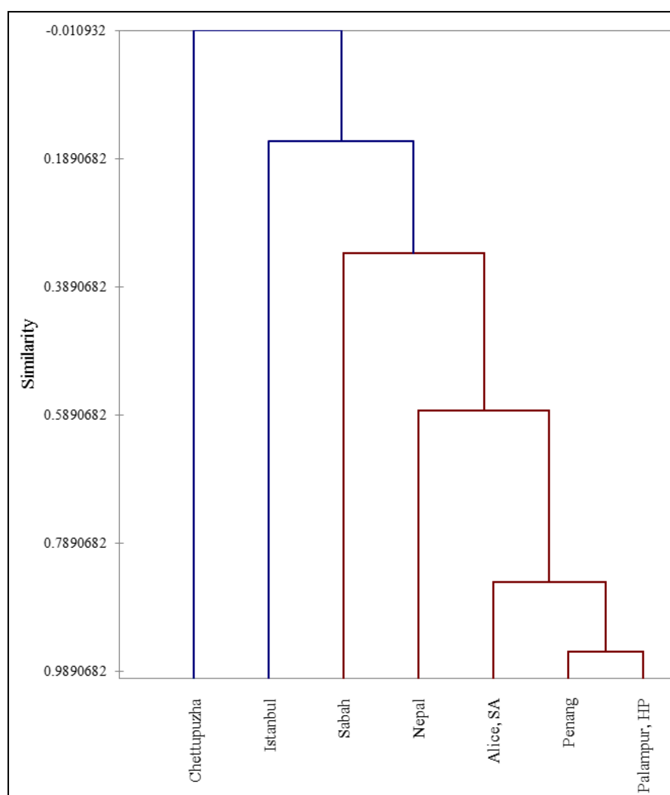

Fig 1: Dendrogram obtained from the agglomerative hierarchical cluster analysis of seven *Centella asiatica* essential oil compositions

4. Conclusions

There is wide variation in the chemical compositions of *Centella asiatica* essential oils, and it would be interesting to see additional compositions from other geographical locations. The biological activities for *C. asiatica* essential oils clearly depend upon the chemical composition, which may play a significant role in the traditional uses and efficacies of this medicinal plant.

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6. References

1. Orhan IE. *Centella asiatica* (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential. Evidence-Based Complementary and Alternative Medicine. 2012; 2012:ID 946259. doi:10.1155/2012/946259.
2. Gohil KJ, Patel JA, Gajjar AK. Pharmacological review on *Centella asiatica*: A potential herbal cure-all. Indian Journal of Pharmaceutical Sciences. 2010; 72(5):546-556. doi:10.4103/0250-474X.78519.
3. James JT, Dubery IA. Pentacyclic triterpenoids from the medicinal herb, *Centella asiatica* (L.) Urban. Molecules. 2009; 14:3922-3941. doi:10.3390/molecules14103922.
4. Adams RP. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry. 4th ed. Allured Publishing, Carol Stream, Illinois, 2007.
5. Wong KC, Tan GL. Essential oil of *Centella asiatica* (L.) Urb. Journal of Essential Oil Research. 1994; 6(3):307-309.
6. Oyedeji OA, Afolayan AJ. Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa. Pharmaceutical Biology. 2005; 43(3):249-252. doi:10.1080/13880200590928843.
7. Joshi VP, Kumar N, Singh B, Chamoli RP. Chemical composition of the essential oil of *Centella asiatica* (L.) Urb. from western Himalaya. Natural Product Communications. 2007; 2(5):587-590.
8. Lee TK, Vairappan CS. Antioxidant, antibacterial and cytotoxic activities of essential oils and ethanol extracts of selected South East Asian herbs. Journal of Medicinal Plants Research. 2011; 5(21):5284-5290.
9. Orhan IE, Atasu E, Senol FS, Ozturk N, Demirci B, Das K, Sekeroglu N. Comparative studies on Turkish and Indian *Centella asiatica* (L.) Urban (gotu kola) samples for their enzyme inhibitory and antioxidant effects and phytochemical characterization. Industrial Crops and Products. 2013; 47:316-322.
10. Santhi CF, Thomas MT. Essential oil profiling of *Centella asiatica* (L.) Urb.: A medicinally important herb. South Indian Journal of Biological Sciences. 2016; 2(1):169-173.
11. Setzer WN, Setzer MC, Hopper AL, Moriarity DM, Lehrman GK, Niekamp KL, Morcomb SM, Bates RB, McClure KJ, Stessman CC, Haber WA. The cytotoxic activity of a *Salacia* liana species from Monteverde, Costa Rica, is due to a high concentration of tingenone. Planta Medica. 1998; 64:583.
12. Wongfhun P, Gordon MH, Apichartsrangkoon A. Flavour characterisation of fresh and processed pennywort (*Centella asiatica* L.) juices. Food Chemistry. 2010; 119:69-74. doi:10.1016/j.foodchem.2009; 05:072.

13. Babu TD, Kuttan G, Padikkala J. Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to *Centella asiatica* (L.) Urban. Journal of Ethnopharmacology. 1995; 48:53-57.