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Chemical composition and biological activities of essential Oil from leaf and bark of *Nyctanthes arbor-tristis* L. from Nepal

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Abstract: The essential oil from the leaves and barks of *Nyctanthes arbor-tristis*, collected from Biratnagar, Nepal, was hydrodistilled and analyzed by GC-MS. A total of 26 compounds were identified in the leaf oil, accounting for 100% of the oil while a total of 20 compounds were identified in the bark oil accounting for only 89.4% of the oil. Both the leaf and bark oil had similar quantities of hexadecanoic acid (26.4% and 34.3%, respectively) and octadecanoic acid (3.9 and 6.2%, respectively). However, the leaf oil also consisted of linalool (11.0%), (*E*)-phytol (13.6%) and (3*Z*)-hexenyl benzoate (11.0%), which were absent in the bark oil. Besides fatty acids, the bark oil exhibited significantly different composition with mostly β-eudesmol and other eudesmol isomers (27.5%). The oil was screened for antimicrobial activity and showed marginal activity against *Bacillus cereus* and *Aspergillus niger* (MIC = 625 µg mL). *N. arbor-tristis* leaf oil was inactive in the brine shrimp lethality test (LC_{50} > 100µg/mL).

Key words: *Nyctanthes arbor-tristis*, essential oil composition, eudesmol, Nepal, antimicrobial, brine shrimp lethality

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Introduction

Nyctanthes arbor-tristis L. (Oleaceae) is widely distributed along subtropical, tropical to sub-Himalayan regions in the South East Asia (Das *et al*., 2010; Khatune *et al*., 2003). It has been extensively used as a therapeutic agent in the Ayurvedic healing traditions of South Asia (Rathee *et al*., 2007; Saxena *et al*., 1986; Tuntiwachwuttikul *et al*., 2003).

Traditionally used to treat sciatica, arthritis and malaria, however, reports from the literature have also indicated that the leaf oil from *N. arbor-tristis* include hepatoprotective, anti-leishmanial, antiviral and antifungal activities (Rathee *et al*., 2007). Local people of Andhra Pradesh, India use the whole tree for cancer, root for fever, sciatica, anorexia and bark as expectorant (Rathod *et al*., 2010). Other research into the leaf extract of the *N. arbor-tristis* have shown considerable immunological activity and water soluble ethanol extracts from the leaves are reported to possess anti-inflammatory activity which, however, accompany development of ulcers in test rats (Rathee *et al*., 2007; Saxena *et al*., 1986). In addition, anti-oxidant studies on the acetone-soluble ethyl acetate leaf extracts have shown significant activity against hydroxy and superoxide radicals, as wells as peroxide scavenging activity (Rathee *et al*., 2007). Likewise, activity-guided isolation of compounds in *N. arbor-tristis* flowers yielded iridoid glucosides that have exhibited antiplasmodial activity against *Plasmodium falciparum* (Tuntiwachwuttikul *et al*., 2003). Besides these compounds, 4-hydroxyhexahydro-benzofuran-7-one (Khatune *et al*., 2003), nyctoside A, arborside C, arborside D, 6-hydroxyloganin, arbortristoside A, arbortristoside B (Sasmal *et al*., 2007) and nyctanthoside (Jensen *et al*., 2002) have been reported.

Besides from these sparse reports into the study of biological activities of leaf and flower extracts of *N. arbor-tristis*, to our knowledge this is the first examination of the leaf and bark essential oils of this medicinal plant from Nepal. The objective of this study is to analyze the chemical compositions, and examine the microbial and brine shrimp lethality activities of leaf and bark oils of *N. arbor-tristis*.

Materials and methods

Plant material

Nyctanthes arbor-tristis was collected from city of Biratnagar (26°28′N, 87°16′E, 72 m above sea level) in Morang district in Koshi Zone in Nepal on 15 May 2011. The plant was identified by Tilak Gautam, and a voucher specimen (1024) has been deposited in the herbarium of the Tribhuvan University, Post-Graduate Campus, Botany Department, Biratnagar. The fresh leaf sample (102 g) and the fresh bark

sample (104 g) were crushed and hydrodistilled using a Clevenger apparatus for 4 h to give clear, colorless essential oils of 0.002 g and 0.005 g, respectively, which were stored at 4°C until analysis.

Gas chromatographic – mass spectral analysis

The leaf and bark essential oils of *N. arbor-tristis* were 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 (Adams 2007) 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 compositions of *N. arbor-tristis* are summarized in Table 1.

Antimicrobial screening

The essential oils were screened for antimicrobial activity against Gram-positive bacteria *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213), and 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 (Setzer *et al*., 2003). Dilutions of the essential oils were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 µL of 1% w/w solutions of oils in DMSO plus 50 µL CAMHB. The oil solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5 ´ 10^8 colony-forming units (CFU)/ mL were added to each well. Plates were incubated at 37 ± 1 °C for 24 hours; the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Gentamicin 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.

Brine shrimp lethality assay

The brine shrimp (*Artemia salina*) lethality test was carried out using a modification (Satyal *et al*., 2012) of the procedure by McLaughlin (1991). *Artemia salina* eggs were hatched in a sea salt solution (Instant Ocean®, 38 g/L) with an incandescent light bulb as the heat source. After 48 hours, the newly hatched nauplii were counted using a micropipette and transferred to 20-mL vials. Nine vials each containing 10 *A. salina* nauplii in 10 mL of sea salt solution (same as the hatching solution) were prepared. Three vials were labeled as controls with first one containing no DMSO, another with 10 µL, and the last one with 100 µL DMSO. Three replicate vials contained 10 µL of 1% essential oil solution in DMSO, and the other three were prepared by adding 100 µL of 1% essential oil solution in DMSO. Surviving *A. salina* was counted after 24 hours.

Results and Discussion

The essential oils of *N. arbor-tristis* were obtained in 0.002% and 0.005% yield for the leaf and bark samples, respectively. A total of 26 compounds were identified in *N. arbor-tristis* leaf oil while only 20 compounds were identified for the bark essential oil.

The composition of the leaf essential oil of *N. arbor-tristis* was notably different from the bark oil. While the leaf and bark oils were mostly composed of similar quantities of fatty acids: palmitic acid (26.4 and 34.3%, respectively) and stearic acid (3.9 and 6.2%, respectively), minor components in leaf oil included (*E*)-phytol (13.6%), (3*Z*)-hexenyl benzoate (11.0%), and linalool (11.0%). The result from this work is in agreement with a previous analysis of

fatty acids of *N. arbor-tristis* bark oil from Bangladesh, which was reported to contain a similar quantity of palmitic acid (16.4%), but additional fatty acids such as oleic acid (13.3%), behenic acid (24.8%), and nervonic acid (12.4%) (Rahman & Shahajan, 2011). However, the bark oil had a considerably different composition with eudesmol isomers (27.5%) along with smaller quantities of *n*-dodecanol (6.8%), elemol (5.8%) and cryptomeridiol (4.8%) also present. Overall, 100% of the leaf essential oil was identified as oppose to only 89.4% for the bark essential oil.

The essential oils of *N. arbor-tristis* were screened for potential antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Aspergillus niger*, but showed no activity against any of the microorganisms (MIC≥ 1250 µg/mL) except for *B. cereus* and *A. niger* (MIC = 625 µg/mL by leaf oil and MIC = 313 µg/mL by bark oil). Additionally, neither *N. arbor-tristis* leaf nor bark oil showed activity in the brine shrimp (*Artemia salina*) lethality test (LC_{50} > 100 µg/mL). As an established traditional medicine and from literature reports suggesting important biological activities, the essential oils from *N. arbor-tristis* may be potential candidates for therapeutic use, and therefore, further research into the biological activities of the oil is currently being pursued.

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Table 1 : Leaf and bark essential oil composition of *Nyctanthes arbor-tristis* from Nepal.

RI[a]	Compound	Leaf (%)	Bark (%)
856	(3Z)-Hexenol	2.5	---
941	α-Pinene	---	tr[b]
1008	(3Z)-Hexenyl acetate	0.5	---
1024	*p*-Cymene	---	tr
1028	Limonene	---	0.2
1031	1,8-Cineole	---	1.3
1043	Phenylacetaldehyde	0.3	---
1065	Acetophenone	0.8	---
1100	Linalool	11.3	tr
1104	Nonanal	0.4	---
1112	2-Phenylethyl alcohol	0.3	---
1143	Camphor	---	0.8
1176	Terpinen-4-ol	0.2	---
1181	*m*-Cymen-8-ol	0.6	---
1184	*p*-Cymen-8-ol	0.5	---
1187	(3Z)-Hexenyl butanoate	0.3	---
1189	α-Terpineol	4.7	---
1192	Methyl salicylate	5.6	---
1226	Nerol	0.7	---
1251	Geraniol	3.7	---
1311	*p*-Vinylquaiacol	0.8	---
1324	(3Z)-Hexenyl tiglate	0.7	---
1337	Methyl anthranilate	0.7	---
1356	Eugenol	1.2	---
1414	(*E*)-β-Damascone	0.4	---
1475	*n*-Dodecanol	5.5	6.8

1487	(*E*)-β-Ionone	1.2	---
1550	Elemol	---	5.8
1564	Dodecanoic acid	---	tr
1569	(3*Z*)-Hexenyl benzoate	11.0	---
1631	γ-Eudesmol	---	1.7
1651	β-Eudesmol	---	17.1
1654	α-Eudesmol	---	8.7
1677	*n*-Tetradecanol	---	tr
1722	(2*Z*,6*E*)-Farnesol	---	0.9
1809	Cryptomeridiol	---	4.8
1920	Methyl palmitate	---	1.8
1957	Hexadecanoic acid	26.4	34.3
2112	(*E*)-Phytol	13.6	---
2123	Methyl stearate	---	1.4
2162	Octadecanoic Acid	6.2	3.9
	Total Identified	100.0	89.4

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