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Composition and bioactivities of the leaf essential oil of *Ficus religiosa* Linn.

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Abstract
The leaf essential oil of *Ficus religiosa* from Nepal was obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry. The major components of the leaf oil were eugenol (27.0%), itaconic anhydride (15.4%), 3-methyl-cyclopenetane-1,2-dione (10.8%), 2-phenylethyl alcohol (8.0%), and benzyl alcohol (4.2%). The leaf oil was screened for antimicrobial activity and was marginally active (MIC = 625 μg/mL) against *Aspergillus niger*, but was inactive (MIC = 2500 μg/mL) against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The oil was marginally active in the brine shrimp lethality test (LC_{50} = 50 μg/mL) and also showed *in-vitro* cytotoxic activity against MCF-7 human breast tumor cell line (80±5% kill at 100 μg/mL).

Keywords: *Ficus religiosa,* peepal, essential oil composition, Nepal.

1. Introduction
The genus *Ficus* (Moraceae) is represented by 800 species and 2000 varieties throughout the world, among them forty-eight species of *Ficus* are found in Nepal [1], those are widely distributed in tropical and sub-tropical regions [2]. *Ficus religiosa* Linn. grows from 150 m to 1550 m above sea level. *F. religiosa* is considered to be the oldest and most sacred tree in the Hindu mythology which is commonly known as 'Peepal tree'. It is a widely branched tree; leaves are heart shaped with long tips and long slender petioles [3]. *F. religiosa* is traditionally used in treatment of many diseases such as skin diseases, diabetes, respiratory disorders, gastric problems, and central nervous system disorders [4].
Previous phytochemical investigations have shown that *F. religiosa* is a good source of the flavonoid quercetin [5]. The bark of this tree contains tannins, saponins, flavonoids, steroids, terpenoids, and cardiac glycosides [6-7]. The leaf oil of *Ficus capensis* from India has carvacrol, (*E*)-caryophyllene, and caryophyllene oxide as the major components [8]. The biological activities and chemical composition of the leaf essential oil of *F. religiosa*, however, have not been previously reported.

2. Materials and Methods
Leaves of *F. religiosa* were collected from Kirtipur, Nepal (27°67′ N 85°28′ E, 1360 m above sea level), and the essential oil was obtained by hydrodistillation in a yield of 0.009% (v/w). The essential oil was analyzed by GC-MS and screened for antimicrobial, cytotoxicity, and brine shrimp lethality as described previously [9].

3. Results and Discussion
The leaf essential oil composition of *F. religiosa* is listed in Table 1. From a total of 44 peaks, 97.20% of the compounds were identified in the oil. The major components of the leaf oil were identified to be eugenol (27.0%), itaconic anhydride (15.4%), 3-methyl-cyclopenetane-1,2-dione (10.8%), 2-phenylethyl alcohol (8.0%), and benzyl alcohol (4.2%).
The leaf oil was screened for antimicrobial activity and was marginally active (MIC = 625 μg/mL) against *Aspergillus niger*, but was inactive (MIC = 2500 μg/mL) against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The oil was marginally active in the brine shrimp lethality test (LC_{50} = 50 μg/mL) and also showed *in-vitro* cytotoxic activity against MCF-7 human breast tumor cell line (80±5% kill at 100 μg/mL).

Table 1: Composition of the leaf essential oil of *Ficus religiosa* Linn. From Nepal

RI	**Compound**	**%**
856	(3*Z*)-Hexenol	1.1
867	(2*Z*)-Hexenol	0.7
869	*n*-Hexanol	0.7
983	Phenol	0.7
995	Adipoin	0.6
999	3-Methylcyclopenetane-1,2-dione	10.8
1018	Itaconic anhydride	15.4
1032	Benzyl alcohol	4.2
1041	Salicylaldehyde	1.5
1043	Phenylacetaldehyde	0.6
1095	Allyl caproate	3.5
1100	Linalool	0.3
1105	*n*-Nonanal	0.3
1112	2-Phenylethyl alcohol	8.0
1131	Unidentified	0.8
1138	Benzeneacetonitrile	1.2
1154	(2*E*,6*Z*)-Nonadienal	0.4
1157	(2*E*)-Nonen-1-ol	0.5
1166	(2*E*,6*Z*)-Nonadienol	0.5
1168	(*E*)-Linalool oxide	0.5
1198	Catechol	1.1
1217	Coumaran	3.4
1299	(*E*)-Cinnamyl alcohol	0.6
1308	*p*-Vinylguaiacol	1.1
1323	(3*Z*)-Hexenyl tiglate	0.6
1358	Eugenol	27.6
1434	(2*E*)-Hexenyl (3*Z*)-hexenoate	0.3
1487	(*E*)-β-Ionone	1.6
1528	Dihydroactinidiolide	0.4
1537	α-Copaene-11-ol	0.4
1569	(3*Z*)-Hexenyl benzoate	0.5
1582	Unidentified	1.7
1596	Unidentified	0.7
1628	*epi*-γ-Eudesmol	0.5
1631	γ-Eudesmol	0.4
1641	*epi*-α-Cadinol	1.0
1650	β-Eudesmol	1.8
1653	α-Eudesmol	1.0
1654	α-Cadinol	0.7
1715	Pentadecanal	0.7
1955	Palmitic acid	0.7
2108	Phytol	0.7
	Total identified	97.2%

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