Essential oil constituents and their biological activities from the leaves of *Cassia fistula* growing in Nepal

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Abstract:

Cassia fistula L. (Fabaceae) fruit is used traditionally in Nepal as an antipyretic and to treat constipation, while the leaves are used to treat jaundice, piles, rheumatism, ulcers, insect bites, facial paralysis and skin eruptions. *C. fistula* leaves are important ingredients in Ayurvedic medicine. This study was undertaken to characterize the volatile constituents of *C. fistula* leaves and to evaluate their antimicrobial and cytotoxic properties. The essential oil from the leaves of *Cassia fistula* collected from Biratnagar, Nepal, was obtained by hydrodistillation and analyzed by GC-MS. Antimicrobial activities (minimum inhibitory concentration) against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Candida albicans*, were determined using the microbroth dilution technique, *in-vitro* cytotoxic activity against MCF-7 human adenocarcinoma cells was determined using the MTT method. *C. fistula* leaf oil was composed of only seven components, all of which were identified: eugenol (25.0%), (*E*)-phytol (21.5%), camphor (13.5%), limonene (11.0%), salicyl alcohol (10.4%), linalool (9.9%), and 4-hydroxybenzyl alcohol (8.7%). The leaf oil showed antifungal activity against *A. niger* (MIC = 78 µg/mL) and *C. albicans* (MIC = 313 µg/mL), but only marginal cytotoxicity against MCF-7 cells (19.63 ± 11.89% kill at 100 µg/mL). All of the individual essential oil components were screened for activity. Eugenol exhibited antifungal properties (MIC on *A. niger* = 78 µg/mL) and limonene and phytol were cytotoxic (IC_{50} = 74.7 and 54.3 µg/mL, respectively).

Key words: essential oil composition, eugenol, phytol, camphor, limonene, salicyl alcohol, linalool, 4-hydroxybenzyl alcohol

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Introduction

The genus *Cassia* is comprised of ten species of yellow, flowering ornamental trees of the Fabaceae (Press *et al.*, 2000). *Cassia fistula* L., locally known as "rajbriksha" in Nepal, is a deciduous tree native to East India, Nepal, Burma, and Malaysia (Press *et al.*, 2000). Traditionally in Nepal, the plant's fruit pulp is used as an antipyretic (Gewali, 2008), it is also used to treat habitual constipation (Mabberly, 1997). The leaf is used to treat jaundice, piles, rheumatism, ulcers, insect bites, facial paralysis and skin eruptions (Wealth of India, 2007), the leaf is also one of the most important ingredients in preparing Ayurvedic medicine (Agrawal *et al.*, 2005). *C. fistula* can attain 6-10 m in height and it has compound leaves of 5-12 cm length (Gupta, 2010). Various pharmacological activities (antitussive, CNS, clastogenic, antipyretic, antioxidant, laxative, anti-inflammatory, hepatoprotective, larvicidal, antitumor, antiparasitic, antifertility, and anti-leishmanial) have been reported for this plant (Danish *et al.*, 2011). Phytochemically, the leaf of the *C. fistula* contains mainly oxalic acid, flavonoids, tannins, and anthraquinones, while the fruits contain anthraquinones, flavonoids, and waxes (Bahorun *et al.*, 2005).

Materials and methods

Plant Material

The plant materials of *Cassia fistula* 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 in May 2011. The plant was identified by Tilak Gautam and a voucher specimen (1703) has been deposited in the herbarium of the Tribhuvan University, Post Graduate Campus, Botany Department, Biratnagar. The fresh leaves (100 g) were crushed and hydrodistilled using a Clevenger-type apparatus for 4 hours, which gave a clear, pale yellow essential oil (0.012 g) that was stored at 4ºC until analysis.

Gas chromatographic – mass spectral analysis

The essential oil of *C. fistula* was analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector as described previously (Satyal *et al.*, 2012). 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 reported are as raw percentages based on total ion current without standardization. The essential oil composition of *C. fistula* from Nepal is summarized in Table 1.

Antimicrobial Screening

The essential oil and the individual essential oil components (Sigma-Aldrich) were screened for antimicrobial activity against *Bacillus cereus* (ATCC No. 14579), *Staphylococcus aureus* (ATCC No. 29213), *Candida albicans* (ATCC No.10231), and *Aspergillus niger* (ATCC No. 16888) using the microbroth dilution technique as reported previously (Setzer *et al.*, 2001). Gentamicin and amphotericin B were used as antibacterial and antifungal controls, respectively. Minimum inhibitory concentrations (MICs) are summarized in Table 1.

Cytotoxicity Screening

Cytotoxic activity of *C. fistula* essential oil and essential oil components against human MCF-7 breast adenocarcinoma cells (ATCC No. HTB-22) was assessed using the MTT assay for cell viability as previously described (Moriarity *et al.*, 2007).

Results and Discussion

Yield of essential oil obtained from *Cassia fistula* leaves was 0.012%. A total of seven compounds accounting for the complete oil composition were identified. The components of the essential oil were eugenol (25.0%), (*E*)-phytol (21.5%), camphor (13.5%), limonene (11.0%), salicyl alcohol (10.4%), linalool (9.9%), and 4-hydroxybenzenemethanol (8.7%). The composition of *C. fistula* leaf oil from Egypt had previously been studied and in this study the leaf oil was dominated by hydrocarbons (54.3%), and (*E*)-phytol (16%); the floral essential oil in the work from Egypt contained (*E*)-nerolidol (38.0%), methyleugenol (7.3%), methyl linoleate (6.3%) and hydrocarbons (31.3%) (Tzakou *et al.*, 2007). Thus, (*E*)-phytol was the only common component found in leaf oils from Nepal and Egypt. Our previous phytochemical study on a related species, *C. tora,* (Satyal *et al.,* 2013) has shown that the major components of the leaf oil were elemol (20.6%), linalool (15.0%), palmitic acid (11.7%), octadecane (10.1%) and eicosane (5.8%). Linalool, therefore, was the only common component in *C. tora* and *C. fistula*.

The essential oil of *C. fistula* and its components were screened for potential antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans. C. fistula* leaf oil showed no antibacterial activity (MIC = 1250 µg/mL), but did exhibit antifungal activity against *A. niger* (MIC = 78 µg/mL) and *C. albicans* (MIC = 313 µg/mL). All components of the essential oil exhibited comparable antifungal activity (Table 1). Consistent with our results, camphor, limonene (Marei *et al.*, 2012), linalool (Pitarokili *et al.*, 2002; Edris & Farrag , 2003; Hammer *et al.* 2003; Nakahara *et al.* 2003; Zhang *et al.* 2006; Khan *et al.* 2010), eugenol (Chami *et al.*, 2004; Gayoso *et al.*, 2005; Wang *et al.*, 2005; Khan *et al.*, 2010) had been previously reported to show antifungal activity against a number of different fungi. Linalool was found to be inactive against *Malassezia furfur*, *Trichophyton rubrum*, and *Trichosporon beigelii* (Adam *et al.* 1998). Crude leaf extracts of *C. fistula* had previously shown antimicrobial activities (Ali *et al.*, 2004; Singh & Karnwal, 2006 ; Duraipandiyan & Ignacimuthu 2007; Panda *et al.*, 2010).

The essential oil of *C. fistula* showed only marginal *in-vitro* cytotoxic activity against MCF-7 cells (19.6 ± 11.9% kill at 100 µg/mL). Interestingly, however, two of the components, limonene (IC_{50} = 74.7 ± 4.1 µg/mL) and (*E*)-phytol (IC_{50} = 54.3 ± 1.6 µg/mL) were active. The concentrations of these two components were apparently not high enough to impart cytotoxicity to the oil itself. Limonene has been shown to have anti-cancer effects through apoptosis induction and modulation of oncogene signal transduction (Tsuda *et al*., 2004; Miller *et al*., 2011). It was found to inhibit the development of rat mammary tumor (Maltzman *et al*., 1989) by inhibiting the association of farnesyl protein transferase and P21ras to the cell membrane (Chen *et al*., 1999; Surh, 2003). (*E*)-Phytol exhibited cytotoxic activity against HT-29 human colon cancer cells, MG-63 osteosarcoma cells and AZ- 521 gastric cancer cells (Lee *et al*., 1999). Previous studies have demonstrated that neither camphor (Cherneva *et al.*, 2012) nor eugenol (Atsumi *et al.*, 2006) have cytotoxic properties.

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Table 1. Chemical composition and biological activities of *Cassia fistula* leaf oil and its major components

RI[a]	Compound	%	Antimicrobial activity (MIC, µg/mL)				MCF-7 Cytotoxicity[b]
			B. cereus	*S. aureus*	*A. niger*	*C. albicans*	
1028	Limonene	11.0	1250	1250	156	313	90.8(9.6)[c]
1100	Linalool	9.9	625	625	156	313	-0-
1143	Camphor	13.5	1250	1250	156	156	13.4(13.0)
1257	Salicyl alcohol	10.4	1250	1250	156	313	11.1(24.8)
1331	4-Hydroxybenzyl alcohol	8.7	1250	1250	156	156	32.2(4.9)
1356	Eugenol	25.0	313	625	78	313	43.4(12.0)
2108	(*E*)-Phytol	21.5	2500	2500	156	313	94.4(1.2)[d]
	C. fistula leaf oil	100	1250	1250	78	313	17.6(9.2)

[a] RI = "Retention Index", determined with reference to a homologous series of *n*-alkanes on an HP-5ms column.

[b] % kill at 100 µg/mL, standard deviations in parentheses.

[c] IC_{50} = 74.7(4.1) µg/mL

[d] IC_{50} = 54.3(1.6) µg/mL