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Coumarin, essential oil and total phenol levels in bark and leaves of *Cinnamomum* species

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Ethnopharmacology, the practice of using indigenous or local medicinal remedies including plants have wide acceptance and currently it is integrated into the main stream medicine. The genus *Cinnamomum* is distributed in South East Asia, China and Australia. It has different applications in medicine, perfumery, flavoring and pharmaceutical industries. Phytochemicals such as eugenol, cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol and volatile substances such as safrole, coumarin and cinnamic acid esters (Dinesh *et al.*, 2015) present in members of the genus *Cinnamomum* provide flavouring and medicinal potential. Many herbs and spices, usually used to flavour dishes, contain lot of phenolic compounds which possess good antioxidant activity (Zheng and Wang, 2001). Cinnamon has a delicate, spicy aroma, which is due to its volatile oil. Composition of essential oil differs between species and also within the species among plant parts (bark, root and leaves). The essential oil of *C. zeylanicum* bark is rich in trans-cinnamaldehyde with antimicrobial effects against animal and plant pathogens, food poisoning and spoilage bacteria and also against fungi (Baratta *et al.*, 1998).

Coumarin (benzo-α- pyrone), a naturally occurring flavouring compound present in cinnamon requires special attention as it is reported to cause hepatotoxicity in animals. Clinical trials in US and Ireland, reported that some patients ingesting coumarin developed signs of drug-induced liver toxicity even at low doses. Hence, the German Federal Institute of Risk assessment (BfR) in 2007, after reviewing the human trial data, affirmed the tolerable daily intake of 0.1 mg kg^{-1} body weight (BfR Health assessment, 2006). It is in this context that the coumarin content in various cinnamon species becomes very critical. Earlier reports show that *C. cassia* (Chinese cassia) has more coumarin content than *C.verum. C. tamala* (the Indian cassia whose leaves are used in flavoring dishes) and *C. camphora* (camphor tree, cultivated for camphor and camphor oil) are other important members of the genus *Cinnamomum*. But no reports are available on the concentration of coumarin in various species and most of the reported studies are on *C. verum* and a few are on cassia cinnamon. Due to the high cost of pure cinnamon *C. verum*, cassia cinnamon is used in the food industry. In some countries substitution of cassia for true cinnamon is prohibited (Blahova and Svobodova, 2012). It is therefore necessary to use analytical methods to discriminate between the *Cinnamomum* species and to detect food adulteration with cassia cinnamon.

Both barks as well as leaf of cinnamon are used as spice and condiment. Most of the reported studies on coumarin content are on bark and there are no reports on the coumarin content in leaf samples. In the present study, we attempted to extract, quantify and compare coumarin, essential oil and phenol contents in four *Cinnamomum* species in bark as well as in leaves. We also tried to estimate coumarin content in a few *C. cassia* accessions and compare

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them with the coumarin content of market samples of cinnamon.

Bark and leaf samples of four cinnamon species were collected from ICAR-IISR experimental farms, Peruvannamuzhi and Chelavoor, Kozhikode, Kerala. Total phenol, essential oil and coumarin content of *C. verum*, *C. cassia*, *C. tamala*, and *C. camphora* were analyzed. Coumarin content of *C. cassia* accessions such as A2, B1, C1, C2, D1, D3, D6 and market samples (collected from retail outlets in Kannur and Kozhikode districts of Kerala) were also analyzed. The high performance liquid chromatography method developed by Sproll *et al*. (2008) was used with slight modification for analyzing the coumarin in ground cinnamon samples. Coumarin content in the sample was calculated as follows,

$$\text{Coumarin content (mg kg}^{-1}) = \frac{\text{X (ng concentration) x 50 mL}}{\text{5 µL x 1 g}}$$

The essential oil of both bark and leaf samples of cinnamon was determined by hydro-distillation in a Clevenger type apparatus, according to the method of ASTA. Total phenols present in the samples were estimated by Folin-Ciocalteau method (Singleton *et al.,* 1999). Two way ANOVA was conducted to compare coumarin content among Cassia accessions and to compare essential oil and phenol contents among *Cinnamomum* species. Student's t-test was conducted to compare coumarin contents between leaves and bark in different *Cinnamomum* species. All these statistical analysis were conducted using mstatc package.

Table 1. Coumarin content in *C. cassia* accessions

Accession	Bark (mg kg^{-1})	Leaf (mg kg^{-1})
A2	146.9 ^{e}	3026.6 ^{d}
B1	34.8 ^{b}	3912.1 ^{f}
C2	27.2 ^{a}	569.5 ^{a}
D6	222.2 ^{g}	3512.2 ^{e}
C1	181.2 ^{f}	1546.5 ^{b}
D1	88.3 ^{d}	2959.3 ^{c}
D3	71.1 ^{c}	5382.9 ^{g}
CV (%)	2.2	1.8
CD (5%)	2.3	43.7

Means followed by different letters are significantly different

The coumarin content in different *C. cassia* accessions is given in Table 1. Both leaf and bark samples showed the presence of coumarin. Among the 7 accessions tested, coumarin content varied from 27.2 to 222.2 mg kg^{-1} in bark samples. Acc. C2 showed the least and Acc. D6 showed the highest. Coumarin content in leaves ranged from 569.5 (*C. cassia* C2) to 5382.9 mg kg^{-1} (*C. cassia* D3). Accession C2 showed the least coumarin content in both bark and leaf. Leaf samples showed higher coumarin concentration than bark. Reports suggest that coumarin, cinnamaldehyde, cinnamic acid and cinnamyl alcohol contents may be used for rapid and reliable authentication and quality assessment of cassia bark (He *et al*., 2005). Our results also suggest that coumarin content of either leaf or bark is sufficient to distinguish cassia cinnamon from true cinnamon. Among the genus *Cinnamomum*, four species *viz., C. verum, C. cassia, C. tamala* and *C. camphora* were selected for the study. Coumarin content in both leaf and bark was studied. The results are presented in Table 2. *C. verum* or true cinnamon has a characteristically different flavour compared to cassia. Our results showed that *C. cassia* bark contained significantly higher levels of coumarin than true cinnamon. *C. cassia* had approximately 11 times higher coumarin content than *C. verum*. *C. cassia* is also the species that is predominantly found in retail cinnamon trade. Coumarin was not detected in *C. tamala* and *C. camphora* bark. Coumarin content in leaf samples of different species was in the order *C. cassia* > *C. verum* > *C. tamala* > *C. camphora* (2987.0>73.9>29.7>6.2 mg kg^{-1}, respectively). In all these species, leaf recorded significantly higher coumarin content than bark (Table 2). This could be due to the fact that coumarin may be synthesized

Table 2. Coumarin content in *Cinnamomum* species

Species	Bark (mg kg^{-1})	Leaf (mg kg^{-1})
C. verum	10.0	73.9 ^{c}
C. cassia	111.4	2987.0 ^{d}
C. tamala	ND	29.7 ^{b}
C. camphora	ND	6.2 ^{a}

Student t-test (between *C. verum* and *C. cassia* bark) (t value 2.77) CV = 1.5%,CD (5%) = 7.72

ND: Not detected; Means followed by different letters are significantly different

in leaf and then transported to the bark through phloem. Reports suggest that coumarin levels even within a single tree in *C. cassia* may vary widely (Woehrlin *et al*., 2010; Lake, 1999), The three market samples analysed showed high coumarin content (65.5, 4612.0 and 6993.1 mg kg^{-1} respectively). Our results (Table 2) revealed that *C. verum* bark contained only about 10.0 mg coumarin per kg cinnamon. Hence, such high coumarin content recorded in traded samples suggests that the traded cinnamon may not be *C. verum.* It could be fully adulterated with the barks of the species such as *C. cassia* and *C. burmanii*, which cost less (Ballin and Sorensen, 2014; Lungarini *et al.*, 2008). Earlier work suggested that coumarin levels in *C. cassia* ranged from 700 to 12200 mg kg^{-1} (Sproll *et al*., 2008). *C. cassia* samples used in this study showed on an average 111.4 and 2987.0 mg coumarin per kg cinnamon bark and leaves, respectively. According to German Federal Institute of Risk assessment, 1 kg of cassia powder contains approximately 2100 to 4400 mg of coumarin (BfR Health assessment, 2006).

In clinical trials coumarin caused liver tumors in rats, lung tumours in rats and mice and clara cell toxicity in mice which indicates its possible carcinogenic nature. Felter *et al*. (2006) reported that coumarin toxicity may be species specific and non-genotoxic and observed that it is directly related to specific metabolism/detoxification capabilities following bolus oral exposure. Also, the observed acute, chronic and carcinogenic effects of coumarin in the rat and mouse are usually based on long-term studies and performed at maximum tolerated doses, where coumarin intake was over 4500 times the estimated human exposure (BfR Health assessment, 2006).

Coumarin compounds have the ability to exert non-covalent interactions with various active sites in organisms, thus displaying varied range of activities such asanti-coagulant, anti-cancer, anti-oxidant, anti-neuro-degenerative and anti-microbial properties (Peng *et al*., 2013). The majority of the natural coumarins are fluorescent in UV light, so it can be used as artificial ion receptors, fluorescent probes for biologically important species and biological stains to monitor timely enzyme activity (Wagner, 2009). Coumarin based bioactive molecules as new drugs have been extensively investigated and some of them like Warfarin 1a (anticoagulant) which is absorbed fast have been marketed and used in clinics. Hydroxyl coumarins specially, 4-hydroxy coumarins have great effect on the formation and scavenging of reactive oxygen species, thus has a great influence on processing involving free radical mediated injury (Kostova *et al*., 2011). Coumarin has also been used to treat patients with advanced cancer or to prevent recurrence of serious cancers in a number of clinical trials (Emami *et al*., 2015; Thakur *et al*., 2015).

In general, there are contrasting reports on the effects of coumarin on human health. But as the quantity of cinnamon used for culinary purposes is too low, it is unlikely that the amount of coumarin consumed through cinnamon exceeds the tolerable daily intake of 0.1 mg kg^{-1} body weight affirmed by the German Federal Institute of Risk assessment (BfR) in 2007 unless the marketed samples are highly adulterated with other cinnamon species such as *C. cassia* which has much higher coumarin content.

Cinnamon bark and its extracts have several medicinal properties. Most bottled or packaged ground cinnamon which is marketed does not mention its type or origin. So, we suggest that manufacturers of such cinnamon based dietary supplements for various medicinal and culinary purposes must ensure that the products contain only true cinnamon because ingesting substantial amount of coumarin on a daily basis may pose health risk to individuals who are more sensitive to coumarin. As suggested, due to differences in coumarin content, as observed in the present study, and also flavor characteristics, it is desirable to have a change in food policy that demands *C. verum* and *C. cassia* to be labeled separately and marketed.

The results presented in Table 3 indicate that *C. cassia* had the highest bark oil and the lowest leaf oil percentage. *C. tamala* had the lowest yield of bark essential oil among the four species of cinnamon. Leaves had higher essential oil than bark in general except in *C. cassia*. Cardoso-Ugarte *et al*. (2015) identified that compounds such as cinnamaldehyde, cinnamyl-acetate, eugenol, linalool and camphor were identified from different Cinnamon varieties as major compounds. Due to its biological activities these compounds are used in the food industry as antioxidants, bactericidal,

Table 3. Essential oil and total phenol contents of *Cinnamomum* species

Species	Bark essential oil (%)	Leaf essential oil (%)	Bark phenol content (% w/w)	Leaf phenol content (% w/w)
C. verum	1.32 [c]	1.56 [d]	4.6 [a]	7.4 [d]
C. cassia	2.20 [d]	0.35 [a]	6.3 [c]	4.3 [a]
C. tamala	0.47 [a]	0.68 [b]	6.7 [d]	6.4 [c]
C. camphora	1.03 [b]	1.41 [c]	6.0	4.3 [b]
CV %	17.63	9.26	3.2	3.5
P value	< 0.01	< 0.01	< 0.01	< 0.01

Means followed by different letters are significantly different

antifungal, and flavor and aroma enhancer. Cinnamaldehyde and its derivatives reduce virulence in *Vibrio* spp. These compounds can interfere with biofilm formation, stress response and virulence in *Vibrio* spp. (Brackman *et al*., 2008). Eugenol is known to possess antioxidant, anti-inflammatory, anti-genotoxic, anti-mutagenic and anti-cancer properties. Mechanism (molecular) of eugenol-induced apoptosis in melanoma, leukemia, skin tumors, osteosarcoma, gastric and mast cells has been well documented (Jaganathan and Suprianto, 2012).

Total phenol content ranged from 4.3 to 6.8 per cent in bark and 4.1 to 7.4 per cent in leaves of different *Cinnamomum* species (Table 3). Bark had more phenols than leaves in general except in *C. verum*, which had the highest leaf phenol content, followed by *C. tamala*. Studies show that a diet rich in polyphenolic compounds may result in a positive health effect attributed to their antioxidant properties (Hertog *et al.*, 1993). Plant phenolics can function as metal chelators, reducing agents, and also as singlet oxygen quenchers (Mathew *et al.*, 2006). The most common plant phenolic antioxidants include flavanoid compounds, cinnamic acid derivatives, coumarins, tocopherols and polyfunctional organic acids (Hertog *et al*., 1993). Dudonné *et al*. (2009) compared antioxidant activity of different plant extracts which included *C. zeylanicum* also and reported that the cinnamon extracts possessed highest antioxidant activity and high phenolic content.

In conclusion, HPLC technique was found to be a reliable and useful analytical tool for the

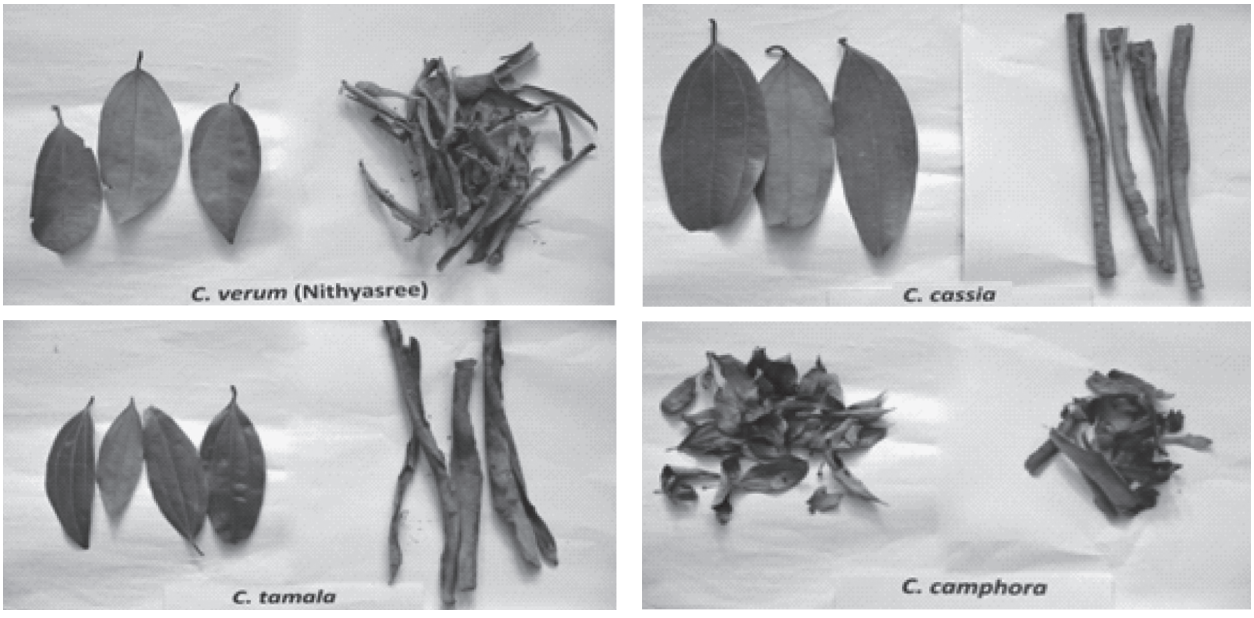

Fig. 1. Bark and leaf samples of *Cinnamomum* species

quantification of coumarin in cinnamon samples. *C.verum* had very low levels of coumarin in both bark and leaves compared to *C. cassia*. Leaf had higher coumarin and essential oil content than the bark, in general, while total phenol content was more in bark than in leaves. The present study is the first attempt to quantify coumarin in leaf samples of *Cinnamomum* species and also in both leaf and bark of *C. tamala* and *C. camphora*. As the coumarin content obtained for *C. cassia* bark samples is low in the present study, it can be safely used when compared to the maximum daily intake fixed by German Federal Institute of Risk assessment. But the market samples of cinnamon bark had higher coumarin content than experimental samples indicating that the market samples may not be true cinnamon (*C. verum*) indicating the possibility of adulteration. However, further confirmation of data by LC-MS makes it more authentic.

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