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Short communication

Comparative analysis of cytotoxic and antioxidant potential of edible *Cinnamomum verum* (bark) and *Cinnamomum tamala* (Indian bay leaf)

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ABSTRACT

Background: *Cinnamomum verum* bark (Cinnamon or *dalchini* in Hindi) and *Cinnamomum tamala* leaf (Indian bay leaf or *tejpatta* in Hindi) are commonly used spices in the Asian subcontinent. In the present study, we compared the bioactive potential of these spices.
Method: The plants were collected and extracted with different solvents. The extracts were analysed for *in vitro* cytotoxicity against human cancer cell lines by SRB assay. Antioxidant activity was measured in terms of DPPH radical scavenging and chelation power on ferrous ions. Relative phenolic content of extracts was also measured.
Results: Comparative analysis demonstrated that *C. verum* (bark) has much greater cytotoxic as well as antioxidant potential than *C. tamala* (leaves). The bark methanol extract showed potential activity against prostrate (PC-3) and glioblastoma (T98G) cancer cell lines with 90% and 78% growth inhibition at 100 μg/ml concentration respectively. The bark methanol extract also showed good DPPH free radical scavenging activity with IC_{50} 111.5 ± 0.62 μg/ml and moderate chelating power on ferrous ions IC_{50} 108.7 ± 0.53 μg/ml. The total phenolic content of bark methanol extract was highest with 210 ± 0.81 mg/g GAE.
Conclusion: This study highlights the relative greater bioactive potential of *C. verum* (bark) and it can be deduced that it is a highly effective cytotoxic, antioxidant spice than Indian bay leaf.

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Spices are dried parts of herbs that are commonly used as flavouring agents in foods. It has been experimentally documented that several common spices can also exert health beneficial physiological effects including digestion stimulation, hypolipidemic, antidiabetic, anti-lithogenic, antioxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic and antimicrobial effects.[1] These flavour enhancing spices can also protect against a wide range of cancers, heart diseases and other chronic diseases.[2] Reactive oxygen species (ROS), which include free radicals like superoxide radicals, hydroxyl radicals, singlet oxygen species have been related to numerous health disorders such as inflammation, artherosclerosis, stroke, arteriosclerosis, diabetes and cancer.[3,4] The imbalance between ROS production and antioxidant defence mechanisms leads to oxidative modification in cellular membrane or intracellular molecules.[5]

Herbs and spices are sources of natural antioxidants, such as flavonoids, phenolic, diterpenes, tannins and phenolic acids. These compounds have antioxidant, anti putrefaction and anticancer properties. Therefore, there has been a considerable interest in the food industry to find natural antioxidants to replace synthetic compounds in food applications, and a growing trend in consumer preferences for natural antioxidants, all of which has given more impetus to explore natural sources of antioxidants.

In this study, we compared the two species of family Lauraceae namely *Cinnamomum verum* bark (dalchini) and *Cinnamomum tamala* leaf (Indian bay leaf) that have been used for several thousand years as culinary herbs and in traditional Eastern and Western systems of medicine. *C. verum* is also traditionally used for anorexia, bloating, dyspepsia with nausea, flatulence, colic, and spastic conditions of the gastrointestinal tract.[6] The bark of cinnamon yields an essential oil containing cinnamaldehyde and eugenol and possesses significant antiallergic, antiulcerogenic, antipyretic and antioxidant properties.[7] The oil isolated from the leaves of *C. tamala* known as Tejpatta oil is medicinally used as a carminative, antiflatulent, diuretic ant it has been reported to show antibacterial and

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Table 1
Comparative yield, antioxidant potential and phenolic content of *Cinnamomum verum* bark and *Cinnamomum tamala* leaf.

Extracts	% Yield		DPPH radical scavenging IC_{50} (μg/ml)		Chelation power IC_{50} (μg/ml)		Total phenolic content (GAE mg/g)	
	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf
Methanol	18.92	9.2	111.5 ± 0.62	175 ± 0.32	108.7 ± 0.53	114.2 ± 0.46	210 ± 0.81	161 ± 0.58
Chloroform	11.94	8	289.5 ± 0.4	299 ± 0.21	287.2 ± 0.35	140 ± 0.24	15.4 ± 0.52	78 ± 0.34
Aqueous	5.97	5	122 ± 0.5	245.9 ± 0.4	254.5 ± 0.47	247 ± 0.47	113.4 ± 0.4	53 ± 0.47

Data is represented as mean ± standard deviation.

hypoglycaemic activity.[8] Essential oil constituents of leaves have four chemotypes i.e. eugenol type, cinnamaldehyde-or cinnamaldehyde/linalool type, trans-sabinene hydrate type.[9]

Keeping in view the significance of these spices, the study was done to compare these two species i.e. *C. verum* bark, and *C. tamala* leaf for their relative cytotoxicity, antioxidant and antimicrobial properties and phenolic content.

2. Materials and methods

2.1. Preparation of crude extracts

The dried cinnamon bark and Indian bay leaf were obtained from local market and ground in a blender to make fine powder. Total of 100 g of each of bark and leaf powder were extracted with 500 ml of three different solvents i.e. chloroform, methanol and water. Methanolic and chloroform extracts were obtained by continuous stirring at 40 °C for 6 h whereas water extract was prepared at 60 °C for 6 h. This treatment was repeated thrice and the extracts were pooled, filtered and evaporated using rotary vacuum evaporator.[10]

2.2. In vitro cytotoxicity

All the extracts of each species were evaluated for antiproliferative activity against human cancer cell lines in 96 well microplates. Cell suspension (100 μl) containing 10^5–2×10^5 cells/ml and 100 μl of tested sample were added into each well. After incubation in 5% CO_2 at 37 °C for 72 h, the cytotoxicity was determined by colorimetric method as described by Skehan and his coworkers.[11]

2.3. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

In this assay, free radical scavenging activity was determined according to the method of Blois et al with modifications.[12] A total of 1 ml from a 0.5 mM methanol solution of the DPPH radical was mixed to 2 ml sample and to this 2 ml of 0.1 M sodium acetate buffer (pH 5.5) was added. The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a double beam UV–VIS spectrophotometer. The radical scavenging activity (RSA) was calculated as a percentage of DPPH radical discolouration, using the equation:

$$\%\mathrm{RSA} = [(A_0 - A_s)/A_0] \times 100$$

where A_0 is the absorbance of the control and A_s is the absorbance of the test compound.

2.4. Chelation power on ferrous (Fe^{2+}) ions

The chelating effect on ferrous ions of the prepared extracts was estimated by the method of Dinis with slight modifications.[13] Briefly, 200 μl of different concentrations of each extract and 740 μl of methanol were added to 20 μl of 2 mM $FeCl_2$. The reaction was initiated by the addition of 40 μl of 5 mM ferrozine into the mixture, which was then left at room temperature for 10 min before determining the absorbance of the mixture at 562 nm. The ratio of inhibition of ferrozine-Fe^{2+}complex formation was calculated using the equation:

$$\%\ \text{inhibition} = ([\text{absorbance of control} - \text{absorbance of test sample}]/\text{absorbance of control}) \times 100.$$

2.5. Determination of total phenols

Total phenolic content of bark and leaf were determined according to Folin–Ciocalteau method.[14] Briefly, 0.5 ml of extract solution was mixed with 0.5 ml of 1 N Folin–Ciocalteau reagent. The mixture was kept for 5 min, followed by the addition of 1 ml of 20% Na_2CO_3. After 10 min of incubation at room temperature, the absorbance was measured at 730 nm using double beam UV–VIS spectrophotometer. The concentration of phenolic compounds was calculated according to the following equation obtained from the standard gallic acid (5–50 μg) graph:

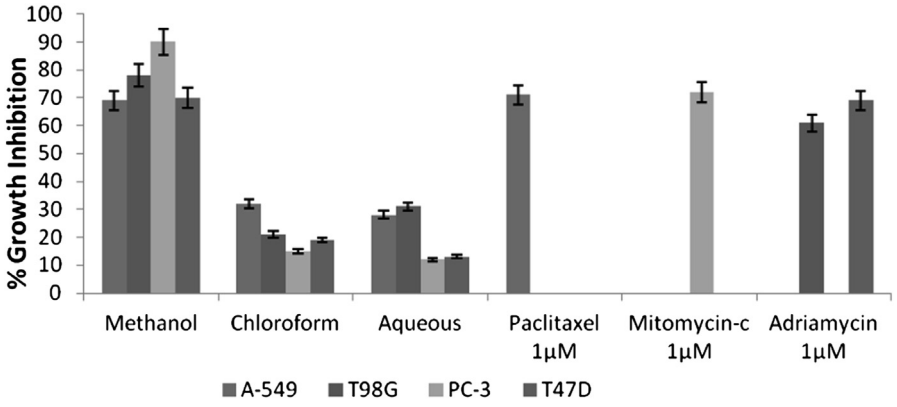

Fig. 1. *In vitro* cytotoxicity of *Cinnamomum verum* bark against human cancer cell lines.

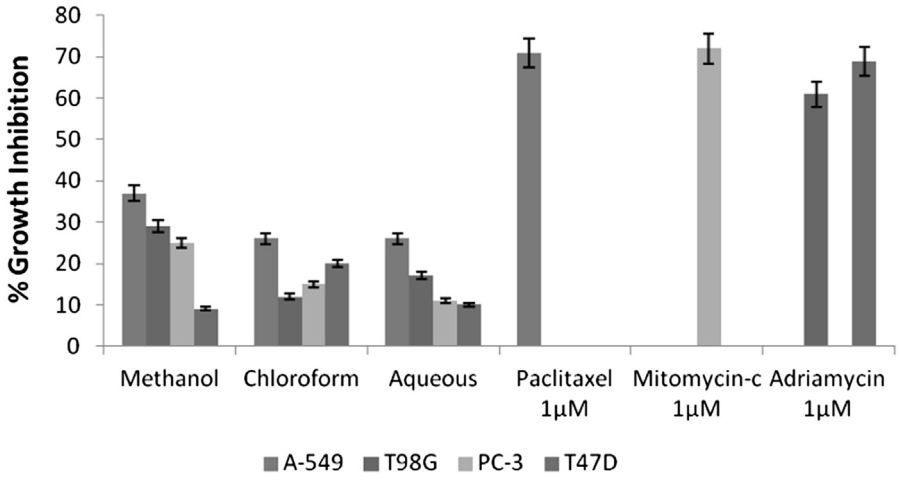

Fig. 2. *In vitro* cytotoxicity of *Cinnamomum tamala* leaf against human cancer cell lines.

$$\text{Absorbance} = 0.0299\ \text{gallic acid}(\mu g) - 0.2451\left(R^2 = 0.998\right)$$

2.6. Statistical analysis

All experiments were carried out in triplicate. Data values are expressed as mean ± standard deviation.

3. Results and discussion

In the present comparative study among cinnamon bark and Indian bay leaf, extracts of each were prepared in different range of solvents (methanol, water, chloroform) and analysed for relative cytotoxic and antioxidant properties. The maximum yield of 18.92% was observed in *C. verum* bark methanolic extract and 9.2% yield was obtained in *C. tamala* leaf (Table 1). Evaluation of *in vitro* cytotoxic potential against four human cancer cell lines i.e. A-549 (lung cancer), PC-3 (prostate cancer), T98G (glioblastoma), T47D (breast cancer) was carried out. Among all the extracts, the methanolic extract of bark showed potential activity against prostrate (PC-3) and glioblastoma (T98G) cancer cell lines with 90% and 78% growth inhibition at concentration of 100 μg/ml respectively (Fig. 1) whereas leaf methanol extract showed insignificant cytotoxic activity at 100 μg/ml with 37% and 29% growth inhibition against PC-3 and T98G human cancer cell lines respectively (Fig. 2). Hence, comparative analysis confirmed that *C. verum* bark has much greater cytotoxic potential than *C. tamala* leaves (Figs 1 and 2). As it has been reported that cinnamon bark possesses very low acute toxicity in the animals.[15] Thus, consumption of cinnamon bark in daily diet is quite safe.

Further, to compare the antioxidant potential, DPPH radical scavenging assay was carried out and it was observed that methanol extracts showed high radical scavenging activity with IC_{50} 111.5 ± 0.62 μg/ml (Cinnamon bark) and IC_{50} 175 ± 0.32 μg/ml (leaf) respectively (Table 1). BHT is a standard antioxidant which shows IC_{50} value of 50 ± 0.62 μM. The result depicted that *C. verum* bark was more effective radical scavenger than Indian bay leaf. This method is commonly used to assess radical scavenging of any antioxidant substance because it is a quick, reliable and reproducible method to search *in vitro* general antioxidant potential of pure compounds as well as plant extracts.[16] The quantification of relative phenolic content in different extracts exhibited that methanolic extracts of both bark and leaf contain high phenolic content i.e. 210 ± 0.81 mg/g GAE and 161 ± 0.58 mg/g GAE respectively in comparison to other extracts. A significant correlation was shown to exist between the phenolic content and DPPH scavenging capacity (Table 1).

The chelation of ferrous ions by extracts was estimated with the method of Dinis et al (1994). Chelation power on Fe^{2+} ions of methanol extract of bark was found to be the highest (IC_{50} 108.7 ± 0.53 μg/ml) whereas leaf methanol extract showed comparatively low chelation activity (IC_{50} 114.2 ± 0.42 μg/ml) (Table 1). Reactive oxygen species (ROS) are often generated as by-products of biological reactions or from exogenous factors.[17] Right dietary sources can provide the much needed antioxidants to control the free radicals from damaging the affected tissues.[18] Dragland and his co-worker speculated that the daily intake of 1 g of various potent antioxidant spices makes a relevant contribution to the total intake of antioxidants in a normal diet.[19]

Our present study supports the fact that *C. verum* bark and *C. tamala* leaf are potential source of antioxidant regime along with antiproliferative properties. The present work is also in accordance with the earlier reports on cytotoxic and antioxidant activity of cinnamon bark.[20,21] Comparative study done on the two species of *Cinnamomum* highlights the better bioactive potential of *C. verum* bark than *C. tamala* leaves.

4. Conclusion

The study demonstrated that among the methanol, chloroform and aqueous extracts of *C. verum* bark and *C. tamala* leaves, methanol extract of *C. verum* bark (Cinnamon) possesses significant higher cytotoxic and antioxidant activity. Therefore, *C. verum* bark (dalchini) extract has more potential than *C. tamala* leaves (tejpatta) and can be used as an easily accessible source of antioxidants. Frequent intake of cinnamon bark in food can help in maintaining balance between ROS generation and the defence system of the body against oxidative stress and other diseases like cancer.

Conflicts of interest

All authors have none to declare.

References

1. Srinivasan K. Role of spices beyond food flavoring: nutraceuticals with multiple health effects. *Food Rev Int*. 2005;21:167–188.

2. Craig WJ. Health-promoting properties of common herbs. *Am J Clin Nutr*. 1999;70:491S–499S.
3. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract Harng Jyur (*Chrysanthemum morefolium* Ramat). *Lebnesmittel-Wiss Technol*. 1999;32: 269–277.
4. El-Habit OHM, Saada HN, Azab KS. The modifying effect of beta carotene on gamma radiation -induced elevation of oxidative reactions and genotoxicity in male rats. *Mutat Res*. 2000;466:179–186.
5. Blumenthal M, Busse WR, Goldberg A, et al. *The Complete Commission E Monographs: Therapeutic Guide to Herbal Medicines*. Boston, MA: Integrative Medicine Communications; 1998:110–111.
6. Torizuka K. Basic lecture of kampo medicine: pharmacological effect of cinnamon. *Kampo Med*. 1998;11:431–436.
7. Kurokawa M, Kumeda CA, Yamamura J, Kamiyama T, Shiraki K. Antipyretic activity of, cinnamyl derivatives and related compounds in influenza virus-infected mice. *Eur J Pharmacol*. 1998;348:45–51.
8. Devi L, Kannappan S, Anuradha CV. Evaluation of *in vitro* antioxidant activity of Indian bay leaf, *Cinnamomum tamala* using rat brain synaptosomes as model system. *Indian J Experimental Biol*. 2007;45:778–784.
9. Hema L, Harish CA, Nirpendra C, Ujjwal B. Variability in volatile constituents of *Cinnamomum tamala* leaf from Uttarakhand Himalaya. *Asian Pac J Trop Biomed*. 2012:S667–S669.
10. Guleria S, Tiku AK, Rana S. Antioxidant and free radical scavenging activity of acetone extract/fractions of *Terminalia bellerica* Roxb fruit. *Indian J Biochem Biophys*. 2010;47:110–116.
11. Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer drug screening. *J National Cancer Institute*. 1990;82: 1107–1112.
12. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl, radical scavengers. *Arch Biochem Biophys*. 1994;315:161–169.
13. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. *J Agr Food Chem*. 2003;51:609–614.
14. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;26:1199–1200.
15. Budavari SB, O'Neil MJ, Smith A, Heckelman PE. *The Merck Index*. Rahway, NJ: Merck and Co; 1989.
16. Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen UP. Determination of DPPH, radical oxidation caused by methanolic extracts of some microalgal species by linear regression, analysis of spectrophotometric measurements. *Sensors*. 2007;7:2080–2095.
17. Pan YM, Wang K, Huang SQ, et al. Antioxidant activity of, Microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel. *J Agric Food Chem*. 2008;106: 1264–1270.
18. Choudhury MK, Venkatraman S, Upadhyay L. Antioxidant and hypo-glycemic property of *Streblus asper* in streptozotocin induced diabetic rats. *J Pharm Res*. 2011;4:1958–1961.
19. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are, important sources of dietary antioxidants. *J Nutr*. 2003;133:1286–1290.
20. Pandey AK, Mishra AK, Mishra A, Kumar S, Chandra A. Therapeutic potential of *C. zeylanicum*, extracts: an antifungal and antioxidant perspective. *Int J Biol Med Res*. 2010;1:228–233.
21. Schoene NW, Kelly MA, Polansky MM, Anderson RA. Water soluble polymeric polyphenols from, cinnamon inhibit proliferation and alter cell cycle distribution patterns of hematologic tumor cell, lines. *Cancer Lett*. 2005;230:134–140.