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HPLC estimation of phenolic acids in leaves of some Central Indian bamboo species

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Abstract: Biochemical estimation for secondary metabolites in leaves of *Dendrocalamus strictus, D. asper, Bambusa nutans* and *B. vulgaris* were carried out by High Performance Liquid Chromatography (HPLC). The results indicate that bamboo leaves are rich in pre-existing secondary metabolites (phenolic acids). Two phenolic acids *viz.* ferulic acid and salicylic acids were found in appreciable amounts in bamboo leaves. Ferulic acid neutralizes free radicals, provides photoprotection and anti-inflammatory effects. Salicylic acid is used as an analgesic and antipyretic agent. Bamboo leaves can thus be used as a potential source in many industrial applications.

Keywords: Biochemical estimation, ferulic acid, phenolic acids, salicylic acid, secondary metabolites.

INTRODUCTION

Bamboo is one of the fastest growing commercial plants and has the greatest contribution in improving the environmental quality. Now-a-days bamboo leaf tea has become an exquisite drink due to its soft, delightful taste and fresh aroma. Recently, Taiwan has developed and innovated "bamboo leaves fragrance" with light bamboo scent without any artificial flavor and bamboo flavored drink. From one ton fresh bamboo leaves 75 kilograms of "bamboo leaves fragrance" and 25 tons of unique flavor "bamboo flavored drink" can be extracted. Studies of Baiyi *et al.* (2006) indicate that a nominal dietary antioxidants of bamboo (AOB) level of 4.30 g/kg body weight (bw) per day has no adverse effect and support the use of AOB as a food additive. Green leaves tea contain polyphenols which greatly reduces cancer causing radicals. Leaves also possess a great deal of flavonoids, bioactive polysaccharide and other effective composition which have the antioxidant function.

Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. The importance

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of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have been the subject of a great number of chemical, biological, agricultural and medical studies. Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential protective role through ingestion of fruits and vegetables, against oxidative damage diseases like coronary heart disease, stroke, and cancers. Phenolic acids form a diverse group that includes hydroxybenzoic and hydroxycinnamic acids. Hydroxycinnamic acid compounds occur most frequently as simple esters with hydroxycarboxylic acids or glucose. Hydroxybenzoic acid compounds are present mainly in the form of glucosides. The major components of bamboo leaves are flavonoids, phenolic acids and lactones. Literature survey revealed that information of phenolic acids of bamboo species *viz. Dendrocalamus strictus, Bambusa nutans* and *B. vulgaris* is scarce. Keeping in view, the present study was undertaken to quantify the total phenolics and phenolic acids present in leaves of these species. *D. asper,* an edible bamboo species of South-East Asia was also analyzed simultaneously.

MATERIALS AND METHODS

Fresh leaves of *D. strictus, D. asper, B. nutans* and *B. vulgaris* were collected from Tropical Forest Research Institute, Jabalpur campus. The leaves were air-dried and subjected to analyses. Three replicates were taken and data were statistically analyzed (Gomez and Gomez, 1984).

Estimation of phenolic acids

Phenolic acids in the sample were determined by Reverse Phase High Pressure Liquid Chromatography. The phenolic acids were extracted from the leaves of *B. nutans, B. vulgaris, D. strictus* and *D. asper* following the procedure of Charpentier and Cowles (1981) with some modification.

Dried powder (5.0 g) was transferred to a conical flask containing 100 ml of 2N HCl. The contents were heated for 30 min over a boiling water bath, cooled to room temperature and filtered. The filtrate was transferred to a separating funnel and extracted with 50 ml (50 x 3) of ether. The combined ether layer was washed with distilled water and dried over anhydrous sodium sulphate. It was then filtered and evaporated to dryness under stream of nitrogen. The residue thus obtained was dissolved in HPLC grade methanol and used for analysis. The sample thus prepared was filtered through 0.5µm filter before injecting it into HPLC column.

Analytical system

The HPLC was a Waters Associates Model 746 equipped with 510 binary pumps, automated gradient controller, absorbance 486 tunable detector at a fixed wavelength of 254 nm and a 746 integrator. The absorbance of the samples, as well as of standards

was recorded at 0.5 sec time constant and AUFS (absorbance units full scale) 0.02. Column used was (3.9 mm x 300 mm) μBondapak C_{18}. The mobile phase was 4 per cent aqueous acetic acid in water and methanol in the ratio of 75:25 (v/v) at a flow rate of 1.0 ml/min. The following phenolic acids obtained from M/s. Sigma Chemicals Co. U.S.A. were used as standards:

Tannic acid, protocatechuic acid, salicylic acid, p-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid and ferulic acid. Chromatographic peak areas of standards obtained were used for calculating the phenolic acids of the sample. The peaks from the sample were identified on the basis of retention time.

Estimation of phenols

Total phenol content in the sample was estimated by Folin-Ciocalteau reagent (Malick and Singh, 1980). Sample (1.0 g) was extracted with 10 times volume of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. Supernatant was saved and the residue was re extracted. All the supernatants were pooled and evaporated to dryness and the residue dissolved in a known volume of distilled water (5 ml); 0.2 ml aliquot was taken into test tubes and volume made up to 3.0 ml with distilled water. Folin-Ciocalteau reagent (0.5 ml) followed by 2 ml of 20 per cent sodium carbonate solution was added. Tubes were placed in a boiling water bath for exactly 1 min, cooled and the absorbance read at 650 nm. Standard catechol solutions of different concentrations were run simultaneously and total phenol content calculated.

RESULTS AND DISCUSSION

Eight major phenolic acids *viz.*, p-hydroxy benzoic acid, salicylic acid, tannic acid, protocatechuic acid, caffeic acid, ferulic acid, vanillic acid and anthranilic acid were identified in bamboo leaves (Table 1). It was observed that among the bamboo species studied, *D. asper* leaves contain considerable amount of phenolic acids except p-hydroxy benzoic acid which was not identified. Tannic acid was not detected in the leaves of *B. nutans*, while tannic acid, vanillic acid and anthranilic acid were not detected in *D. strictus* leaves.

Ferulic acid and salicylic acids were found in appreciable amounts in bamboo leaves. The ferulic acid content in leaves of all the four species ranged from 228.93 μg to 429.50 μg/g, the highest value being observed in *B. nutans* and the lowest in *D. strictus*. Ferulic acid is a ubiquitous plant constituent that arises from the metabolism of phenylalanine and tyrosine. It occurs primarily in seeds and leaves both in its free form and covalently linked to lignin and other biopolymers. Due to its phenolic nucleus and an extended side chain conjugation it readily forms a resonance stabilized phenoxy radical which accounts for its potent antioxidant potential. UV absorption by ferulic acid catalyzes stable phenoxy radical formation and thereby potentiates its ability to terminate free radical chain reactions. By virtue of effectively scavenging deleterious

Table 1. Composition of phenolics in bamboo leaves

Species	Phenolic acids (μg/g)								Phenols (mg/100g)
	p-hydroxy benzoic acid	Salicylic acid	Tannic acid	Proto-catechuic acid	Caffeic acid	Ferulic acid	Vanillic acid	Anth-ranilic acid	
B. nutans	32.80	186.70	0.0	61.77	89.11	429.50	35.42	0.0	551.61
B. vulgaris	30.92	174.51	42.34	73.77	72.92	273.80	21.86	196.13	419.20
D. strictus	79.46	182.63	0.0	85.46	55.54	228.93	0.0	0.0	218.96
D. asper	0.0	276.80	1750.29	105.50	102.91	364.80	34.94	116.32	478.90
SE±	0.4718	38.435	3.2055	8.5892	2.1114	8.4947	6.2294	0.9672	1.3417
CD (0.05)	1.1545	94.047	7.8435	21.017	5.1664	20.7860	15.243	2.3667	3.2830

radicals and suppressing radiation-induced oxidative reactions, ferulic acid may serve an important antioxidant function in preserving physiological integrity of cells exposed to both air and impinging UV radiation. Similar photoprotection is afforded to skin by ferulic acid dissolved in cosmetic lotions. Its addition to foods inhibits lipid peroxidation and subsequent oxidative spoilage. By the same mechanism ferulic acid may protect against various inflammatory diseases. A number of other industrial applications are based on the antioxidant potential of ferulic acid (Sahelian, 2004). Ferulic acid is beneficial to sperm viability and motility in both fertile and infertile individuals and reduction of lipid peroxidative damage to sperm membranes and increase of intracellular cAMP and cGMP may be involved in these benefits. It is possible that ferulic acid may be used for cure of asthenozoospermic infertility (Zheng and Zhang, 1997). The salicylic acid content of bamboo leaves ranged from 174.57 μg to 276.80 μg/g in *B. vulgaris* and *D. asper* leaves respectively. Salicylic acid is analgesic, antipyretic and anti-inflammatory drug, precursor compound to aspirin. Aspirin (acetylsalicylic acid) is still the most commonly used salicylate.

The antioxidant properties of the dietary dihydroxycinnamic acids [caffeic (CA), dihydrocaffeic (DHCA), and chlorogenic (CGA) acids] have been well studied (Moridani *et al.* 2001). p-Hydroxybenzoic acid, tannic acid, protocatechuic acid, caffeic acid, vanillic acid and anthranilic acids were the other phenolic acids found in bamboo leaves. The results reveal that the p-hydroxy benzoic acid content in bamboo leaves showed a great variation ranging from 30.92 μg/g to 79.46 μg/g in *B. vulgaris* and *D. strictus* respectively, while it was not detected in *D. asper* leaves. *D. asper* leaves were found to contain maximum amount of tannic acid. *B. vulgaris* and *D. asper* leaves contained 42.34 μg and 1750.29 μg/g tannic acid respectively. Tannic acid was not detected in *B. nutans* and *D. strictus* leaves. Pirie (1978) reported the use of tannic acid to treat high quality proteins (*e.g.* casein) to render them indigestible in the rumen. The tannin-protein complex passes through (by passes) the rumen and in the intestine the complex dissociates, releasing the protein. This provides a means of supplementing the relatively low quality bacterial protein with a high quality dietary protein that does not get digested in the rumen. Tannic acid has anti-bacterial, anti-enzymatic and

astringent properties. Tannic acid has constringing action upon mucous tissues. The ingestion of tannic acid causes constipation and can be used to treat diarrhoea (in the absence of fever or inflammation). Externally, tannic acid is used to treat ulcers, toothache and wounds. Tannic acid is also used in many industrial applications. The best known is the tanning of leather. Tannic acid is sometimes used to clear wines. Tannic acid reacts with proteins in wine to form insoluble complexes which sediment or can be filtered. Tannic acid has numerous food and pharmacological applications. It is an additive in medicinal products, and is used as a flavouring agent as well as an anti-oxidant in various foods and beverages. It exhibits antimutagenic and anticarcinogenic activities and induces apoptosis in animal cells (Khan *et al*, 2000). The protocatechuic acid content in bamboo leaves ranged from 61.77 µg to 105.50 µg/g. The highest content was recorded in *D. asper* and the lowest in *B. nutans* leaves. Protocatechuic acid and caffeic acid are used as an anticarcinogenic agent (Kaul and Khanduja, 1998). Protocatechuic acid has an inhibitory potential on inducible nitric oxide synthase (iNOS) and hepatic damage induced by lipopolysaccharide (LPS, an endotoxin) (Lin *et al.*, 2003). The caffeic acid content in bamboo leaves varied from 55.54 µg to 102.91 µg/g in *D. strictus* and *D. asper* leaves. Jung *et al.* (2006) reported antihyperglycemic and antioxidant properties of caffeic acid in *db/db* mice. The vanillic acid ranged from 21.86 µg to 35.42 µg/g in *B. vulgaris* and *B. nutans* leaves. Vanillic acid was not detected in *D. strictus* leaves. Vanillic acid can be used as a flavouring agent. It is also an intermediate in the production of vanillin from ferulic acid (Claudio *et al.*, 2000). The concentration of anthranilic acid showed a great variation ranging from 116.32 µg to 196.13 µg/g in *D. asper* and *B. vulgaris*, while it was not detected in *B. nutans* and *D. strictus* leaves (Table 1). Anthranilic acid is used as an intermediate for production of dyes, pigments, and saccharin. Anthranilic acid and its esters are used in preparing perfumes, pharmaceuticals and UV-absorber as well as corrosion inhibitors for metals and mould inhibitors in soya sauce. Anthranilic acid can be used in organic synthesis to generate the benzyne intermediate (Logullo, 1973).

Besides the above eight phenolic acids identified, one major peak with retention time of 27.56 was found in bamboo leaves of all the four species studied, while a peak with retention time of 12.67 was found only in *B. vulgaris* and *D. strictus* leaves. A peak with retention time of 33.61 was found in *D. strictus* and *B. nutans*. Peak with retention time of 6.50 was found in *D. asper*, *B. vulgaris* and *B. nutans*, while it was not detected in *D. strictus*. Peaks with 6.97 and 13.96 retention time were found only in *D. strictus* and *B. vulgaris* leaves. Major peak with retention time of 4.27 was obtained only in *B. vulgaris* leaves.

The distribution of individual simple phenolics among forages is not well established, except for p-coumaric and ferulic acids. These two cinnamic acids are the most abundant phenolics in all forages. In grasses, p-coumaric and ferulic acids constitute 0.5 per cent to 2.0 per cent of the total cell wall, whereas in legumes they account for less than 0.2 per cent of the fibrous cell walls. Within a forage species, the various

plant parts differ in their concentrations of simple phenolics. It is expected that the simple phenolic content of forages be influenced by environmental variations such as temperature, (Ford and Hewitt, 1979).

The phenol content varied from 218.96 mg to 551.61 mg/100g in *D. strictus* and *B. nutans*. Peschel *et al.* (2006) screened vegetable and fruit byproducts for industrial polyphenol exploitation potential and antioxidant activity and found that the preservative effect of the extract was similar to the established anti-oxidants.

CONCLUSION

Antioxidants are intimately involved in the prevention of cellular damage, the common pathway for cancer, aging, and a variety of diseases. In short, green teas such as the bamboo leaf tea can help reduce the free radicals. This study demonstrates the industrial polyphenol exploitation potential of recovering high amounts of phenolics with antioxidant properties from bamboo leaves not only for food, cosmetics but also for pharmaceutical applications.

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