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EXTRACTION AND ANALYSIS OF INDIGENOUS SPICE *Cinnamomum zeylanicum* WIDELY CONSUMED IN BANGLADESH: AN ASSESSMENT TO EXPLORE ITS BIOACTIVE AND ANTIOXIDANT ACTIVITIES

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Cinnamon *(Cinnamomum zeylanicum)* or true cinnamon is the most well-known spice and condiment. It is widely grown and consumed in many countries around the world, such as Sirlanka, China, Vietnam, India, and Bangladesh (Unlu *et al.*, 2010). It is also commonly known as the Bangladeshi word 'Daruchini'. The bark is sold in scrap or powder form and is widely used in many food dishes due to its strong pleasing aromatic taste. The flavoring bark powder is mixed in the preparation of many kinds of desserts, spicy candies, in tea for flavor, and also used in the kitchen and cooking with it makes food a whole lot tastier. In addition, it's used in dentifrices, confectionery, perfumes, liqueurs, pharmaceuticals, and alternatives to traditional food preservatives (Paliwal *et al.,* 2018). Cinnamon primarily contains bioactive components, such as cinnamaldehyde, cinnamylacetate, cinnamic acid, phenylpropyl acetate, eugenol, linalool, α- pinene, terpineol etc. The major bioactive component of cinnamon is cinnamaldehyde, which is widely used in the flavorings or aroma, food processing, cosmetics, confectionaries and pharmaceuticals (Abdelwahab *et al.,* 2017). Moreover, it helps people with type-II diabetes and improves their ability to respond to insulin, thus normalizing their blood sugar levels (Khan *et al.,* 2003). In addition, cinnamaldehyde has health protecting effect that helps to prevent unwanted clumping of blood platelets (Abdelwahab *et al.,* 2017). Cinnamon bark contains high-value protein, fibre, vitamins, and minerals that are acknowledged to promote health facilities and prevent disease (Gul and Safdar, 2009). The antioxidant activity of cinnamon species has been extensively investigated. In recent years, during the COVID-19 pandemic, the use of cinnamon bark has increased noticeably in Bangladesh to boost immunity. However, only a few scientific or in our countries first report about the bioactive compounds, phytochemicals, and antioxidant compounds and their activities have been reported. Hence, this investigation aimed to estimate the major constituents' such as bioactive (cinnamaldehyde for aroma), phytochemicals, and antioxidant composition of indigenous cinnamon variety which can give more comprehensive information.

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The experiment was implemented at the Central Laboratory, Research Wing, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. A cinnamon sample was collected from the Spices Research Centre, BARI, Gazipur. Among them, the Cinamon var. BARI Daruchini-1 was considered in the present study. Fresh cinnamon barks were cleaned, washed, and dried at room temperature and ground to powder by using a grinder. Finally, sieves with a 60 micron opening were used to pass the powder, and stored in sealed containers for further research. The extracted essential oil from cinnamon bark to measure of bioactive elements from samples according to the following protocol with some modifications describe by Hossain *et al.*, (2023). The powdered sample (250 g) was added ethanol (750 ml) which was connected to the condenser in a heating mantle. Initially, the contents were heated $60^{O}C$ to $100^{O}C$ for 12 h and the essential oil content of each sample was determined on a dry weight basis and stored in glass vials at $4^{O}C$ until analysis. The bioactive compound of essential oils of cinnamon was estimated by gas chromatography. Cinnamon essential oil sample (200 ul) was treated with 5 ml of ethylate reagent and vortex and put in overnight at normal temperature. Then, 5 ml salt solution was added and shaken. The upper oily layer was collected and injected with a Gas Chromatograph (Shimadzu, GC-2010, Japan). The proximate composition (protein, fibre, fat, and carbohydrate) of dry powder of cinnamon bark samples was determined using the standard Association of Official Analytical Chemists (AOAC, 2000) method. The contents of minerals in cinnamon powder samples were estimated according to the method Petersen *et al.,* (2002) with slight modification. The total phenolic and flavonoid content were determined spectrophotometrically using the folin-ciocalteau and aluminum chloride colourimetric assay method John *et al.,* (2014). The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assay were determined according to a method suggested by Brand-Williams *et al.,* (1995). Data obtained for each analysis were expressed in triplicate. Mean values and standard deviation (SD) were used .

Bioactive composition of cinnamon bark oil

Essential oils and their bioactive constituents of cinnamon have out-standing antioxidant properties. The present study has therefore evaluated the chemical constituents of cinnamon bark oil by using gas chromatography (GC). BARI Daruchini-1 bark oil obtained or identified 15 bioactive compounds (Table 1). The most abundant components were *trans*-cinnamaldehyde (72.91%), eugenol (2.73%), eucalyptol (8.17%), limonene (2.42%), cinnamyl ester (2.07%), and α-Humulene (3.22%). The other important components were *p*-cymene (1.10%), myrcene (1.51%), linalool (1.17%), β-Caryophyllene (1.04%), and cinnamyl acetate (1.65%) (Table 1). The present result revealed that BARI Daruchini-1 contained a high amount of *trans*-cinaamaldehyde (72.91 %). The spicy and fragrance or aroma characteristics of cinnamon are mainly due to cinnamaldehyde. It is used traditionally to provide aroma, medicinal purposes, and essence compounds (Rao and Gan, 2014). In addition, its active constituents and other related this phenylpropanoid compound enhance defense against ROS contributed hyperglycemia and protect cells by reducing lipid peroxidation (Zhao *et al*., 2021). Another is eugenol (4-allyl-2-methoxy phenol) prevents ROS formation under conditions related to cancer, diabetes, inflammation diseases by breaking peroxidation cascade and scavenging radicals (Aminzare *et al*., 2018). In accordance with results, several studies have reported that cinnamaldehyde is the major chemical compound of *C. zeylanicum* bark essential oil (Unlu *et al*., 2010; Behbahani *et al*., 2020).

Table 1. Chemical constituents of BARI Daruchini-1 bark essential oil.

Name of compounds	Retention time (min)	Content (%)
		BARI Daruchini-1
α- pinene	1.265	0.39 ± 0.09
Benzaldehyde	1.575	0.71 ± 0.03
Eucalyptol	1.737	8.26 ± 0.70
Myrcene	2.187	1.51 ± 0.20
p-Cymene	2.272	1.17 ± 0.21
Limonene	2.375	2.41 ± 0.19
trans-cinaamaldehyde	2.460	72.64 ± 0.74
Terpinene	3.017	0.17 ± 0.02
Linalool	3.417	1.18 ± 0.02
Isoborneol	3.642	0.16 ± 0.03
Eugenol	3.738	2.74 ± 0.06
β- Caryophyllene	4.193	1.07 ± 0.11
Cinnamyl ester	4.712	2.09 ± 0.04
α-Humulene	5.230	3.22 ± 0.09
Cinnamyl acetate	6.457	1.63 ± 0.09

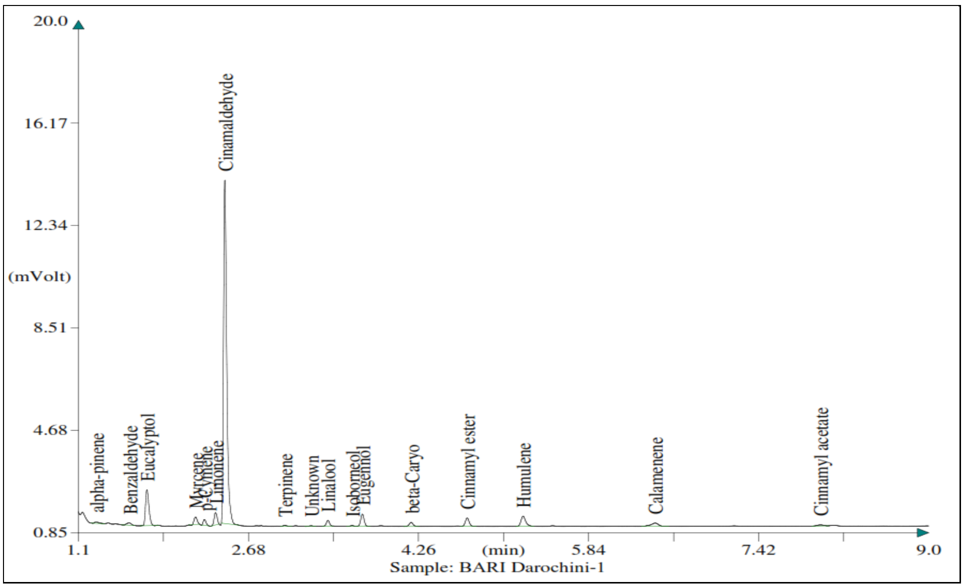

Fig. 1. Chromatogram of the chemical composition of BARI Darochini-1 bark oil.

Proximate and mineral composition of cinnamon bark

The nutrient content of cinnamon bark is shown in Table 2. The crude fibre, crude fat, crude protein, and total carbohydrate content of BARI Daruchini-1 are 24.76, 4.17, 5.02, and 58.73%, respectively. The present study observed that carbohydrate and fiber content represent the major nutrients constituent in Bangladeshi cinnamon, which is almost like the fiber and carbohydrate values of cinnamon reported by Lartey *et al.*, (2023) and Sana *et al.*, (2019). The protein composition of cinnamon (5.02%) in this study compares with that similar by Sana *et al.*, (2019). Furthermore, the cinnamon lipid or fat or oil result was higher than most of the values from previous studies (Gul and Safdar, 2009). Indeed, dietary fat is established to be very important in ensuring the absorption and retention of aroma or flavour, thereby increasing the palatability of food (Lartey *et al.*, 2023). The differences seen in the percentage composition of the parameters between different studies are suspected to be due to the length of drying time, the variation due to differences in varieties, growth conditions, harvesting times, soil properties, climate, origin, environmental conditions, and geographic parameters. Minerals are inorganic chemicals that are required in specific amounts for human cells to operate correctly. The composition of minerals in cinnamon is presented in Table 2. The average results indicated that 100 g of cinnamon bark powder contains calcium (661.19 mg), potassium (777.20 mg), phosphorus (195.27 mg), iron (11.06 mg), and zinc (8.79 mg), respectively . The results from the mineral analysis revealed potassium and calcium as the most predominant inorganic constituents in cinnamon, whilelowest values in iron and zinc composition. This agrees with the findings of Sana *et al.*, (2019) in which potassium and calcium constituted the majority of the mineral composition of cinnamon. Another highest occurring mineral component in cinnamon is phosphorus which was corroborative by Goel and Mishra, (2020). Its occurrence in such a quantity makes cinnamon a good addition to food for the maintenance of regular heart contraction, osteosynthesis, general body cell growth, and regulation of blood sugar levels as indicated by the role of phosphorus (Indrayan *et al.*, 2005). The nutritional composition of plant materials varied across geographical locations due to the differences in climate and growth conditions.

Table 2. Nutrient compositions of cinamon var. BARI Daruchini-1 bark

Nutrients	BARI Daruchini-1	Nutrients	BARI Daruchini-1
Content (%)		Amount in “mg/100g”	
Crude Fibre	24.76 ± 1.76	Calcium	661.19 ± 3.17
Crude Fat	4.17 ± 0.25	Potassium	777.20 ± 3.05
Crude Protein	5.02 ± 0.61	Phosphorus	195.27 ± 2.64
Carbohydrate	58.73 ± 0.81	Iron	13.06 ± 0.54
		Zinc	8.79 ± 0.24

Values are expressed as %, mean of three replicates ± standard deviation.

Total phenolic (TPC) content and total flavonoid (TFC) content of *C. zeylanicum*

Plant secondary metabolites, such as phenolic compounds, as a major group of phytochemicals, play an important role in plant protection, and these are responsible for antioxidant activity. Phenolic compounds and their activities are defined by their structure (reactive benzene rings), which is directly linked with quenching radicals in biological systems. The amounts of TPC in extracts of cinnamon bark are shown in Table 3. The TPC of BARI Daruchini-1 was 163.62 mg GAE/100g DW. The total phenolics might be the main substances that contribute the antioxidant activities for cinnamon. Besides, the total flavonoids have been reported to have multiple biological effects, including antioxidant activity. The capacity of flvonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. As shown in the Table 3, the TFC of bark extract is 97.83 mg QE/100g DW. This result agrees with the findings of Paliwal *et al.*, 2018. Phenolic and flavonoid compounds in plants characterize as a reducing agents by the ability to donate electrons to oxidant species, scavenge free radicals, and chelate metal ions (Khan *et al.*, 2003; Zhao *et al.*, 2021).

Table 3. Phytochemicals and antioxidants content in cinamon var. BARI Daruchini-1 bark

Compounds	BARI Daruchini-1
Phenolic compounds	
Total phenolic (mg GAE/100g DW)	163.62 ± 0.72
Total flavonoids (mg QE/100g DW)	97.83 ± 0.26
Antioxidant capacity	
DPPH radical activity inhibition (%)	57.29 ± 1.67
FRAP (mg GAE/100g)	39.35 ± 1.75

Values are expressed as %, mean of three replicates ± standard deviation.

Antioxidant Activities of cinnamon bark

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity assay was performed on the cinnamon sample and is given in Table 3. The DPPH content of BARI Daruchini-1 is 57.29%. DPPH free radicals react with antioxidant compounds and are inhibited. The reduction of DPPH molecules is directly related to the number of hydroxyl groups. Hydroxyl groups by denoting H into free DPPH radicals converts them from purple diphenyl pyrilyl-hydrazine to colorless compound; thus, the discoloration degree indicates the free radical scavenging activity of the antioxidant agent (Aminzare *et al.*, 2018), and finally, the results are expressed as the percentages of a reduction in the adsorption of DPPH solutions in the presence of essential oils. Our results indicate that DPPH activity of the essential oil of BARI Daruchini-1 was found to be 57.29%, indicating the strong ability of the oil to neutralize DPPH free radicals *via* either hydrogen atom or electron donation mechanisms (Aminzare *et al.,* 2018).

The ferric-reducing antioxidant power (FRAP) activity of cinnamon oil is measured by the reduction of Fe^{3+} TPTZ complex to ferrous (Fe^{2+}) form (intense blue colour) using electron-donating antioxidants at low pH, measuring FRAP values by absorbance change with the increase in absorbance of blue Fe^{3+} concentrations, which forms an important mechanism for antioxidant action. Increasing the optical absorption in the mixture caused by the intensity of the green color produced by the production of iron II (ferruginum) will mean increasing the reducing (antioxidant) power. The present results of the reducing power test are shown in Table 3. The FRAP content of BARI Daruchini-1 is 39.35 mg GAE/100g. According to the amount of reported absorption if the concentration of oils is increased, the reducing power is increased; as well as the reported values (39.35 mg GAE/100g) indicate that the reducing activity of cinnamon was higher. Indeed, it has been proven that antioxidant power is directly related to the percentage of phenolic compounds in essential oils (Abdelwahab *et al*., 2017).

C. zeylanicum is used worldwide as a spice in daily life with a positive impact on human health. The major bioactive constituents of BARI Daruchini-1 were found to be *trans*-cinnamaldehyde (72.91%), eugenol (2.73%), eucalyptol (8.17%), limonene (2.42%), cinnamyl ester (2.07%), and α-Humulene (3.22%). These compounds can affect the human body in many different ways and can be applied to treat a wide range of diseases and metabolic disorders. Among the most valuable bioactive element is cinnamaldehyde, which is widely used in the aroma or flavorings, food processing, cosmetics, confectionaries and pharmaceuticals. The nutrient analysis revealed cinnamon as a rich sources of fibre (24.76%), carbohydrate (58.73%), and lipid (4.17%) and mineral content also revealed calcium (661.19 mg/100g), potassium (777.20 mg/100g), phosphorus (195.27 mg/100mg), and iron (11.06 mg/100g) of BARI Daruchini-1 variety. The cinnamon oil contained remarkable levels of phytochemicals such as phenolic (163.62 mg GAE/100g), flavonoid (97.83 mg QE/100g), and antioxidants, DPPH radical scavenging activities (57.29%), and FRAP activities (39.35 mg GAE/100g). Due to their antioxidant properties these compounds indirectly affect the receptor-mediated mechanism. Overall, nutritional, chemical profiles and antioxidants could be effectively applied for monitoring cinnamon which plays significant roles in human body.

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