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ANTIDIARRHOEAL ACTIVITY, NITRIC OXIDE SCAVENGING AND TOTAL TANNIN CONTENT FROM THE BARK OF *CERIOPS DECANDRA* (GRIFF.) DING HOU

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ABSTRACT

The present study was designed to investigate the antidiarrhoeal, nitric oxide scavenging and total tannin content from the ethanolic extract of the bark of *Ceriops decandra* (Griff.) Ding Hou (Family: Rhizophoraceae). The ethanol extract at graded doses (200 & 400 mg/kg body weight) was investigated for antidiarrhoeal activity in term of reduction in the rate of defecation and consistency of faeces in castor oil induced diarrhoea. At the dose of 400 mg/kg body weight, the extract showed a significant anti-diarrhoeal activity showing 61.57% reduction in diarrhoea ($P<0.05$) comparable to that of standard drug loperamide 68.55%. Two complementary test systems, namely nitric oxide scavenging and total tannin contents were used for screening antioxidant activities. The ethanol extract showed maximum scavenging activity of 67.42 ±0.83% at 100 µg/ml, where as ascorbic acid at the same concentration exhibited 81.13±0.74% inhibition. The IC_{50} value for ethanolic extract was found fairly significant (63.59 µg/ml) while compared to the IC_{50} value of the reference standard ascorbic acid (36.81 µg/ml). The total tannin content was also quite significant and high in ethanolic extract (276.52 mg/g of tannic acid equivalent). The results of the present study confirm antidiarrhoeal activity, nitric oxide scavenging and total tannin content from the bark of *Ceriops decandra*, thus provide the scientific basis for the traditional uses of this plant as the modality for diarrhoea and other pathological conditions where free radicals are implicated.

INTRODUCTION: *Ceriops decandra* (*C. decandra*) (Griff.) Ding Hou (Family: Rhizophoraceae) locally known as Goran and Cuttia (Bengali) is a medium-sized straight, columnar, evergreen tree, under favorable conditions reaching up to 35 m in height distributed widely throughout the east coast of Bangladesh and India, southwestern Thailand and western part of the Malay peninsula.

This mangrove species *C. decandra* has long been used as a traditional folk remedy for angina, diabetes, diarrhea, dysentery, wounds and boils [1, 2]. A new epoxy ent-kaurene diterpenoid ceriopsin E was isolated from *C. decandra* [3]. Two diterpenoids, ceriopsins F and ceriopsins G have been isolated from the bark of *C. decandra* [4]. The leaf, bark and pneumatophore of *C. decandra* possess antinociceptive, antidiabetic and anti-inflammatory activities [5, 6, 7].

Recently, antioxidant properties from the bark of *C. decandra* have been investigated [7, 8]. But no data is available to confirm the nitric oxide scavenging and total tannin content from the bark of this plant.

Diarrhoeal disease is a leading cause of mortality and morbidity, especially in children in developing countries [9]. A vast majority of the people of developing countries relies on herbal drugs for the management of diarrhoea. Considering this fact the World Health Organization has constituted a diarrhoeal disease control programme, which includes studies of traditional medicinal practices, together with the elevation of health education and prevention approaches [10].

Nitric oxide (NO) has emerged as one of the more intriguing molecules in vertebrate biology in recent years [11]. NO is a lipophilic and highly diffusible solute, forms within the cell and its actions are concentration dependent [12]. NO scavengers have been considered including dithiocarbamate derivatives that chelate iron and thus, bind NO, but these two have some adverse effects [13].

Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytoconstituents due to their well known abilities to scavenge free radicals (i.e. antioxidant power) [14].

Since no literature is currently available to substantiate antidiarrhoeal activity, nitric oxide scavenging and total tannin content from the bark of *C. decandra*, the present study was designed to provide scientific evidence for its use as a traditional folk remedy [2] by investigating the antidiarrhoeal activity as well as nitric oxide scavenging and total tannin content that also confirm its use in diarrhoeal remedy and other pathological conditions where free radicals are implicated.

MATERIALS AND METHODS:

Collection and identification of Plant Material: The plant *C. decandra* was collected from Sathkhira area of Sundarban forest, Bangladesh in December 2010 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession number-DACB-39541).

Preparation of ethanolic extract: The collected barks were separated from undesirable materials and then were washed with water and sun-dried for one week. The dried plant materials were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powdered sample was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 400g of powered material was taken in a clean, flat-bottomed glass container and soaked in 1200 ml of ethanol.

The container along with its contents was sealed and kept for a period of 7 days with occasional shaking or stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. It was then filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated by using rotary evaporator at reduced pressure (Buchi, Switzerland) and dried. It rendered a 40.96g of gummy concentrate (10.24%) and was designated as crude ethanol extract.

Test for different Chemical Groups: The crude ethanolic extract was tested for its different chemical groups (phytoconstituents) as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins [15, 16]. 10% (w/v) solution of the extract in ethanol was used for each of the above test.

Test for Antidiarrhoeal Activity:

Test animals & drugs: White albino mice (Swiss-webstar strain, body weight: 20-25 gm) of both sexes were used for *in vivo* Antidiarrhoeal activity. They were housed in standard environmental conditions at animal house of Pharmacology Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong. Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°C), relative humidity: 55-65% and 12hrs light/12 hrs dark cycle) and had free access to feed and water ad libitum. The cages were cleaned once daily. This study was carried out following approval from the ethical committee comprising pharmacologist and toxicologist expert on the use and care of animals of the BCSIR. Loperamide (Square Pharmaceuticals Ltd, Bangladesh) was used as standard drug for this study.

Castor oil-induced Diarrhoea: The experiment was performed according to the method described by Shoba and Thomas [17]. Briefly, mice fasted for 24 h were randomly allocated to four groups of five animals each. The animals were all screened initially by giving 0.5 ml of castor oil. Only those showing diarrhoea were selected for the final experiment. Group I received 1% tween 80 (10 ml/kg, p.o.). Group II was given Loperamide (10 mg/ kg, p.o.). Groups III and IV received the drug extract orally 200 and 400 mg/kg respectively. After one hour each animal was given 0.5 ml of castor oil by oro-gastric polyethylene catheter and placed in separate cages, the floor of which was lined with adsorbent paper which was changed every hour, observed for 4 h and the characteristic diarrhoeal droppings were recorded.

Chemicals: L-ascorbic acid, Folin-ciocalteu phenol reagent, Griess reagent and tannic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium nitroprusside, tween 80, phosphate buffer saline, castor oil, ferric chloride and sodium carbonate were of analytical grade and purchased from Merck (Darmstat, Germany).

Nitric oxide (NO) Scavenging Activity: Nitric oxide scavenging activity was measured spectrophotometrically [18]. Sodium nitroprusside (5 mmol) in phosphate buffered saline was mixed with different concentrations of the extract (5–100 µg/ml) dissolved in methanol and incubated at 25 °C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubation solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride).

The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm with a double beam Analykjena UV/Visible spectrophotometer (Model 205, Jena, Germany). The nitric oxide (NO) radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I\ (\%) = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound with all reagents. IC_{50} value is the concentration of sample required to scavenge 50% nitric oxide free radical and was calculated from the plot of inhibition (%) against extract concentration. All the tests were carried out in triplicate and average of the absorptions was noted. Ascorbic acid was used as positive control standard for this study.

Total Tannin Content Determination: The tannins were determined using the Folin-ciocalteu Phenol reagent as reported by Amorim [19]. Briefly, 0.1 ml of the sample extract is added with 7.5 ml of distilled water and then added 0.5 ml of Folin-ciocalteu Phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of tannic acid is read against a blank. The results of tannins are expressed in terms of tannic acid in mg/g of extract.

Total tannin content was determined as mg of tannic acid equivalent per gram using the equation obtained from a standard tannic acid calibration curve $y=4.5692x-0.2538$, $R^2=0.9953$.

Statistical Analysis: Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. *p* values < 0.05 were considered to be statistically significant.

RESULTS:

Chemical Group Test: Preliminary phytochemical screening of the ethanol extract of *Ceriops decandra* bark revealed the presence of reducing sugars, gums, saponins and significantly presence of flavonoids and tannins (**Table 1**).

TABLE 1: RESULTS OF DIFFERENT GROUP TESTS OF ETHANOLIC EXTRACT OF *C. DECANDRA* BARK

Phytoconstituents	Ethanol extract of *C. decandra*
Alkaloid	-
Reducing sugars	+
Tannins	++
Gums	+
Flavonoids	++
Saponin	+
Steroid	-

+: Positive result; - : Negative result; ++: significantly positive

Test for Antidiarrhoeal Activity*:* The ethanol extract was found to be effective against castor oil induced diarrhoea on experimental mice at the dose of 400 mg/kg body weight (**Table 2**). At the dose of 400 mg/kg body weight, the extract produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation and consistency of faeces in albino mice. At the same dose, the extract showed a significant antidiarrhoeal activity (P<0.05) showing 61.57% reduction in diarrhoea comparable to that of standard drug loperamide 68.55%.

TABLE 2: EFFECT OF *C. DECANDRA* BARK EXTRACT ON CASTOR OIL-INDUCED DIARRHOEA IN MICE

Groups	Treatment	Dose (p.o)	No. of faeces in 4h	% Inhibition of defaecation
Group-I	1% Tween 80 in water	10 ml/kg	22.9±0.86	-
Group-II	Loperamide	10 mg/kg	7.2±0.91	68.55*
Group-III	EE of *C. decandra*	200 mg/kg	15.6±0.65	31.87
Group-IV	EE of *C. decandra*	400 mg/kg	8.8±1.28	61.57*

Values are presented as mean ± SEM, (n = 5); * $p < 0.05$, Dunnet test as compared to control; p.o: per oral; EE: Ethanol extract

Nitric oxide (NO) Scavenging Assay: The scavenging of NO by the ethanol extract of *C. decandra* was increased in dose dependent manner. A significant decrease in the NO radical due to scavenging ability of the extract and ascorbic acid is showing at **Table 3**. The ethanol extract showed maximum scavenging activity of 67.42 ±0.83% at 100 µg/ml, where as ascorbic acid at the same concentration exhibited 81.13±0.74% inhibition. The IC_{50} value for ethanolic extract was found fairly significant (63.59 µg/ml) while compared to the IC_{50} value of the reference standard ascorbic acid (36.81 µg/ml).

Total Tannin Content: The total tannin content was calculated as quite high in ethanolic crude extract 276.52 mg/g of tannic acid equivalent that are shown at **table 4**.

TABLE 3: NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF THE ETHANOLIC EXTRACT OF *C. DECANDRA* AND ASCORBIC ACID (STANDARD)

Concentration (µg/ml)	% NO inhibition of the extract and standard at different concentration	
	Ethanol Extract of *C. decandra*	Ascorbic acid (standard)
5	10.26 ±0.92	27.55 ± 0.29
10	16.38 ±0.88	35.24±0.38
20	25.29 ±0.62	47.75±0.59
40	36.34 ±0.76	56.49±0.46
60	48.12 ±0.79	66.26±0.73
80	59.29 ±0.46	72.11 ± 0.68
100	67.42 ±0.83	81.13±0.74
IC_{50} (µg/ml)	63.59 ±0.71	36.81 ± 0.63

The values are expressed as mean ± standard deviation (n=3)

TABLE 4: TOTAL TANNIN CONTENT OF ETHANOL EXTRACT OF *C. DECANDRA* BARK

Extract	Avg. absorbance at 725 nm	Total tannin content
		mg of tannic acid equivalent (TAE) per gm of dry extract
Ethanol extract of *C. decandra* bark	1.01±0.08	276.52±0.65

The values are expressed as mean ± standard deviation (n=3)

DISCUSSION: Several mechanisms have been proposed to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na^+, K^+-ATPase activity to reduce normal fluid absorption [20], activation of adenylate cyclase or mucosal cAMP mediated active secretion [21] , stimulation of prostaglandin formation [22], platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil [23]. However, it is well evident that castor oil produces diarrhoea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [24].

Since the ethanol extract of *C. decandra* successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action via antisecretory mechanism which was also evident from the reduction of total number of wet faeces (not shown separately) in the test groups in the experiment. Again, flavonoids present in the plant extract are reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil [25]. The antidiarrhoeal activity of the extract may also be due to denature proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion.

Nitric oxide (NO) scavenging capacity of the extract may help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions [26]. NO works as an atypical neural modulator that is involved in neurotransmitter release, neuronal excitability and learning and memory. Besides its role in physiologic processes, it also participates in pathogenic pathways underlying a large group of disorders including muscle diseases, inflammatory bowel disease, sepsis and septic shock, primary headaches and stroke.

Additionally, increasing evidence shows that NO modulates neurotoxin induced cell damage and is involved in neuronal cell death in Parkinson's disease (PD) and other neurodegenerative disorders such as Alzheimer disease [27]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals [28]. The high inhibition value of *C. decandra* ethanol extract may due to the presence of significant amount of flavonoids and tannins in the extract as phytochemical. Flavonoids and tannins, commonly found in plants have been reported to have significant antioxidant activity [29].

Various phytochemical components, especially polyphenols (such as flavonoids, phyenyl propanoids, phenolic acids, tannins etc) are known to be responsible for the free radical scavenging and antioxidant activities of plants. The total tannin amount was calculated as quite high in ethanolic crude extract (276.52±0.65 mg/g of tannic acid equivalent) (table 6). In generally, polyphenols all share the same chemical patterns, one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals [30].

In conclusion, the present study demonstrates that the ethanol extract of *C. decandra* bark contains pharmacologically active substance(s) possessing significant antidiarrhoeal activity, nitric oxide scavenging activity and significant amount of tannins content. The present data provided a scientific support for the traditional use of this plant as diarrhoeal remedy [2] as well as significant activity on nitric oxide scavenging and total tannins content also confirm its use in diarrhoeal treatment [23]. The nitric oxide scavenging activity of the ethanol extract might be due to the presence of significant amount of flavonoids and tannins as phytoconstituents.

However, more detailed phytochemical analysis will be necessary to isolate and characterize the active compounds which are responsible for the antidiarrhoeal and nitric oxide scavenging activities and to understand exact mechanisms of action of these activities.

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