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ANALGESIC AND ANTHELMINTIC ACTIVITIES IN COMMON FRUITS OF THE SUNDARBANS MANGROVE FOREST, BANGLADESH

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Abstract

The analgesic and anthelmintic activities of the common fruits in the Sundarbans forest, Bangladesh were evaluated. Acetic acid-induced writhing test and hot plate test in mice model were demonstrated for examining the analgesic activity whereas an anthelmintic test was performed using a flatworm, *Paramphistomum cervi* of livestock ruminants. Among the fruits, *Ceriops decandra* showed the strongest analgesic activity through inhibiting acetic acid-induced writhing (45%) and increasing response time (16.8 sec) on a hot plate in mice at 250 mg extract/kg body weight (b.w.). Diclofenac sodium (25 mg/kg b.w.), a positive control, demonstrated writhing inhibition of 51.3% whereas morphine (10 mg/kg b.w.), a well-known centrally active analgesic drug, increased response time of 18.2 sec. The fruits showed inhibition of acetic acid-induced writhing in mice with strong correlations to both the total polyphenols ($r^2 = 0.85$) and the total flavonoids ($r^2 = 0.81$) contents. Similar results were also observed for an increase of response time in mice on hot plate test. Therefore, both the peripheral and the central analgesic activities of the fruits were profoundly related to their total contents of polyphenols as well as flavonoids. Extract of *Aegiceras corniculatum* fruit displayed the strongest anthelmintic activity with the lowest CDT_{50} (concentration for death time 50) of 0.69 mg extract/ml whereas albendazole, a positive control, had CDT_{50} of 5.60 mg/ml for the parasite. The anthelmintic activity of the fruits was not dependent on their contents of polyphenols ($r^2 = 0.30$) or flavonoids. The chromatograms of HPLC-DAD analysis detected caffeic acid, (+)-catechin hydrate, *p*-coumaric acid, (-)-epicatechin, rutin hydrate, and syringic acid in *C. decandra* fruit extract and quantified as 10.6, 598.4, 1.5, 28.5, 2 and 1.3 mg/100 g extract, respectively. Results revealed that *C. decandra* and *A. corniculatum* fruits are the potentials to treat pain and helminthiasis, respectively.

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage as defined by the International Association for the Study of Pain (IASP) (Merskey and Bogduk 1994). Pain is involved in impairments in attention control, working memory, mental flexibility, problem-solving, and information processing speed (Hart *et al.* 2003) and it also increases depression, anxiety, fear and anger (Bruehl *et al.* 2009). Various analgesic drugs are useful in treating pain but their prolonged use elicits side effects such as gastrointestinal ulceration, bleeding disorders, hepatopathy and immunosuppression (Kahn 2002).

Humans, livestock and domestic animals are frequently infected with various types of helminths. Reportedly, more than two billion people are affected by helminths, particularly in the tropical and subtropical regions of the world. Helminthiases are involved in anemia, blindness, elephantiasis, fatigue, food intake reduction, growth stunting, indigestion, inflammation, malabsorption, maternal morbidity and mortality, memory and cognition impairment, and neonatal

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underweight (Christian *et al.* 2004, Hotez *et al.* 2008). Moreover, each year parasitic nematodes are the cause of US$ 157 billion loss in agriculture (Abad *et al.* 2008). Therefore, it is of utmost importance to control these diseases. Though various synthetic drugs are available to combat pain and helminthiases they show severe side effects and hence, demands for natural medicines are increasing day by day because they are free from adverse effects.

Fruits in the Sundarbans mangrove forest provide various nutrients and beneficial bioactive components to the coastal rural people of Bangladesh. Physicochemical properties, antioxidant, antibacterial and antidiarrheal activities of the fruits were reported (Hossain *et al.* 2013, 2016, 2017, Hosen *et al.* 2020, 2021). Preparations of different food recipes using *Avicennia* sp., *Nypa fruticans*, *Bruguiera gymmnorrhiza* fruits were also reported (Brown 2006). However, it showed that the consumption of fruits is associated with the reduction of pain (Han *et al.* 2017). Though forest products such as edible fruits are an inseparable part of tropical coastal people, little scientific attention on edible mangrove fruits has been paid until today. To fulfill food security as well as primary health care of coastal vulnerable people, it is paramount important to explore the edible fruits in the Sundarbans mangrove forest, and therefore, the present research was conducted.

Materials and Methods

The mature fruits namely *Aegiceras corniculatum* (L.) Blanco., *Avicennia officinalis* L., *Bruguiera gymnorrhiza* (L.) Lamk., *Ceriops decandra* (Griff.) Ding Hou, *Heritiera fomes* Buch.-Ham., *Nypa fruticans* Wurmb., *Phoenix paludosa* Roxb., *Sarcolobus globosus* Wall., *Sonneratia apetala* Buch.-Ham., *Sonneratia caseolaris* (L.) Engl., and *Xylocarpus mekongensis* Pierre were collected from the Sundarbans mangrove forest in Bangladesh from July to September 2017. Collected fruits were washed in distilled water and then, the separated edible parts were cut into small pieces and shed dried. Using a grinder machine dried fruit was pounded into a fine powder, and stored at room temperature in an air-tight container.

Ten grams of powder of each fruit was mixed with 200 ml of solvent (methanol:ethanol, 1:1). The mixture was shaken vigorously and then, kept overnight in a shaking incubator at 30^{o}C (150 rpm). The filtrate was collected from the mixture after filtration through the filter paper (Whatman No. 1). The collected filtrate was then air-dried, and the obtained extract was stored at 4^{o}C in a refrigerator.

The Folin-Ciocalteu's method (Ough and Amerine 1988) was followed to determine the total polyphenols in the extracts, and the amount was expressed as mg of gallic acid equivalent (mg GAE) since it was used as positive control. The colorimetric assay (Zhishen *et al.* 1999) was performed to measure the total flavonoids contents in the extracts and was expressed as mg of (+) - catechin equivalent (mg CE) because of using (+)- catechin as positive control.

Swiss-albino mice (18-22 g) were purchased from the International Centre for Diarrheal Disease Research, Dhaka, Bangladesh (icddr'b). Following the guidelines of the Animal Ethics Committee, Khulna University, Bangladesh (Research Ref. No.: KUAEC-2017/07/02), the experiments were conducted. The analgesic test was performed on Albino mice using acetic acid-induced writhing (Koster *et al.* 1959) and on a hot plate as described (Eddy and Leimbach 1953). The anthelmintic activity of the fruits was examined following the method described by Tandon *et al.* (1997) with some modifications. Parasites, *Paramphistomum cervi* (n = 10) were incubated at room temperature with the treatments and observed for the paralysis time and the time until death. The concentration of the fruit extracts for death time 50 (CDT_{50}) of the parasite was calculated using the following equation:

$$CDT_{50} = \frac{1}{n}\sum_{i=1}^{n}\frac{\text{(Treatment concentration)x (mean death time of the treatment)}}{\text{½(mean death time of the control)}}$$

where n = number of treatment; i = initial treatment number. Selected phenolic compounds in the extract of *C. decandra* were detected and quantified using HPLC-diode array detector (DAD) analysis (Ahmed *et al*. 2021).

Mean ± standard deviation was used to express the results. Statistical difference in several groups was analyzed by one-way analysis of variance (ANOVA) where p values < 0.05 were considered significant.

Results and Discussion

The contents of total polyphenols in *A. corniculatum*, *A. officinalis*, *B. gymnorrhiza*, *C. decandra*, *H. fomes*, *N. fruticans*, *P. paludosa*, *S. globosus*, *S. caseolaris* and *X. mekongensis* fruits were 16.3, 17.1, 22.6, 58.1, 47.1, 4.6, 31.2, 9.3, 22.1 and 19.2 mg GAE/g powder whereas total flavonoids contents were 3.2, 27.1, 9.2, 86.4, 58.7, 1.2, 35, 3.8, 13.9 and 19.7 mg CE/g powder, respectively. Polyphenol is the largest group of consumed bioactive phytochemicals and they are involved in preventing the pathogenesis of many metabolic and infectious diseases.

Abdominal constrictions in mice induced by acetic acid were significantly ($p < 0.05$) inhibited when treated with the extracts (methanol:ethanol, 1 : 1) orally at 250 mg/kg b.w. (Fig. 1). Extract of *C. decandra* fruit showed the highest inhibition (%), which was similar to diclofenac sodium (25 mg/kg b.w.), a standard drug used as positive control, followed by *H. fomes*, *A. corniculatum* and *P. paludosa* (Fig. 1A). Control group revealed the average writhing numbers of 47.2 ± 2.6. Since extracts of *C. decandra* and *H. fomes* fruits demonstrated strong activity, their dose-dependent inhibitions of writhing numbers in mice were also shown (Fig. 1B). Fruit extract of *C. decandra* (500 mg/kg b.w.) showed a stronger inhibitory effect (63%) than the positive control (51%) on acetic acid-induced writhing in mice. Inhibitions (%) of acetic acid-induced writhing by the fruit extracts (250 mg/kg b.w.) displayed strong correlation with their contents of total polyphenols ($r^2 = 0.85$, Fig. 1C) and flavonoids ($r^2 = 0.81$, Fig. 1D). Figure 2 showed the response time (paw licking/jumping) increasing effects of different fruit extracts on mice. The fruit extracts at 250 mg/kg b.w. increased the response time significantly ($p < 0.05$) than that of control mice at 60, 90, 120 and 150 min on the hot plate. All the fruits exhibited the highest response time at 90 min and then started to decline. Fruits of *C. decandra* showed the highest response time of 16.8 sec followed by *H. fomes* (14.2 sec) and *A. corniculatum* (13.6 sec) whereas a positive control, morphine (10 mg/kg b.w.) showed a response time of 18.2 sec and that for control was 9.2 sec (Fig. 2A). The dose-dependent increase of response time was studied for the fruit extracts of *C. decandra* and *H. fomes* (Fig. 2B, 2C). Even at 50 mg/kg b.w., *C. decandra* had a prolonged analgesic effect that was significantly started from 30 min and continued over 150 min. The correlation coefficient between response time at 90 min of the fruit extracts at 250 mg/kg b.w. and total polyphenols contents was 0.82 (Fig. 2D) and so did total flavonoids.

The acetic acid-induced writhing and hot plate tests were utilized to investigate the peripheral (Julius and Basbaum 2001) and the central (Yaksh and Rudy 1977) analgesic activities, respectively. The fruit extracts showed a strong correlation between the polyphenols or flavonoids contents and analgesic activity in mice. Since the fruits of *C. decandra* and *H. fomes* had high contents of polyphenols and flavonoids, they showed strong analgesic activity mediated through both the peripheral and the central nervous systems. Polyphenols such as (+)-catechin showed an antinociceptive effect by opening the ATP-sensitive K^+ channel and inhibiting the release of histamine, serotonin, and bradykinin (Islam *et al*. 2019a). Moreover, different studies showed the anti-histaminic and/or anti-inflammatory activities of citrus fruit peels, and various edible fruits (Hossain *et al.* 2008, Mubassara *et al.* 2011, Tsujiyama *et al.* 2013).

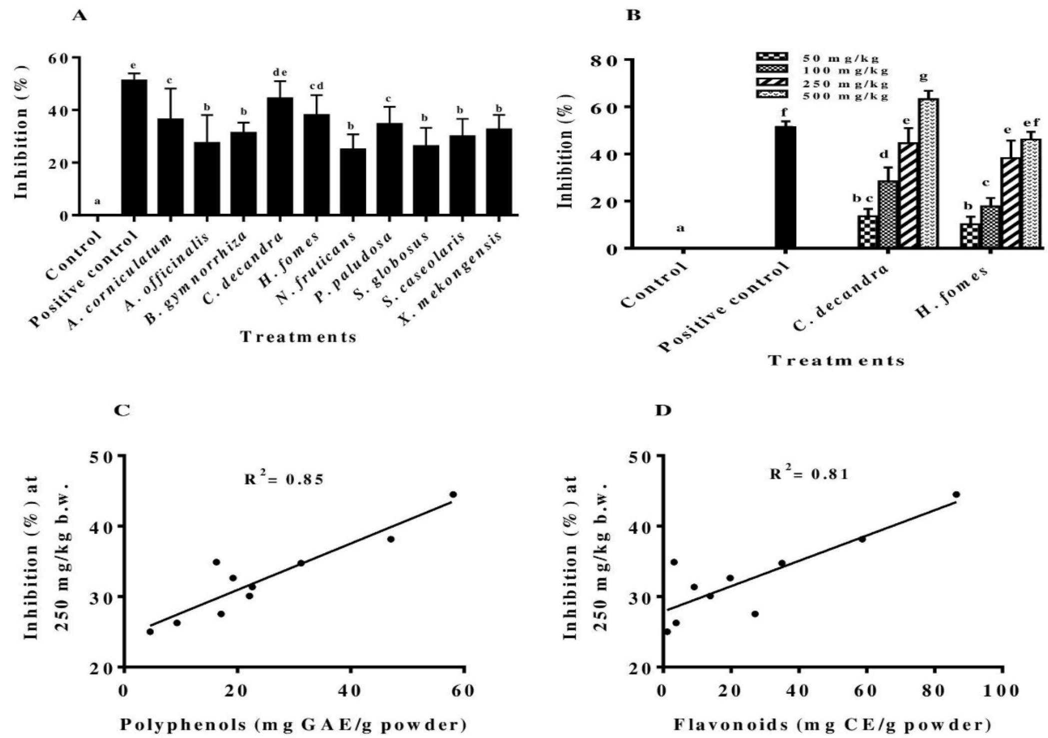

Fig. 1. Effects of edible fruit extracts (methanol:ethanol, 1 : 1) on acetic acid-induced writhing in mice. (A) Inhibition (%) of writhing at 250 mg extract/kg body weight (b.w.); (B) dose-dependent effects of *Ceriops decandra* and *Heritiera fomes* fruits on writhing inhibition (%). Correlation of writhing inhibition (%) at 250 mg extract/kg b.w. to (C) total polyphenols contents; and (D) total flavonoids contents of the fruits. Data are expressed as mean ± SD (n = 6). Different letters (a-g) indicate significant differences compared with each other group using one-way analysis of variance (ANOVA) at $p < 0.05$. Positive control, diclofenac sodium 25 mg/kg b.w. GAE, gallic acid equivalent; CE, (+)-catechin equivalent.

The parasite, *Paramphistomum cervi* was used to examine the anthelmintic activity of the fruit extracts. The time required for paralysis was designated as paralysis time whereas the time needed until the death of the parasites was termed as death time. Since the anthelmintic activity of *S. apetala* fruit was not reported, it was included in the present study. Among the eleven fruits, *A. corniculatum* showed the strongest anthelmintic activity because of the minimum time required for both the paralysis (42 min) and the time until death (66 min) of parasites at 10 mg/ml extract whereas albendazole, a standard drug (positive control, 15 mg/ml) showed the same at 78 min and 107 min, and that were at 322 min and 376 min for the control, respectively (Fig. 3A). The dose-dependent anthelmintic effects of potential fruit of *A. corniculatum*, *P. paludosa* and *S. apetala* are presented in Fig. 3B. Figure 3B also showed the stronger anthelmintic effect of *A. corniculatum* fruit than albendazole, the positive control. From the dose-dependent curves or using the equation mentioned in the methodology, the concentrations for death time 50 (CDT_{50}, the concentration of the extract that was needed to reduce 50% of the death time of parasites) of the fruit extracts were calculated. Figure 3C showed CDT_{50} values of the fruits where *A. corniculatum* showed the lowest

CDT_{50} (0.69 mg/ml) and therefore, it had the strongest anthelmintic activity followed by *P. paludosa* (CDT_{50}, 7.93 mg/ml), and CDT_{50} for the positive control was 5.6 mg/ml. A poor correlation ($r^2 = 0.30$) was observed between the anthelmintic activity of the fruit extracts at 10 mg/ml and total polyphenols contents (Fig. 3D). The anthelmintic activity of the fruits might be due to the presence of other components rather than polyphenols or flavonoids since they showed a small correlation. Different classes of compounds- lipids, phenolics, saponins, terpenoids, and alkaloids were mentioned as anthelmintic (Liu *et al.* 2020). Moreover, the antinematodal peptide was also reported from bacteria (Jang *et al.* 2004). However, the majority of anthelmintic drugs target ion channel proteins in the nematode (Liu *et al.* 2020).

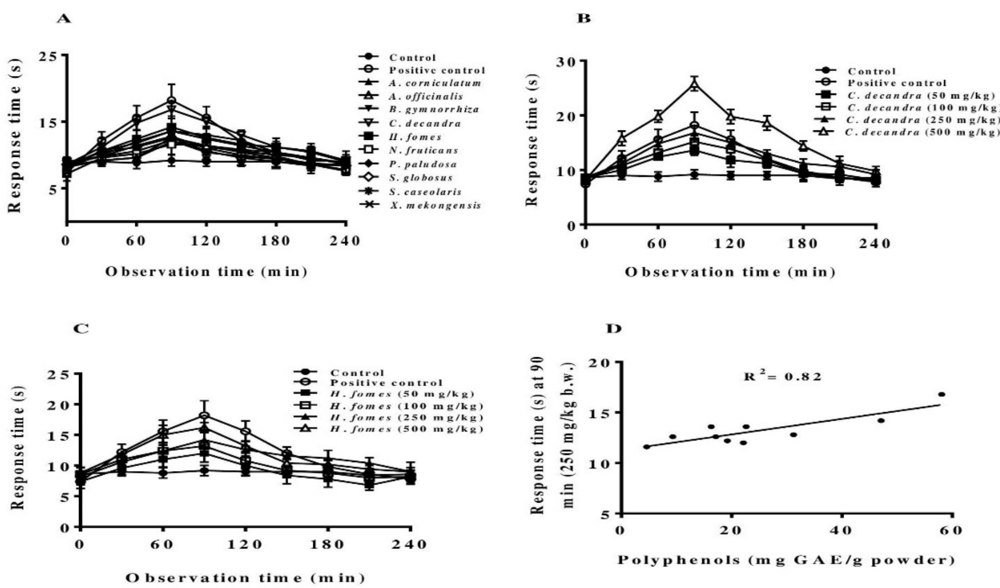

Fig. 2. Effects of fruit extracts (methanol:ethanol, 1 : 1) on the increase of response time (S, seconds) in mice. (A) Response time of mice treated with 250 mg extract/kg body weight (b.w.), observed at different times (min, minutes). Dose-dependent effects of (B) *Ceriops decandra*, and (C) *Heritiera fomes* on response time. (D) Correlation of the response time at 90 min by 250 mg extract/kg b.w. to total polyphenols contents of the fruits. Data are expressed as mean ± SD (n = 6). All data are significant ($p < 0.05$) at 60, 90, and 120 min when compared to the control group using one-way analysis of variance (ANOVA). GAE, gallic acid equivalent; positive control, morphine 10 mg/kg b.w.

Polyphenolic compounds in the extract of *C. decandra* fruit were detected and quantified with the HPLC-DAD system (Fig. 4) and they were caffeic acid, (+)-catechin hydrate, *p*-coumaric acid, (-)-epicatechin, rutin hydrate and syringic acid. Among them, (+)-catechin hydrate was the highest (598.4 mg/100 g extract) followed by (-)-epicatechin (28.5 mg/100 g extract) and caffeic acid (10.6 mg/100 g extract) (Table 1). Caffeic acid, (+) -catechin, (-) -epicatechin, ellagic acid, gallic acid and quercetin were also reported from the mangrove fruit of *S. apetala* (Hossain *et al.* 2016). Honey produced in the Sundarbans showed strong analgesic and anthelmintic activities (Islam *et al.* 2019b), and polyphenols- (+)-catechin, (-)-epicatechin, *p*-coumeric acid, syringic acid, *trans*-cinnamic acid, and vanillic acid were quantified (Islam *et al.* 2017). Reportedly the identified polyphenols are strong antioxidants, and they could effectively ameliorate the inflammatory responses through the reduction of oxidative stress and the inhibition of pro-inflammatory enzyme activities.

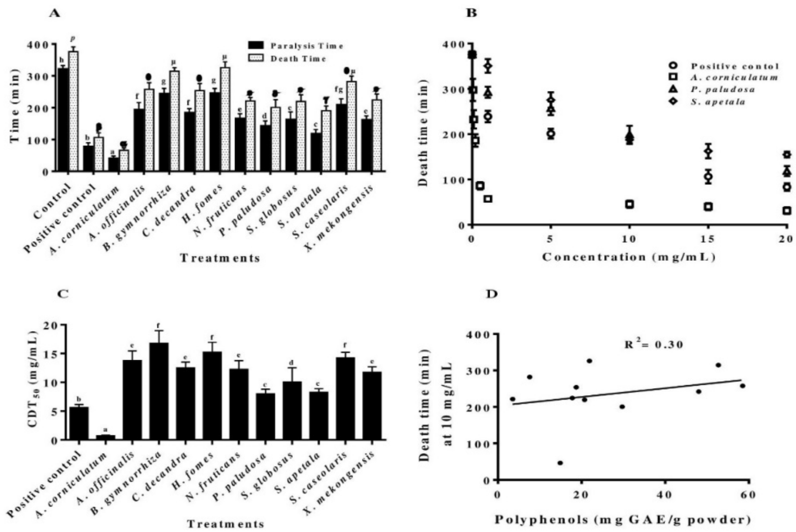

Fig. 3. Anthelmintic activity of fruit extracts (methanol:ethanol, 1:1) on *Paramphistomum cervi*. (A) Paralysis time (min, minutes) and death time (min) of the parasite at 10 mg/ml; (B) dose-dependent anthelmintic activities of *Aegiceras corniculatum*, *Phoenix paludosa* and *Sonneratia apetala* on death time of the parasite; (C) CDT_{50} (concentration for death time 50) values of the fruits; and (D) correlation of death time of the parasite at 10 mg/ml to total polyphenols contents of the fruits. Data are expressed as mean ± SD (n = 10). Different letters (a-f; α-ω) indicate significant differences compared with each other group using one-way analysis of variance (ANOVA) at $p < 0.05$. GAE, gallic acid equivalent; positive control, albendazole 15 mg/ml.

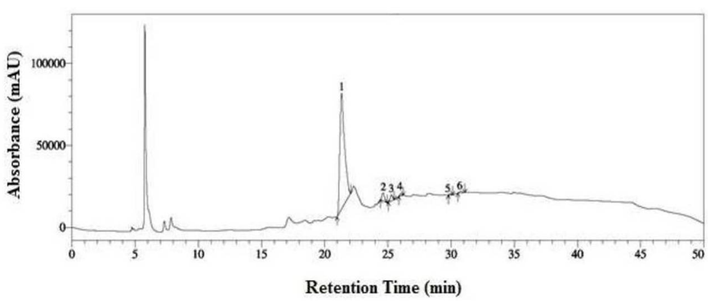

Fig. 4. HPLC chromatogram of methanol:ethanol (1 : 1) extract of *Ceriops decandra* fruit. Peaks: 1: (+)-catechin hydrate; 2: (-)-epicatechin; 3: caffeic acid; 4: syringic acid; 5: rutin hydrate; and 6: *p*-coumaric acid.

Table 1. Amount of polyphenolic compounds in the *C. decandra* fruit extract.

Polyphenolic compounds	Content (mg/100 g of dry extract)	% RSD
(+)-Catechin hydrate	598.4	1.7
(-)-Epicatechin	28.5	1.3
Caffeic acid	10.6	0.8
Syringic acid	1.3	0.1
Rutin hydrate	2.0	0.2
p-Coumaric acid	1.5	0.1

% RSD: % of relative standard deviation.

The results revealed that consumption of *C. decandra* and *A. corniculatum* fruits can prevent pain and helminthiasis, respectively. Results also showed that the analgesic effect of 289 mg extract produced from 1.7 g powder or 3.7 g fresh fruit of *C. decandra* was equivalent to diclofenac sodium of 25 mg/kg b.w. In Bangladesh to control intestinal worms 500 mg albendazole tablet is used that was equivalent to 8.3 mg extract produced from 43 mg powder or 76 mg fresh fruit of *A. corniculatum.* Thus, consumption of a few grams of *C. decandra* and *A. corniculatum* fruits will produce strong analgesic and anthelmintic activities, respectively. In the future, research should be concentrated to isolate and identify potential bioactive compounds from these two fruits and explore their utilization in dietary supplements, preventive medicine, and drug development.

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