Comparative Pharmacological Potential of *Ceriops decandra* (Griff.) and *Ceriops tagal* Linn: Medicinal Plants of the Sundarbans

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**Abstract**

**Background:** *Ceriops decandra* (Griff.) Ding Hou is used conventionally for managing a number of therapeutic conditions including diabetes, haemorrhage, pain, diarrhoea, angina, and dysentery etc. Furthermore, *Ceriops tagal* Linn is used to treat hemorrhage, acariasis, wound infection, malaria, malignant diabetes and ulcers etc in folk medicine. The present study was designed to evaluate the comparative pharmacological activities of leaves of these two medicinal plants of the Sundarbans.

**Methods:** Antioxidant activity was assessed by total phenolic, total flavonoids, total tannin content, and DPPH free radical scavenging assays. Acetic acid-induced test for analgesic, oral glucose tolerance test for antihyperglycemic, open field test for neurobehavioral, prothrombin time test for anticoagulant, anthelmintic potential on *Paramphistomum cervi*, and *in vivo* cytotoxicity test on brine shrimp nauplii were used for appraising pharmacological activities of both extracts.

**Results:** *C. decandra* extract significantly (*P* < 0.05) reduced writhing inhibition of 54.55% and 66.37% at 500 mg/kg and 250 mg/kg, respectively. In oral glucose tolerance test, *C. decandra* significantly lowered the blood glucose level for pretreated mice with glucose by 25.76%, 2272%, and 31.88%, 38.71% respectively, at 60 min and 120 min at the 250 mg/kg, and 500 mg/kg dose level. But, the reduction of blood glucose level by *C. tagal* extract was less than that of *C. decandra*. Furthermore, *C. decandra* produced a sedative effect at both doses starting from 30 min to 120 min of experimental period. The crude extract of *C. decandra* and *C. tagal* delayed the prothrombin time in a dose dependently. The *C. decandra* and *C. tagal* produced dose-dependent paralysis and death time. The LC50 values obtained from brine shrimp lethality bioassay were 94.69, 114.29 μg/mL for *C. decandra* and *C. tagal*, respectively.

**Conclusion:** *C. decandra* revealed prominent pharmacological activities than that of *C. tagal*. Also, the present study confirmed the scientific evidence of both plants.

**Keywords:** antioxidant, analgesic, anticoagulant, antihyperglycemic, anthelmintic, cytotoxic

1. Introduction

Bangladesh is one of the rich sources of diverse medicinal plants among the South Asian countries, where about 250 plants (out of 500) are being used as a source of raw materials for preparing different complementary medicines. Major percentages of them have not yet been subjected to scientific investigation and isolation of active constituents. Systematic scientific studies always help to discover any drug molecule especially based on ethnopharmacological and ethnobotanical approach [1]. This process accelerates the discovery process by collecting initial information from the ethnic population [2, 3]. Consequently, assessment of medicinal plants having promising bioactive potential has emerged as an important and revolutionary sector. Discovery of novel drug lead using natural sources consist of several unique features including availability, convenience, cheap, diversity and safety. Therefore, the study of medicinal plants could attract keen interest for scientists worldwide since last some years. *Ceriops decandra* (Griff.) Ding Hou is a shrub from family Rhizophoraceae family and grown in the mangrove forest. Traditionally this plant has been known for trading a number of disease such as angina hemorrhage, hepatitis wounds, boils, diarrhoea, ulcers, pain and dysentery, diabetes, [4-7]. Very few pharmacological reports are literally available on this plant.
The antinociceptive, antioxidant, antidiabetic, antifungal, antibacterial, chemopreventive properties of *C. decandra* has been reported earlier [8, 9, 6]. On scientific investigations, the leaves of *C. decandra* yielded many important chemical constituents such as lupenone, lupeol, betulinaldehyde, 3β-Z-coumaroylglucose, 30-nor-lup-3β-ol-20-one, 3-epi-betulinic acid, 3β-E-coumaroylglucose, betulin, betulinic acid, 3β-E-feruloylbetulin, oleanolic acid, 3β-E-cafeyloylglucose, lup-20(29)-en-3β,30-diol, 3β-hydroxylupan-29-oic acid, 3β,20-dihydroxylupeol, ursolic acid, and 3β-E-feruloylglucose and 3β-Z-coumaroylglucose which are of relevance to our present report. Another study reported that the leaf extracts afforded a-amyrin, β-amyrin, lupeol, oleanolic acid, and ursolic acid. The bark of *C. decandra* yielded decidrin A-K; 2-(9-hydroxy-3,5a,5b,8,8,11a-hexamethylicosahydro-1-hecylpenteno[a]chrysene-1-y1) propanoic acid (3β-hydroxylupan-29-oic acid), d-catechin, leucoanthocyanidins; procyanidin; decidrinin; (−)-syringaresinol, (−)-pinoresinol, β-sitosterol, stigmasterol, palmic acid, and 3,4-dihydroxybenzoic acid Et ete; and 7,13-abietadien-3β-ol, 7,13-oxodehydro-abietaol, marginocin, 3β-hydroxy-abieta-8,11,13-trien-7-one, 15,18-dihydroxyabieta-8,11,13-trien-7-one, 17β,18-dihydroxydehydriabietaol, 4-epiiriptobezene L, 7a, 18 dihydroxydehydriabietaol, sabipereone E, 13β,18-dihydroxy-abiet-8-[14],ene-7-one, ent-labd-8(17),13E-dien-15-ol, ent-8(14)-pinamere-15R,16-diol, and (5S*,8S*,9S*,10R*,13S*)-3-hydroxy-16-nor-2-oxodolar-3-en-15-oeic acid. Furthermore, the wood extract of *C. decandra* yielded 3β, 13β-dihydroxy-8-abietaen-7-one, and 3β-hydroxy-8,13-abietaen-7-one. Other studies reported the isolation of ceriopsin A-D, ceriopsin E, and ceriopsin F and G from the roots [5, 9, 10, 11,12].

*Ceriops tagal* Linn is also a mangrove shrub under Rhizophoraceae family. The bark of *C. tagal* is used in the treatment of hemorrhage in defecation and wounds due to its powerful astringent property, especially in Thailand and Philippines. The oil collected from this class has antipruritic property and thus used in the treatment of chillblain and acarasis. The decoction of its leaves is effective in treating malaria (substitute for quinine) and diabetes in China. In Bangladesh, it is used topically to treat malignant ulcers and abdominal ailments [13, 14]. Previous studies have reported the Cytotoxic, anticaner, antitumor, antibacterial, fungicidal, antidiabetic properties of *C. tagal* [15, 16, 17]. Previously some chemical constituents like terpenoids (mono, di and tri), flavonoids, polyphenolics alkaloids, saponins, glycosides, and sterols have been isolated from this plant [13, 18, 19]. Therefore, based on the traditional importance and application, a comparative assessment of the pharmacological potential of *C. decandra* and *C. tagal* leaves extracts was designed and carried out in this investigation.

### Materials and Methods

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, were procured from Sigma–Aldrich (St. Louis, MO, USA). Standard drugs Diclofenac sodium, Albendazole, Glibenclamide, Warfarin; and Vincristine sulfate were obtained from Square Pharmaceuticals Ltd. and Beacon Pharmaceuticals Ltd. Bangladesh, respectively. All other chemicals were reagent grades.

### Experimental animals

Swiss albino mice of ages 6–7 weeks and weight 22–28g were collected from Jahangirnagar University, Bangladesh. They were adapted for the experimental environment maintained at suitable conditions (55-65% relative humidity, 27 ± 1 °C temperature and 12:12 h light-dark cycle for two weeks).

### Collection of plant materials and extraction

The leaves of *Ceriops decandra* and *Ceriops tagal* were collected from the Sundarban, Bangladesh. The plant samples were authenticated by experts in Bangladesh National Herbarium, Mirpur, Dhaka, (Accession No.: DACB-43819 and DACB-43823, respectively) where voucher specimens have been deposited as reference.

### Preparation of crude and fractionated extract

The collected leaves were detached from undesirable materials, washed with distilled water and dried under shade and finally ground into a coarse powder using a suitable grinder (Capacitor start the motor, Wuhu motor factory, China). About 450 g ground powders were macerated in 1000 ml of ethanol (95%) for 14days accompanying routine shaking and stirring. The yield was obtained as 3.16% and 4.55% for *C. decandra* and *C. tagal*, respectively followed by filtration and evaporation of the solvent. Then the crude extracts were fractionated using n-hexane, ethyl acetate, butanol, and water. And finally, 0.85 g, 2.47 g, 2.06 g, 3.17 g extract were obtained for n-hexane, ethyl acetate, butanol, and water fraction for *C. decandra* and 0.92 g, 2.35 g, 1.96 g, 3.25 g for *C. tagal* leaves extract.

### Phytochemical screening

Diverse phytochemical groups such as tannins alkaloids reducing sugar, combined reducing sugar, xanthoprotein, saponins, gum, steroids, glycosides, flavonoids terpenoids and acidic compounds were identified by characteristic visual/physical change applying standard chemical tests procedure [20].

### Total phenol content

Folin-Ciocalteu’s method was used with minor modifications for determining the total phenolic content of the test extracts [21]. Briefly, test sample (1 mg/mL) was mixed with 5 mL of 10% (v/v) Folin-Ciocalteu reagent and 4 mL of sodium carbonate (75 g/L). The reaction mixture was left at 40 °C for 30 mins. After that, the absorbance of the reaction mixture was taken at 765 nm in a UV spectrophotometer. A standard calibration curve of gallic acid for different concentrations (0.1–0.5 mg/ mL) was prepared from where total phenol content was determined (unit: mg gallic acid equivalent (GAE) per gram of dry extract).

### Total flavonoids content

Aluminum chloride colorimetric assay was applied for estimating total flavonoid content [22]. In 1 ml of the extract solution (1 mg/mL), 4 mL of distilled water and 0.3 mL NaNO₂ (5% w/v) were added. Five minutes later, 0.3 mL AlCl₃ (10% w/v) and 2 mL NaOH (1M) were added and adjusted the volume up to 10 mL. Then it was kept for 15 minutes at room temperature and absorbance was recorded at 510 nm. Here, a standard calibration curve was prepared using quercetin (0.25-1 mg/mL) and the total flavonoid content of the extract was expressed in mg quercetin equivalent (QE)/g of dried extract.

### Total tannin content

Folin Ciocalteu method was used here to determine the tannin content of the extracts [23]. In 0.1 ml of the extract solution, 7.5 mL of distilled water and 0.5 mL of Folin Ciocalteu
phenol reagent, 1 mL of 35% (w/v) Na₂CO₃ solution was added and diluted up to 10 mL adding distilled water. After thorough mixing by proper shaking, the reaction mixture was stored at room temperature for half an hour. A calibration curve was prepared using tannic acids solutions as standard (20-100 μg/mL). Absorbance was taken for both samples and standard solutions at 725 nm and the resultant tannin content were recorded as mg of TAE/g of the dry extract.

**DPPH radical scavenging assay**

The ability of the test extract to scavenge the free radical 2,2-diphenyl-1-picryl hydrazyl (DPPH) was applied as the basic principle for this quantitative experiment [24]. Initially, a stock solution of 1024 μg/mL concentration of the test samples was prepared. Following serial dilution method, test samples of varying concentrations ranging from 512–1 μg/mL were prepared using that stock solution. Then, to each test tube containing 1 mL of each concentration, 3 mL DPPH alcoholic solution (0.1 mM) was added and allowed 30 min of incubation period keeping in dark at room temperature. After that absorbance was taken using a UV spectrophotometer at 517 nm. The solution of Ascorbic acid served as standard here and the DPPH free radical scavenging activity was calculated as Percent radical scavenging activity \( = \frac{[(A_0 - A)]}{A_0} \times 100 \) Where, \( A_0 \) is the absorbance of the control solution with all reagents except plant extract, A is the absorbance reaction mixture.

Finally, the IC₅₀ value (concentration of the sample required to scavenge 50% DPPH free radical) was calculated using trend line from the plot of inhibition (%) vs the concentration of test extract.

**Analgesic activity**

This test was conducted based on acetic acid induced writhing inhibition in mice model [21, 25]. 30 animals were randomly divided into six groups having 5 in each. Each animal of the set group received a particular treatment orally. Each mouse was weighed properly and the dose of the test samples, standard and control materials were calculated accordingly. A thirty minutes was time relapsed for complete absorption of the administered test solutions and then the acetic acid solution (0.7%, 15 mL/kg body weight) was administrated intraperitoneally to each of the animals of all groups. Five minutes later (adequate time for absorption of acetic acid), a number of squirms (writhing) given by each mice was counted for the next 15 minutes.

**Antihyperglycemic Activity Test**

Twenty-five experimental animals (overnight fasted) were divided into six groups consisting of five mice in each group. Group-I (control group) received 2% Tween 80 in water (10 mL/kg body weight). Group-II (standard group) received Glibenclamide at a dose of 5 mg/kg body weight. Group-III to VI received the crude extracts at a dose of 250 mg/kg and 500 mg/kg body weight [26, 27]. The fasting blood glucose level of each mouse was measured using a glucometer. The tail tips of tested mice were cut with a sharp blade and blood sample was collected. For avoiding any possible infection/inflammation on the cut tail end, the povidone-iodine ointment was applied on the affected area. The control, standard, and extracts were given orally with the help of a feeding needle in the respective treatment group. Thirty minutes later, glucose (2 g/kg body weight) was administered orally to all groups. Blood glucose levels were measured at 60 minutes and 120 minutes after glucose administration.

**Neuropharmacological Activity Test**

Albino mice of weight between 20 and 25 g were used to assess neuropharmacological test using the open-field method. Animals of either sex were grouped into four (I-IV), each having five animals. Group I (control) received vehicle orally (1% tween 80), Group II (standard) was treated with diazepam (1mg/kg, orally). Group III, IV, V, VI were given crude extracts orally at the doses of 250 and 500 mg/kg body weight. After respective treatment, animals were placed individually in one of the corner squares and the number of squares crossed by the animals was recorded for three minutes on 0, 30, 60, 90, and 120 min during the study period. The experiments were carried out in a sound-attenuated room [28].

**Anticoagulant activity test**

Anticoagulant activity of both crude extracts was measured by the prothrombin time test method [29]. Blood samples were collected in trisodium citrate (3.8%) containing tube from healthy volunteers (age 20-25years) free from cardiovascular diseases, diabetes, dyslipidemic disorders, nonsmokers, and normal prothrombin time. It was centrifuged at 3000rpm for 15 mins and plasma was separated and stored at 4°C until use. Different concentration of extracts (350, 175, 87.5, 43.25 mg/mL), 0.2mL plasma and 0.3mL CaCl₂ (25 mM) were mixed together in a test tube. 0.1 mL sodium chloride (9%), 0.2 mL plasma and 0.3 mL CaCl₂ (25 mM) were added to the second test tube for control. And, 0.1 mL warfarin, 0.2 mL plasma, and 0.3 mL CaCl₂ (25 mM) were added to the third test tube for standard. Each tube was incubated at 37°C and visually inspected for any signs of clotting by tilting the test tube every 5 seconds. This test was carried out three times and clotting time was recorded using a stopwatch and the average time was noted.

**Anthelmintic activity test**

This activity was conducted on live pathogenic parasites named *Paramphistomum cervi* collected from the cattle intestine (30). Live parasites, divided into four groups of six in each Petri dish, were treated with test extracts (25, 50, and 100 mg/kg) and Albendazole (1.5 mg/mL) as the positive control, respectively. Each Petri dish contained 10 mL of phosphate buffer saline (PBS) in which extracts and Albendazole were dissolved upon treatment with 0.2% Tween-80. The control group was administered with 0.1% Tween-80 in PBS. Anthelmintic activity was presented as the time consumed for paralysis and death of the test helminthes. The paralysis time was considered when no movement observed unless vigorously shaken. Death time was recorded when the parasites could not move even after shaking strongly, dipped in hot water (50 °C), or subjected to outer stimuli. The result was presented as the required time for both paralysis and death of helminthes as compared to control.

**Cytotoxic Activity Test**

This test was performed following the Meyer method [31, 32] where Brine shrimp nauplii hatched from *Artemia salina* leach eggs were used as the test organism. Simulated seawater was used as the hatching medium with constant oxygen supply and two days were required for complete hatching to matured nauplii. At first, the extracts (50 mg) were dissolved in dimethylsulfoxide (DMSO) and solutions of different concentrations (640, 320, 160, 80, 10, 40, 20μg/mL) were prepared using simulated seawater. Every time it was ensured that the concentration of DMSO in these test tubes did not exceed 10μl/mL. The solutions were then transferred to the
pre-marked vials having 10 live brine shrimps nauplii in 5 mL simulated seawater. After 24 h interval, the number of living nauplii in each vial was counted was visually inspected and the percent of the lethality of the brine shrimp nauplii was calculated. The median lethal concentration LC50 of the test samples after 24 hr was obtained by a plot of the percentage of the shrimps died against the sample concentration (toxicant concentration) and the best fit line was obtained from the curve data using regression analysis. In this assay, vincristine sulfate served as standard.

**Result**

**Phytochemical Screening**

After finishing a wide scope of qualitative chemical tests for the proof of therapeutically significant phytochemical group, different fractions of both extracts revealed the presence of different classes of compounds (Table 1). Although several phytochemical groups were present or absent in some cases; glycosides, gum, xanthoprotein, and acidic compounds were absent in all fractions of both extracts. Furthermore, saponins were absent in C. decandra but present in C. tagal. Besides, terpenoids were present in fractions of the former extract but absent in all fractions of the latter extract (Table 1).

**Total phenolic content**

Total phenolic content of the different fractions of both extracts varied depending on the solvent of extraction and unit was written as milligrams of gallic acid equivalents (GAE). In both cases, crude extract showed the highest content of phenolic compounds. Among the fractions of C. decandra, ethyl acetate fraction significantly showed the highest (55.99 mg/g) amount of phenolic contents followed by the butanol, water, and hexane fraction (Table 2). On the other hand, for C. tagal fractions, the butanol-1 extract showed the highest (92.52 mg/g) content of polyphenol followed by ethyl acetate, water, and hexane fraction (Table 2).

**Total flavonoids content**

A well-known calorimetric method was used to estimate the flavonoid contents of the C. decandra and C. tagal crude extracts and their fractions. In the case of C. decandra, water fraction significantly showed the highest (293.31 mg/g) quantity of flavonoids followed by the butanol, ethyl acetate, and hexane fraction (Table 2). In contrast, among the fractions of C. tagal the ethyl acetate fraction exhibited the highest (498.99 mg/g) amount of flavonoids content followed by water, butanol, and hexane fraction (Table 2).

**Total Tannin Content**

Furthermore, the total tannin content of C. decandra and C. tagal extracts was estimated using Folin Ciocalteu method. Among the different polarities extracts of C. decandra, ethyl acetate fraction showed the top (Table 2) tannin content while water fraction of C. tagal disclosed maximum (Table 2) tannin content. Other fractions of both extracts divulged very little content of tannin content (Table 2).

**DPPH radical scavenging assay**

The antioxidant activity of C. decandra and C. tagal extracts was determined by DPPH scavenging assay. Crude extract along with all other fractions of both extracts exhibited varying degrees of DPPH scavenging capacity that were dose-dependent. The ethyl acetate fraction of C. decandra showed highest DPPH radical scavenging activity (IC50 = 323.56 µg/mL) comparing to other fractions. Similarly, the ethyl acetate fraction of C. tagal was superior to other fractions in DPPH scavenging activity (Table 2).

**Analgesic Activity Test**

The results of C. decandra and C. tagal crude extracts on the acetic acid-induced writhing in mice has been presented in Table 3. The result showed that the C. decandra extract at the dose of 250 and 500mg/kg significantly (P < 0.05) reduced abdominal writhing compared to the control group and dose-dependently. The inhibition was found as 54.55% at 250 mg/kg to 66.37% at 500mg/kg. But, C. tagal extract at both doses showed negligible writhing inhibition.

**Antihyperglycemic Activity Test**

The effect of crude C. decandra and C. tagal extract on the fasting blood glucose level of normal mice has been summarized in Table 4. In oral glucose tolerance tests, crude extract of C. decandra significantly and dose-dependently reduced blood glucose levels (25.76%, 2272%, and 31.88%, 38.71% respectively, at 60 min and 120min at doses of 250, and 500 mg each per kg body weight in mice), whereas standard antihyperglycemic drug, glibenclamide reduced blood glucose levels by 47.75% and 57.72% at a dose of 5 mg per kg at two different times. Additionally, the reduced blood glucose level by C. tagal extract was less than that of C. decandra extract at both doses in the same time period.

**Neuropharmacological Activity Test**

In the open field test, the number of squares traveled by each mouse was recorded after 0, 30, 60, 90, and 120 min and the result was represented in Table 5. From the result, it was evident that C. decandra produced a sedative effect (decrease locomotor activity) at both doses. The sedative effect was found for the period of 30 to 120 min of the experiment. But, in comparison to control, C. tagal extract at both doses showed an insignificant sedative effect.

**Anticoagulant activity test**

The crude extract of C. decandra and C. tagal at 87.5, 175, and 350 mg/mL prolonged the PT compared to the control (Table 6). Both extracts shorten the prothrombin time in a concentration-dependent manner.

**Anthelmintic activity test**

Anthelmintic activity of C. decandra and C. tagal was conducted at doses of 25, 50, and 100 mg/mL. Both extracts revealed dose-dependent and significant anthelmintic activity (Table 7). The C. decandra extract at the concentration of 100 mg/mL demonstrated paralysis and death time of worms double than that of albendazole. Both extracts produced a dose-dependent loss of motility (paralysis) and loss of response to external stimuli and consequent death of Paramphistomum cervi.

**Cytotoxic Activity Test**

The LC50 values obtained from brine shrimp lethality bioassay were 94.69, 114.29µg/mL for C. decandra and C. tagal, respectively (Figure 1, 2). Both the extract showed comparable brine shrimp larvicidal activity with that of standard drug vincristine sulphate (LC50 0.40 µg/mL; Figure 3).

**Discussion**

Native communities in Bangladesh utilize many indigenous medicinal plants for remedial purposes since ages. These
plants contain a complex mixture of chemical compounds known to play important role in several biological activities. In the present study, both C. decandra and C. tagal confirmed the presence of these phytochemicals. These tested plant species possess most importantly alkaloids, carbohydrates, flavonoids, phenols, tannins, terpenoids, saponins, etc. (Table 1). Every phytochemical exhibit some sorts of biological potential and they may be new/alternative source of drugs. A number of in vitro methods are literally available for rapid evaluation of medicinal plants having antioxidant activity. Free radicals are responsible for many pathological symptoms [33]. Antioxidants counteract free radicals and protect our body cell from being damaged. They do so either by neutralizing the reactive oxygen species or protecting the body’s self-antioxidant defensive property. In the laboratory, this scavenging efficacy can be measured as directly proportional to the donation of the electron by antioxidative natural products to synthetic free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Here the original purple color of DPPH turns to bleach and the intensity of color change is proportional to the antioxidants present in the test extract. The ethyl acetate fraction of C. decandra showed the highest DPPH radical scavenging activity (IC\textsubscript{50} = 323.56 µg/mL) comparing to other fractions. Similarly, the ethyl acetate fraction of C. tagal was superior to other fractions in DPPH scavenging activity (Table 2). By analyzing the results found here, it may be thought that the plant extract must contain one or more phytochemical constituents which can reduce free radical by donating electron to them. Flavonoids a group of chief Phyto-constituents can scavenge almost all free radicals including reactive oxygen species, singlet oxygen and consequently fight against several diseases [33, 34, 35].

The writhing inhibition method has appeared as one of the versatile and popular tools where the peripheral antinociceptive activity is assessed. The C. decandra extract at the dose of 250 and 500mg/kg significantly (P < 0.05) and dose dependently reduced (34.55% at 250 mg/kg to 66.37% at 500mg/kg) abdominal writhing in mice compared to the control group. But, C. tagal extract at both doses showed negligible writhing inhibition. Acetic acid is reported to elevate the PGE2 and PGF2α in peritoneal fluid during pain sensation [36, 37]. Phytochemical screening of C. decandra revealed the presence of flavonoids, terpenoids, alkaloids, tannins, and phenols. Several reports have shown the analgesic and anti-inflammatory properties of flavonoids, triterpenoids, tannins, and other polyphenolic compounds in different experimental animal models [38]. Moreover, flavonoids, triterpenoids and tannins are reported to lower prostaglandin bio-synthesis and this pain inhibition could be due to the presence of these classes of components.

For detection of hyperglycemia or intermediate diabetes, the oral glucose tolerance test (OGTT) is a popular laboratory technique. In principle, OGTT determines the absorption pattern of glucose by the body which in turn may be dependent on insulin secretion and insulin resistance [39]. In the present study crude extract of C. decandra significantly and dose-dependently lowered blood glucose levels diabetic induced mice by 25.76%, 2272%, and 31.88%, 38.71% respectively, at 60 min and 120min at doses of 250, and 500 mg each per kg body weight in mice. In the same experiment the lowering was47.75% and 57.72% by glibenclamide, a standard antihyperglycemic drug at a dose of 5 mg per kg at two different times. Additionally, the reduction of blood glucose level by C. tagal extract was less than that of C. decandra extract at both doses in the same time period. That means the mice treated with test extract showed better glucose utilization capacity. Mechanistically, this effect may be facilitated through a number of bio-ways including the embarrassment of glucose absorption, stimulation of peripheral glucose utilization, reduction in glycogenolysis, and gluconeogenesis [40]. Therefore, the present study suggests that C. decandra extract is capable of minimizing hyperglycemia-related complications of diabetes. Some earlier studies also reported that antidiabetic property of medicinal plants is due to the presence of phenolic compounds, alkaloids, terpenoids, and flavonoids [41]. Thus, it may be suggested that the antihyperglycemic activity of C. decandra may be due to the presence of such different secondary metabolites which may act individually, as adjunct or synergistic.

The open-field test is used to evaluate the animal’s emotional state. In this test, any agents having sedative property will reduce their locomotor behavior. Locomotor activity is an indicator of mental stimulation or alertness and decreases locomotion is the sign of calmness and sedation at the end [42]. Results obtained here suggest that C. decandra decreased spontaneous locomotion at both experimental doses. The sedative effect was started at 30 min and sustained up to 120 min. But, in comparison to control, C. tagal extract at both doses showed an insignificant sedative effect. A wide variety of agents can stimulate CNS activity and thus opposing the CNS depression activity. Locomotion is mediated through dopamine hormone and related pathway and other neural mechanisms. Several phytoconstituents have been previously reported to have sedative, hypnotic or anxiolytic effects. Terpenoids, flavonoids, alkaloid, different saponins were previously reported to have depressive effects [43]. These phytochemicals were present in C. decandra extract. So, it can be suggested that the inhibitory effect of C. decandra extract on locomotor activity could be arbitrated by interference in the GABA neurotransmission of CNS.

Both plants showed a significant decrease in prothrombin time in a concentration-dependent manner i.e. have anticoagulant properties. Prothrombin time may be prolonged by the presence of clotting inhibitor in the test extracts. Mechanistically, the clotting inhibitors perform their action by interfering with the integrity of coagulation proteins, (factor VII) in the extrinsic coagulation trail. In in-vitro anthelmintic activity test, the ability of plant materials to paralyse (loss of movement) or kill (death) the parasites is measured. Here both C. decandra and C. tagal extract significantly and dose-dependently shorten the paralysis and death time of parasite Paramphistomum cervi compared with standard drug albendazole. Plant’s secondary metabolites such as flavonoids essential oils, terpenoids, alkaloids, or polyphenols such as condensed tannins demonstrate anthelmintic effects [44]. Chemically, tannins are non-nitrogenous plant constituents having astringent action on mucous membranes. Mechanistically they precipitate protein from the cells of mucous membranes. Also, some synthetic phenolic anthelmintics block energy generation in helminth parasites and they do so by uncoupling oxidative phosphorylation [30].

The brine shrimp lethality bioassay has appeared as an easy and sensitive facile method for forecasting important cellular activities including cytotoxic activity, ion channel interference, and enzyme inhibition. The National Cancer Institute (NCI, USA) successfully showed a correlation between the brine shrimp lethality bioassay and in vitro growth inhibition of rapidly growing human tumor cell lines...
In the present study, the LC$_{50}$ values were 94.69, 114.29 µg/mL for *C. decandra* and *C. tagal*, respectively. In comparison to standard drug vincristine sulphate (LC$_{50}$ 0.40 µg/mL), both extracts showed good brine shrimp larvicidal activity. Near past, plant phenolic compounds such as flavonoids, polyphenols, anthraquinones, coumarins, alkaloids has been reported as very effective antitumor agents [45, 46]. The presence of alkaloids, tannins, and flavonoids in the experimental extracts could be responsible for its possible cytotoxic properties.

**Fig 1:** Cytotoxic effect of *C. decandra* extract

**Fig 2:** Cytotoxic effect of *C. tagal* extract
Fig 3: Cytotoxic effect of Vincristine sulphate

Table 1: Qualitative phytochemical screening of Ceriops decandra and Ceriops tagal

| Phytochemical Group | Ceriops decandra | Ceriops tagal |
|---------------------|------------------|---------------|
|                     | Crude            | n-Hexane      | Ethyl Acetate | Butanol-1 | Water | Crude | n-Hexane | Ethyl Acetate | Butanol-1 | Water |
| Reducing sugar      | +                | +             | +             | +         | +     | +     | +         | +         | +     | +     |
| Combined reducing sugar | +             | +             | +             | +         | +     | +     | +         | +         | +     | +     |
| Tannins             | +                | +             | +             | +         | +     | +     | +         | +         | +     | +     |
| Flavonoids          | +                | +             | +             | +         | +     | +     | +         | +         | +     | +     |
| Saponin             | -                | -             | -             | -         | -     | -     | -         | -         | -     | -     |
| Gums                | -                | -             | -             | -         | -     | -     | -         | -         | -     | -     |
| Steroids            | +                | +             | +             | -         | -     | -     | -         | -         | -     | -     |
| Alkaloids           | +                | -             | -             | +         | +     | +     | +         | +         | +     | +     |
| Glycoside           | -                | -             | -             | -         | -     | -     | -         | -         | -     | -     |
| Xanthoproteins      | -                | -             | -             | -         | -     | -     | -         | -         | -     | -     |
| Terpenoids          | +                | +             | +             | -         | -     | -     | -         | -         | -     | -     |
| Acidic compounds    | -                | -             | -             | -         | -     | -     | -         | -         | -     | -     |

Table 2: Total phenolic, flavonoids and tannin content and antioxidant assay of C. decandra and C. tagal

| Sample name | Fraction | Total phenolic content (mg GAE/g) | Total flavonoid content (mg QE/g) | Total tannin content (mg GAE/g) | DPPH IC50 (μg/mL) |
|-------------|----------|----------------------------------|----------------------------------|--------------------------------|-------------------|
| C. decandra | Crude    | 69.47                            | 326.75                           | 18.37                          | 184.13            |
|             | Water    | 31.01                            | 293.31                           | 19.10                          | 477.97            |
|             | Ethyl Acetate | 55.99                           | 152.84                           | 9.97                           | 323.56            |
|             | Butanol-1 | 46.00                            | 253.17                           | 6.12                           | 355.37            |
|             | n-Hexane | 22.76                            | 26.08                            | 3.56                           | 519.99            |
| C. tagal    | Crude    | 144.17                           | 907.85                           | 6.26                           | 228.62            |
|             | Water    | 34.20                            | 329.43                           | 9.08                           | 499.13            |
|             | Ethyl Acetate | 83.85                           | 498.99                           | 8.04                           | 171.77            |
|             | Butanol-1 | 92.52                            | 72.57                            | 7.45                           | 533.00            |
|             | n-Hexane | 18.69                            | 52.34                            | 1.15                           | 452.58            |
| Standard    | Ascorbic Acid | -                              | -                                | -                              | 14.36             |
From the above results and discussions, it may be concluded that the ethanol leaf extracts of *C. decandra* and *C. tagal* possess significant antioxidant, antihyperglycemic, anticoagulant, sedative effect, anthelmintic and cytotoxic potential. Also, from the present study, the traditional uses of these plants in the treatment of various diseases can be scientifically validated. Therefore, further investigation is recommended to interrogate the meticulous phytoconstituents and appropriate mechanisms of the action that are liable for the abovementioned pharmacological activities.

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**Reference**

1. Shaikh J Uddin, Darren GI, Evelyn Tiralongo. Cytotoxic Effects of Bangladeshi Medicinal Plant Extracts. Evidence-Based Complementary Altern. Med. 2011. doi:10.1093/ecam/nep111.

2. Atanassov AG, Waltenberg B, Pferschy-Wenzig EM al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015;33(8):1582-1614. doi:10.1016/j.biotechadv.2015.08.001

3. Ulysses PA, Joabe GM, Maria FM, Irwin RM, Geraldo JM et al. Natural Products from Ethnodirected Studies: Revisiting the Ethnobiology of the Zombie Poison. J. Evidence-Based Complementary Altern. Med. 2012. doi:10.1155/2012/202508

4. Murugesan G, Sundaram R, Muthusamy A. Hepatoprotective activity of Ceriops decandra (Griff.) Ding Hou mangrove plant against CCl4 induced liver damage. J Taibah Univ Sci. 2017;11:450-457.

5. Judy P, Chien-chang S, Consolacion YR. Triterpenes from *Ceriops decandra* (GRIFF.) W. THEOB. Asian J Pharm Clin Res. 2017;10(1):244-246.

6. Aritra S, Amit R. Analysis of and correlation between phytochemical and antimicrobial constituents of *Ceriops decandra*, a medicinal mangrove plant, from Indian

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**Table 3:** Effect of *C. decandra* and *C. tagal* extract in acetic acid induced writhing in mice

| Treatment       | Mean writhing | % writhing | % writhing inhibition |
|-----------------|---------------|------------|-----------------------|
| Control         | 22 ± 3.0      | 100        | -                     |
| Diclofenac Na (25 mg/kg) | 3.8±0.84 | 17.27       | 87.72*                |
| *C. decandra* (250 mg/kg) | 10±1.58 | 45.45       | 54.55*                |
| *C. decandra* (500 mg/kg) | 7.4±1.14 | 33.63       | 66.37*                |
| *C. tagal* (250 mg/kg) | 20±2.0 | 90.91       | 9.1                   |
| *C. tagal* (500 mg/kg) | 17.4±2.19 | 79.09       | 20.91*                |

* P < 0.05 vs. Control

**Table 4:** Effect of *C. decandra* and *C. tagal* in oral glucose loaded mice

| Treatment       | Blood glucose level | 0min | 60min | 120min |
|-----------------|---------------------|------|-------|--------|
| Control         | 5.38 ± 0.84         | 13.1 ± 1.17 | 9.14 ± 2.17 |
| Glibenclamide   | 4.38±0.64           | 6.8±0.40 (47.75%) | 3.8±0.70 (57.72%) |
| *C. decandra* (250 mg/kg) | 7.28±0.72*  | 9.72 ± 1.11* (25.76%) | 7.06 ± 0.23 (22.72%) |
| *C. decandra* (500 mg/kg) | 7.88±1.23*  | 8.92±0.43* (31.88%) | 5.6 ± 0.50 (38.71%) |
| *C. tagal* (250 mg/kg) | 7.42±0.79*  | 11.32 ± 0.75 (13.56%) | 7.86 ± 0.27 (13.98%) |
| *C. tagal* (500 mg/kg) | 7.36±0.49*  | 10.02 ± 1.14* (23.48%) | 6.36 ± 0.35* (30.38%) |

* P < 0.05 vs. Control

**Table 5:** Neurological effect of *C. decandra* and *C. tagal* extract on prothrombin time of experimental mice

| Treatment       | No. of square crossed by the mice | 0min | 30min | 60min | 90min | 120min |
|-----------------|----------------------------------|------|-------|-------|-------|--------|
| Control         | 117.4 ± 4.72                     | 88.8 ± 5.26 | 76.2 ± 3.7 | 71.2 ± 5.4 | 78.6± 4.39 |
| Diazepam 1mg/kg | 113.4 ± 5.08                     | 37.4± 3.85* | 31.6 ± 3.85* | 28.6 ± 3.65* | 31.4± 3.13* |
| *C. decandra* (250 mg/kg) | 119.2 ± 5.07 | 68.8± 2.86* | 57.8± 3.56* | 48.8±0.92* | 62.8±3.19* |
| *C. decandra* (500 mg/kg) | 120.8 ± 3.27 | 58.2± 4.15* | 47.6 ± 2.79* | 42.6±0.36* | 51.8±2.39* |
| *C. tagal* (250 mg/kg) | 113.6 ± 6.69 | 122.2±5.76 | 137.6±5.5 | 123.4±5.18 | 117.2±4.6 |
| *C. tagal* (500 mg/kg) | 115.2 ± 5.59 | 130±7.07 | 149.2±5.26 | 136.4±6.5 | 122±8.66 |

* P < 0.05 vs. Control

**Table 6:** Effect of *C. decandra* and *C. tagal* on prothrombin time of human plasma

| Treatment | Concentration | Average time (min) |
|-----------|---------------|--------------------|
| Control   | 0.9% NaCl     | 3.57 ± 0.19        |
| Warfarin  | 5mg/mL        | 59.00 ± 2.28       |
| *C. decandra* | 87.5 mg/mL | 35.81 ± 2.24       |
|           | 175 mg/mL     | 25.93 ± 5.38       |
|           | 350 mg/mL     | 9.33 ± 2.21        |
| *C. tagal* | 87.5 mg/mL   | 8.60 ± 0.63        |
|           | 175 mg/mL     | 5.08 ± 0.65        |
|           | 350 mg/mL     | 4.0 ± 0.5          |

* P < 0.05 vs. Control

**Table 7:** Anthelmintic activity of *C. decandra* and *C. tagal* on *P. cervi*

| Treatment | Concentration | Mean paralysis time (min) | Mean Death time (min) |
|-----------|---------------|---------------------------|-----------------------|
| Control   | 0.2% Tween-80 in water | - | -                  |
| Albendazole | 15 mg/mL   | 15±2.61 | 23.17±2.48 |
|           | 25mg/mL      | 50±4.73 | 73.67±6.56 |
|           | 50mg/mL      | 42.33±2.88 | 57.67±2.66 |
|           | 100 mg/mL    | 29.83±2.86 | 51±2.61 |
| *C. decandra* | 25mg/mL | 57±5.4 | 85.83±1.72 |
|           | 50mg/mL      | 45.67±4.37 | 62±2.37 |
|           | 100 mg/mL    | 36.33±3.08 | 58.17±2.86 |

**Conclusion**

From the above results and discussions, it may be concluded that the ethanol leaf extracts of *C. decandra* and *C. tagal* possess significant antioxidant, antihyperglycemic, anticoagulant, sedative effect, anthelmintic and cytotoxic potential. Also, from the present study, the traditional uses of these plants in the treatment of various diseases can be scientifically validated. Therefore, further investigation is recommended to interrogate the meticulous phytoconstituents and appropriate mechanisms of the action that are liable for the abovementioned pharmacological activities.
Phytochemical Investigations of the Mangrove Plant Ceriops tagal and its Bioactive Polyphenols by HPLC-DAD. Evidence-Based Complementary Altern Med 2016. DOI:10.1155/2016/3874516.

Anisuzzaman M, Nazmul MHZ, Sharmin SK, Asaduzzaman M, Golam MH. Antioxidant, antibacterial potential and HPLC analysis of Dioscorea alata bulb. Indonesian J Pharm 2016;27(1):9-14.

Imtiaz M, Zilani MNH, Nripendra NB, Bishwajit B. Bioactivities of Bruguiera gymnorrhiza and profiling of its bioactive polyphenols by HPLC-DAD: Clinical Phytoscience 2017;3(1):1-11.

Farhana Y, Mahin H, Amit S. Antalgesic, Antidiarrheal, Antioxidant, Cytotoxic and Oral Glucose Tolerance Activities of Ethanolic Leaf Extract of Aristolochia indica L. Biosci Bioeng Commun 2016;2(4):137-143.

Abdulwali A, Jamaludin M, Khalijah A, Jamil AS, Aditya A. Evaluation of anti diabetic and antioxidant properties of Brueceavania seed. Sci World J. 2014;1:786130. doi:10.1155/2014/786130.

Wahid H, Nazmul HZ, Nazneen AS, Mahmoud I, Anisuzzaman M et al. Neuropharmacological potential of Cerisoides turgida (Ros.) leaf and root in mice. Clinical Phytoscience 2019;5:5. DOI: 10.1186/s40816-019-0099-x.

Anisuzzaman M, Mahedi H, Amit KA, Asish KD, Sinthia R. In Vivo and In Vitro Evaluation of Pharmacological Potentials of Secondary Bioactive Metabolites of Dalbergia cadenatensis Leaves. Evidence-Based Complementary Altern Med 2017. doi:10.1155/2017/5034827.

Saha S, Shilpi JA, Mondal H, Govur F, Billah M, Nahar L, et al. Bioactivity studies on Musa seminifera Lour. Phcog Mag 2013;9:315-22.

Meyer S. Phytochemical methods (a guide to modern techniques to plant analysis), Chaman and Hall, USA 1982.

Nripendra NB, Amit KA, Shams A, Shamsunnahar K, Bishwajit B. Screening of Natural Bioactive Metabolites and Investigation of Antioxidant, Antimicrobial, Antihyperglycemic, Neuropharmacological, and Cytotoxicity Potentials of Litsea polyantha Juss. Ethanolic Root Extract: Evidence-Based Complementary Altern Med, 2017.

Rajni D, Rachna V, Dinesh K, Sunil P, Ashwani T et al. Bioactive Compounds of Edible Fruits with Their Anti-Aging Properties: A Comprehensive Review to Prolong Human Life. Antioxidant 2020;9(11):1123. doi:10.3390/antioxid1111123.

Shashank Kumar, Abhay K Pandey. Chemistry and Biological Activities of Flavonoids: An Overview. Sci World J 2013. doi:10.1155/2013/162750.

Sarker Ramproshad, Riya Das, Banani Mondal, Labani Saha. Phytochemical Evaluation and Antioxidant Activities of Spilanthes Calva DC. Pharmacologyonline. 2019;1:289-300.

George JA, Joseph K. Antinociceptive and Anti-Inflammatory Activities of Leaf Methanol Extract of Cotedylon orbiculata L. (Crassulaceae). Adv Pharmacol Sci 2012. doi:10.1155/2012/866265.

Achinta S, Mohammad AM, Sitesh CB, Joydeb KK, Bidyut KD et al. The Analgesic and Anti-Inflammatory
Activities of the Extracts of Phyllanthus reticulatus. Pharm Biol 2007;45(5):355-359.

38. Asie S, Majid M, Sima N, Manijeh M. Evaluation of Anti-inflammatory and Analgesic Activity of the Extract and Fractions of Astragalus hamosus in Animal Models. Iran J Pharm Sci 2015;14(1):263-269.

39. Napapach C, Sanong C, Chusri T. Toxicology and Oral Glucose Tolerance Test (OGTT) of Thai Medicinal Plant Used for Diabetes control, Phyllanthus acidus L. (Euphorbiaceae). Pharmacogn J 2017;9(1):58-61.

40. Zemene DK, Jibril SY, Seyfe AA. Evaluation of in vitro and in vivo Anti-Diabetic, Anti-Hyperlipidemic and Anti-Oxidant Activity of Flower Crude Extract and Solvent Fractions of Hagenia abyssinica (Rosaceae). J Exp Pharmacol 2020;12:151-167.

41. Nazir N, Zahoorn M, Nisar M. et al. Phytochemical analysis and antidiabetic potential of Elaeagnus umbellata (Thunb.) in streptozotocin-induced diabetic rats: pharmacological and computational approach. BMC Complement Altern Med 2018;18(1):332. doi:10.1186/s12906-018-2381-8.

42. Hossain MF, Talukder B, Rana MN, Tasnim R, Nipun TS, Uddin SM. et al. In vivo sedative activity of methanolic extract of Sterculia villosa Roxb. leaves. BMC Complement Altern Med 2016;16(1):398. doi:10.1186/s12906-016-1374-8.

43. Kukuia KKE, Mensah JA, Amoateng P, Aponsoah SK, N'Guessan BB, Asiedu-Gyekye JJ. Antidepressant Potentials of Components from Trichilia monadelpha (Thonn.) J.J. de Wilde in Murine Models. Evid Based Complementary Altern Med 2018. doi:10.1155/2018/6863973.

44. Williams AR, Ropiak HM, Fryganas C, Desrues O, Mueller-Harvey I, Thamsborg SM. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of Oesophagostomum dentatum. Parasit Vectors 2014;7:518. doi:10.1186/s13071-014-0518-2.

45. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004;74(17):2157-2184. doi:10.1016/j.lfs.2003.09.047.

46. Rex JRS, Muthukumar NMSA, Faulraj M Se. In vitro anthelmintic activity of leaf extracts of four different types of Calamus species. Pharm Pharmacol Int J 2017;5(2):72-76.