



Biological assessment on *Cerbera manghas* (linn.)

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Abstract

In the present biological studies, several pharmacological investigations were carried out to ascertain analgesic, and antioxidant activity of the ethanol extract of the leaves of *Cerbera manghas* Linn. (Family-Apocynaceae). The presence of reducing sugars, tannins, steroids, alkaloids, glycosides, and flavonoids were indicated by the phytochemical analysis of the extract. To evaluate analgesic activity, acetic acid induced writhing method was utilized. The ethanol extract exhibited statistically significant ($p < 0.001$) analgesic effect in acetic acid induced writhing test in white Swiss-Albino mice (Swiss-webster strain). The extract produced 17.75% and 30.64% writhing inhibition at the doses of 250 and 500 mg/kg-body weight, respectively in dose dependent manner. To evaluate *in vitro* antioxidant activity, free-radical-scavenging assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used. The extract exhibited the IC_{50} value of 292 $\mu\text{g/ml}$ in DPPH scavenging assay.

Key words: *Cerbera manghas*, Apocynaceae, Swiss-Albino mice, writhing test, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH)

Introduction

Cerbera manghas Linn. (Family- Apocynaceae), locally known as dabor and dagor. It is a small evergreen coastal tree growing up to 12 m tall, and the shiny dark-green leaves are alternate, and ovoid in shape. *C. manghas* is naturally and abundantly distributed from the Seychelles Islands in the Indian Ocean eastward to French Polynesia. It attributes lowland and coastal habitats, and is often associated with mangrove forests like Sundarbans. In Bangladesh, it is distributed mainly in the Sundarbans and southern region.

The leaves and the fruits of the plants contain the potent cardiac glycoside cerberin, which is extremely poisonous in nature if ingested orally. People in the ancient times used the sap of the plant as a poison for animal hunting, especially in aboriginal community. A potent drug, cerberin, has been extracted from the poisonous seeds, and it has some resemblance to digitalis in its activity on the heart, and has been used in the medicine system in very small amounts (1).

In the previous study, the bark of *C. manghas* showed cytotoxic activity against HepG2, MCF-7, and HeLa cell lines (2). 2'-Epi-2'-O-acetylthevetin B extracted from the seeds induces cell cycle arrest and apoptosis in human hepatocellular carcinoma HepG2 cells (3). β -D-Glucosyl-(1-4)- β -D-thevetosides of 17 β -digitoxigenin (GHSC-73) is a cardiac glycoside isolated from the seeds showed the inhibition of the growth of HepG2 cells through caspase dependent and independent apoptosis pathways (4). Neriifolin, a cardiac glycoside isolated from *C. manghas* reduced the viability of HepG2 cells, induced S and G2/M phase arrests of the cell cycle, and stimulated apoptosis of HepG2 cells, and induced activation of caspase-3, -8, and -9, and up-regulated expression of Fas and FasL proteins (5).

As part of ongoing research in the field of phytochemistry and pharmacology research (6-10), we have tried to justify the traditional uses of *C. manghas* through these bioactivity studies. In the present investigation, analgesic and antioxidant activities of the ethanol leaves extract of *C. man-*

ghas were investigated in different *in vivo* and *in vitro* established models.

Materials and Methods

Plant Material

The leaves of *C. manghas* was collected from Dumuria upazilla in Khulna district, Bangladesh. The plant was collected in 12th February, 2011. Any type of adulteration was strictly avoided during collection. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession no: DCAB-35569), and a voucher specimen was also deposited.

Preparation of Plant Extract

The leaves were subjected to shade drying to avoid any photochemical degradation. After proper drying, the leaves were grinded into coarse powder with a suitable mechanical grinder. To avoid any possible fungal attack the powder was stored in an air-tight container, and kept in a cool, dark, and dry place. The leaves were extracted by cold extraction method. 100 gm of grinded leaves powder was soaked in 500 mL of 98% of ethanol in a glass container for eight days accompanying regular shaking and stirring. The extract was separated from the plant debris by filtration by a piece of clean, white cotton plug and it was performed twice. At room temperature, the solvent was evaporated with an electric fan to get the dried crude extract (yield value 7.34%). The crude extract was deep purple in colour. The crude extract was stored in refrigerator at 4 °boC to avoid any possible fungal attack.

Experimental Animals

Swiss-Albino mice of either sex (age 4-5 weeks, weight 20-25 gm) were procured from the animal resources department of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The animals were kept in animal house,

Pharmacy Discipline, Khulna University under the standard laboratory condition (relative humidity 55-60%, room temperature $25\pm 2^\circ\text{C}$, and 12 hours light: dark cycle) for period of one week prior to the pharmacological experiment. The experiment was conducted according to the animal ethics guidelines of Institutional Animal Ethics Committee (11).

Chemicals, Reagents, and Standard Drugs

Acetic acid and ascorbic acid were purchased from Merck, Germany. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, USA. Tween-80 was collected from Loba Chemie Pvt Ltd, India. All other reagents and solvents were of analytical grade. Diclofenac sodium was obtained from Beximco Pharmaceuticals Ltd, Bangladesh.

Phytochemical Screening

The crude ethanol extract was subjected to different preliminary phytochemical screening to identify major phytochemical groups (12,13). The presence of reducing sugars, tannins, steroids, alkaloids, glycosides, and flavonoids were indicated by the phytochemical analysis of the extract.

Evaluation of Analgesic Activity

Analgesic activity of the ethanol extract of *C. manghas* was performed using the model of acetic acid induced writhing in mice (14). The experimental mice were selected randomly, and divided into four groups denoted as control, positive control, and test group I and II, consisting of 5 mice in each group. Test group I and II were treated with the test sample at the doses of 250 and 500 mg/kg body weight. Control group received 1% tween-80 in distilled water at the dose of 10 mL/kg body weight, and positive control group received diclofenac sodium at the dose of 25 mg/kg body weight. Test samples, control, and diclofenac sodium were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered treatments. Then the writhing

inducing chemical, acetic acid solution (0.6%, 10 mL/kg) was administered intraperitoneally to each mouse of every group. After an interval of 5 minutes, which was given for absorption of acetic acid, number of squirms (writhing) was counted for 10 minutes. For the assessment of analgesic activity, the percent inhibition of writhing for each group was calculated, and compared with the control group.

In Vitro Antioxidant Activity Test

Using stable free radical DPPH (1,1-Diphenyl-2-picrylhydrazyl) the antioxidant activity of the ethanol leaves extract was estimated both qualitatively and quantitatively (15).

Qualitative Analysis

For the qualitative assessment, Thin Layer Chromatographic (TLC) technique was applied (15). TLC plates were developed in non-polar, medium polar, and polar solvent systems to resolve compounds of different polarities. TLC plates were sprayed with 0.02% DPPH in ethanol solution. After the interval of 30 min, the bleaching of DPPH (yellow on purple background) radical was observed and noted.

Quantitative Analysis

Based on their scavenging activity of the stable DPPH radical, the anti-oxidant potential of the ethanol extract was estimated (16). At first, 10 test tubes were taken to make aliquots of 9 concentrations (1.57, 3.13, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml) for the plant extract. Ascorbic acid was used as positive control and prepared in same concentrations as plant extract. Plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentrations by dilution technique. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used. After making the desired concentrations, 6 ml of 0.004%

DPPH solution was applied on each test tube by pipette and then 2 ml of different concentrations was mixed in each test tube. Test tubes were kept for 30 minutes in dark to complete the reactions. DPPH was also applied on the control test tubes at the same time where only 2mL ethanol was taken as control. After 30 minutes, absorbance of each test tube was measured by UV spectrophotometer at 517 nm. Percent inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample or standard}) / \text{Absorbance of control}] \times 100}{1}$$

IC₅₀ value was determined from % inhibition vs. concentration graph.

Statistical Analysis

Student's t-test was used to determine significant differences between the control and test groups. Results were considered as statistically significant when $P < 0.001$.

Results

Phytochemical Tests

The phytochemical screening of the ethanol extract was performed for the detection of different biologically active chemical groups and the results are summarized in the Table 1.

Phytochemical groups	Results
Reducing sugars	+
Alkaloids	+
Glycosides	+
Steroids	+
Gums	-
Saponins	-
Flavonoids	+
Tannins	+

+ = Presence - = Absence

Table 1: Phytochemical screening of *C. manghas*

Acetic Acid-Induced Writhing Test

The ethanolic extract exhibited statistically significant ($p < 0.001$) analgesic effect in acetic acid

induced writhing in Swiss-Albino mice. The extract produced 17.75% and 30.64% writhing inhibition at the doses of 250 and 500 mg/kg-body weight, respectively, which was highly comparable to the diclofenac sodium, exhibited inhibition of writhing of 79.03% ($P < 0.001$) at the dose of 25 mg/kg body weight.

see Table 2.

DPPH Scavenging Activity

The extract showed DPPH radical scavenging activity in concentration dependent manner. The ethanol extract showed the IC₅₀ value of 292 µg/ml, which was compared with the ascorbic acid (IC₅₀ value of 15 µg/ml).

see Figure 1.

Discussion

The present biological assessment was conducted to assess some phytochemical and pharmacological properties of the ethanol leaves extract of *C. manghas*. To get preliminary idea about the phytochemicals present in the extract, phytochemical screening was performed, which revealed the presence of reducing sugars, tannins, steroids, alkaloids, glycosides, and flavonoids.

Acetic acid induced writhing test is a simple and widely used method for evaluating *in vivo* analgesic activity in mice, especially for evaluating peripheral analgesic activity. In this test, sensitization of pain receptors by prostaglandins release is used to evaluate the analgesic activity of the sample (17,18). Extract might possess the ability to inhibited prostaglandin release to reduce pain. The phytochemicals present in the extract may be responsible for this promising analgesic activity. It is already established that terpenoids, reducing sugar, gums, xanthoprotein, flavonoids, and tannins are responsible for different biological activities including peripherally acting analgesic activity (19-21).

In vitro antioxidant activity study of the extract

was carried out by the most widely used DPPH scavenging assay. In this test, the ethanol extract converted the free radical DPPH into stable DPPH-H form by donating electron, or hydrogen radical. As a result the deep violet colour of the DPPH radical was converted to light yellow colour. Amount of unconverted DPPH was measured by UV spectrophotometer at 517 nm against blank. Flavonoids and tannins present in the extract may be responsible for the antioxidant properties (22,23). The extract showed concentration dependent DPPH radical scavenging activity that was comparable to the standard antioxidant ascorbic acid.

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Conclusion

The ethanol leaves extract of *C. manghas* showed potential antioxidant and analgesic activity. Further investigations like LC-MS are required to identify bioactive compounds responsible for this bioactivity.

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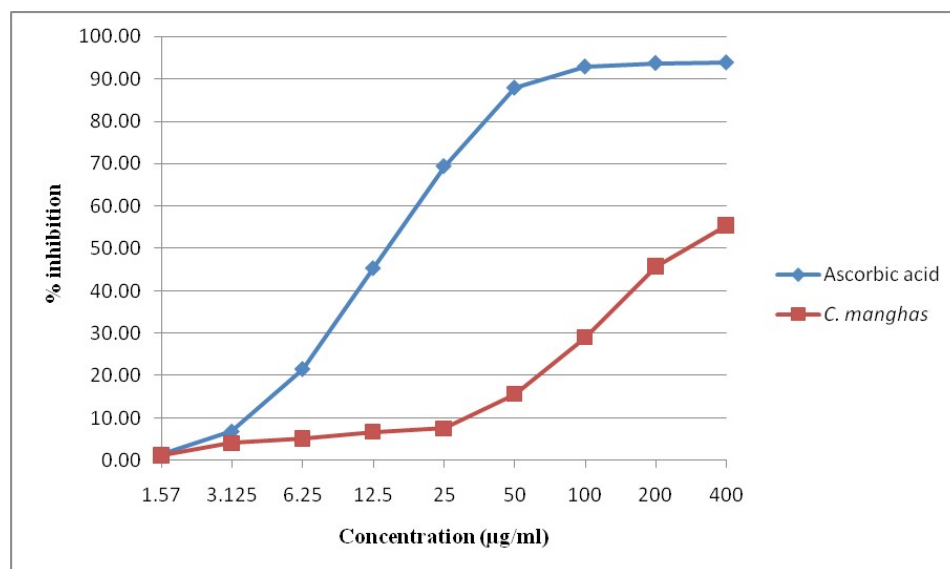


Figure 1: DPPH scavenging activity of *C. manghas*

Treatment (n = 5)	Dose (mg/kg)	No. of writhes	% Inhibition
Control	---	24.80±0.60	---
Diclofenac sodium	25	5.20±0.90*	79.03
Extract	250	20.40±1.40**	17.75
	500	17.20±1.05*	30.64

Table 2: Effect of *C. manghas* leaves on acetic acid induced writhing in Swiss-Albino mice

Results are expressed as mean ± SEM, SEM= Standard error of mean, *P < 0.001, **P < 0.01 versus control, Student's t-test.