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# Antibacterial, cytotoxic and neuropharmacological activities of *Cerbera odollam* seeds

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## SUMMARY

The MeOH extract of seeds of *Cerbera odollam* Gaertn. (Apocynaceae) was screened for its antibacterial, cytotoxic and neuropharmacological activities. The extract showed moderate anti-bacterial activity against *Salmonella typhi, Streptococcus saprophyticus,* and *Streptococcus pyogenes.* It exhibited high level cytotoxicity against brine shrimp ($LC_{50}$: 3 μg/ml). The extract potentiated pentobarbital induced sleeping time in mice which was further supported by the exploratory behavior test at dose of 25 mg/kg. The overall results tend to suggest the antibacterial, cytotoxic and CNS depressant activities of the extract.

**Key words:** *Cerbera odollam*; Antibacterial activity; Cytotoxic activity; CNS depressant activity

## INTRODUCTION

*Cerbera odollam* Gaertner (pink-eyed *cerbera*, yellow-eyed *cerbera*, *odollam* tree), also sometimes called *Cerbera manghas* L, belongs to the notoriously poisonous Apocynaceae family, which also includes the yellow oleanders (*Thevetia peruviana* and *Thevetia nerifolia*) and common oleanders (*Nerium oleander* and *Nerium indicum*). The *C. odollam* tree grows to a height of 6 - 15 m and has dark green fleshy lanceolate leaves. The large white flowers have a delicate perfume, reminiscent of jasmine. The fruit, when still green, looks like a small mango, with a green fibrous shell enclosing an ovoid kernel measuring approximately 2 cm × 1.5 cm and consisting of two cross-matching white fleshy halves. On exposure to air, the white kernel turns violet, then dark grey, and ultimately brown or black. The plant as a whole yields a milky white latex. The tree grows in coastal salt swamps and creeks in south India (particularly abundantly near the canals and backwaters of Kerala) and along riverbanks in southern and central Vietnam, Cambodia, Sri Lanka, Bangladesh and Myanmar. In Bangladesh the plant is known as Dabur, Dhakur or Um-dabur. The seeds have a long history as an ordeal poison in Madagascar, due to the highly toxic cardiac glycosides they contain. The seeds contain non-siccative oil, producing a shining flame with a pleasant nut-like odour. The Burmese use it for lighting, as a cosmetic, or mixed with other oils as an insecticide or insect-repellent (Chopra *et al*., 1956). The bark and leaves of the plant are traditionally used as emetic and cathartic; kernels are used as emetic; fruit is used as a cure for hydrophobia (Kirtikar and Basu, 1987). Its bark and fruits are purgative and used

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for the treatment of rheumatism (Rollet, 1981). Previously, we studied and reported the antinociceptive and sedative effects of its barks (Ahmed *et al*., 2006). Also some other research works have been performed to evaluate its biological activities as cytotoxic activity (Laphookhieo *et al*., 2004), effect on central nervous system (Hien *et al*., 1991), purgative and antirheumatic activity (Yamauchi and Abe, 1987), cardiac stimulant activity (Chen and Zheng, 1987), neurological activities (Iyer and Narendranath, 1975), cardiotoxic activity (Kini and Pai, 1965), etc.

The main objective of this study was to evaluate the antibacterial, cytotoxic and neurophamacological activities of MeOH extract of *C. odollam* seeds.

## MATERIALS AND METHODS

**Plant material collection and extraction**

The plants were collected from Sundarbans Mangrove forest of Bangladesh in September 2006 and were identified at National Herbarium of Bangladesh (Accession no.: 29788). About 750 g of pulverized seeds were taken in a clean, flat-bottomed glass container and soaked in 950 ml of 80% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by filtration through whatmann filter paper. The filtrate thus obtained was concentrated under ceiling fan followed by vacuum desiccation.

**Tests for different Chemical groups**

The crude MeOH extract was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins (Evans, 1989). In each test 10% (w/v) solution of the extract in MeOH was taken unless otherwise mentioned in individual test.

**Animals**

Young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for neuropharmacological activity tests. The animals were kept at animal house of Pharmacy Discipline, Khulna University, for adaptation under standard environmental condition and fed with standard diets (ICDDRB formulated).

**Microorganisms**

Both gram positive and gram-negative bacterial strains were taken for antibacterial test. The bacterial strains used for the investigation are listed in Table 2.

**Drugs**

Pentobarbital sodium (Sigma Chemicals, USA).

**Antibacterial activity**

Antibacterial activity of the seed extract was tested against some most common pathogenic gram positive and gram negative bacteria by agar well diffusion method (Rios *et al*., 1987). In this method measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentration (50 μg/ml). Then three wells were made through the media of the seeded plates, one for the sample, one for the blank and another for the standard (Gentamicin) by using sterile cork borer. Then the samples, blank and standard were applied to the holes by using micropipette with desired amount. The plates were then kept in refrigerator for about 2 h

**Table 1. C**hemical constituents of *C. odollam*

| Plant Extract | Alkaloids | Reducing Sugars | Tannins | Gums | Flavonoids | Saponins |
|---|---|---|---|---|---|---|
| MeOH extract | + | - | + | - | - | + |

+: Positive result; -: Negative result

**Table 2.** *In vitro* antibacterial activity of MeOH extract of *C. odolla*

| Bacterial strains | Diameter of zone of inhibition in mm | |
|---|---|---|
| | MeOH extract (500 μg/hole) | Gentamycin (30 μg/hole) |
| **Gram positive** | | |
| *Staphylococcus aureus* | 06 | 27 |
| *Staphylococcus epidermis* | 00 | 22 |
| *Streptococcus saprophyticus* | 16 | 30 |
| *Streptococcus pyogenes* | 11 | 28 |
| *Gram negative* | | |
| *Salmonella typhi* | 15 | 28 |
| *Shigella boydii* | 00 | 22 |
| *Shigella sonnie* | 00 | 21 |
| *Shigella flexneri* | 06 | 28 |
| *Shigella dysenteriae* | 06 | 26 |

at 4 °C to allow the material to diffuse into a considerable area of the medium. Finally the plates were incubated upside down at 37 °C for 18 - 24 h. After proper incubation, the antibacterial activity was determined by measuring the diameter of zone of inhibition in terms of millimeter with a slide calipers.

**Cytotoxic activity**

Cytotoxicity of the extract was tested by using brine shrimp lethality bioassay (Meyer *et al*., 1982). Test solution (MeOH extract in DMSO) of different concentrations as 2, 4, 8, 16, 32 and 64 μg/ml was applied to the test tubes containing hatched brine shrimp naupli in sea water followed by counting the survived naupli after 24 h.

**Neuropharmacological activity**

**i. Pentobarbital induced hypnosis:**

Pentobarbital induced hypnosis test was carried out by the method of Tedeschi and Tedeschi (1968). The test animals were divided into two groups consisting of ten mice in each. Group I was the control group and group II was the test group. Test groups were administered with the MeOH extract at dose of 25 mg/kg, while the control group animals were supplied with distilled water containing 0.1% (v/v) tween-80 at dose of 10 ml/kg. Control vehicle and test sample were administered 60 min prior to the i.p. injection of pentobarbital at dose of 45 mg/kg. The total sleeping time was recorded for both controls as well as for test group. Total sleeping time represents the time between the loss and regain of righting reflex.

**ii. Exploratory behavior:**

This experiment was performed by i) Open field test (Gupta *et al*., 1971) and ii) Hole cross test (Takagi *et al*., 1971). The test animals were divided into two groups consisting of five mice in each. Group I was the control group and group II was the test group. Test group was administered with the MeOH extract at dose of 25 mg/kg (i.p.), while the control group animals were supplied with 0.1% (v/v) Tween-80 (i.p.) at dose of 10 ml/kg. The observations were made on 0 min before injection and 30, 60, 120 and 240 min after injections (i.p.) of the test sample and control vehicle.

**Statistical analysis**

Student's *t*-test was used to determine a significant difference between the control group and test group.

## RESULTS

**Preliminary phytochemical analysis**

Results of different chemical tests on the methanol

extract of seeds of *C. odollam* showed the presence of alkaloids, tannins and saponins (Table 1).

**Antibacterial activity**

Table 2 showed the antibacterial activity of *C. odollam* relative to that of the standard drug gentamycin. It showed moderate antibacterial activity against *Salmonella typhi, Streptococcus pyogenes* and *Streptococcus saprophyticus* where the zone of inhibition was 15, 11 and 16 mm respectively while mild activity against *Staphylococcus aureus, Shigella flexneri* and *Shigella dysenteriae* where the zone of inhibition was 06 mm in each case.

**Cytotoxic activity**

In this bioassay, the extract showed lethality against the brine shrimp nauplii. The extract showed different mortality rate at different concentrations. The plot of percent mortality versus log concentration on the graph paper produced an approximate linear correlation between them. From the graph, the concentrations at which 50 and 90% mortality occurred were obtained by extrapolation ($LC_{50}$: 03 μg/ml; $LC_{90}$: 32 μg/ml) (Table 3).

**Neuropharmacological activity**

**i. Pentobarbital induced hypnosis:**

Table 4 showed the effects of MeOH extract of *C. odollam* on pentobarbital induced sleeping time. The extract increased total sleeping time. The average duration of sleep was about 56.45 min at

**Table 3.** Cytotoxic activity of *C. odollam* against brine shrimp

| Test sample | Conc. (μg/ml) | Log (Conc.) | Avg. no. of alive shrimp | % mortality | $LC_{50}$ (μg/ml) | $LC_{90}$ (μg/ml) |
|---|---|---|---|---|---|---|
| MeOH extract | 2 | 0.30 | 07 | 30 | 03 | 32 |
| | 4 | 0.60 | 04 | 60 | | |
| | 8 | 0.90 | 03 | 70 | | |
| | 16 | 1.20 | 02 | 80 | | |
| | 32 | 1.50 | 01 | 90 | | |
| Me. extract of *C. odollam* | 64 | 1.80 | 00 | 100 | 10 | 39.81 |

**Table 4.** Effect of *C. odollam* on pentobarbital induced hypnosis

| Animal group | Treatment(i.p.) | Time of onset of sleep (min) | Total sleeping time (min) |
|---|---|---|---|
| I. Control | 0.1% Tween 80 solution10 ml/kg | 3.36 ± 0.457 | 49.09 ± 2.44 |
| II.Test group | MeOH Extract 25 mg/kg | 4.27 ± 0.304* | 56.45 ± 5.61* |

Values are expressed as mean ± S.E.M. (n = 10); *indicates $P < 0.02$ vs control.

**Table 5.** Effect of *C. odollam* on exploratory behavior in mice

| Group | Response at | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120 min | 240 min |
| | Open Field Test | | | | |
| I. Control | 113 ± 16.27 | 94 ± 23.19 | 83 ± 22.3 | 83 ± 18.51 | 86 ± 22.85 |
| II. MeOH extract 25 mg/kg | 107 ± 20.78 | 82 ± 10.63 | 63 ± 9.45 | 27 ± 4.63* | 17 ± 3.60* |
| | Hole Cross Test | | | | |
| I. Control | 1 ± 0.26 | 3 ± 0.58 | 4 ± 1.0 | 4 ± 1.29 | 5 ± 0.97 |
| II. MeOH extract 25 mg/kg | 0.33 ± 0.21 | 1.16 ± 0.74 | 2.5 ± 1.147 | 1.5 ± 0.50 * | 0.33 ± 0.21** |

Values are expressed as mean ± S.E.M. (n = 5) * indicates $P < 0.02$; ** indicates $P < 0.01$ vs control.

dose of 25 mg/kg where as in control group it was about 49.09 min. Thus the results showed that the MeOH extract of *C. odollam* potentiated the pentobarbital induced sleeping time in mice.

**ii. Exploratory behavior:**

It was observed that the extract caused a significant ($P < 0.02$) decrease in the open field score (Table 5) and decrease in the number of hole crossed from one chamber to another chamber significantly ($P < 0.001$) (Table 5) in mice at the dose of 25 mg/kg.

## DISCUSSION

*C. odollam* is a poisonous tree, which is responsible for about 50% of the plant poisoning cases and 10% of the total poisoning cases in Kerala, India. It is used both for suicide and homicide. Interestingly, the same species available in Coastal region of Bangladesh is not too much poisonous, and even the local people use the fleshy portion of the fruit as food. Previously we reported that its barks were not toxic to albino mice ($LD_{50}$: 750 mg/kg). In the present study, MeOH extract of seed kernels were screened for its biological activities. The $LD_{50}$ value was found to be 500 mg/kg. Odollam Seeds were more toxic compared to its barks, and was carefully used. Due to its toxicity lower doses were used in antibacterial, cytotoxic and neuropharmacological activity tests.

*In vitro* antibacterial activity of the extract was determined by agar-well diffusion method (Rios *et al*., 1987). Agar-well diffusion method is widely acceptable for the preliminary screening of antimicrobial activity. It is essentially a qualitative or semi quantitative test indicating the sensitivity or resistance of microorganisms to the test materials (Roland, 1982). On the basis of the result of agar-well diffusion method, it can be concluded that the extract possesses mild to moderate antibacterial activity.

The cytotoxic activity of extract was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. (Anderson *et al*, 1988). The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

Neuropharmacological activity was tested by pentobarbital induced sleeping time test and the tests for exploratory behavior. Pentobarbital shorten the onset of sleep and increases sleep duration. The MeOH extract of *C. odollam* potentiated the pentobarbital induced sleeping time in mice, which suggests its central depressant activity (Perez *et al*. 1998), thus suggesting the probable tranquilizing action (Capasso *et al*. 1996). The extract also made mice to reduce their behavioral exploration, which further supports the central sedative properties of the extract. The overall results tend to predict the central nervous system depressant action of the extract.

In conclusion, it can be suggested that the MeOH extract of *C. odollam* seeds possesses antibacterial, cytotoxic and CNS depressant effects, which correlate well with the traditional use of the plant. The activity of the seed extract may be due to the presence of alkaloid and/or saponins present in it. Further researches, however, are essential to find out the active principles responsible for these activities.

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