

# Forensic Evaluation of Phytochemical Constituent and Antibacterial Activity of Pong-Pong (Cerbera Odollam) Leaves

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## Abstract

In this study, Cerbera odollam called Pong-Pong a poisonous tree belongs to family Apocynaceae. Cerbera odollam leaves were extracted by using Cold Extraction Method- Cold Maceration Method with different solvent-Hexane, Methanol and Chloroform. Hexane a low-polar solvent while Chloroform medium polar solvent and Methanol highly polar solvent. Solvent Extract of leaves of Cerbera odollam were examined for Phytochemical Screening and Antibacterial Activity. Phytochemical constituents were analyzed for both Qualitative Analysis for (Alkaloids, Flavonoids, Cardiac glycosides, Saponins, Tannins, Carbohydrates, Phenols, Amino Acids, Sterols, Terpenoids, Proteins, Fatty Acids, Lipids, Phytosterols through chemical tests) and Quantitative Analysis for total Alkaloid and total Cardiac glycosides calculated under UV Spectrophotometry. Antibacterial Activity was determined by Agar-Well Diffusion Method for Hexane, Chloroform and Methanol leaves extracts. Study carried out for gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) Bacteria. Minimum Inhibitory Concentration (MIC) calculated by using Tube Dilution method for 100mg leaf extract sample.

**Keywords:** Pong-Pong, Cerbera odollam, Extraction, Maceration method, Phytochemical, Quantitative Analysis, Qualitative Analysis, Spectroscopy, Antibacterial, MIC.

## INTRODUCTION

Pong-pong (Cerberaodollam), an angiospermic tree, belongs to the toxic Apocynaceae. Pong - pong is because of its hanging similitude with mango. Pong-Pong (Cerbera odollam) is understood for prevalence in self-poisoning cases from southern states of India, that is attributed to regarding five thousand of the plant poisoning cases in Kerala, India. It was reportable that the tree contributed regarding five thousand of the plant poisoning

and 100% of the entire poisoning incidences in Kerala, India. 537 recorded destruction from 1989 to 1999 within Kerala alone and one out of ten of usually deadly poisonings. The Suicide tree, self-destruction tree, buta-butua, kepongpong, mintolla, othalam whereas in some places it's referred to as poison apple or yellow saucer-eyed cerbera. It bears a natural product called othallanga<sup>1</sup>.

### Medicinal Importance of Pong-Pong Tree

The tree is likewise regarded for various medicative residences. The latex is believed in India for its nauseant, purgative and infliction consequences.

- The oil from the seeds as a treatment for, itch or enforced to the hair as associate degree pesticide.
- The bark and leaf of the plant square measure historically used as nauseant and cathartic.
- Kernels square measure used as a treatment for hydro - phobic neurosis.

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- Its bark and end result square measure purgative and used for the remedy of Rheumatism.

### ***Symptoms of Pong-Pong Poisoning***

The reported presenting symptoms of Odollam toxicity include Headache, Muscle weakness, dizziness, altered mental status, nausea, vomiting, abdominal pain, chest pain, and palpitations. Bradycardia, hyperkalemia, and thrombocytopenia are often present<sup>2</sup>.

### ***Disease and Disorder caused due to Pong-Pong***

Cancer, Cardio-Vascular disease, Neural disorders, Alzheimer's disease, mild cognitive impairments, Parkinson's disease, ulcerative colitis, Ageing and Atherosclerosis<sup>3</sup>.

### ***Fatal Suicide Cases***

In the coastal state of Kerala in India, there were 537 suicide deaths from 1989-1999 due to Cerbera odollam poisoning. Among the victims, 70%-75% are females. The availability of Cerbera odollam fruits is the main factor in most of the suicide cases. The social and educational status of the victims plays a role although there has no distinction among the different castes of people in Kerala. It has been reported that, out of 38 patients admitted to the Medical College Hospital of Trivandrum in Kerala for C. odollam poisoning, nine patients died<sup>4</sup>. The poisoning causes hyperkalemia with higher plasma potassium at greater toxicity. Out of 12 patients with plasma potassium above 8 mmol/L, eight died. There is no positive correlation between the amount of fruit kernel consumed and the mortality rate. From 2001-2002, out of 315 post-mortems performed at the Batticaloa Teaching Hospital in eastern Sri Lanka, seven deaths were due to C. manghas poisoning<sup>5</sup>.

### ***Non-Fatal Suicides Cases***

After eating Cerbera fruits or seeds, there have been some reported suicide deaths; also, cardiac intoxication recovery is not unheard of. Both Taiwan and the US have recorded occurrences of this kind. A fifty-year-old man tried to end his life by eating three C fruits. Manghas was admitted to the Chang-Gung Hospital in Keelung, Taiwan upon arrival at the hospital he had shortness of breath, dizziness and vomited with diarrhea. The a ventricular rate of 30 bpm and a sinus rate of 78 bpm, the patient had full atrioventricular block and pacing of the heart beat and other supportive treatments, all abnormalities subsided

after 3day, and then he was discharged with no complications. Another case of acute Cerbera toxicity happened in the United States, far from the natural habitats of these trees. A 51-year-old woman, with a history of depression and suicides from drug overdose, ingested. Codollam seeds purchased from an online website<sup>6</sup>. She presented herself to the Walter Reed National Military Medical Center in Bethesda, Washington DC, complaining of nausea, vomiting, and diarrhea and chest tightness. The patient was lethargic and had an irregular pulse. Her heart rate was 30 bpm and blood pressure 90/60mmHg. She received atropine, which caused her heart rate to increase to 91 bpm. Given two doses of digoxin-Fab, she soon recovered and was discharged under a stable condition.

## **MATERIAL AND METHOD**

### ***Place of Work***

The present study was administrated in 3 phases, Extraction of leaves sample and Phytochemical analysis were performed at Research laboratory in Forensic Department, Shuats, Quantitative analysis of leaf extract at MCE Research lab, MCE Department, JIBB, Shuats and Antibacterial Activity of leaf extract and MIC at Biogen Research lab, Chaka, Naini in Prayagraj.

### ***Collection & Preservation of Samples***

Green leaves sample of Pong-Pong were Provided by the Department of Forensic Science, SHUATS. The samples after collection were washed with tap water, dried under shed and then grinded into fine powdery form, the grinded leaf sample was preserved in air tight plastic container.

### ***Extraction of Sample***

Maceration Method (Cold Extraction Method)

***Phytochemical Screening (Qualitative Analysis)***  
In Phytochemical screening for qualitative analysis for polarity of different test (Alkaloid, Amino Acid, Phenol, lipid etc.)

***Phytochemical Screening (Quantitative Analysis)***

Antibacterial Activity Preparation of Media  
- Liquid Nutrient Broth, Nutrient Agar Media  
- Preparation of Inoculums, MIC - (Minimum Inhibitory concentration)<sup>7</sup>.

## RESULT AND DISCUSSION

### Phytochemical Screening (Qualitative Analysis)

In Phytochemical screening for qualitative analysis Methanol leaf extract (high polar solvent) possess highest concentration of these Phytochemicals while Hexane (low polar solvent) leaf extract found lowest concentration and Chloroform (medium polar solvent) leaf extract show medium concentration. Amino acids and proteins are found completely absent in all solvent extract. This shows as polarity increases, concentration of these Phytochemicals also increases.

### Phytochemical Screening (Quantitative Analysis)

In Phytochemical screening, quantitative analysis calculated for only Alkaloids and Cardiac glycosides because these two are highly toxic compounds found in Pong-Pong. Alkaloids content was found more than Cardiac glycosides content. Alkaloids content were found highest in Hexane, lowest in Methanol and intermediate in Chloroform while Cardiac glycosides content was found in opposite nature i.e. highest in methanol and lowest in

hexane and chloroform in intermediate amount. The result also shows difference when there is change in polarity.

### Antibacterial Activity

In Antibacterial Activity *Staphylococcus aureus* (Gram +ve) showed better zone of inhibition than *Escherichia coli* (Gram -ve) bacteria. Methanol showed highest zone of inhibition while Hexane showed least zone of inhibition (0 mm zone of inhibition in *Escherichia coli*) and Chloroform showed intermediate zone of inhibition. High polar compounds show better result than low polar compounds.

### MIC - (Minimum Inhibitory Concentration)

Minimum Inhibitory Concentration were determined only for those samples which had shown zone of inhibition as hexane extract of pong-pong did not show Zone of inhibition in *Escherichia coli* thus it was not considered for MIC. Least MIC value was shown by Methanol extract that's why it shows highest Antibacterial activity. Highest value of MIC was shown by Hexane extract that's why it showed least zone of inhibition and found intermediate MIC and Antibacterial activity of Chloroform extract.

## ANTI BACTERIAL ACTIVITY

Table 1: Comparative table for antibacterial activity

Micro organism	Media Control	(Gram-Ve) Control	(Gram+Ve) Control	Test Plate 1 <sup>st</sup>	Test Plate 2 <sup>st</sup>
<i>Escherichia Coli</i> (Gram - Ve)	0 mm	0 mm	CL-12mm TET - 18 MM	H - 0mm C - 3mm M - 6mm	H - 0mm C - 4mm M - 6mm
<i>Staphylococcus</i> (Gram - Ve)	0 mm	0 mm	CL- 8mm TET - 20 MM	H - 6mm C - 7mm M - 8mm	H - 5mm C - 7mm M - 9mm

### MCI - Minimum Inhibitory Concentration

Table 2: MCI Value

Bacterial Strain	Solvent	MCI Value
<i>Escherichia Coli</i>	Chloroform	25 mg
<i>Escherichia Coli</i>	Methanol	12.5 mg
<i>Staphylococcus Aureus</i>	Hexane	50 mg
<i>Staphylococcus Aureus</i>	Chloroform	25 mg
<i>Staphylococcus Aureus</i>	Methanol	12.5 mg

## CONCLUSION

From present study it was concluded that Pong-Pong (*Cerbera odollam*) leaf extract possess higher amount of phytochemicals, and possess Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in solvents i.e. low polar Hexane, medium polar Chloroform and high polar Methanol solvent leaf extract of pong-pong. It can be deciphered from the above that higher polarity shows better results than lower polarity solvent extract. However it is observed that the polarity is

highly responsible for the mentioned study. Pong-Pong is a plant, which can delay the rate of putrefaction of corpus delicti against *Staphylococcus aureus* and *Escherichia coli*. This study can be useful for forensic scientist as well as other organizations who are involved in investigation of Microbial study and deal with plant poison as well as poisoning caused by pong-pong (Cerbera odollam).

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