

Phytochemical And Pharmacological Screening Of Ethanolic Extract Of Clerodendrumplomidis

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I. Introduction

Cancer is one of the most life-threatening diseases in which deregulating proliferation of abnormal cells invades and disrupts surrounding tissues. It constitutes serious public health problems in both developed and developing countries. About 12.7million people were diagnosed with cancer across the world in 2008, and 7.6million people died from the cancer during the same year. Lung cancer, breast cancer, colorectal cancer and stomach cancer accounted for two-fifths of the total cases of cancers diagnosed worldwide. More than 70% of all cancer deaths occurred in low-and middle-income countries, Deaths due to cancer are projected to continuously increase and it has been estimated that there will be 11.5million deaths in the year 2030 and 27million new cancer cases and 17.5million cancer deaths are projected to occur in the world by 2050. Natural compounds have been expected to play an important role either as chemo preventive or chemotherapeutic agents to fight against cancer. Some herbs reduce the toxic side effects of chemotherapy and radiotherapy.

Cancer is a complex family of diseases, and carcinogenesis, the events that turn a normal cell in the body into a cancer cell, is a complex multi step process. From a clinical point of view, cancer is a large group of diseases, perhaps up to a hundred or more, that vary in their age of onset, rate of growth, state of cellular differentiation, diagnostic detectability, invasiveness,

metastaticpotential, responsetotreatment,andprognosis.Clerodendrumplomidis(Lamiaceae) is an important and well known medicinal plant extensively used in Ayurveda and Siddha system of medicine for treatment of various ailments. The popular therapies include on inflammation ,diabetes, nervousdisorder, asthma, rheumatism, digestivedisorders and urinary disorders as well as a bitter tonic. Moreover, the presence of glycoside moieties like saponins,anthraquinones,steroidal glycosides and flavonoids could inhibit tumor growth.

II. Methodology

Collection of Plant:

The leaves of Clerodendrumplomidis was collected from the local region, TamilNadu and authenticated by Taxonomist. The herbarium of the plant was been kept in our institution. The leaves were dried in the shade for 7days at room temperature (28C).

Extraction of Plant materials:

The fresh plant materials were washed with running tap water and shade dried. The samples were crushed to coarse powder by grinder. These coarse powders (25g) were then subjected to defatted with petroleum ether and then extracted with ethanol till exhaustion by using Soxhlet apparatus. The collected extracts were stored and then taken up for further investigations. The DMSO (Dimethylsulfoxide) is act as dissolved solvents for these extracts.

Preliminary Qualitative Biochemical Tests for detection ofchemical constituent

Preliminary phytochemicals analysis was carried out for ethanol extract as per standard methods.

Detection of Alkaloids

The powder of ethanol extract was dissolved in dilute hydrochloricacid and filtered. The filtrates were used to test the presence of alkaloids.

Mayer's test: Filtrate was treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

a)Wagner's test: Filtrate was treated with wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Leadacetate test: Ethanol extract powder was treated with few drops of leadacetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

a) Sulphuric acid test: Extract was treated with few drops of sulphuric acid. Formation of orange color indicates the presence of flavonoids.

Detection of Steroids

2ml of acetic anhydride was added to 0.5g of the powder ethanol extract, with 2ml of H₂SO₄. The colour changed from violet to blue or green in sample indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration of the inner face indicates the presence of terpenoids.

Detection of Anthraquinones

Borntrager's test

About 0.2g of the powdered extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrate. Few drops of 10% NH₄ were added to the mixture and heated. Formation of pink colour indicates the presence of anthraquinones.

Detection of Phenols

a) Ferric chloride test: Extract was treated with few drops of ferric chloride solution.

Formation of bluish black color indicates the presence of phenol.

b) Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

Detection of Saponins

About 0.2g of the ethanol powder extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

Detection of Tannins

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Detection of Carbohydrates

Extract was dissolved in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Detection of Oils and Resins

Test solution was applied on filter paper. It develops transparent appearance on the filter paper. It indicates the presence of oils and resins.

To evaluate the cytotoxic studies by leaf extract

Brine Shrimp Lethality Assay (BSLA)

(Artemiasalina) eggs were hatched in artificial sea water prepared from commercial sea salt 38g/L. A lamp was placed above the open side of the tank to attract the hatched shrimps close to the tank wall. After 24 hours, the shrimps matured as nauplii (Artemiasalina) and were ready for the assay. The brine shrimp lethality bioassay was carried out on the ethanol extract using the standard. Twenty milligrams of the extract was dissolved in 1ml of Propylene glycol/Tween 80/water (4:1:4) to give a crude extract concentration of 20mg/mL. A two-fold serial dilution was carried out with salt water to obtain a test solution in the range of 0.1-10mg/mL. Each concentration was tested in triplicate. A test tube containing propylene glycol/Tween 80/water (4:1:4) in 5ml of salt water was used as the negative control. Ten milligrams of potassium dichromate (as positive control) was dissolved in propylene glycol/Tween 80/water (4:1:4) and serially diluted, to obtain test concentrations ranging from 0.01 to 5mg/mL. A suspension of larvae (0.1ml), containing about 10-15 larvae, was added into each test tube and incubated for 24 hours. The test tubes were then examined and the number of dead larvae in each bottle. The total number of shrimps in each bottle was counted and recorded. The death percentage and lethal concentration (LC₅₀) were determined using statistical analysis.

Equation:

Percentage of Death (%): $(\text{Total naupii} - \text{Alive naupii}) \times 100\% / \text{Total naupii}$

III. Results

Plant extracts

The percentage yield of the ethanolic leaf extract of Clerodendrum phlomidis (Lamiaceae) was found to be 68.78 % obtained by soxlet extraction process.

Qualitative phytochemical analysis of Clerodendrum phlomidis leaves (Lamiaceae)

Table 1: Phytochemical characteristics of ethanolic leaves extract of Clerodendrum Phlomidis leaves (Lamiaceae)

Sl.No	Phytochemical test	Ethanolic leaf extract of Clerodendrum phlomidis	Ethanolic leaf extract of Clerodendrum phlomidis
1	carbohydrates	+	+
2	Steroids	+	+
3	Alkaloids	-	-
4	saponins	+	+
5	flavanoids	+	+
6	Tannins	+	+
7	phenolics	+	+

Brine Shrimp Lethality Test

Table 2: Effect of ethanolic leaves extract of Clerodendrum phlomidis on brine shrimp lethality assay

Sl No	Time (hrs)	Conc	No of Naupliitaken	Total no of surviving nauplii	% mortality		
				Leaf Extract of Clerodendrum phlomidis			
1	3	0.2%	20	10	10	10	10%
2	6	0.4%	20	10	10	10	10%
3	9	0.6%	20	10	10	10	10%
4	12	0.8%	20	10	10	10	10%

As shown in the Table 2, ethanolic leaves extract of Clerodendrum phlomidis was subjected to Brine Shrimp lethality bioassay for possible cytotoxic action was carried out against at different concentrations of 1µg, 10µg, 100 µg and 1000 µg. The suspensions of larva were examined after 24 of the incubation period. At 100µg/ml concentration of leaves extract of Clerodendrum phlomidis was showed 50 percent of mortality rate and 1000µg/ml concentration of leaves extract of Clerodendrum phlomidis were showed 100 percent of mortality rate. In this study, ethanolic leaves extract of Clerodendrum phlomidis was found to be toxic to Brine Shrimp nauplii, with LC50 value of 100µg/ml.

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