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Phytochemical and pharmacological investigation of *Plumbago indica* L

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Abstract

This study was conducted with the leaves of plant *Plumbago indica* L. (Family: Plumbaginaceae) to investigate the pharmacognostical standards and the biological activities especially the possible cytotoxic effects.

The powder from the leaves of *Plumbago indica* L was soaked with methanol and kept for 15 days. After then it was filtered and kept in open air for evaporation. After evaporation the concentrated methanol extract was then stored for further uses. Testing of different chemical groups present in extract represents the preliminary pharmacognostical studies. The chemical group tests are performed by 10% (w/v) solution of the extract of *Plumbago indica* L. In methanol. Reducing sugar, alkaloids, flavonoids, gum and steroids are found.

In the brine shrimp lethality bioassay, the LC₅₀ and LC₉₀ value were found to be 4.57µg/ml and 9.87µg/ml for crude methanolic extract of *Plumbago indica* L. Based on these results we can say that this plant may have uses for traditional medicine. This is only a preliminary study and to make final comment, the drug should thoroughly investigated phytochemically and pharmacologically to explore their medicinal and pharmaceutical potentialities.

Keywords: Phytochemical, Pharmacological, *Plumbago indica* L., Brine shrimp and Lethality bioassay.

Introduction

From thousands of years a remarkable number of modern drugs have been obtained from the natural source and in primary health care those are very. Variety of plants exhibit phytochemical and pharmacological activity. Some active compounds which are obtained from natural source play an important role for maintaining human health [1]. Natural products are very helpful drug developing programmes in the pharmaceutical industry [2]. In traditional healing there are a few reports on the use of plants [3-5].

The effectiveness of traditional herbs against microorganisms is found by many reports due to become the plants one of the bases of modern medicine [6]. Many researchers had investigated the antimicrobial activity of medicinal plants for developing alternative form of health care. They also tried to found microbial resistance against the available antibiotics [7-10]. Silver and Bostian [11] have documented the use of natural products as new antibacterial drugs.

It is may be emergency to identify novel substances which are active against strong resistant pathogens [12, 13]. Herbal remedies can combine with many other substances easily and give superior effect. In developing countries, synthetic drugs are so expensive than herbal remedies [14]. *Plumbago indica* L. which was known *P. rosea* before is a species of flowering plant in the family Plumbaginaceae. It is native to south East Asia. It grows to 2m (7 ft.) tall by 1m (3 ft.) wide. It is a spreading evergreen shrub with oval leaves and racemes of deep pink or scarlet flowers in winter. It is known as Indian leadwort, scarlet leadwort and whorled plantain. In Bengali, it is known as Agnichita.

Medicinal plants are good source for curing of diseases. Most of the people are dependent on medicinal plants due to free from side effects caused by synthetic chemicals. Ecofriendly and bio-friendly plant based products are very helpful for the prevention and cure of different human diseases. For this reason people attention are on this products. Synthetic drugs contain lots of adverse effects. For this reason the Western population is searching natural remedies because those are safe and effective. It is documented that most of the World's population has taken in traditional medicine, particularly plant drug for the primary healthcare.

Here are some medicinal uses of different parts of *Plumbago indica* L.

Its roots are abortifacient; used in hepatitis, dyspepsia, flatulence, piles, leucoderma, leprosy and anasarca; locally as vesicant in rheumatism, paralytic affections and enlarged glands. Root contains "Plumbagin" which have antifertility properties and Juice is useful in ophthalmia and scabies. Chakma of Rangamati use roots for jaundice and leaves for dysentery. In Chittagong Hill Tracts leaves of this plant are used to make pills and given as a contraceptive by the Marma tribe [15].

The principle aim of the present study was to investigate the scientific basis of the traditional uses of the plant *Plumbago indica* L., in the same time find the chemical groups present in the active plant parts to get preliminary idea about the active constituent and also to know whether it possesses any cytotoxic activity.

Material and Methods

Collection of Plant material

The plants selected for this present research *Plumbago indica* L. (Family: Plumbaginaceae) and collected from Naramuk, Rajasthali of Rangamati district. After collection, suitable herbarium sheets were prepared for each plant which contain all basic information of that plant and were send to Bangladesh Council of Scientific and Industrial Research (BCSIR), Baluchara, Chittagong. After identify, the provided us the scientific name of the plant.

Extraction

At first the collected plants particularly the leaves and stems were separated from unwanted materials of plants or any of their parts. Then the plants were shed dried at 35-40°C. The fully dried plants were ground into a coarse powder by using a suitable grinder. The coarse powder was carefully stored in an airtight container which is made by inert compound as though it does not react with powder and it kept in a dry, cool, and dark place until extraction commenced. About 185 gm of powder material of *Plumbago indica* L. (Family: Plumbaginaceae) was taken in a clean, dry and flat bottomed glass container and dissolved it with 1700 ml of methanol. The container with its contents was labeled and kept for 10 days with episodic shaking and stirring. After 7 days the whole mixture then filtered by a piece of clean, dry, dustless, white cotton material. Then these coarse mixtures further filtered through Whatman filter paper and the solvent was give up to evaporate at the room temperature. The remaining extract was collected and the residues were preserved in a refrigerator for further studies.

Pharmacognostic Study

Plumbago indica L. (Family: Plumbaginaceae) was subjected to pharmacognostic study. Various methods are used in this study including organoleptic study and preliminary phytochemical studies.

Organoleptic study

The coarse powder of *Plumbago indica* L. was used for studies.

Chemical group tests of *Plumbago indica* L.

About 20 gm of the coarse powder of *Plumbago indica* L. (Family: Plumbaginaceae) was weighed accurately and dissolved with 250 ml of fresh, hot water. After 1 hour it was filtered and the supernatant was used as the extract. For detecting of different plant constituents these whole extracts were subjected to qualitative chemical tests.

Tests for reducing sugar

A. Fehling's Test

2ml of an aqueous extract of the plant *Plumbago indica* L. (Family: Plumbaginaceae) was added in 1ml of a mixture of equal volumes of Fehling's solutions A and B. The mixed well and oiled for few minutes. A brick red or red colour precipitate was formed with the reducing sugar.

B. Benedict's Test

In a test tube 0.5 ml of aqueous extract of the plant material with 5 ml of Benedict's solution was taken. They mixed well and boiled for 5 minutes and kept it to cool spontaneously. A red colour precipitate of cuprous oxide was formed with the reducing sugar.

Tests for Alkaloids

A. Mayer's Test

2ml solution of the extract taken in a test tube and 0.2 ml of dilute hydrochloric acid were added to it. Farther 1 ml of Mayer's reagent also added. A yellow colour precipitate was formed in the presence of alkaloids.

B. Dragendroff's Test

2 ml aqueous solution of the extract taken in a test tube and 0.2 ml of dilute hydrochloric acid added to it. Farther 1ml of Dragendroff's reagent was added. An Orange brown precipitate was formed. It indicates the presence of alkaloids.

C. Wagner's Test

2 ml aqueous solution of the extract taken in a test tube and 0.2ml of dilute hydrochloric acid added to it. Farther 1ml of Wagner's reagent was added. Reddish brown precipitate was formed. This precipitation indicates the presence of alkaloids.

D. Hager's Test

2ml aqueous solution of the extract taken in a test tube and 0.2ml of dilute hydrochloric acid added to it. Farther 1 ml of picric acid solution (Hager's reagent) also added to it. A yellowish precipitate was formed with the alkaloids.

Tests for Tannins

A. Ferric Chloride Test

5ml aqueous solution of extract and 1ml of 5% Ferric chloride solution were taken in a test tube. No greenish black precipitate was formed. It declared the absence of tannins.

B. Potassium Dichromate Test

In a test tube 5ml aqueous solution of the extract was taken. Then 1 ml of 10% Potassium dichromate solution was added to it. No yellow precipitate was formed. So tannin is absent.

Test for Flavonoids

In a test tube a small amount of alcoholic extract of the plant material was taken. Few drops of concentrated hydrochloric acid added to it. Immediate development of a red colour. This red colour indicates the presence of flavonoids.

Test for Saponins

1 ml aqueous solution of the extract was diluted with distilled water and fill up to 20 ml. Then the solution shaken in a graduated cylinder for 15 minutes. No foam layer was formed which indicates that the saponin is not present.

Test for Gum

In a test tube 5ml aqueous solution of the extract was taken. Then A few amount of molish reagent and sulphuric acid were

added into it. Red violet ring was formed at the junction of two liquids. Which indicates the presence of gums.

Test for Steroids

Liebermann Bur chard Test

1 ml aqueous solution of chloroform extract was taken in a test tube. Then 2 ml Liebermann Burchard reagent added to it. A reddish purple colour was formed. Which indicates the presence of steroids.

Biological Investigation

Preparation of sample

5 mg of dried methanol extract was taken in a 80 ml beaker. Then 500 μ l DMSO was added to it. The volume was filled up to 5 ml by methanol. The concentration of this sample solution was 1000 μ g/ml.

Hatching of Brine shrimp

Sea water was taken in a particular divided small tank and shrimp eggs were taken in the one side of the divided tank. Then the one side of the tank which contain shrimp eggs were covered. In this condition the shrimps were allowed to hatch for 36 hrs and to mature as nauplii. During this process constant oxygen supply and around 37 $^{\circ}$ c temperature was maintained. The hatched nauplii shrimps were attracted to the lamp through the prick in the dam and they were selected for bioassay.

Application of test sample to the test tube containing brine shrimp nauplii

36 clean and uniform test tubes were taken and marked at 10ml by using a permanent marker. Here, 18 test tubes were taken for the samples with six different concentrations (three test tubes are used for each concentration) and 18 test tubes for control (three test tubes are used for each concentration). Then sample solutions with different concentration were shifted to the test tubes. 10 living shrimps were taken in each of the test tubes by using Pasteur pipette. For control, the DMSO and methanol of definite volume were shifted into the control tubes. The concentration of DMSO should not be above of 10 μ l/ml, because above this concentration of DMSO can be toxic to the nauplii.

Preparation of control group

Control group was added in cytotoxic activity to validated the test method and obtained result during the cytotoxic activity of the test agent. Then an anticancer drug, Methotrexate, was added to marked glass container, which contain 5 ml of sea water and 10 shrimp nauplii for using as control groups. In the preparation of control solution no extract was added. If the mortality rate of these brine shrimps of the vials was very rapid, then the test method considered as invalid as the nauplii died rapidly due to some reason other than the cytotoxicity of the compound.

Counting of nauplii

After 24 hours, inspected the vials by using a magnifying glass and counted the numbers of survived nauplii in each vial and calculated the values of LC₅₀ and LC₉₀.

Results and Discussion

Results of Organoleptic Study

Colour, odour and taste characters are indicated by organoleptic study. Aerial powder's colour showed green

colour. Then the taste and odour of the aerial powders were also tested but the taste is bitter and on analysis the aerial powder gives a characteristic odour (Table-1).

Table 1: Organoleptic study of the powder

1	Colour	Green
2	Odour	Characteristics
3	Taste	Mucilaginous and slightly bitter

Results of Chemical Group Tests

For chemical group tests some method had performed in this study and every method performed two or three for getting accurate result which has been represented in Table-2.

Table 2: Results of different Chemical group tests of *Plumbago indica* L.

Test groups	Name of the test	Presumption
Reducing sugar	Fehling's test	+
	Benedict's test	+
Alkaloids	Mayer's test	+
	Dragendroff's test	+
	Wagner's test	+
	Hager's test	+
Tannins	Ferric chloride test	-
	Potassium dichromate test	-
Flavonoids		+
Saponins		-
Gum		+
Steroids	Liebermann Burchard test	+

+ = Presence - = Absence

Results of Brine Shrimp Lethality Bioassay

Brine shrimp nauplii, methanolic extract of *Plumbago indica* L were used in in brine shrimp lethality bioassay and positive result was found. For this reason it can be assumed that the extract is pharmacologically active. An approximate linear correlation was found by plotting the log of concentration (log C) versus (%) mortality for all test samples. For checking the toxic level of the extract the median lethal concentrations (LC₅₀, the concentration at which 50% mortality of brine shrimp nauplii occurred) were determined and LC₉₀ values were also determined from the graph. Cytotoxic activity against brine shrimp nauplii and LC₅₀ value was 4.57 μ g/ml (Table-3 and Figure-1) were also found by the crude extract of *Plumbago indica* L. The 90% mortality rate (LC₉₀) was also calculated to get the therapeutic index and the value was 9.87 μ g/ml (Table-3 and Figure-1). Methotrexate was used to validate the test method.

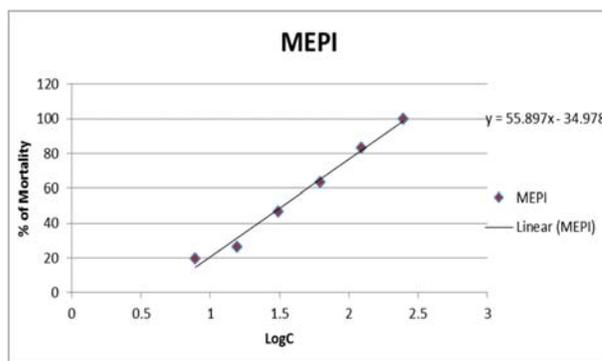


Fig 1: Determination of LC₅₀ and LC₉₀ of methanolic extract of *Plumbago indica* L.

Table 3: Brine shrimp lethality bioassay of MEPI (Methanolic extract of *Plumbago indica* L.):

Test groups	Conc. (µg/ml)	Log C	No. of alive shrimp			Mean alive	% mortality	LC ₅₀ (µg/ml)	LC ₉₀ (µg/ml)
			T1	T2	T3				
	7.81	0.89	8	8	8	8	20		
	15.63	1.19	7	7	8	8.33	26.66		
MEPI	31.25	1.49	6	5	5	5.33	46.7	4.57	9.87
	62.5	1.79	4	4	3	3.66	63.4		
	125	2.09	2	1	2	1.66	83.4		
	250	2.39	0	0	0	0	100		
	7.81	0.89	10	10	10	10	0		
	15.63	1.19	10	10	10	10	0		
Control	31.25	1.49	10	10	10	10	0		
	62.5	1.79	10	10	10	10	0		
	125	2.09	9	10	9	9.33	6.7		
	250	2.39	8	8	9	8.33	16.7		

Conclusion

The results of organoleptic study offer a scientific basis for the traditional use of *Plumbago indica* L. which possess characters like green, characteristic odour, mucilaginous and slightly bitter taste. The aerial part of the *Plumbago indica* L. has been tested for the identification of the chemical group present in that plant. It has been found that Reducing sugars, Alkaloids, Steroids, Flavonoids and Gums were present.

From the brine shrimp lethality bioassay study, it can be concluded that *Plumbago indica* L. can be investigated as a source of anti-tumor agent. This is only a preliminary study and to make final comment the drug should thoroughly investigated phytochemically and pharmacologically to explore their medicinal and pharmaceutical potentialities.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ZTR, MA and MH collected the plant. MA, ZTR and MSI carried out conception and design of the study. MA, RI and FF wrote the manuscript. MA, MH, MAHK, RI and SP revised the manuscript. All authors read and approved the final manuscript.

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