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Research article

PHYTOCHEMICAL AND ACUTE TOXICITY STUDY OF LEAVES OF *ARTOCARPUS HETEROPHYLLUS LAM*

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ABSTRACT

Artocarpus heterophyllus Lam commonly known as jack fruit widely distributed in north east India, West Bengal and south Karnataka. In the present study intended with various phytochemical screening and toxicity studies were carried out on the leaves of *Artocarpus Heterophyllus.* The phytochemical study shows the presences of flavonoids, tannins, saponins and carbohydrates in methanolic and aqueous extracts. In acute toxicity study both the extract were found safe on a dose of 2000 mg/Kg.

KEYWORDS: *Artocarpus heterophyllus,* Phytoconsituents, Acute toxicity.

INTRODUCTION

Since the tribal native practitioner of Manipur claimed that the leaves of *Artocarpus heterophyllus* are highly useful in many diseases and we were in search for an alternative medicine, this claim has attracted our attention and selected the plant for present study. On the basis of current literature survey leaves of Artocarpus *heterophyllus* Lam, was taken for phytochemical investigation and toxicological evaluation. The phytochemicals present in a plant indicates the possible pharmacological action whereas the toxicological study indicates the possible dose to carry out the pharmacological screening models.

MATERIAL AND METHODS

PLANT MATERIAL

Leaves of *Artocarpus heterophyllus* Lam were collected from the forest of Manipur. The plant was authenticated by Dr. Bisehwori Thongam (IBSD/MPHRD/M/1008). The shade-dried leaves were course powdered and this powder were packed in soxhlet column and extracted successively with petroleum ether, chloroform, methanol and aqueous. The extracts were concentrated under reduced pressure (bath temperature 50°C). The dried extracts were stored in air tight container in refrigerator below 10°C.

EXTRACTION WITH PETROLEUM ETHER

At first the finely ground leaves are placed in a 'thimble' made by a strong filter paper in a chamber of soxhlet (2000ml). The powders are extracted at 55℃ using round bottomed flask for 72 hrs. The extracting solvent in round bottomed flask is heated and its vapour condenses in condenser. The condensed extractant drips into the thimble containing the crude drug and extract it by contact. After completion of extraction petroleum ether is filtered and concentrated to dry mass. The extract is air dried to remove all traces of the solvent and the percentage yield was calculated. Petroleum ether extraction was done to defeating the powder.

EXTRACTION WITH CHLOROFORM

The marc left after petroleum ether extraction, is dried and subsequently extracted with 1200ml of chloroform (61℃) in a soxhlet using round bottomed flask for 72 hrs. Then the extract is

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concentrated by using rotary evaporator and dried to get the extract.

EXTRACTION WITH ETHANOL

The marc left is again packed in the soxhlet. The solvent is heated using isomentle and began to evaporate. For ethanol extraction the temperature used is 78℃. The extraction had for 18-20 hrs and after completion of extraction solution was evaporated to dryness under reduced pressure and controlled temperature by using rotary evaporator.

EXTRACTION WITH DISTILLED WATER

The marc left after ethanol extraction is placed in a stopper container with the distilled water (1176ml) and chloroform (24ml) and allowed to stand at room temperature for a period of 7 days with frequent agitation until the soluble matter has dissolved. Then the mixture is strained, the marc is pressed and the combined liquids are clarified by filtration. At last the solution is dried using rotary evaporator.

PRELIMINARY PHYTOCHEMICALS SCREENING

The preliminary phytochemical Screening was carried out on petroleum ether, chloroform, Ethanolic and aqueous extracts of *Artocarpus heterophyllus* for qualitative identification of type of phytoconstituents present.

DETECTION OF ALKALOIDS

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

MAYER'S TEST

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

WAGNER'S TEST

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

DRAGENDROFF'S TEST

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

HAGER'S TEST

Filtrates were treated with Hager's reagent (saturated picric acid solution). Presences of alkaloids are confirmed by the formation of yellow coloured precipitate.

DETECTION OF FLAVONOIDS

ALKALINE REAGENT TEST

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

LEAD ACETATE TEST

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

DETECTION OF PHYTOSTEROLS

SALKOWSKI'S TEST

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

LIBERMANN BURCHARD'S TEST

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

DETECTION OF TANNINS

GELATIN TEST

- To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.
- About 0.5 g of the individual extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride (FeCl3) was added and observed for brownish green or a blue-black coloration.
- The alcoholic or aqueous extract is treated with lead acetate (10%) solution. White precipitate is formed.

DETECTION OF SAPONINS

FROTH TEST

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

FOAM TEST

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

DETECTION OF PROTEINS AND AMINOACIDS

XANTHOPROTEIC TEST

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

NINHYDRIN TEST

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

DETECTION OF CARBOHYDRATES

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

MOLISCH'S TEST

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

BENEDICT'S TEST

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

FEHLING'S TEST

Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

EXPERIMENTAL ANIMALS

Albino mice (20-30gm) of either sex were procured from Sri Venkateshwara enterprises, Bangalore. After procuring the animals were acclimatized for 10 day's under standard husbandry conditions as follows;

Room temperature - 27 ± 3°C

Relative humidity - 65 ± 10 %

12 hours light / dark cycle -

The animals were fed with feed gold mohr, Lipton India Ltd., Bangalore and water was given ad libitum under strict hygienic conditions. This project was approved by Institutional Animal Ethical Committee (IAE/SKIPS/2011/MAY15/I/12/RATS-96/MICE-36).

ACUTE TOXICITY

The acute toxicity for methanolic and aqueous extracts of leaves of *Artocarpus heterophyllus* was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies.

RESULTS AND DISCUSSION

PREPARATION OF EXTRACTS

The yield, colour and consistency of extracts of leaves of *Artocarpus heterophyllus*, were shown in the table no 1.

Table No. 1: Colour, Consistency and Percentage yield of extracts of leaves of *Artocarpus heterophyllus*

Sl. No.	Solvent	Colour and Consistency	Percentage yield
1	Pet. Ether	Dark Brown Sticky	5.40%
2	Chloroform	Dark Brown Solid	2.67%
3	Methanol	Very Dark Brown Sticky	7.50%
4	Aqueous	Brown Solid	10.83%

PRELIMINARY PHYTOCHEMICAL STUDIES OF DIFFERENT EXTRACT

Preliminary phytochemical studies revealed that leaves of different extracts contain flavonoids, tannins, saponins and carbohydrates in ethanolic and aqueous extracts where as steroids are found to be present in pet. ether and extract of leaves. The results are compiled in table no 2.

Table No.2: Preliminary phytochemical studies of different extract of leaves of *Artocarpus heterophyllus*

Chemical constituents	**Solvent Used**			
	Pet. Ether	**Chloroform**	**Methanol**	**Aqueous**
Alkaloids	–	–	–	–
Flavonoids	–	–	+	+
Steroids	+	–	–	–
Tannins	+	+	+	+
Saponin	–	–	+	+
Proteins	–	–	–	–
Carbohydrate	_	_	+	+

+ Present
- Absent

ACUTE TOXICITY

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