Ind. J. Pharm. Edu. Res., 2026; 60(2s):s541-s549.
https://www.ijper.org

Original Article

Artocarpus heterophyllus Lam.: Pharmacognostic, Phytochemical Study and Its Evaluation for Antimitotic Potential by Modified *Allium cepa* Bioassay

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ABSTRACT

Background and Objectives: *Artocarpus heterophyllus* Lam. commonly known as jack tree reported to have various traditional applications, attributed by every part, for the treatment of various ailments. Although various research studies suggested the valuable importance of jack tree and still under explorations; still much is waiting to hidden out. Some of these areas include the determination of antimitotic potentials of various extracts of its leaves as a direction for heading towards anticancer efficacy and any variations occurred with that of earlier studies along with some controversies reported by the previous researchers as an important objective. **Materials and Methods:** This study was investigated stepwise with pharmacognostic evaluation-collection and authentication of plant materials, macroscopic evaluation, microscopic evaluation, physical evaluation followed by phytochemical screening of petroleum ether, chloroform, acetone, and ethanol extracts of plant leaves for the presence of various phytoconstituents groups. For the determination of antimitotic activity of leaves extracts, the *Allium cepa* bioassay with some modifications was used. **Results and Discussion:** Our study revealed that the majority of the pharmacognostic and phytochemical characteristics of *A. heterophyllus* Lam. are comparable, as supported by prior literature. While, in the assessment of antimitotic potential using a modified *Allium cepa* bioassay, the petroleum ether extract demonstrated a notable antimitotic effect. **Conclusion:** On analysis of the results, besides the common features, some interesting pharmacognostic findings includes presence of large amount of cystoliths which might responsible for higher ash value, higher moisture content, presence of both anomocytic and actinocytic stomata, both hooked and non-hooked covering trichomes. In the antimitotic bioassay, the petroleum ether extract of leaves showed higher antimitotic activity with mitotic index 27.8±0.03 ($p<0.05$) followed by chloroform, acetone and ethanol extracts, probably due to presence of various phytoconstituents in it as revealed by phytochemical screening.

Keywords: *A. heterophyllus*, *Allium cepa* Bioassay, Antimitotic, Pharmacognostic, Phytochemical.

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Received: 16-05-2025;
Revised: 24-07-2025;
Accepted: 05-09-2025.

INTRODUCTION

Artocarpus heterophyllus Lam. (Family-Moraceae) commonly known as jackfruit tree or jack tree is native to Western Ghats of India and widely distributed among tropical and subtropical countries like Brazil, Indonesia, Malaysia, Thailand and Sri Lanka.[1,2] It's a medium sized, evergreen tree reaching the height of about 9-25 m.[3,4] Almost every part of jack tree like leaves (fever, boils, wounds), young fruits (carminative, astringent), ripe fruits (aphrodisiac, laxative, brain tonic), seeds (diuretic), wood (antidiabetic, sedative), roots (skin diseases, asthma) along with latex (ophthalmic disorders, pharyngitis) have traditional medicinal applications.[5] Various scientific investigations carried out have proven the importance of *A. heterophyllus* in the upcoming modern therapeutic era not only due to richness in the nutrients like vitamins (Vitamin A, Vitamin C and other), minerals (Calcium, iron and other), proteins, fats, carbohydrates, fibres[6-8] but also due to plenty of phytoconstituents like alkaloids, saponins, flavonoids, triterpenoids, tannins and carotenoids. Various scientific studies shown that the *A. heterophyllus* leaves extract have potential pharmacological actions like antioxidant, antidiabetic, antibacterial, anthelmintic, anti-inflammatory and wound healing. Although some studies about anticancer activities of *A. heterophyllus* wood, seeds and fruits have noted but its leaf extract still is a field of new explorations.[9-12]

Cancer is one of the most concerning and alarming disease, nowadays, resulting the death of humans despite modernization of life style and medical and pharmaceutical sciences. It involves the abnormal cell division resulting to malignancy. The prime goal for the treatment and management of cancer is to suppress the

DOI: 10.5530/ijper.20263421

abnormal cell divisions. For this purpose, the cytotoxic drugs are employed in which natural products namely placitaxel, vincristine and combretastatin hold an utmost importance by inhibiting the cancerous cell division (antimitotic action).

Various models have been used for the preliminary screening of substances as cytotoxic agents. One of such plant-based models is *Allium cepa* bioassay. In this assay, the divisions in the roots of *A. cepa* (onion roots) meristematic cells have to be studied, resembling to cancer cell division in human, for the antimitotic activity. This bioassay has been proven beneficial for the early predictions of anticancer activity of drug substances. It is reliable, easy, rapid and inexpensive method. Although, it can be the matter of doubt whether, the antimitotic results obtained in the plant model will show similar results in animal-b as e d model and finally in human but various studies have concluded that, the plant cells are 1000 times more resistant to the colchicine showing good correlation of cytotoxic tests in mammalian systems.[13,14] Although few preliminary pharmacognostic studies of *A. heterophyllus* leaves have been carried out,[15,16] but some controversies have been noted among the authors while studying the *Artocarpus* species.[17] Similarly, some factors like geographical locations of the plant during its growth might affect its physical properties,[18] biosynthesis of phytoconstituents[19] which can directly affect the pharmacological actions it expresses. As far as our literature survey concerned, no such study of *A. heterophyllus* involving pharmacognostic, phytochemical and antimitotic investigations have been carried out. So, our study here deals with the investigation of antimitotic potential of leaf extracts of *A. heterophyllus* along with pharmacognostic and phytochemical investigation.

MATERIALS AND METHODS

Chemicals and Reagents

For this study, Vinblastine sulphate was procured from Yucca Enterprises, India. All the solvents and chemicals/reagents (analytical grade) were purchased from CDH Fine Chemicals, India. Throughout the experimental process, double distilled water (prepared in the laboratory) was used.

Collection and Authentication of Plant Material

To use the leaves of jack tree for this study, the twig of the tree of about 11 years old bearing flower and fruit is collected from Anjora town (21.19664514682418°N, 81.21988931490479°E) of Chhattisgarh state in the month of July and was authenticated from Department of Botany, Govt. T.V.Y.PG. Autonomous College, Durg (C.G.).

Macroscopic Evaluation

For the macroscopic evaluation of leaves, firstly the collected leaves were clean with the water and surface was dried gently by using tissue paper. The colour, odour, taste, size, shape and texture were examined.

Microscopic Evaluation

Preparation and observation of Transverse Section (TS)

For the microscopic examination, TS of leaves were taken using sharp razor blade via the midrib and lamina. The said TS were treated with absolute alcohol for 15-20 min to remove pigments. Further, the sections were treated with Pholoroglucinol: Conc. HCl (1:1) solution as a staining agent, observed under the microscope with various magnifications and pictures were recorded using HD camera.

Quantitative Microscopy

Determination of stomatal number and stomatal index

For the determination of stomatal number and stomatal index, the jack tree leaf was peeled out by hands and the white colour epidermal layer was separated. Further, the epidermal layer was treated with absolute alcohol for 5-10 min, placed on clean slide and mounted using glycerine water. The micrometric study using camera lucida (mirror type) was performed as per the standard procedure. In this part of study, the type of stomata along with epidermal cells, stomatal number and stomatal index (average values) were determined. For the determination of stomatal index, following formulae was used.

$$SI = \frac{S}{E + S} \times 100$$

SI-Stomatal Index.

S-Number of stomata per unit area.

E-Number of epidermal cells in the same unit area.

Determination of vein-islet and veinlet termination number

For this study, desired leaf piece was kept in the maceration fluids (Glacial acetic acid: Hydrogen peroxide: Water-4:1:5) for 25-30 min (Here we faced difficulty in clearing the leaf section by using organic solvents, hence we used the maceration fluids). The average number of vein-islet and veinlet terminations per sq mm of leaf in the midway between midrib and margin was determined using camera lucida as per the standard procedure.

Determination of palisade ratio

For the determination of average number of palisade cells present beneath each epidermal cell, the clearing of leaf was carried out as per the above mentioned process and determined using camera lucida as per the standard procedure.

Physical Evaluation

Determination of ash values

The accurately weighed powdered drug was incinerated using incinerator with increasing temperature upto 650°C until the carbon free ash obtained as 'total ash'. The resulted total ash was further used for the determination of 'water soluble' and 'acid

insoluble' ash using dilute hydrochloric acid. The process was repeated thrice and average value was calculated.

Determination of extractive values

For the determination of the extractive values, accurately weighed 5 g of drug powder was macerated with 100 mL alcohol (90%) for 24 hr with occasional shaking for first 6 hr, evaporated, dried at 105ºC and percentage w/w of alcohol soluble extractive value was calculated. For the determination of water soluble extractive value, instead of alcohol, chloroform water was used as a solvent in the above procedure.

Determination of foaming index

After confirmation of presence of saponin glycosides by the preliminary phytochemical screening given subsequently, the foaming index was determined. The test for foaming index was performed as per the standard procedure using decoction of plant material. We used the parameter as, by measuring the height of each test tube (number of test tube used were 10), if the height of foam in every test tube is less than 1 cm then foaming index become less than 100. If it's more than 1 cm in every test tube then it will be over 1000 and if the height of the foam in any one test tube become 1 cm, then following formulae was used for the determination of foaming index.

$$\text{Foaming index} = \frac{1000}{a}$$

a-Volume of plant material's decoction (mL) in the test tube showing 1 cm height.

Determination of crude fibres

For the determination of crude fibres, the 'Dutch method' was used. In this process, the accurately weighed drug powder was treated with dilute acid (10% nitric acid) followed by dilute alkali (2.5% sodium hydroxide) and the percentage of residue of resistant tissues as crude fibres were determined.

Determination of Loss on Drying (LOD)

For the determination of LOD, the gravimetric method was used. In this method, the drying of accurately weighed powder drug was carried out at 105ºC using hot air oven until the constant weight obtained and the percentage of volatile substance along with moisture was determined.

Preparation of Plant Extracts

For the preparation of extracts, the soxhlet extraction method was used. Firstly, the collected *A. heterophyllus* leaves were dried in shed. Further it was subjected to pulverization to coarse powder and successively extracted with solvents petroleum ether, chloroform, acetone and ethanol. The liquid extracts were filtered through Whatman filter paper no. 1 and the organic solvents were recovered by using rotary vacuum evaporator (Acculab, India). The extracts were further concentrated and dried using water bath and subjected to phytochemical evaluation and antimitotic study.

Phytochemical Evaluation

Preliminary phytochemical screening was carried out for all four extracts by various standard procedures to determine the presence of carbohydrates, proteins, alkaloids, anthraquinone glycosides, saponin glycosides, steroids, flavonoids, terpenoids, tannins and amino acids.

Antimitotic Study

Allium cepa bulbs (weighing each of about 50 g) were procured from the spice and condiment market of Durg city, Chhattisgarh state, India. Moldy, dried onions were discarded and healthy were taken for the study.

Antimitotic assay was performed as per the method published by Fiskesjo[20] with some modifications by us, in detailed, the onion bulbs were allowed to germinate in the dark room over some beakers containing potable water until the uniform growth of roots, approximately 5 cm occurred, with regular replacement of water at an interval of 24 hr (Figure 1). Further, the selected onions were dipped (roots to be dipped) into distilled water (control), vinblastine sulphate (100 µg/mL; standard) and extracts solution (prepared in distilled water)-petroleum ether, chloroform, acetone and ethanol (50 mg/mL; tests) for 24 hr. After 24 hr, the roots were clean with the distilled water and surface content was washed using tissue paper. A small portion of root (near the root tip) was cut by the blade, put on the slide and pressed under the cover slip. The pressed tissue was further treated with ethanol followed by water. The tissue was further stained using methylene blue, excess stain was removed by washing with water and observed under the trinocular microscope with various magnifications and photographs were taken using HD camera. The total number of cells along with dividing cells of the tissue were counted and mitotic index was calculated using following formulae.

$$\text{Mitotic index} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells}} \times 100$$

RESULTS AND DISCUSSION

The plant specimen was authenticated and identified as *Artocarpus heterophyllus* Lam. belonging to family Moraceae. A herbarium specimen (No: ACP/HER/M.Ph-2009/01/22) was deposited to Govt. T.V.Y.PG. college along with Pharmacognosy and Phytochemistry Department of Apollo College of Pharmacy, Durg (C.G.) for future reference.

The macroscopic study revealed that, the young leaves are dark green (upper) and pale green (underside), 4-25 cm long and 2-8 cm broad, elliptic to oval in shape, entire, alternate with glossy appearance. Apex is blunt, pointed, short with pinnate venation

and thick texture. The odour of leaf is characteristic, resembling somewhat with banana leaf (*M. acuminata*) with agreeable, slight astringent taste resembling to jamun fruit (*E. jambolana*) (Figure 2).

The transverse section of *A. heterophyllus* leaf revealed the dorsiventral feature with single layered epidermis, several cystoliths, three layers of palisade cells, 5-9 layers collenchyma on both sides, several layers of spongy parenchymatous cells of variable sizes and collateral vascular bundles. As discussed in the introduction section about the controversies existed about some microscopical features, we found the presence of both anomocytic and actinocytic stomata unlike some researchers who had reported the presence of only anomocytic but not the actinocytic. Both glandular and covering trichomes, rarely to be seen, were observed. Interestingly, the covering trichomes were of both types (hooked and non-hooked). The hooked trichomes were of collapsed cell at the tip. However previous study reported the presence of only non-hooked covering trichomes (Figure 3).[17] Beside these microscopical features, the various leaf constants found are-stomatal number (7-9), stomatal index (13.8-15.6), vein-islet number (4-5), veinlet termination number (2-4) and palisade ratio (9.5-10.1). The physical evaluation results of *A. heterophyllus* leaves represented the total ash value as 15.2±0.3%, water soluble ash as 11.66±0.1% and acid soluble ash as 6.3±0.3% which explains about higher percentage of inorganic constituents present in it. One of its reasons might be the higher amount of cystoliths (deposits of calcium carbonate in enlarge epidermal cells) as observed in the microscopical studies. The extractive values,

Figure 1: Antimitotic assay using A. cepa. (A)-Distilled water/control; (B)-Vinblastine sulphate/standard, 100 µg/mL; (C)-Petroleum ether extract, 50 mg/mL; (D)-Chloroform extract, 50 mg/mL; (E)-Acetone extract, 50 mg/mL; (F)-Ethanol extract, 50 mg/mL.

as a first hand representative of nature of phytoconstituents especially about its solubility in the specific solvents, were found to be-alcohol soluble (1.25±0.4%) and water soluble (11.66±1.3%). The foaming index of *A. heterophyllus* leaves extract was found to be 125 which gives idea about the presence of higher percentage saponins in it, somewhat quantitatively, besides preliminary phytochemical screening. The crude fibres value by Dutch method was found to be 5.52±0.12% representing the resistant tissues important for providing mechanical strength and holding the leaves properly. The loss on drying of powdered drug was 9.3±0.1%, as the volatile components are not reported in the leaves; such higher moisture content can be the cause of deterioration by fungal or bacterial growth.

On phytochemical investigation of various extracts, carbohydrates, proteins were found present in ethanol extract; alkaloids, saponin glycosides and terpenoids in petroleum ether, chloroform and acetone extract; flavonoids in petroleum ether extract; tannins were reported in all extracts and steroidal compounds in petroleum ether and chloroform extracts (Table 1).

The results of antimitotic assay of different *A. heterophyllus* extracts using *Allium cepa* bioassay are summarized in Table 2. It was observed that petroleum ether extract shown the significant antimitotic action ($p<0.05$) with mitotic index 27.8±0.03 in addition to the reduction in root length and numbers, as compared to chloroform extract (44.3±0.03), acetone extract (49.02±0.01) and ethanol extract (62.2±0.03) while it was 63.4±0.02 for distilled water (control) and 1.6±0.02 for vinblastine sulphate (standard) (Figures 4 and 5).

Table 1: Phytochemical analysis.

Phytoconstituents	Tests	Petroleum ether extract	Chloroform extract	Acetone extract	Ethanol extract
Carbohydrates	Molisch's	-	-	-	+
	Fehling's	-	-	-	+
	Barfoed's	-	-	-	+
	Bial'sOrcinol	-	-	-	+
	Selwinoff's	-	-	-	+
	Tollen's phloroglucinol	-	-	-	+
Proteins	Biuret	-	-	-	+
	Million's	-	-	-	+
	Sulphur containing	-	-	-	+
Saponin glycosides	Foam	+	+	+	-
	Haemolysis	+	+	+	-
Alkaloids	Mayer's	+	+	+	-
	Wagner's	+	+	+	-
	Hager's	+	+	+	-
	Dragendroff's	+	+	+	-
Anthraquinone glycosides	Borntrager's	-	-	-	-
Steroids	Salkowski's	+	+	-	-
	Legal's	+	+	-	-
Flavonoids	Shinoda	+	-	-	-
Terpenoids	Salkowaski's	+	+	+	-
	Liebermann-Burchard's	+	+	+	-
Tannins	Ferric chloride	+	+	+	+
	Lead acetate	+	+	+	+
	Potassium dichromate	+	+	+	+
	Gelatin	+	+	+	+
Amino acids	Ninhydrin	-	-	-	-

+ represents "present" and – represents "absent".

Figure 2: Macroscopic observations of *A. heterophyllus* Leaf.

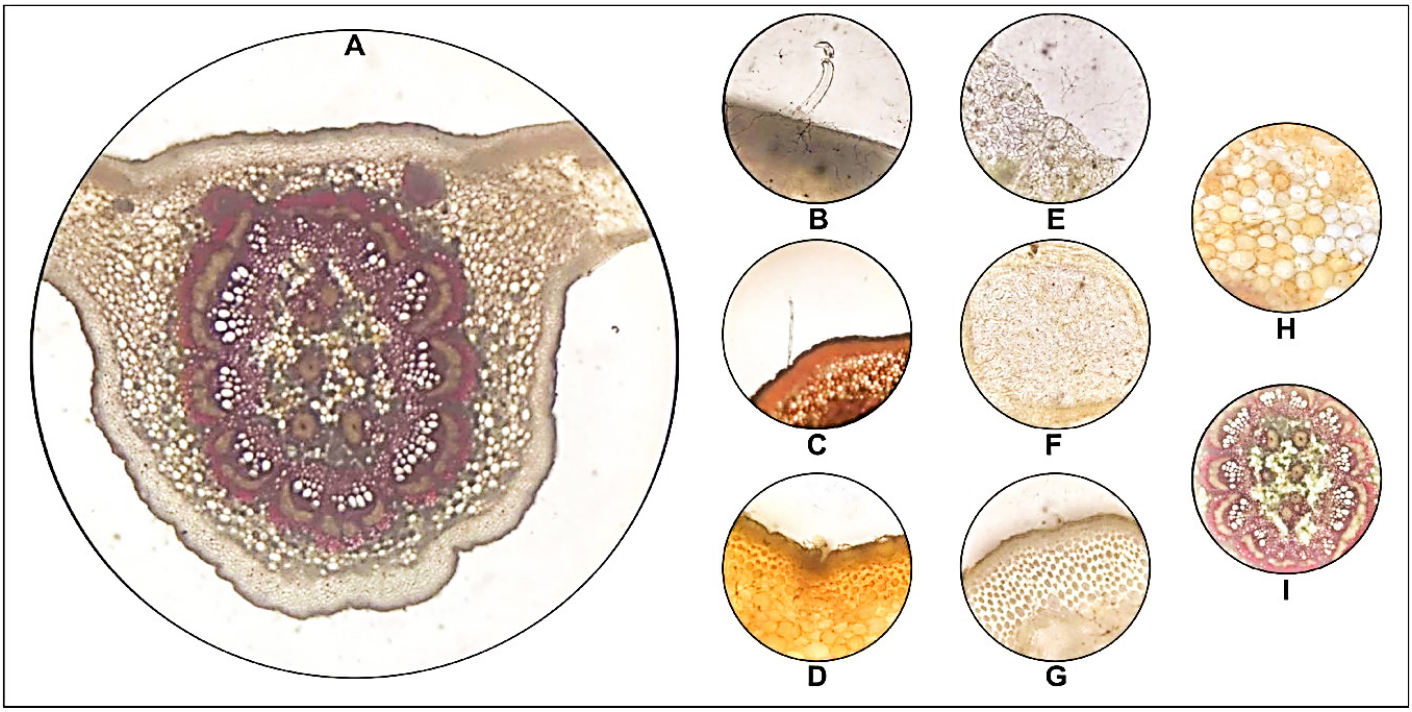

Figure 3: Microscopic observations of *A. heterophyllus* Leaf. (A)-Transverse section; (B)-Hooked covering trichome with collapsed cell; (C)-Non-hooked covering trichome; (D)-Glandular trichome; (E)-Cystolith; (F)-Anomocytic and actinocytic stomata; (G)-Collenchyma; (H)-Parenchyma; (I)Vascular bundles.

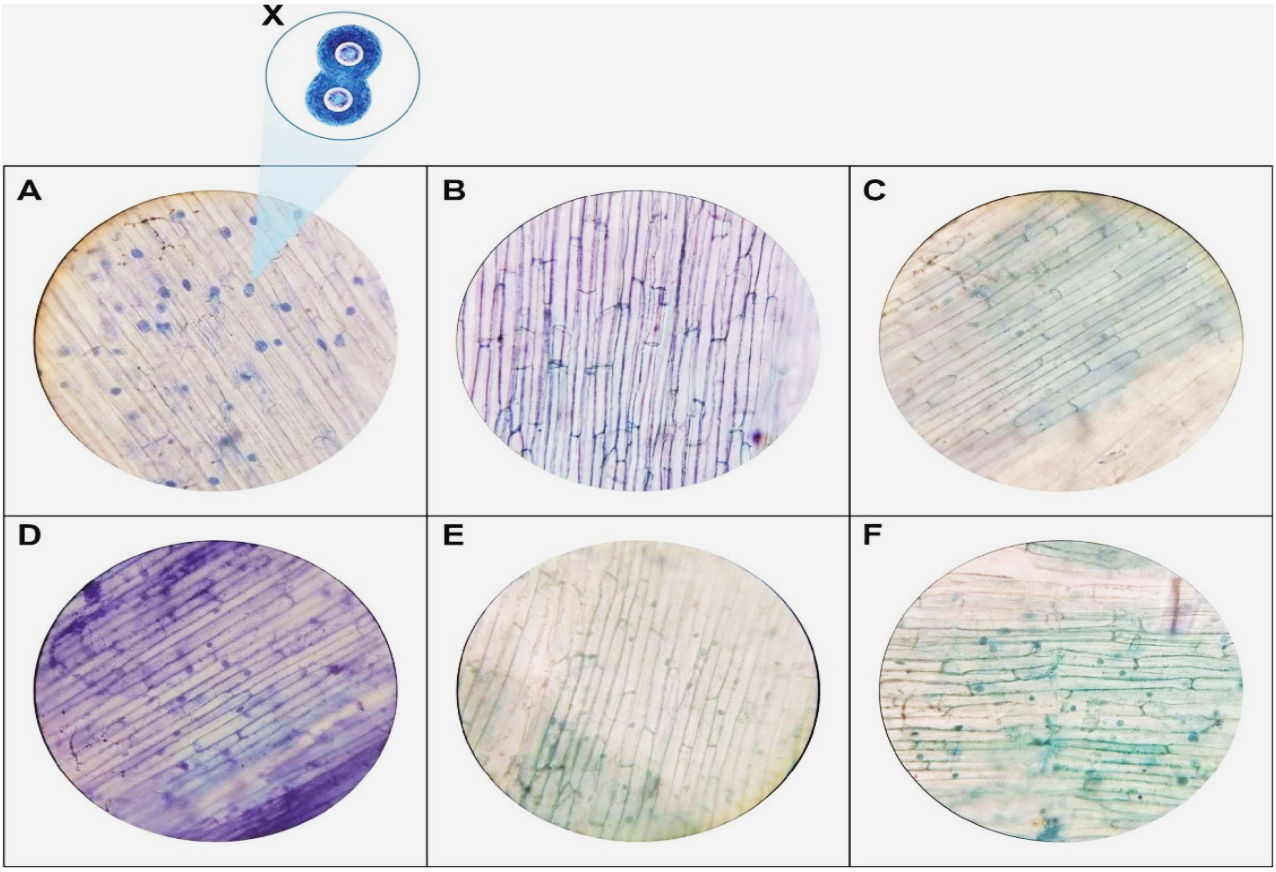

Figure 4: Microscopic observation of *A. cepa* meristematic cells treated with: (A)-Distilled water/control; (B)-Vinblastine sulphate/standard; (C)-Petroleum ether extract; (D)-Chloroform extract; (E)-Acetone extract; (F)-Ethanol extract. X-Represents dividing cells.

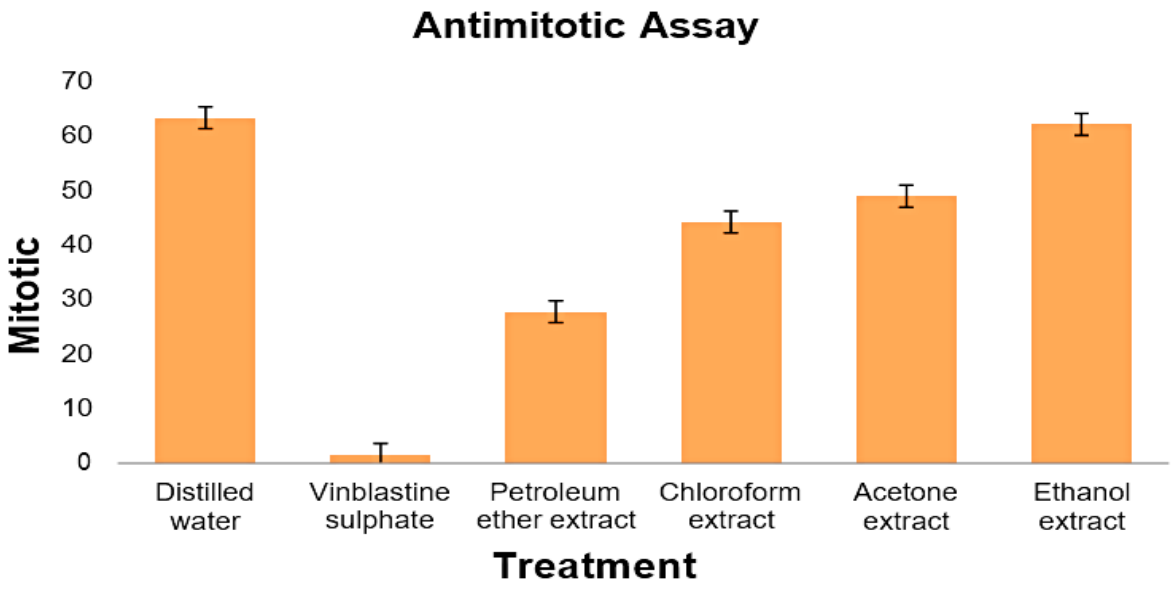

Figure 5: Graphical comparison of mitotic index shown by various extract in antimitotic assay.

Table 2: Mitotic index (MI)%values obtained in antimitotic assay.

Treatments	Mitotic index (MI) %
Distilled water	63.4±0.02
Vinblastine sulphate	1.6±0.02
Petroleum ether extract	27.8±0.03
Chloroform extract	44.3±0.03
Acetone extract	49.02±0.01
Ethanol extract	62.2±0.03

Values are expressed as Mean±SD (*n*=5).

CONCLUSION

Concluding the study, the pharmacognostic, phytochemical and antimitotic evaluation of *A. heterophyllus* Lam. leaves were carried out. In the pharmacognostic study, various macroscopic, microscopic and physical factors along phytochemical evaluations were studied. Some important findings of our study were, presence of large amount of cystoliths that might be the reason of higher ash value, higher LOD, presence of both anomocytic and actinocytic stomata which have various controversies, presence of both types i.e., hooked and non-hooked covering trichomes which were not reported in earlier studies. Some of the factor responsible for such variations might be the geographical locations, rainfall, soil contents and others which need to be evaluated comparatively. Also, the antimitotic evaluation represents the potential of petroleum ether extract to restrict the continuous division of cells. The reason for such arrest of cells division probably because of presence of saponin glycosides, alkaloids, flavonoids, steroids, terpenoids and tannins compounds in the extract as revealed by the phytochemical screening. Although the concentration of extracts as compared to standard drug was higher but as already known fact that, the extract is a mixture of large number of constituents and if we could process further especially, the fractional separation assisted isolation of particular components of said petroleum ether extract, the breakthrough molecule for treatment of cancer can surely open the door of future.

ACKNOWLEDGEMENT

The authors would like to acknowledge Central Instrumentation Facility (CIF) of Apollo College of Pharmacy, Durg, India for the provided instrumental facilities and financial support during course of work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

A. heterophyllus: *Artocarpus heterophyllus*; **E. jambolana:** *Eugenia jambolana*; **LOD:** Loss on Drying; **M. acuminata:** *Musa acuminata*; **SD:** Standard Deviation; **TS:** Transverse Section.

SUMMARY

In this study, the pharmacognostic, phytochemical and in vitro cytotoxic activity of Artocarpus heterophyllus Lam. leaves by using Allium cepa bioassay was carried out. The pharmacognostic study, beside already reported characteristics, has revealed some interesting features which mainly included presence of large amount of cystoliths, both anomocytic and actinocytic stomata, both hooked and non-hooked covering trichomes. While, in the antimitotic bioassay, the petroleum ether extract has revealed the potency to arrest the mitosis in onion root tips with mitotic index 27.8±0.03 ($p<0.05$) as compared to other extracts.

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Cite this article: Verma V, Wadibhasme P, Banarase N, Pounikar Y. *Artocarpus heterophyllus* Lam.: Pharmacognostic, Phytochemical Study and Its Evaluation for Antimitotic Potential by Modified *Allium cepa* Bioassay. Indian J of Pharmaceutical Education and Research. 2026;60(2s):s541-s549.