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SCREENING OF ETHANOLIC EXTRACT OF *ARTOCARPUS HETEROPHYLLUS* L. BARK FOR ANTHELMINTIC ACTIVITY

Afreen*[1], K. Hemamalini[1], S. Bharath Reddy[1], K. Manasa[1], K. Vidya[1], K. Madhuravani[1]

[1]*Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally, Yadagirigutta, Yadadri-Bhongir, Telangana, India.

ABSTRACT

The aim of the present study was to evaluate anthelminthic activity of crude ethanolic extract of Artocarpus heterophyllus bark by using test worm. Albendazole was included as standard. The results indicated that ethanolic extract was significantly paralysis and also caused death of worm in short time compared to that of the standard drug. Various concentrations like 5, 10, 15, 25, 50mg/ml of ethanolic extract was tested, against the paralysis and death, but 50mg/ml showed less time (03.88±0.19) for paralysis and (23.57±0.38) for death of worm. So we conclude, that the traditional use of *Artocarpus heterophyllus* has anthelminthic activity and further studies are suggested to isolate the active principles which are responsible for the activity. The observations showed the anthelminthic activity of *Artocarpus heterophyllus* by paralysis and death of earth worms.

KEYWORDS

Albendazole, *Artocarpus heterophyllus*, Helminthiasis and Worms.

Author for Correspondence:

Afreen,

Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally, Yadagirigutta,

Yadadri-Bhongir, Telangana, India.

Email: afreen796@gmail.com

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus*) is a congener of (i.e., member of the same genus as) Breadfruit (*Artocarpusaltilis*), as well as a number of other culturally and economically important trees[1-3] (e.g., *A. mariannensis*, *A. camansi*, *A. integer*, *A. lakoocha*, *A. odoratissima*, and *A. lingnanensis*)[4-6]. The Chinese consider jackfruit pulp and seeds tonic, cooling and nutritious, and to be "useful in overcoming the influence of alcohol on the system". The seed starch is given to relieve biliousness and the roasted seeds are regarded as aphrodisiac. The ash of jackfruit leaves, burned with corn and coconut shells, is used alone or mixed with coconut

oil to heal ulcers and anthelmintic[7-10]. The dried latex yields artostenone, convertible to artosterone, a compound with marked androgenic action. Mixed with vinegar, the latex promotes healing of abscesses, snakebite and glandular swellings. The root is a remedy for skin diseases and asthma. An extract of the root is taken in cases of fever and diarrhea. The bark is made into poultices. Heated leaves are placed on wounds. The wood has a sedative property its pith is said to produce abortion. Literature survey shows that analgesic, anti-inflammatory[11], haemolytic[12], antioxidant[13], Antiulcer[14], wound healing[15], Hypoglycemic[16-18], antibacterial, anti diabetic[19], antimicrobial, antifungal[20] and anti-proliferative activity.

The research work was mainly focused to evaluate helmintholytic activity of crude ethanolic extract of *Artocarpus heterophyllus* using *Pheretima Posthumaas* test worms.

MATERIAL AND METHODS

Collection and authentication of *Artocarpus heterophyllus* bark

Artocarpus heterophyllus bark were collected in Vangapally (Village), Yadadri-Bhongiri (District) and authenticated by a botanist Dr. S. Srinivasrao, Swami Degree College, Bhongir (Mandal), Yadhadri-Bhongir (District). The bark were dried in shade and made into coarse powder.

Preparation of Ethanolic extracts of Artocarpus heterophyllus bark

The bark was separated from plant and it was washed with absolute ethanol to avoid the microbial growth, the bark were dried at open air under the shade, cut in to small pieces and powdered mechanically, then 50 gm of powder *Artocarpus heterophyllus* bark was extracted with 250ml ethanol in a soxhlet apparatus for 12 hrs. The extract obtained was concentrated by recovery of ethanol. The concentrated product was used as ethanolic extract of *Artocarpus heterophyllus bark.*

Phytochemical investigation on a tropical *Artocarpus heterophyllus*

Test for carbohydrates

Molisch's test

The test is positive with soluble.

Reduction of fehling's solution

To the solution of sample, equal quantity of Fehling's solutions A and B is added. After heating, brick red precipitate is obtained.

Test for amino acids

Millon's test

To the test solution add 2ml of millions reagent, white precipitate indicates presence of amino acid.

Ninhydrin test

To the test solution add ninhydrin solution, boil, violet colour indicates presence of amino acid.

Test for proteins

Warming test

The test solution take in a test tube and heat in boiling water bath, proteins get coagulated.

Test with trichloro acetic acid

To the test solution add Trichloroacetic acid, precipitate is formed.

Biuret test

To the test solution (2ml) add Biuret reagent (2ml), violet colour indicates presence of proteins.

Hydrolysis test

Hydrolyze the test solution with hydrochloric acid or sulphuric acid. Then carry out the ninhydrin test for amino acid.

Xanthoproteic test

Orange colour is formed.

Test for alkaloids

Dragendorff's test

To 2-3 ml filtrate, add few drops of dragendorff's reagent. Orange brown precipitate is formed.

Mayer's test

2-3 ml filtrate with few drops of Mayer's reagent gives precipitate.

Hager's test

2-3ml filtrate, add few drops of Hager's reagent gives yellow precipitate.

Wagner's test

2-3 ml filtrate with few drops of Wagner's reagent gives reddish brown precipitate.

Test for Glycosides

Keller kiliani test

A reddish brown layer acquiring bluish-green colour after standing is observed.

Legal test

The extract is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline-pink or red colour is produced.

Baljet test

To the section of sample, sodium picrate solution is added. It shows yellow to orange colour.

Test for cardiac glycosides

Kedde's test

The colour reaction with 3, 5 Di nitro benzoic acid depends on the presence of alpha, beta unsaturated lactones in the aglycone.

Keller-killiani test (Test for deoxy sugars)

Acetic acid layer shows blue colour.

Raymonds test

Treat the test solution with hot ethanolic alkali, violet colour is produced.

Legals test

Treat the test solution with pyridine and alkaline sodium nitroprusside solution, blood red colour appears.

Baljet test

Treat the test solution with picric acid or sodium picrate, orange colur is formed.

Test for tannins

Ferric chloride test

Blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

Phenazone test

To the 5ml of aqueous extract add 0.5 gm of sodium acid phosphate. Then warm it and filter. To the filtrate add 2% Phenazone solution, precipitate is formed which is often colored.

Gelatin test

To the test solution add 1% gelatin solution containing 10% sodium chloride. Precipitate is formed.

Test for flavonoids

Shinoda test

To dry powder or extract, add 5 ml 95% ethanol, few drops concentrated Hcl and 0.5 gm magnesium turnings. Pink colour observed. To small quantity of extract, add lead acetate solution. Yellow colored precipitate is formed.

Test for saponins

Foam test

Take 2ml of drug solution in a test tube. To it add small amount of water, shake well, stable froth (foam) is formed.

Helmintholytic activity

The activity are performed on Indian earth worms due to its anatomical and physiological resemblance with the intestinal round worm parasite of human intestine. Indian adult earth worm (pheretima posthuma) of 5-8 cm in length and 0.23-0.3 cm in width were used. Eight groups of approximately equal sized earth worms each containing six earth worms were selected. All the earth worms were placed in a standard and extracts and time of paralysis (P) and time of death (D) were calculated. The time of paralysis was noted when no movement of any sort could be observed expect when the worms were shaken vigorously. The time of death were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50^0c).

RESULTS AND DISCUSSION

Collection and authentication of *Artocarpus heterophyllus* bark

Bark of *Artocarpus heterophyllus* was collected and authenticated. The collected bark was dried in shade and made into coarse powder. The coarse powder of *Artocarpus heterophyllus* bark was used for further process.

Preparation of ethanolic extracts of *Artocarpus heterophyllus* bark

The ethanolic extract of *Artocarpus heterophyllus* bark was prepared according to the procedure discussed in materials and methods. The concentrated product was used in phytochemical and pharmacological screening.

Phytochemical investigation on a tropical tree *Artocarpus heterophyllus* bark

The preliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the ethanolic extract of *Artocarpus heterophyllus* bark according to the procedure.

In the above chemical test the ethanolic extract of *Artocarpus heterophyllus* gives positive results for Saponins, Tannins, Amino acids, Proteins, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids. The results of preliminary test of ethanolic extract of *Artocarpus heterophyllus* bark were shown in Table No.1.

Helmintholytic activity of *Artocarpus heterophyllus* bark

1. Primary phytochemical screening of crude alcoholic extract detects some secondary plant metabolites like steroids, alkaloids, saponins and flavanoids. Helmintholytic activity of the ethanolic extracts of bark of *Artocarpus heterophyllus (B*) was shown in.
2. From the experimental work standard drug albendazole at *Pheretima posthuma* are 80.36±0.05 and 96.02±0.57 mins respectively. Albendazole involves inhibition of glucose uptake system leading decrease in energy reserves in the helminthes to cause paralysis so that they are expelled in the feaces of man and animals.
3. Ethanolic extract of bark of *Artocarpus heterophyllus* was found to pocesses interestingly good level of helmintholytic activity.
4. Ethanolic extracts of bark of *Artocarpus heterophyllus* and its different fractions exhibited helmintholytic activity in dose-dependent manner giving shortest time of paralysis (03.88±0.19 to 10.64±0.38min) and death (10.64±0.38min) with 50 mg/ml concentration as compared to that of other concentrations.
5. Ethanolic extracts *Artocarpus heterophyllus* 25mg/ml 15min for paralysis and death 30 min, (15mg/ml 18.66 min for paralysis and death 31.87), 10mg/ml 23.78 min for paralysis and death time 44.23 min, 5mg/ml 28 mins for paralysis and death time 50mins, shown in Table No.2.
6. The bark of *Artocarpus heterophyllus* (B) not only resulted paralysis but also caused death of worms especially at higher concentration (50 mg/ml) of ethanolic extracts in shorter time as compared to reference drug Albendazole.

All values represent Mean ± SEM; n - 6 in each group; P: Paralysis time, D: Death time; SEM – Standard error of mean; control worms were alive up to 24 hrs of the experiment; n= number of animals (earth worms).

1. This study has revealed that this plant *Artocarpus heterophyllus* has many secondary metabolites (Phytoconstituents) and shows dose dependent helmintholytic activity and albendazole shows less paralysis and death time as compared to others.
2. Various concentrations of (5, 10, 15, 25, and 50 mg/ml) of ethanolic and aqueous extracts were tested in the bioassay, which involved the determination of time of paralysis (P) and time of death (D) of the worms.
3. Ethanolic extract shows less time (03.88 min for paralysis and 23.64 min for death of worm with 50 mg/ml of ethanolic extract of (*Artocarpus heterophyllus*) for paralysis and death of the worms as compared to the other concentrations in the study. Further, it would be interesting to isolate the phytoconstituents responsible for helmintholytic potential.
4. The present research work was providing a basis for further detailed investigations in the direction of isolation of other active compounds.

Table No.1: Phytochemical screening results of *Artocarpus heterophyllus* bark

S.No	PHYTOCONSTUTIENT	RESULTS
1	Carbohydrates	+
2	Proteins	+
3	Amino acids	+
4	Alkaloids	+
5	Glycosides	+
6	Tannins	+
7	Saponins	+

Table No.2: Helmintholytic activity of the ethanolic and aqueous extracts of *Atrocarpus heterophyllus* bark

S.No	Test Substance	Concentration(mg/ml)	Time taken for paralysis(p) and death (d) of worms	
			P(min)	d(min)
1	Albendazole	25	80.36±0.05	96.02±0.57
2	Ethanol extract	5	28	50
		10	23.78	44
		15	18.66	31
		25	15.11±0.25	30±0.57
		50	03.94±0.28	10.46±0.57
3	Control	Water	Alive	Alive

Figure No.1: *Artocarpus heterophyllus* plant for bark collection

Figure No.2: Bark of *Artocarpus heterophyllus*

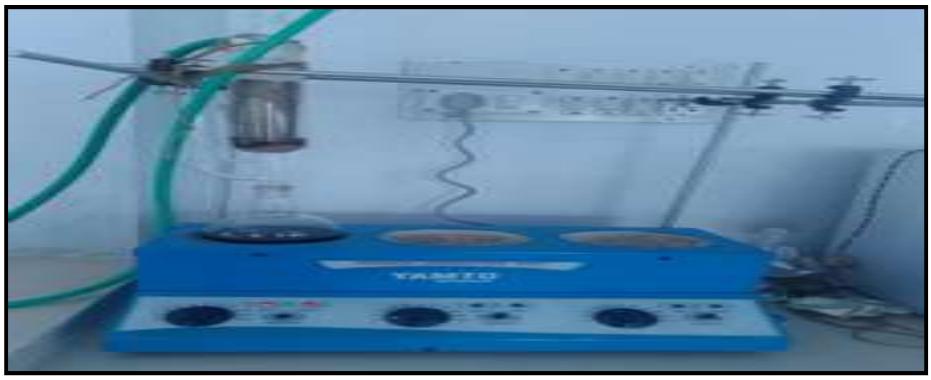

Figure No.3: Powder of *Artocarpus heterophyllus* Bark

Figure No.4: Soxhlation of *Artocarpus heterophyllus* bark

Figure No.5: Powder after soxhalation of *Artocarpus heterophyllus*

Figure No.6: Phytochemical screening of *Artocarpus heterophyllus*

SUMMARY AND CONCLUSION

Pheritima posthuma (Indian earthworm) are used as test worms which resemble helminthes in physiology. In the experimental model, the ethanolic extract of Artocarpus heterophyllus bark (5, 10, 15, 25, 50 mg) with the standard Albendazole (25 mg) is used. As per experimental model concentration of 50 mg showed best in having helmintholytic activity. The bark is having potency to be used in the treatment of helminthiasis. Further studies on usage of this extract helmintholytic without any adverse effects are needed to collected.

This study has revealed that this plant *Artocarpus heterophyllus* has many secondary metabolites and shows dose dependent helmintholytic activity and albendazole shows less paralysis and death time as compared to others. This study has revealed that this plant *Artocarpus heterophyllus* B. as many secondary metabolites and shows dose dependent helmintholytic activity. Various concentrations of (25, 50, 100 mg/ml) of ethanolic extracts were tested in the bioassay, which involved the determination of time of paralysis and time of death of the worms. Ethanolic extract of *Artocarpus heterophyllus* B. shows less paralysis time (80:36 min) and death time (96:02 min) as compared to standard marketed dose of 25 mg of Albendazole. Ethanolic extract shows less time for death (10 min) of worms with 50mg/ml of ethanolic extract of *Artocarpus heterophyllus* B. for paralysis and death of worms as compared to the other concentrations in the study. The present research work was providing a basis for further detailed investigations in the direction of isolation of other active compounds.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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