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ORIGINAL RESEARCH ARTICLE

ANTIPROTOZOAL ACTIVITY OF THE CRUDE EXTRACT OF *FLACOURTIA INERMIS* FRUIT BY MICROSCOPIC COUNT METHOD

SHIBUMON GEORGE AND BENNY P.J.*

Department of Zoology, St.Thomas College Palai , Arunapuram , Kottayam, Kerala

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ABSTRACT

New, effective antiprotozoal drugs are necessary to combat protozoal diseases. Based on this objective, a preliminary study was made by microscopic count method for assessing the antiprotozoal activity of the acetonic extract of *Flacourtia inermis* fruit against free-living protozoa and endo-commensal rectal ciliates of frog. Study shows that the fruit extract has excellent antiprotozoal activity against the tested organisms. Antiprotozoal study by microscopic count method can be considered as a preliminary test for detecting the antiprotozoal activity of the unknown sample.

Key Words: - *Flacourtia inermis*, Loika, antiprotozoal, microscopic count method.

INTRODUCTION

For centuries, man has been confronted with pathogenic microorganisms. Of them protozoa form a major group and stand the first place for developing human diseases. Protozoal attacks are common in different parts of the world population and account for the major cause of mortality in developing countries [1]. On considering protozoal disease, *amoebic dysentery* or *amoebiasis* caused by *Entamoeba histolytica* infects more than 50 million people around the world and up to 110,000 die every year [2]. Giardiasis caused by *Giardia lamblia*, Balantidiasis caused by *Balantidium coli* and Malaria caused by *Plasmodium* species are some of the other common protozoal diseases of the tropical countries. *Trichomonas vaginalis*, a protozoa causing STD- Trichomoniasis, also shows the medical significance of protozoa [3, 4].

Although a good number of antiprotozoal drugs are available, most of them are toxic or less efficient [5, 6]. Therefore, efforts should be made to invent new and more efficient drugs from natural sources. Based on this objective, crude extract of fruit of Flacourtia inermis Roxb was tested for its antiprotozoal activity. It belongs to Flacourtiaceae family and is very common in the household areas of Kerala State-India where its vernacular name is Loika or Lavalolika. Its edible fruits are not exploited economically or medicinally because its fruits are not studied for its medicinal significances. However, few previous studies show that its fruits are excellent agent for antifungal and antioxidant activities [7, 8]. In the present study, acetonic crude extract of its fruits was tested for antiprotozoal activity by a microscopic count method, using free-living and endo-commensal protozoa.

MATERIALS AND METHODS

One of the main practical difficulties of studying antiprotozoal activity of pathogenic protozoa is that it is not possible to culture them in an ordinary microbiological laboratory. More over, most of these organisms are viable only in a highly complex media. Creating such a suitable *in–vitro* condition is also a hindrance to antiprotozoal study. Because of the above said disadvantages, free-living protozoa, those are easy to culture in ordinary laboratory and rectal ciliates of frog, which are easy to isolate, were taken into

***Corresponding Author: Dr. Benny P.J**, **Email:** shibugeorgepala@gmail.com, **Contact No:** 09249138946

consideration. The free-living species selected for the present study was *Paramecium caudatum* and *Vorticella campanula*. Rectal ciliates were *Opalina ranarum*, *Nyctotherus cordiformis* and *Balantidium coli*.

Preparation of the extract:

Crude acetonic extract of the fruit of *Flacourtia inermis* was used for the antiprotozoal study. Fresh fruits were collected from rural areas of Kerala State-India and washed thoroughly, cut into small pieces, dried in hot air oven at 60 °C and were powdered in a mixer grinder. 250 gram of the dried powder was serially extracted with 500 ml each of petroleum ether, chloroform and acetone using a Soxhlet extractor. From the acetone extract stock, aliquot was taken and 5ml of distilled water was added, gently heated until the samples were completely dissolved. The final concentration of the sample prepared was 20mg/ml. This aqueous solution of acetonic extract was used for testing antiprotozoal activity.

Preparation of the culture media for free-living protozoa:

Undefined complex medium was used to culture protozoa. In this method, few leaves of submerged weeds from a pond were collected and kept in a 1-liter jar having distilled water. It was covered and allowed to rot. Within a few days large numbers of protozoa appeared. In order to grow them, hay infusion was prepared by autoclaving hay in tap water and then the supernatant was collected. A few grains of wheat were added to it and were kept undisturbed for four days, in order to get bacterial growth that serves as a source for protozoal nutrition. Then about 5 ml of the inoculum was transferred to the infusion and incubated for two days. This was used for testing the antiprotozoal activity.

Isolation of non-pathogenic intestinal protozoa of frog:

Endo-commensals were used as experimental organisms for testing the inhibitory activity of fruit extract against intestinal protozoa. For this purpose, ciliated protozoa of frog were used. The following ciliated protozoa-*Opalina ranarum*, *Nyctotherus cordiformis* and *Balantidium coli* were isolated into 0.9% NaCl solution from the rectum of a pithed frog.

Antiprotozoal test:

It was made by *microscopic count method*. 1 ml of aqueous solution of acetonic extract was added to 4ml of protozoal inoculum, to get a final concentration of 4 mg/ml. After two minutes, 0.02 ml was transferred onto a glass slide. In control experiment, only 1 ml of distilled water, instead of aqueous extract, was added to the 4ml of inoculum. Both the test and control samples were examined under a compound microscope and motile and non-motile organisms were counted. Non-motile organisms were considered as non-viable due to its susceptibility towards the extract and motile were considered as resistant to the extracts. Tests were repeated four times and the average number of motile/non-motile organisms was recorded.

RESULTS AND DISCUSSION

Table1 shows the antiprotozoal activity of the crude extracts of *Flacourtia inermis* fruit against free-living protozoa and **Table 2** shows the activity against endo-commensal protozoa of frog. Analysis of the results showed that in the test sample almost all the protozoa were non-motile while in the control sample they were alive. This indicates that the fresh water protozoa and endo-commensal rectal ciliates of frog were completely susceptible to the fruit extract even at a crude concentration of 4 mg/ml. The study revealed that the fruit extract of *Flacourtia inermis* has some antiprotozoal principle active against free living and endo-commensal protozoa.

This method can be considered as a preliminary method for identifying antiprotozoal activity of natural products or plant extracts. Due to the practical difficulties for culturing pathogenic protozoa, limited antiprotozoal research activities are going on in the world. Most of the present day antimicrobial studies are concentrated only for antibacterial and antifungal studies. In this context, this preliminary method will be helpful for identifying the antiprotozoal potency of unknown plant extracts or compounds, which can be used for further detailed studies.

Because the cellular machinery of both parasitic and non-parasitic protozoa are similar in many respects, antiprotozoal study with non-pathogenic protozoa has significances because the action of test material is more or less similar in pathogenic and non-pathogenic protozoa. Therefore, those test material having antiprotozoal activity against non-pathogenic protozoa can be considered for

confirming the activity against pathogenic protozoa.

Present study can be concluded that all the tested protozoa were completely susceptible to the extract at a concentration of 4 mg/ml. There is no previous report on its antiprotozoal activity of its fruits. This is the first report on the antiprotozoal activity of *Flacourtia inermis* fruit and the present study suggests that its fruit contains significant antiprotozoal principle.

Table 1. Antiprotozoal effect of the crude acetonic extract of *Flacourtia inermis* fruit against fresh water protozoa

Tested organism	Type of Sample	Total no. of protozoa counted	Observation of protozoa after 2 min for sensitivity/resistance	
			No. of motile/ resistant organisms	No. of non-motile/ sensitive organisms
Paramecium caudatum	Test sample	6 ± 2	0	All
	Control sample	7 ± 1	All	0
Vorticella campanula	Test sample	8 ± 1	0	All
	Control sample	7 ± 2	All	0

Table 2. Antiprotozoal effect of the crude acetonic extract of *Flacourtia inermis* fruit against endo-commensal rectal ciliates of frog

Tested organism	Type of Sample	Total no. of protozoa counted	Observation of protozoa after 2 min for sensitivity/resistance	
			No. of motile/ resistant organisms	No. of non-motile/ sensitive organisms
Opalina ranarum	Test sample	5± 1	0	All
	Control sample	4 ± 1	All	0
Nyctotherus cordiformis	Test sample	4 ± 2	0	All
	Control sample	5 ±1	All	0
Balantidium coli	Test sample	6 ± 1	0	All
	Control sample	4 ±1	All	0

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