

Anticancer Activity Of *Praecitrullus Fistulosus* Fruit Extracts Against Eac Tumor Bearing Mice

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ABSTRACT

Object: To evaluate the anticancer activity of *Praecitrullus fistulosus*

Materials and methods: The different extracts of *Praecitrullus fistulosus* were studied for anticancer activity EAC tumor bearing mice The inhibitory properties of these extracts are compared with standard 5-Fluoro Uracil for EAC tumor bearing mice. The various extracts of *Praecitrullus fistulosus* treated animals significantly inhibited the tumor volume, Packed cell volume, tumor (viable) cell count.

Results: The ethanolic extract of *Praecitrullus fistulosus* shows the better results as compared to other extracts. The Phytochemical investigation showed the presence of phenol, flavonoid, glycosides, alkaloids Saponins.

Conclusion: The existence of phenolic and flavonoid compounds in the extract may be accountable for the anticancer activity. Thus this activity can be contributed to the phytochemicals present in it. The ethanolic extract of *Praecitrullus fistulosus* can be concluded to possess highest amounts of phenolic and flavonoid from the present studies.

Keywords: *Praecitrullus fistulosus*, Anticancer

INTRODUCTION

Cancer is a multifaceted disease, extremely variable in its presentation, development and outcome. It is well established that cancer is a multifactorial disease caused by a complex mixture of genetic and environmental factors. However the knowledge of the genetic, molecular, and cellular basis of cancer can provide new targets and strategies for therapy. Many anticancer drugs are unable to reach their target site in sufficient concentrations and efficiently exert the pharmacological effect without causing irreversible unwanted injury to healthy tissues and cells¹⁻³

Plants have been used for centuries to treat diseases. In various parts of the world, several plants are consumed for their health benefits as a part of traditional folk medicine. The increase in the incidence of various types of cancer creates a need for new anticancer drugs. For example, in 2017, 1,688,780 new cancer cases and 600,920 cancer deaths are projected to occur in the United States⁴. Numerous anticancer drugs isolated from plant materials are tested on cells (including various cancer cell lines) and

experimental animals after purification and then sent to clinical trials. In recent years, there has been a dynamic increase in the number of newly discovered natural compounds. In 2006, about 50,000 such substances were known, whereas, in 2014, the number of the newly discovered molecules increased to approximately 326,000. Among these, there were approximately 170,000 compounds in the toxicity class. In addition, there are 195,000 pharmacologically active compounds for which the interactions are quantitatively known⁵. Plants that have been used in traditional medicine for centuries have found application as sources of materials that possess high biological activity⁶. There are still a number of plants that have an anticancer potential but they have not yet been fully investigated. Thus, the alternate solution for the harmful effects of synthetic drugs is the use of complementary alternative medicines as very few studies have been reported on the use of herbal medicine in treatment of cancer⁷.

Praecitrullus fistulosus is an important plant of Cucurbitaceae which contains high amount of moisture and is rich in nutritional value. Cucurbitaceae are vegetable crops, which belong to the family Cucurbitaceae. Cucurbits are an excellent fruit in nature having composition of all the essential constituents required for good human health⁸. Cucurbits are among the largest and most diverse plant families, cultivated worldwide in a variety of environmental condition. The fruit of cucurbits is used in terms of human health, i.e., purification of blood, give energy, and removal of constipation⁹. The optional metabolites are together or independently may act by restraint of tubulin polymerization and blocking glucose take-up any harm to the mucopolysaccharide film of worms will uncover the external layer confining their development which at long last may cause loss of motion and at last demise of parasite. That why this plant organic product chose for the investigation of restorative properties of *Praecitrullus fistulosus* for the anthelmintic movement. The accompanying goals are chosen for biological study.

MATERIALS AND METHODS

Collection of Plant Material:

The fresh fruits of *Praecitrullus fistulosus* were procured from the local market of Agra in month of September –October (2017).

Identification and Authentication:

The collected plant parts were identified and authenticated from the department of botany, University of Rajasthan, Rajasthan. A voucher specimen [RUBL 21098] (*Praecitrullus fistulosus*)

Extraction of *Praecitrullus fistulosus*:

Powdering: The fresh and semi –ripped fruits were sliced using a home slicer and the obtained slices were shade dried, followed by powdering manually using mortar and pestle.

Sieving: The dried powdered plant material was passed through a 20 mesh sieve to remove excessive mucilaginous hair.

Soxhlation: The dried, powder plant material were extracted with different solvents at 60°C for 24 h using a soxhlet apparatus. The collected mass was subject to drying to evaporate the excess of solvent. The collected material was termed as extract of *Praecitrullus fistulosus* fruit.

The extraction was carried out with following solvents successively.

- 1) Petroleum ether
- 2) Chloroform,
- 3) Ethyl acetate,
- 4) Acetone,
- 5) ethanol

PHYTOCHEMICAL INVESTIGATION

Chemical test were carried out on all extracts for the qualitative determination of phytochemical constituents¹⁰.

PHARMACOLOGICAL INVESTIGATION

Anticancer Activity (In Vivo)¹¹:

Tumor cell: The EAC (Erlich Ascites Carcinoma) cells were maintained in swiss albino mice, by intraperitoneal (ip) transplantation on every 9th day. The ascetic fluid was collected by syringe and the tumor cell count was performed in the Neubauer hemocytometer and 2×10^7 cells/ml was obtained by dilution with normal saline. Tumor cell suspension show more than 90% viability (checked by trypan blue dye (0.4%) exclusion assay) was used for transplantation.

Treatment schedule: Healthy swiss albino mice were weighted and divided into eight group (n=6). EAC cells (2×10^6 cells/ mouse) were injected i.p. to each mouse of each group except normal saline group. This was taken as day 0. Extract and reference drug treatment were continued for subsequent 9th days starting from day 1. On 10th day 24 hour after the last dose, mice were sacrificed from each group. After sacrificing the animals blood was collected to evaluated the hematological and biochemical parameter.

S.No.	Group	Treatment
1.	Group I	2% Tween -80 (5ml (0.9%w/v)/kg b.wt, i.p.)
2.	Group II	EAC (2×10^6 cells/mouse) + 2% Tween-80 (5ml Kg-1 b.wt, i.p.)

3.	Group III	EAC (2×10 ⁶ cells/mouse) + Petroleum ether extract of <i>Praecitrullus fistulosus</i> (400mg Kg ⁻¹ b.wt, I.p.)
4.	Group IV	EAC (2×10 ⁶ cells/mouse) + chloroform extract of <i>Praecitrullus fistulosus</i> (400mg Kg ⁻¹ b.wt, I.p.)
5.	Group V	EAC (2×10 ⁶ cells/mouse) + ethyl acetate extract of <i>Praecitrullus fistulosus</i> (400mg Kg ⁻¹ b.wt, I.p.)
6.	Group VI	EAC (2×10 ⁶ cells/mouse) + acetone extract of <i>Praecitrullus fistulosus</i> (400mg Kg ⁻¹ b.wt, I.p.)
7.	Group VII	EAC (2×10 ⁶ cells/mouse) + ethanol extract of <i>Praecitrullus fistulosus</i> (400mg Kg ⁻¹ b.wt, I.p.)
8	Group XIII	EAC (2×10 ⁶ cells/mouse) + 5-fluorouracil (20 mg Kg ⁻¹ b.wt, I.p.)

RESULT OF PHYTOCHEMICAL SCREENING OF *Praecitrullus fistulosus* FRUIT

The Preliminary phytochemical investigation revealed the presence of various phytoconstituents in various extracts of *Praecitrullus fistulosus* fruits. The results of phytochemical screening were found as given in table below.

Table 2:Result of Preliminary phytochemical screening of various extracts of <i>Praecitrullus fistulosus</i>, fruits						
Phytochemicals		Petroleum Ether	Chloroform	Ethyl Acetate	Acetone	Ethanol
Alkaloids	Mayer's Reagent test	+	+	+	+	+

Carbohydrates (Monosaccharides, Oligosaccharides and Polysaccharides)	General Test	+	+	+	+	+
	Monosaccharides	+	+	+	+	+
	Disaccharides	+	+	+	+	+
	Non Reducing Polysaccharides	-	-	-	-	-
	Gums	-	-	-	-	-
	Mucilage	+	+	+	+	+
Proteins and Amino acids	Proteins	-	-	-	-	-
	Amino Acids	-	-	-	-	-
Glycosides	General Test	+	+	+	+	+
	Cardiac Glycosides	+	+	+	+	+
	Anthraquinone Glycosides	-	-	-	-	-
	Saponins Glycosides	+	+	+	+	+
	Cyanogenetic Glycosides	-	-	-	-	-
Flavonoids	Alkaline reagent test	+	+	+	+	+
Tannins and Phenolic Compounds	Ferric Chloride Test	+	+	+	+	+
Steroids		+	+	+	+	+
Volatile Oils		-	-	-	-	-
Fats and Oils		-	-	-	-	-

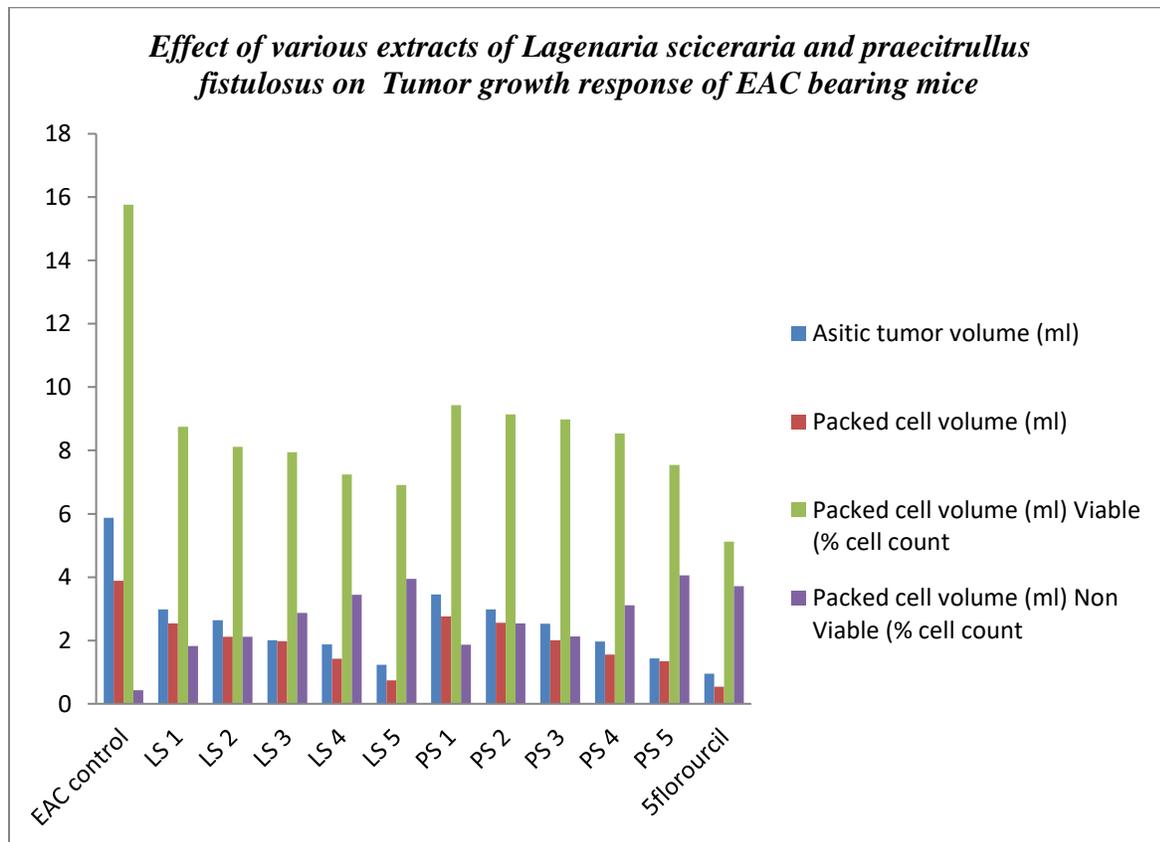
Note: + sign indicate the presence; - sign indicate the absence

Anticancer Activity (In Vivo)¹²:

Antitumor activity of extracts against EAC tumor bearing mice was assessed by the parameters such as tumor volume, packed cell volume, viable and non-viable cell. In case of tumor growth response study, Extracts treatment significantly ($p < 0.01$) reduced tumor volume, packed cell volume and viable cell count compared to those of EAC control mice while nonviable cell count was found to be increased significantly in the treated groups.

Table 2: Effect of various extracts of *Praecitrullus fistulosus* on Tumor growth response of EAC bearing mice

Group	Asitic tumor volume (ml)	Packed cell volume (ml)	Tumor cell count (×10 ⁷ ml ⁻¹)	
			Viable (% cell count)	Non Viable (% cell count)
EAC control	5.87±0.13	3.89±0.19	15.76±0.13	0.43±0.17*
PF1	3.45±0.21*	2.76±0.44*	9.43±0.43*	1.87±0.21*
PF 2	2.98±0.42*	2.56±0.43*	9.14±0.23*	2.54±0.32*
PF3	2.53±0.24*	2.01±0.36*	8.98±0.29*	2.13±0.19*
PF 4	1.97±0.31*	1.55±0.28*	8.54±0.54*	3.11±0.18*
PF 5	1.43±0.16**	1.34±0.15**	7.54±0.52**	4.06±0.53**
5florourcil(20mg/kg)	0.95±0.19**	0.54±0.17**	5.12±0.42**	3.71±0.42**



The present investigation was carried out to evaluate the antitumor activity of various extracts of praecitrullus fistulosus in EAC tumor bearing mice. The various extracts of praecitrullus fistulosus treated animals significantly inhibited the tumor volume, Packed cell volume, tumor (viable) cell count. In EAC

tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be the means to meet the nutritional requirement of tumor cells. Treatment with various extract of *Lagenaria sciceraria* and *Praecitrullus fistulosus* inhibited the tumor volume, packed cell volume and viable tumor cell count, increasing the non viable cell count.

Experimental result revealed that ethanolic extract of *praecitrullus fistulosus* posses highest anticancer activity which may be due to its cytotoxicity.

The present study thus explores the potent anticancer activity of various extracts of *Lagenaria praecitrullus fistulosus* which may be either because of a direct cytotoxic effect of the extract on tumor cells or due to its indirect local effect which may involve macrophage activation and vascular permeability inhibition. Along with this, the significant antioxidant property of the extract probably potentiates its anticancer activity further. This relevant pharmacological activity may be attributed to the presence of polyphenolics, flavonoids or the protein in the extract. Flavonoids such as quercetin, kaemferol and their glycosides have shown to possess antimutagenic and antimalignant effect. The anticancer activity is probably due to its flavonoids content.

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