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Phytochemical Profiling and Anticancer Potential of Praecitrullus fistulosus Fruit Extracts on Ehrlich Ascites Carcinoma (EAC) Cell Lines

Rajesh Sharma[1], Vignesh Annam[1], Dr. Nidhi Tyagi[*1], Dr. Nitin Kumar[1]

[1]Saraswathi College of Pharmacy, Anwarpur, Pilkhuwa, Hapur-245304 UP India.

Corresponding Author:

Dr. Nidhi Tyagi

Email Id: nidhityagi029@gmail.com

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ABSTRACT

The global incidence of cancer has prompted extensive research into finding novel, selective anticancer agents that target tumor cells while minimizing harm to normal cells. Traditional medicine, especially the use of plant-based compounds, has emerged as a promising source for discovering such therapeutic agents. This study investigates the potential anticancer activity of Praecitrullus fistulosus, a plant used in traditional medicine. The fruits of P. fistulosus were extracted using various solvents, including petroleum ether, chloroform, ethyl acetate, acetone, and ethanol, and were evaluated for their total phenolic and flavonoid contents. The ethanolic extract demonstrated the highest concentrations of both phenolic (24 mg/g) and flavonoid (21.28 mg/g) compounds. In vitro and in vivo assays were performed to assess the anticancer potential of these extracts. The in vivo study on Erlich Ascites Carcinoma (EAC) tumor-bearing mice showed a significant reduction in tumor volume, packed cell volume, and viable tumor cell count in treated groups, with the ethanol extract showing the most potent effect. The results suggest that the anticancer properties of P. fistulosus may be attributed to its high content of bioactive compounds, particularly polyphenolics and flavonoids, which likely exert both direct cytotoxic and antioxidant effects on tumor cells. This research highlights Praecitrullus fistulosus as a promising candidate for the development of novel anticancer therapies, warranting further investigation into its therapeutic potential.

Keywords: *Praecitrullus fistulosus, anticancer activity, Erlich Ascites Carcinoma, bioactive compounds, phenolic content, flavonoid content, in vitro, in vivo, tumor reduction, traditional medicine, natural products, chemotherapy, cancer therapy*.

1. INTRODUCTION

The development of cancer registries worldwide has driven the search for innovative anticancer drugs that specifically target cancer cells without causing harm to normal cells. Traditionally, anticancer drugs have been highly toxic, affecting not only tumor cells but also the healthy cells surrounding the cancer. In response, the search for new, more selective anticancer agents has expanded to include natural sources, particularly terrestrial plants and marine environments. Plants, long used in traditional medicine across different cultures, offer a promising avenue for the discovery of therapeutic compounds with high biological activity [1].

With the increasing global incidence of cancer—illustrated by the projected 1.7 million new cancer cases and 600,000 deaths in the U.S. in 2017—the need for novel treatments is pressing [2]. Recent years have seen a significant surge in the number of natural compounds discovered, with 50,000 known in 2006, expanding to 326,000 by 2014. Among these compounds, many show pharmacological activity, particularly in anticancer research. In some cases, extracting these substances from natural sources is more cost-effective than synthesizing them chemically [3].

One notable example is Praecitrullus fistulosus, a plant that has recently garnered attention for its potential anticancer properties. Known for its traditional uses in various regions, Praecitrullus has been identified as a promising candidate for further research into its bioactive compounds. The plant's ability to produce substances that might inhibit cancer cell growth is currently under investigation, contributing to the broader effort to harness plant-based compounds for therapeutic purposes [4].

Natural compounds, such as alkaloids, diterpenes, purine-based compounds, peptides, and cyclic depsipeptides, have been isolated and tested for their antitumor properties. This approach—either through direct extraction or by using biotechnology to synthesize these compounds—represents a dynamic and expanding frontier in cancer treatment, one that could yield more effective therapies with fewer side effects compared to conventional treatments [5]

been shown to induce apoptosis in cancer cell lines, including lung cancer, leukemia, and colon adenocarcinoma [7,8]. Gallic

2. MATERIALS AND METHODS

Collection of Plant Material

The fresh fruits of Paecitrullus fistulosus were procured from the local market of agra in month of September -October.

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) were deposited in herbarium of Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.

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.

Soxlation: The dried, powder plant material were extracted with different solvents at 60^0C 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

Estimation of total phenolic content in various extracts of Praecitrullus fistulosus fruit:

The total phenolic content of the extracts were estimated according to the method described by Singleton and Rossi. (Singleton and Rossi, 1965) From the stock solution (1 mg/ml) of the various extracts of plants, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of Folin-ciocalteu's reagent. After 5 min, 4 ml of 20% (w/v) sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. After 30 min, the absorbance was recorded at 765 nm. Percentage of total phenolic was calculated from calibration curve of gallic acid (50-500 µg) plotted by using same procedure and total phenolics were expressed as % equivalent to gallic acid.

Absorbance of standard (Gallic acid)

S.No	Concentration(µg/ml)	Absorbance of STD (Gallic acid)
10	0.137±0.020	
20	0.225±0.025	
30	0.317±0.029	
40	0.438±0.020	
50	0.550±0.018	
60	0.597±0.043	
70	0.713±0.021	
80	0.806±0.007	
90	0.899±0.010	
100	0.994±0.002	

Data presented in (Mean ± SD), n=3

Total phenolic content of various extracts of Praecitrullus fistulosus

S.No	Conc.	Absorbance

(µg/ml)	Petroleum Ether	Chloroform	Ethyl acetate	Acetone	Ethanol
1. 50	0.177± 0.012	0.197± 0.010	0.234± 0.010	0.288± 0.021	0.298± 0.022
2. 100	0.305± 0.010	0.323± 0.030	0.347± 0.020	0.387± 0.022	0.388± 0.030
3. 200	0.416± 0.009	0.446± 0.019	0.436± 0.020	0.498± 0.009	0.499± 0.010
4. 300	0.518± 0.027	0.555± 0.018	0.576± 0.019	0.606± 0.007	0.665± 0.022
5. 400	0.694± 0.020	0.716± 0.023	0.745± 0.020	0.778± 0.020	0.787± 0.023
6. 500 0.795± 0.020		0.824± 0.009	0.883± 0.017	0.906± 0.018	0.935± 0.010

Data presented in (Mean ± SD), n=3

5.6. Estimation of total flavonoid content in various extracts of Praecitrullus fistulosus fruits.

The total flavonoid content was determined with aluminium chloride ($AlCl_3$) according to the method of Zhishen et al, 1999 (Zhishen et al, 1999) using rutin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 0.03 ml $NaNO_2$ (5%) and incubated for 5 min at 25°C. Later 0.03 ml $AlCl_3$ (10%) was added and further after 5 min, the reaction mixture was treated with 0.2 ml (1mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The flavonoid content was calculated from a rutin standard curve.

Absorbance of standard (Rutin)		
S.No	Concentration (µg/ml)	Absorbance of STD (Rutin)
1.	10	0.197±0.019
2.	20	0.288±0.010
3.	30	0.385±0.020
4.	40	0.487±0.010
5.	50	0.566±0.020
6.	60	0.664±0.010
7.	70	0.744±0.021
8.	80	0.815±0.017

9.	90	0.899±0.011
10.	100	0.986±0.098

Data presented in (Mean ± SD), n=3

Total flavonoid content of various extracts of Praecitrullus fistulosus

S.No	Con. (µg/ml)	Absorbance				
		Petroleum ether	*Chloroform*	*Ethyl acetate*	*Acetone*	*Ethanol*
1.	50	0.220± 0.023	0.248± 0.019	0.266± 0.021	0.287± 0.020	0.319± 0.022
2.	100	0.304± 0.040	0.356± 0.019	0.343± 0.016	0.400± 0.012	0.414± 0.010
3.	200	0.471± 0.010	0.454± 0.011	0.493± 0.019	0.516± 0.017	0.533± 0.014
4.	300	0.586± 0.004	0.620± 0.005	0.561± 0.014	0.622± 0.016	0.684± 0.015
5.	400	0.675± 0.020	0.745± 0.019	0.741± 0.021	0.746± 0.020	0.790± 0.024
6.	500	0.795± 0.019	0.835± 0.010	0.855± 0.021	0.892± 0.017	0.918± 0.013

Data presented in (Mean ± SD), n=3

ANTICANCER ACTIVITY (IN VIVO): (SAHA, 2011) Tumor Cell Preparation:

Erlich Ascites Carcinoma (EAC) cells were maintained in Swiss albino mice through intraperitoneal (i.p.) transplantation every 9 days. Ascitic fluid was collected via syringe, and the tumor cell count was determined using a Neubauer hemocytometer. A concentration of 2×10^7 cells/mL was achieved by diluting the ascitic fluid with normal saline. The viability of the tumor cell suspension was assessed by the trypan blue dye exclusion assay (0.4%), which confirmed more than 90% viability. This suspension was then used for transplantation.

Treatment Schedule:

Healthy Swiss albino mice were weighed and randomly assigned to thirteen experimental groups (n=6). EAC cells (2×10^6 cells/mouse) were injected intraperitoneally into each mouse, except for the normal saline control group, on Day 0. Following this, the respective extracts or reference drugs were administered daily for 9 consecutive days, starting from Day 1. On Day 10, 24 hours after the final dose, the mice were sacrificed. Blood was then collected from each group for the evaluation of hematological and biochemical parameters.

Experimental Design:

The experimental groups were as follows

Group of Anticancer Activity (In Vivo)

S.No.	Group	Treatment
	Group I	2% Tween -80 (5ml (0.9%w/v)/kg b.wt, I.p.)
	Group II	EAC (2×10^6 cells/mouse) + 2% Tween-80 (5ml $Kg^{-}1$ b.wt, I.p.)
	GroupIII	EAC (2×10^6 cells/mouse) + Petrolium ether extract of Praecitrullus fistulosus (400mg $Kg^{-}1$ b.wt, I.p.)
	Group IV	EAC (2×10^6 cells/mouse) + chloroform extract of Praecitrullus fistulosus (400mg $Kg^{-}1$ b.wt, I.p.)
	Group V	EAC (2×10^6 cells/mouse) + ethyl acetate extract of Praecitrullus fistulosus (400mg $Kg^{-}1$ b.wt, I.p.)
	Group VI	EAC (2×10^6 cells/mouse) + acetone extract of Praecitrullus fistulosus (400mg $Kg^{-}1$ b.wt, I.p.)
	Group VII	EAC (2×10^6 cells/mouse) + ethanol extract of Praecitrullus fistulosus (400mg $Kg^{-}1$ b.wt, I.p.)
	Group VIII	EAC (2×10^6 cells/mouse) + 5-flurouracil (20 mg $Kg^{-}1$ b.wt, I.p.)

Tumor growth response: The effect of various extracts on tumor growth cell was examined by studying the various parameter such as tumor volume, packed cell volume, tumor cell count, viable and non viable tumor cell count.

Tumor volume and packed cell volume: The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube. Packed cell volumes were determined by centrifuging at 1000 rpm for 5 min.

Tumor cell count: The ascetic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the neubauer chamber and the numbers of cell in the 64 small squares were counted.

Viable and non viable cell count: The cells were then stained with trypan blue (0.4% in a normal saline dye). The cells that did not take up the dye were viable and those that took the stain are nonviable. These viable and non viable cells were counted.

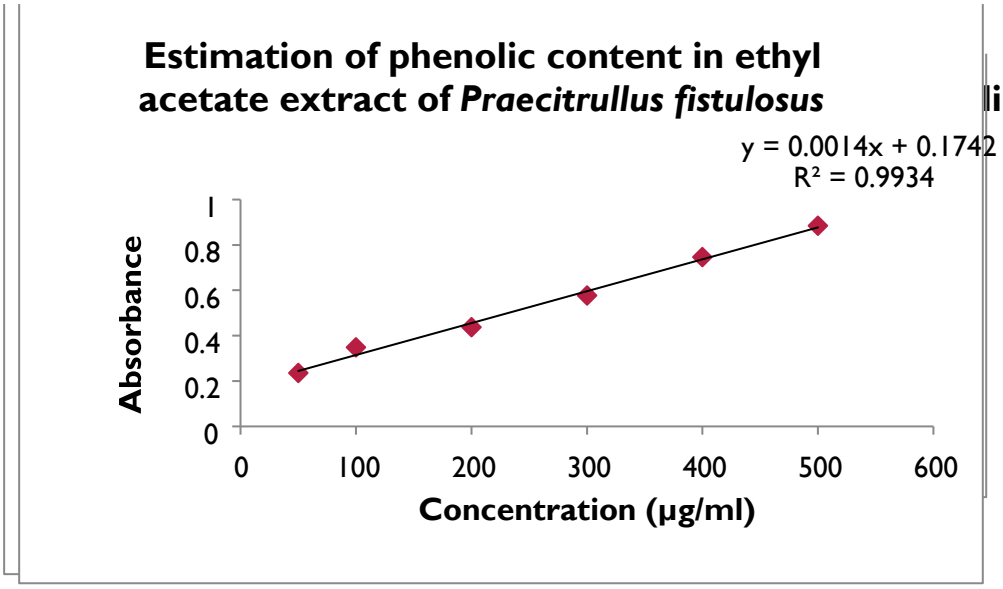
3. RESULT AND DISCUSSION

TOTAL PHENOLIC CONTENT

The various extracts of Paecitrullus fistulosus fruits contained high content of phenols. The amount of phenols varied in all the extracts. The total phenols varied from 12.25 mg/g, 14.875 mg/g, 16.5 mg/g, 23.5 mg/g to 24.1 mg/g in petroleum ether, chloroform, ethyl acetate, acetone and ethanol extracts of Praecitrullus fistulosus respectively. The maximum phenolic content was found in ethanolic extract of Praecitrullus fistulosus.

Paecitrullus fistulosus (mg/g Gallic acid equivalent)

Paecitrullus (mg/g equivalent)

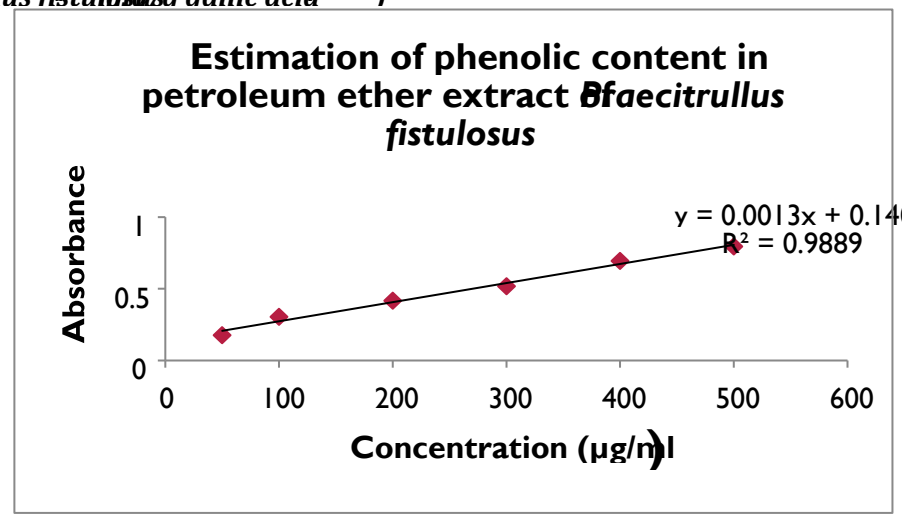


lic fistulosus Gallic acid

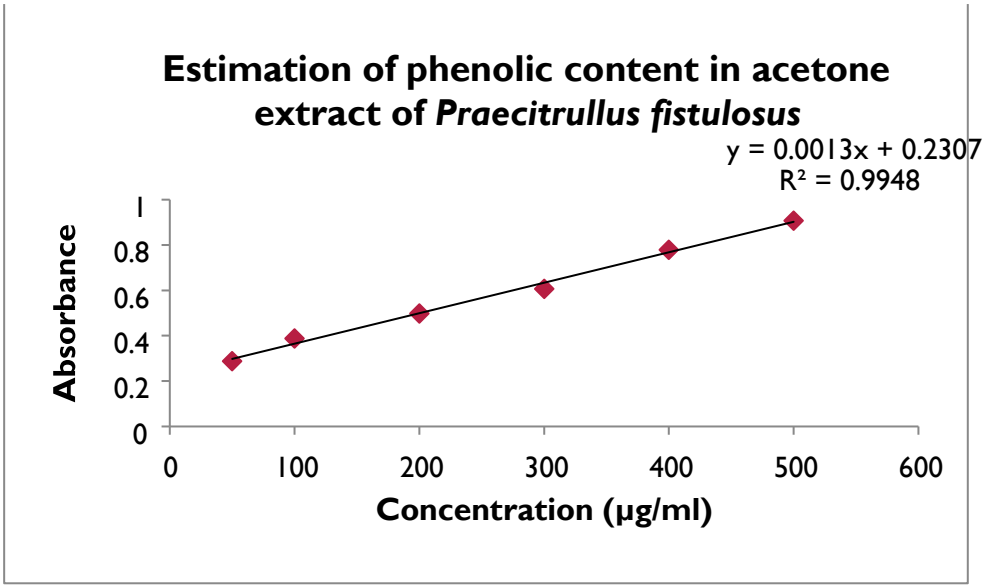
Paecitrullus (mg/g equivalent)

fistulosus Gallic acid

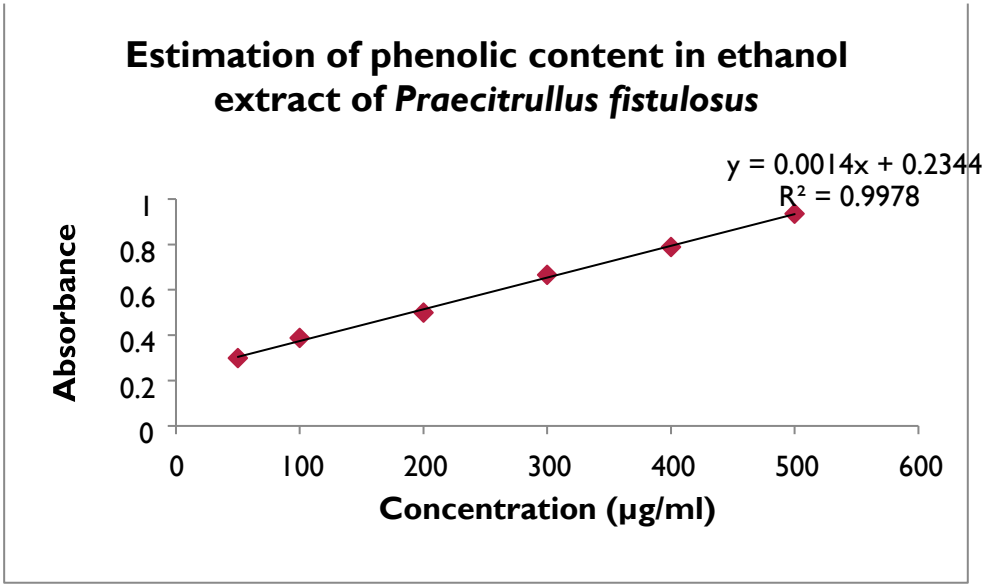
Total phenolic content in chloroform extract of Praecitrullus fistulosus and gallic acid)



Total phenolic content in petroleum ether extract of



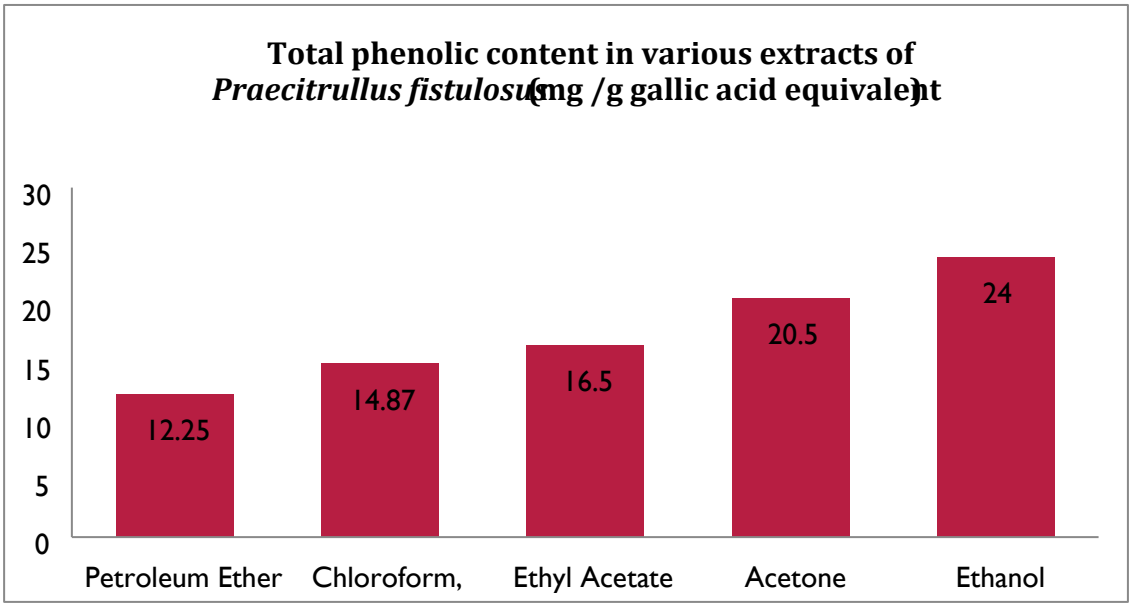
Total phenolic content in acetone extract of
Paecitrullus fistulosus (mg/g Gallic acid equivalent)



Total phenolic content in ethanolic extract of
Paecitrullus fistulosus (mg/g Gallic acid equivalent)

Total phenolic content in various extracts of		
S.No	***Praecitrullus fistulosus*** **)**	**Total phenolic content (mg /g gallic acid equivalent)**
1.	Petroleum Ether	12.25
2.	Chloroform,	14.87
3.	Ethyl Acetate	16.5

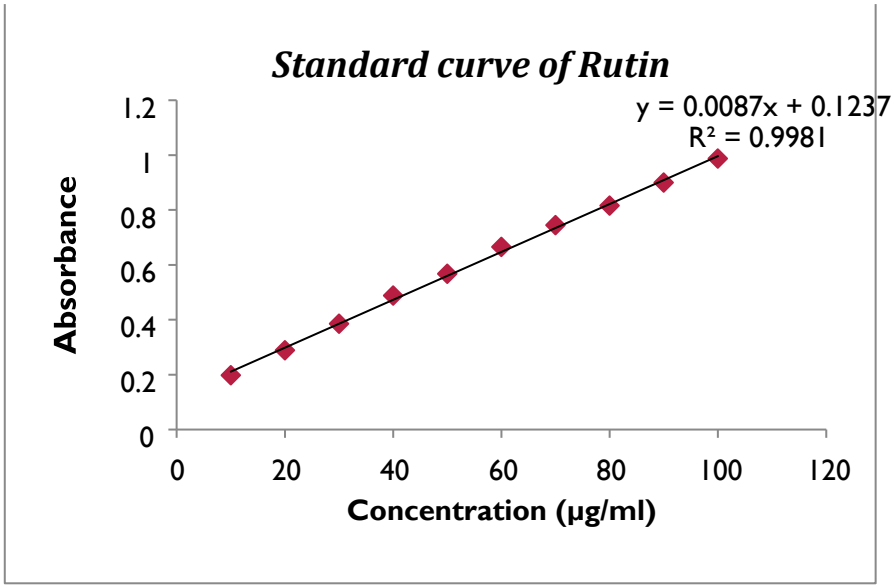
4.	Acetone	20.5
5.	Ethanol	24



Total phenolic content in various extracts of
Praecitrullus fistulosus (mg /g gallic acid equivalent)

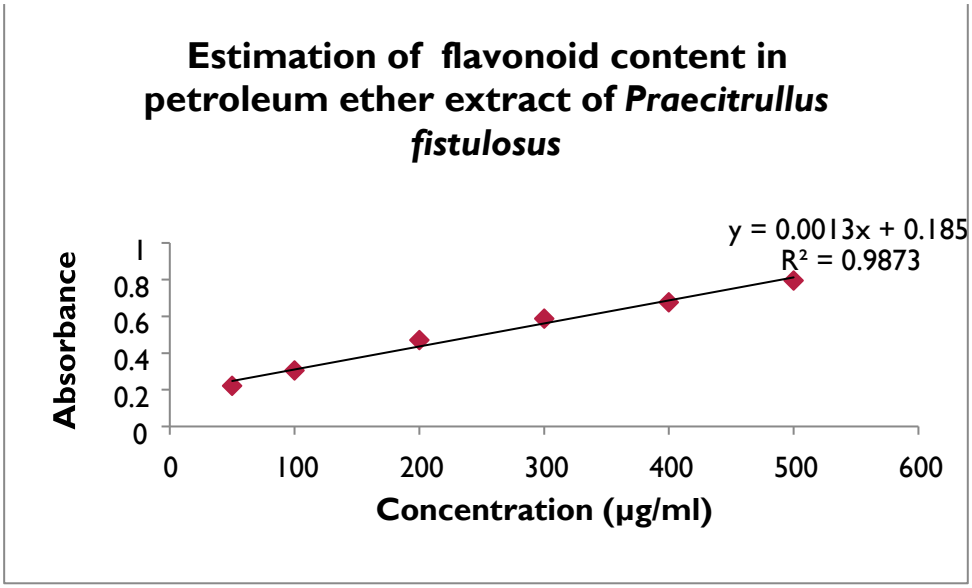
FLAVONOID CONTENT

The various extracts of Paecitrullus fistulosus fruits contained high flavonoid content. the total flavonoid varied from 8.86 mg/g, 11.86 mg/g, 12.28 mg/g, 17.85 mg/g to 21.28 mg/g in petroleum ether, chloroform, ethyl acetate, acetone and ethanol extracts of Paecitrullus fistulosus respectively. The maximum flavonoid content was found in ethanolic extract of Paecitrullus fistulosus .



Standard curve of Rutin

Praecitrullus fistulosus (mg/g Rutin equivalent)



Total flavonoid content in petroleum ether extract of
Paecitrullus fistulosus (mg/g Rutin equivalent)

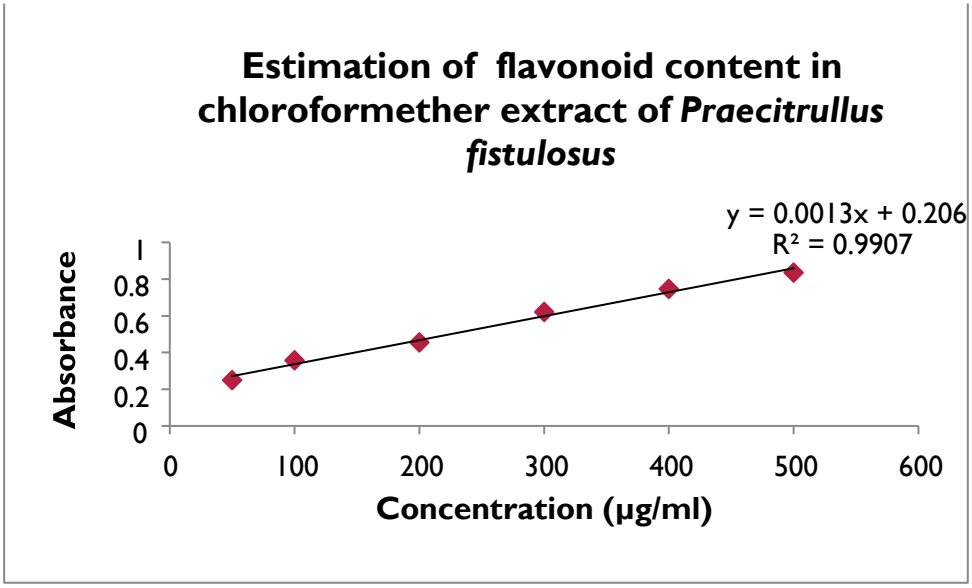


Fig 29: Total flavonoid content in chloroform extract of
Paecitrullus fistulosus (mg/g Rutin equivalent)

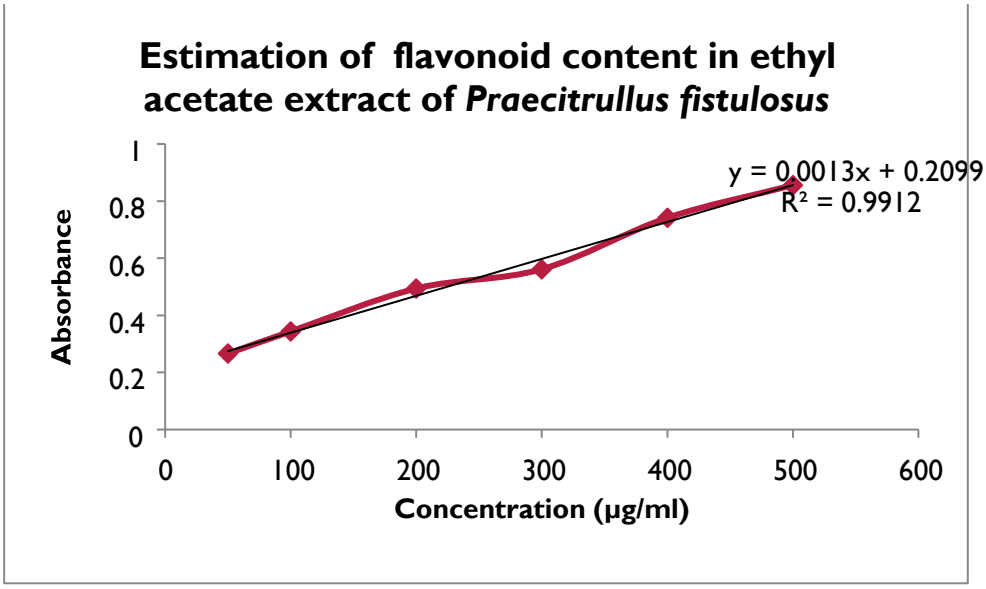
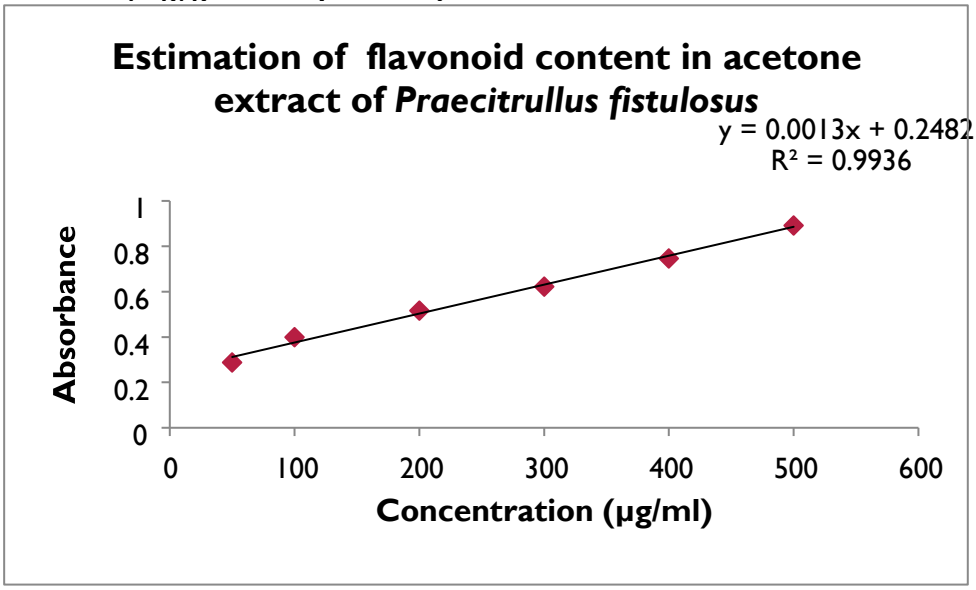
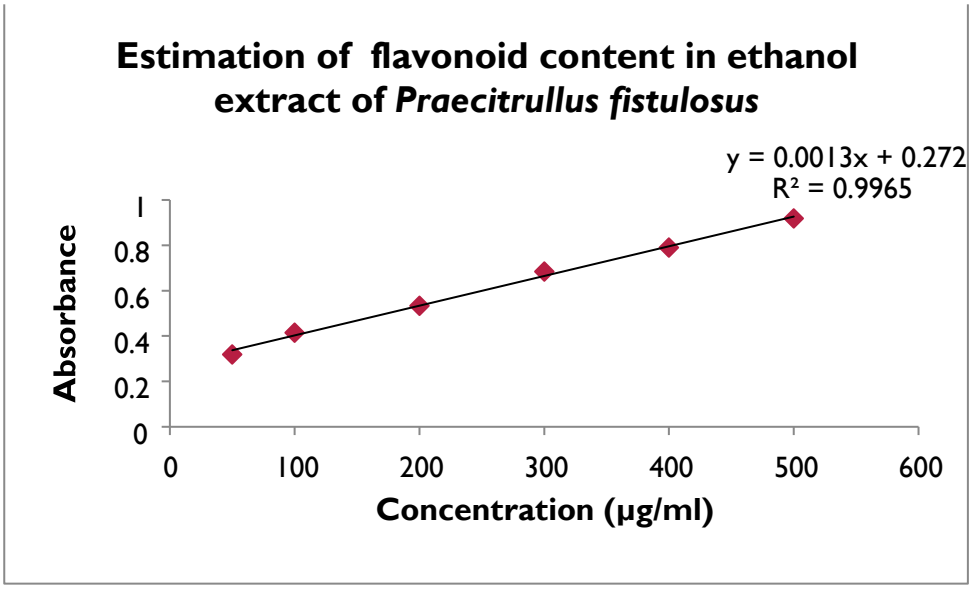


Fig 30: Total flavonoid content in ethyl acetate extract of Paecitrullus fistulosus (mg/g Rutin equivalent)



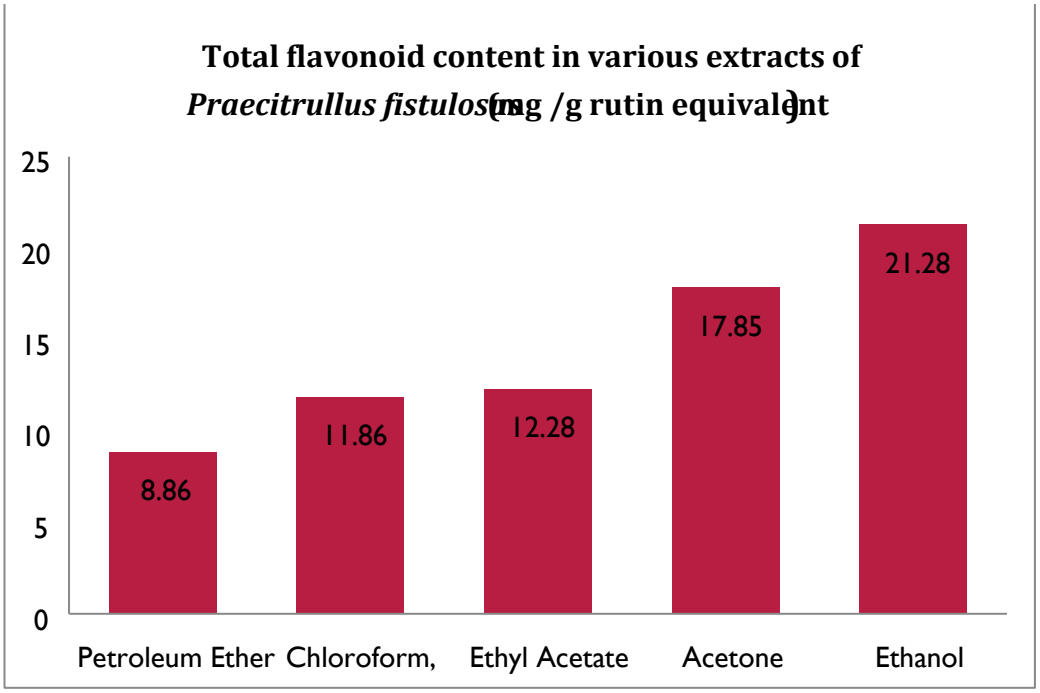
Total flavonoid content in acetone extract of
Paecitrullus fistulosus (mg/g Rutin equivalent)



Total flavonoid content in ethanolic extract of Paecitrullus fistulosus (mg/g Rutin equivalent)

Total flavonoid content in various extracts of Praecitrullus fistulosus

S.No	Praecitrullus fistulosus (Extract)	Total Flavonoid content (mg /g rutin equivalent)
1.	Petroleum Ether	8.86
2.	Chloroform,	11.86
3.	Ethyl Acetate	12.28
4.	Acetone	17.85
5.	Ethanol	21.28



Total flavonoid content in various extracts of Paecitrullus fistulosus

(mg/g Rutin equivalent)

Anticancer Activity (In Vivo):

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.

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 ($\times 10^7$ 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*
PS 1	3.45±0.21*	2.76±0.44*	9.43±0.43*	1.87±0.21*
PS 2	2.98±0.42*	2.56±0.43*	9.14±0.23*	2.54±0.32*
PS 3	2.53±0.24*	2.01±0.36*	8.98±0.29*	2.13±0.19*
PS 4	1.97±0.31*	1.55±0.28*	8.54±0.54*	3.11±0.18*
PS 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**

Values are expressed as mean ± SEM; n=6; *P<0.01, **P<0.001; one way ANOVA followed by Dunnett's multiple comparisons test.

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 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 and antioxidant property.

The present study thus explores the potent anticancer activity of various extracts of 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…

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