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Antioxidant And Anticancer Activity of Praecitrullus Fistulosus Fruit Extracts on Mcf-7 Cancer Cell Lines

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ABSTRACT

Objective: This study aims to evaluate the antioxidant and anticancer potential of Praecitrullus fistulosus fruit extracts, particularly their effectiveness against the MCF-7 human breast cancer cell line.

Methods: Fresh fruits of Praecitrullus fistulosus were collected, authenticated, and extracted using sequential Soxhlet extraction with different solvents (petroleum ether, chloroform, ethyl acetate, acetone, and ethanol). The antioxidant activity was assessed using the DPPH free radical scavenging assay, while the anticancer activity was evaluated against the MCF-7 breast cancer cell line using the Sulforhodamine B (SRB) assay.

Results: The ethanolic extract exhibited the highest DPPH scavenging activity (63.157%), demonstrating strong antioxidant potential. The cytotoxicity assessment showed a dose-dependent inhibition of MCF-7 cells, with the ethanolic extract displaying the highest anticancer activity (50.71% inhibition at 80 µg/ml). The cytotoxic effect of the extracts was attributed to their rich content of polyphenols, flavonoids, and saponins, which are known to induce apoptosis and inhibit cancer cell proliferation.

Conclusion: The results suggest that Praecitrullus fistulosus fruit extracts, particularly the ethanolic extract, possess significant antioxidant and anticancer activities. These findings highlight the plant's potential as a natural source of bioactive compounds for cancer therapy. Further studies are required to isolate and characterize the active compounds responsible for these effects.

Keywords: *Praecitrullus fistulosus, Antioxidant activity, DPPH assay, Anticancer activity, MCF-7 cell line, SRB assay, Polyphenols, Flavonoids, Herbal medicine.*

1. INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality worldwide. In 2018, it accounted for approximately 18.1 million new cases and 9.6 million deaths. Among 36 distinct types of cancer, men are predominantly affected by colorectal, liver, lung, prostate, and stomach cancers, whereas women most commonly suffer from breast, cervical, colorectal, lung, and thyroid cancers [1]. Despite significant advancements in cancer treatment, the disease remains a major global health challenge.

Current cancer treatment modalities, including chemotherapy, radiation therapy, and surgery, are widely used but present substantial limitations. These conventional therapies often lead to severe side effects, systemic toxicity, and the development of treatment resistance, ultimately reducing their effectiveness [2,3]. Therefore, there is an urgent need for alternative and more effective treatment strategies that minimize side effects and improve patient outcomes.

Herbal medicine has gained increasing attention as a promising alternative in cancer therapy, offering natural, bioactive compounds with potential anticancer properties. Medicinal plants are known to exert therapeutic effects through various mechanisms, including antioxidant, anti-inflammatory, antiproliferative, and apoptosis-inducing activities [4]. Among the many plants studied for their anticancer potential, Praecitrullus fistulosus (commonly known as Tinda or Indian round gourd) has emerged as a notable candidate due to its rich phytochemical composition and medicinal value [5,6].

Recent studies have demonstrated that active metabolites derived from Praecitrullus fistulosus possess significant anticancer activity. In particular, gallic acid, a polyhydroxy phenolic compound found in the fruit extract of Praecitrullus fistulosus, has been shown to induce apoptosis in cancer cell lines, including lung cancer, leukemia, and colon adenocarcinoma [7,8]. Gallic acid is a potent antioxidant with known anticancer, anti-inflammatory, and antimicrobial properties. It plays a critical role in

preventing malignant transformation, inhibiting tumor growth, and enhancing the efficacy of conventional cancer therapies [9,10].

Given the growing interest in plant-derived anticancer agents, the present study aims to investigate the extraction and potential therapeutic applications of *Praecitrullus fistulosus* fruit extract, with a particular focus on its gallic acid content

2. MATERIALS AND METHODS

Collection of Plant Material

Fresh fruits of *Praecitrullus fistulosus* were procured from the local market in Agra during the months of September and October. The fruits were selected based on their ripeness and quality to ensure optimal phytochemical content.

Identification and Authentication

The collected plant specimens were identified and authenticated by experts at the Department of Botany, University of Rajasthan, Rajasthan. A voucher specimen (*Praecitrullus fistulosus*, RUBL 21098) was deposited in the herbarium of the Department of Botany, University of Rajasthan, Jaipur, India, for future reference.

Extraction of *Praecitrullus fistulosus* Preparation of Plant Material

Fresh and semi-ripe fruits were washed thoroughly with distilled water to remove any surface impurities.

The fruits were sliced using a home slicer and shade-dried for 7–10 days to preserve their bioactive compounds.

The dried slices were then manually ground using a mortar and pestle to obtain a fine powder.

Sieving

• The powdered plant material was passed through a 20-mesh sieve to remove excessive mucilaginous hair and obtain a uniform particle size.

Soxhlet Extraction

The dried powdered plant material was subjected to Soxhlet extraction using different solvents in increasing polarity to ensure comprehensive extraction of phytochemicals.

The extraction was performed at 60°C for 24 hours in a Soxhlet apparatus.

After extraction, the solvent was evaporated using a rotary evaporator under reduced pressure to obtain a concentrated extract.

The final extract was stored at 4°C until further analysis.

The extraction was carried out sequentially using the following solvents:

Petroleum ether: Used for the extraction of non-polar compounds such as lipids and essential oils.

Chloroform: Extracts slightly more polar compounds, including some alkaloids and flavonoids.

Ethyl acetate: Effective in extracting medium-polarity compounds such as flavonoids and certain phenolics.

Acetone: Facilitates the extraction of a wide range of phenolic compounds and some glycosides.

Ethanol: A polar solvent used to extract bioactive compounds such as polyphenols, tannins, and gallic acid.

3. ASSESSMENT OF IN VITRO ANTIOXIDANT ACTIVITY

DPPH Free Radical Scavenging Activity (Gautam and Shivhare, 2011)

Principle

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is widely used to assess antioxidant activity. This stable free radical exhibits a strong absorption maximum at 517 nm and appears purple in color. Upon interaction with an antioxidant, the odd electron in the DPPH radical pairs with hydrogen, leading to the formation of reduced DPPH-H. This reaction results in a color change from purple to yellow, accompanied by a decrease in absorbance at 517 nm. The degree of decolorization is directly proportional to the antioxidant capacity of the sample being tested.

Preparation of Reagents

DPPH Solution

A **100 mM DPPH solution** was prepared by dissolving **33 mg of DPPH** in **100 ml of methanol.** From this stock solution, **10 ml** was taken and diluted to **100 ml** using methanol to obtain a **100 µM DPPH solution**. The solution was stored in a test tube wrapped with aluminum foil to protect it from light.

Ascorbic Acid Standard Solution

A stock solution of 100 µg/ml was prepared by dissolving **10 mg of ascorbic acid in 100 ml of distilled water**. From this stock, different concentrations **(10, 20, 40, 60, 80, and 100 µg/ml)** were prepared for use as a standard antioxidant.

Preparation of Test Solutions

A stock solution of 1 mg/ml was prepared by dissolving **10 mg of extract** in **10 ml of methanol.** From this, serial dilutions were prepared to obtain test solutions of the following concentrations: **50, 100, 200, 400, 800, and 1000 µg/ml. Procedure**

1. Sample Preparation:

o **100 µl** of each extract solution at varying concentrations **(50–1000 µg/ml)** was mixed with **100 µl** of DPPH solution **(100 µM in methanol)** in a test tube.

2. Incubation:

o The reaction mixture was incubated at **37°C for 30 minutes** in the dark.

3. Measurement:

The absorbance of the reaction mixture was measured at **517 nm** using a spectrophotometer.

A control sample containing **an equal volume of methanol and DPPH** was used as a reference.

4. Replication and Calculation:

The experiment was performed in **triplicate** to ensure accuracy.

The percentage radical scavenging activity was calculated using the following formula:

$$\% \text{ of Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

In Vitro Anticancer Activity

Assessment of Cytotoxicity Against Human Breast Cancer Cell Line (MCF-7)

Experimental Procedure: Sulforhodamine B (SRB) Assay (Vanicha and Kanyawim, 2006)

The cytotoxic activity of *Praecitrullus fistulosus* fruit extract was evaluated using the Sulforhodamine B (SRB) assay against the human breast cancer cell line **MCF-7**. The SRB assay is a colorimetric method used to measure cell density based on protein content, making it a reliable tool for assessing cell proliferation and cytotoxicity.

Cell Culture and Experimental Setup

• Cell Line Maintenance:The MCF-7 human breast cancer cells were cultured in RPMI-1640 medium supplemented with:

o 10% fetal bovine serum (FBS) o 2 mM L-glutamine

• **Incubation Conditions:**The cells were maintained under controlled conditions:

Temperature: 37°C o **CO_2 Concentration:** 5%

Air Composition: 95% air, 100% relative humidity

• Cell Seeding:

The cells were seeded into **96-well microtiter plates** at an appropriate density.

The plates were incubated for **24 hours** to allow cell attachment before the addition of test compounds.

Preparation of Experimental Drug Solutions

• Stock Preparation:

The extract was initially dissolved in **dimethyl sulfoxide (DMSO)** at a concentration of **100 mg/ml.**

This was diluted to **1 mg/ml** using water and stored at **-20°C** until use.

• Working Solution Dilutions:

At the time of treatment, the frozen stock was thawed and further diluted to **100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml** in complete medium.

From these solutions, **10 µl** was added to wells containing **90 µl** of medium, achieving final concentrations of:

▪ 10 µg/ml

▪ 20 µg/ml ▪ 40 µg/ml

- 80 µg/ml

SRB Assay Procedure

1. Drug Treatment:

The prepared drug solutions were added to the **MCF-7-**containing wells.

Plates were incubated under standard conditions **(37°C, 5% CO_2)** for **48 hours.**

2. Cell Fixation with Trichloroacetic Acid (TCA):

The assay was terminated by adding **50 µl of cold 30% (w/v) TCA** to each well (final concentration: **10% TCA).**

Plates were incubated at **4°C for 60 minutes** to allow cell fixation.

The supernatant was discarded, and plates were **washed five times with tap water** and air-dried.

3. Staining with Sulforhodamine B (SRB):

o 50 µl of 0.4% (w/v) SRB solution in 1% acetic acid was added to each well. o Plates were incubated at room temperature for 20 minutes. o Excess dye was removed by washing five times with 1% acetic acid.

o Plates were air-dried.

4. Elution and Absorbance Measurement:

The bound stain was dissolved in **10 mM Trizma base.**

The absorbance was measured using a microplate reader at **540 nm,** with a reference wavelength of **690 nm.**

Calculation of Cytotoxicity

Percentage growth was calculated for each test well relative to the control wells using the formula: [Ti/C] x 100 %

4. RESULTS AND DISCUSSION

% yield of various extracts of *Praecitrullus fistulosus* fruits

% yield of various extracts of *Praecitrullus fistulosus* fruits

***Praecitrullus fistulosus* (Extracts)**	**Yield of Extracts (g)**	**% yield**
Petroleum Ether	1.6	2.7%
Chloroform,	3.2	5.3%
Ethyl Acetate	4.7	7.8%
Acetone	6.1	10.1%
Ethanol	7.2	12%

Result of Antioxidant activity-

The DPPH assay provides a reliable and straightforward method to assess the free radical scavenging ability of *Praecitrullus fistulosus* extracts. A higher percentage of DPPH scavenging activity indicates a stronger antioxidant potential, suggesting the plant extract's efficacy in neutralizing free radicals. The results of this study may contribute to understanding the therapeutic potential of *Praecitrullus fistulosus* in oxidative stress-related diseases, including cancer. Free radicals scavenging activity of DPPH has been widely used to evaluate the antioxidant activity of natural products obtained from plant and microbial sources. In DPPH scavenging activity model it was observed that extracts significantly scavenged DPPH in a concentration dependent manner. However extracts showed weak scavenging activity in lower concentrations; the higher concentrations exhibited promising DPPH scavenging activity ranging from 46.650%, 50.239%, 54.066%, 58.373% to 63.157% in petroleum ether, chloroform, ethyl acetate, acetone and ethanol extracts of *Paecitrullus fistulosus* respectively.

DPPH is a relatively stable free radical and the assay determines the ability of extracts to reduce DPPH to the corresponding hydrazine by converting the unpaired electrons to form pairs. This conversion is the action of the antioxidant.

Absorbance of standard(ascorbic acid)

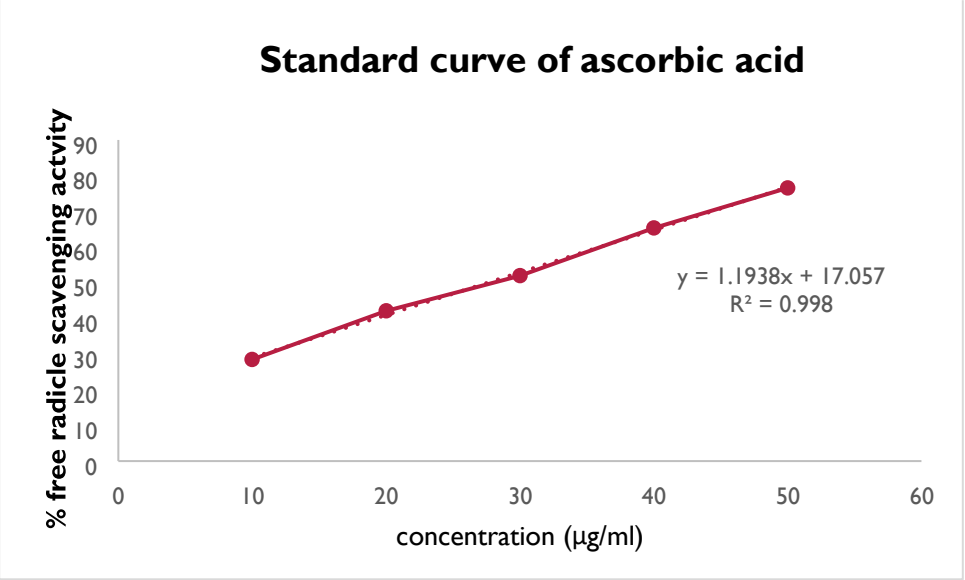
S.No	Concentration (µg/ml)	Absorbance
1.	10	28.468 ±0.006
2.	20	42.105 ±0.014
3.	30	51.913 ±0.005
4.	40	65.311 ±0.008
5.	50	76.555 ±0.007

Data presented in (Mean ± SD), n=3

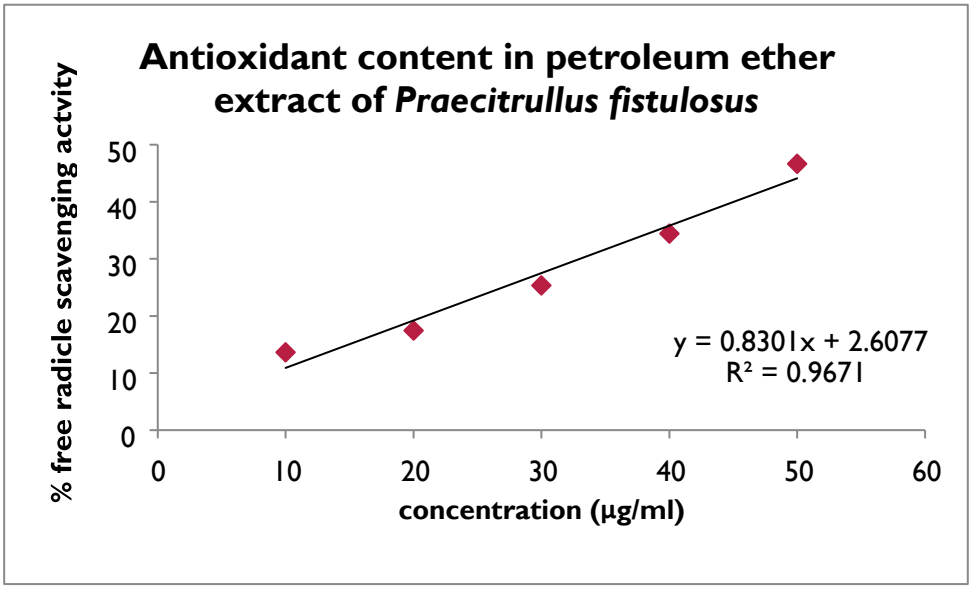
Total Antioxidant content of various extracts of Praecitrullus fistulosus

S.No	Con. (µg/ml)	Absorbance				
		Petroleum ether	*Chloroform*	*Ethyl acetate*	*Acetone*	*Ethanol*
1	10	13.636 ± 0.187	14.354 ± 0.114	16.507 ± 0.112	19.377 ± 0.119	21.770 ± 0.201
2	20	17.464 ± 0.143	19.856 ± 0.143	25.358 ± 0.109	33.253 ± 0.126	34.449 ± 0.153
3	30	25.358 ± 0.195	35.167 ± 0.197	30.861 ± 0.143	38.516 ± 0.101	40.909 ± 0.134
4	40	34.449 ± 0.397	39.473 ± 0.125	41.387 ± 0.133	49.521 ± 0.121	53.827 ± 0.132
5	50	46.650 ± 0.164	50.239 ± 0.101	54.066 ± 0.153	58.373 ± 0.119	63.157 ± 0.143

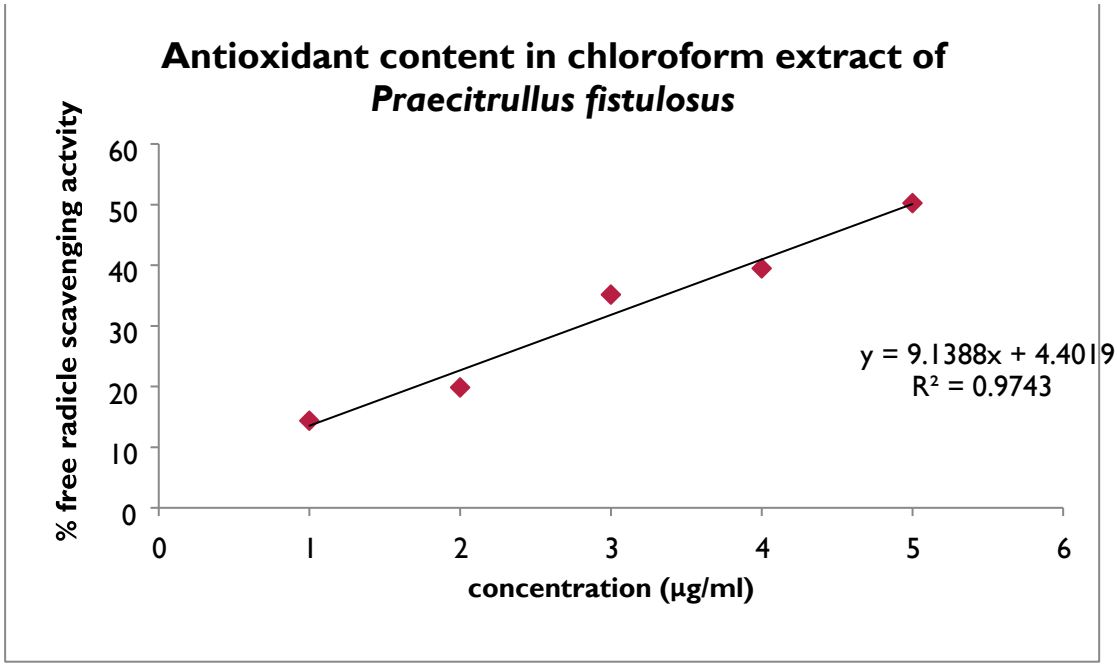
Data presented in (Mean ± SD), n=3



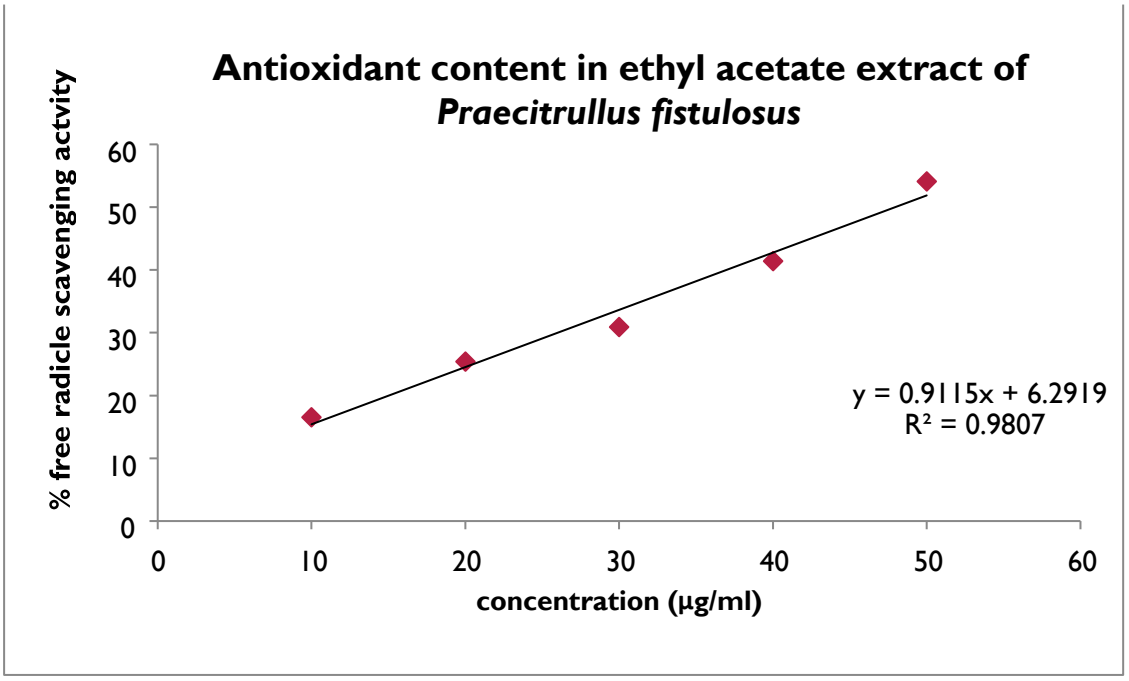
Standard curve of ascorbic acid



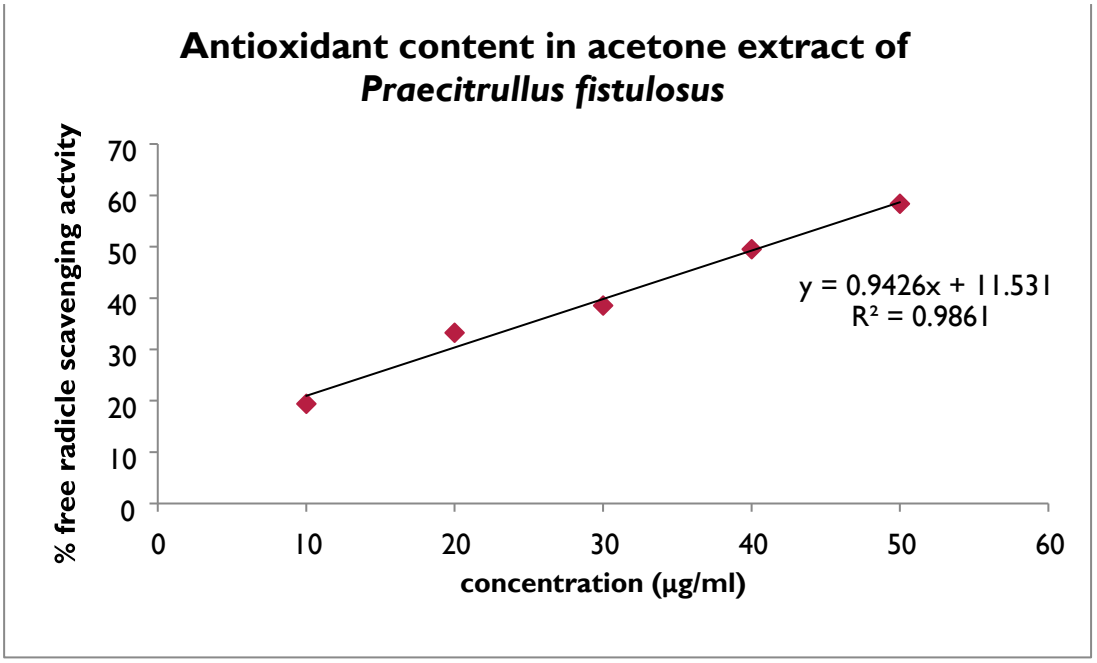
Total antioxidant content of petroleum ether extract of Praecitrullus fistulosus



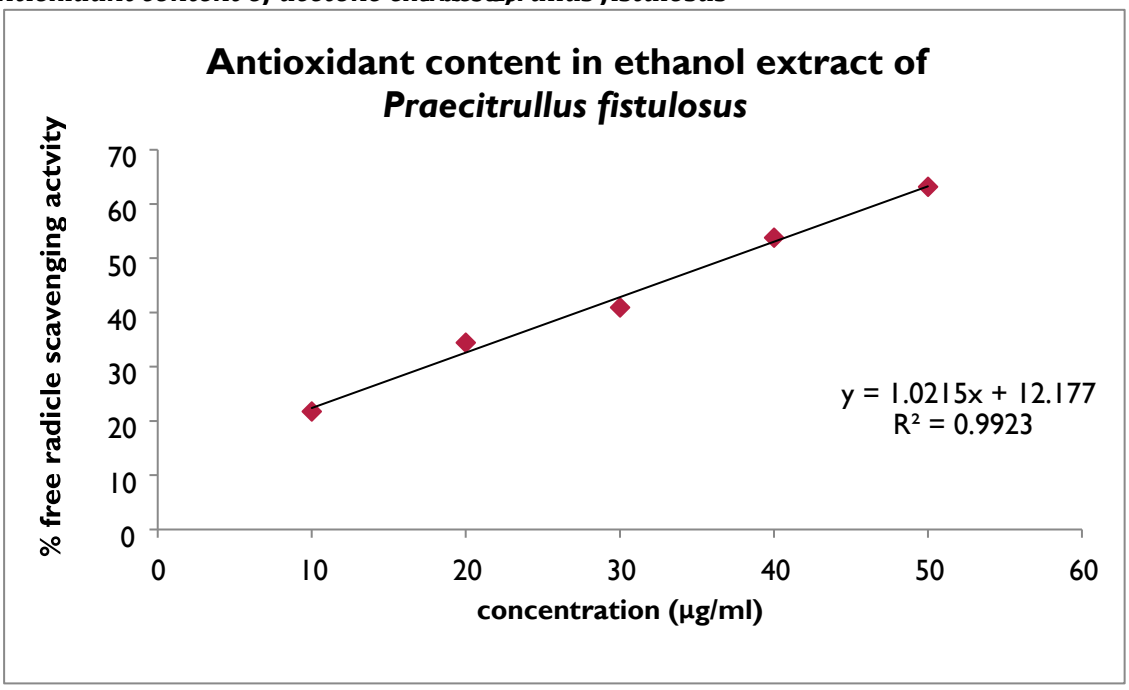
Total antioxidant content of chloroform extract of Praecitrullus fistulosus



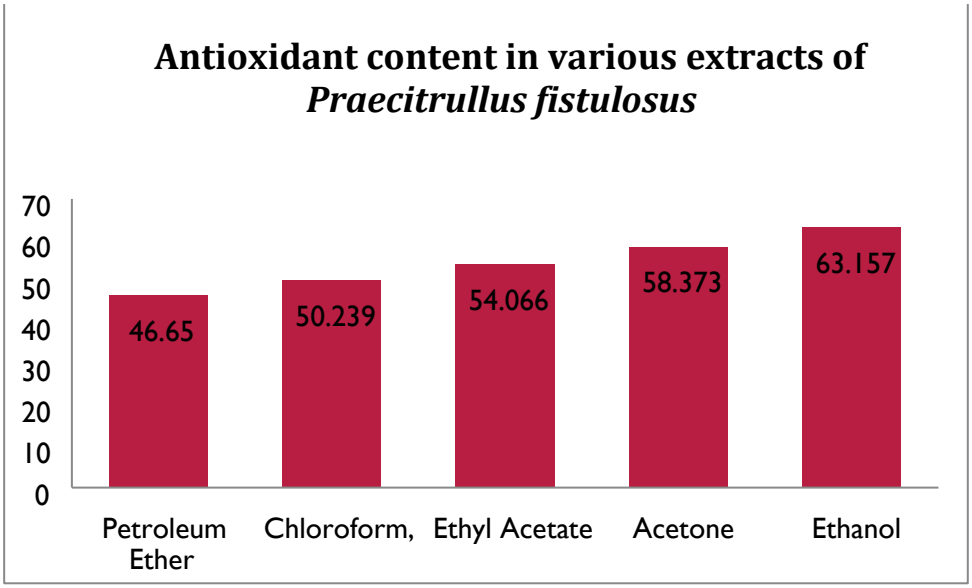
Total antioxidant content of ethyl acetate extract of Praecitrullus fistulosus



Total antioxidant content of acetone extract of Praecitrullus fistulosus



Total antioxidant content of ethanol extract of Praecitrullus fistulosus



Antioxidant content in various extracts of Praecitrullus fistulosus

Anti cancer activity:

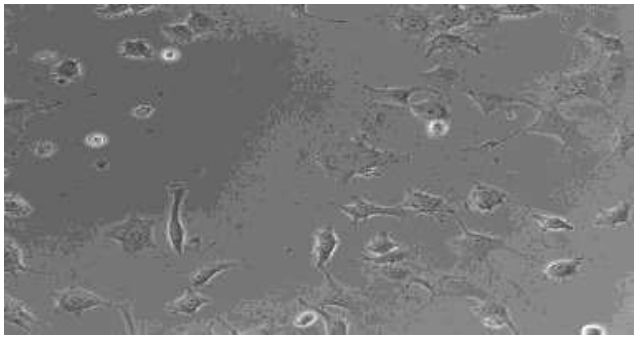
Screening of various extracts of *Paecitrullus fistulosus* resulted in moderate anticancer activities against MCF-7. The inhibitory properties of these extracts are compared with standard 5-Fluoro Uracil for MCF-7 cell line. The percentage cancer cell inhibition profiles were found to be concentration dependent. The maximum concentration (µg/ml) used in the study was 80 µg/ml. Following results were obtained when anticancer activities of plant extracts were studied.

Effect of various extracts of praecitrullus fistulosus on **Human Breast Cancer Cell Line MCF-7 (SRB Assay)**

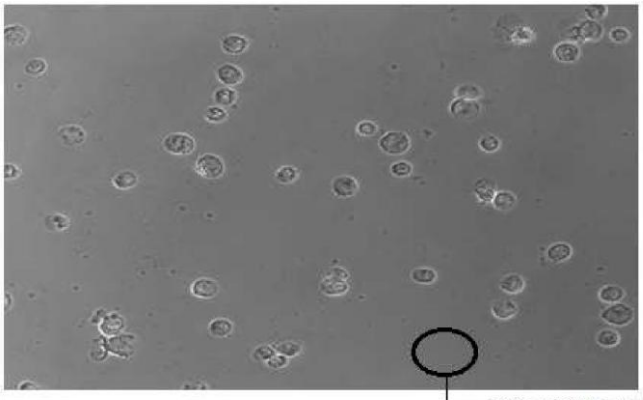
% Inhibition of cell

Group	Conc 10**(µg/ml)**	Conc 20**(µg/ml)**	Conc 40**(µg/ml)**	Conc 80**(µg/ml)**
PS 1	8.56±0.21*	15.06±0.44*	20.98±0.43*	24.67±0.21*
PS 2	12.45±0.42*	19.09±0.43*	26.68±0.23*	29.90±0.32*
PS 3	17.78±0.24*	24.13±0.36*	30.89±0.29*	35.56±0.19*
PS 4	20.03±0.31*	28.34±0.28*	35.46±0.54*	42.56±0.18*
PS 5	24.43±0.16**	33.76±0.15**	44.67±0.52**	50.71±0.53**
5florourcil(20mg/kg)	28.46±0.19**	38.27±0.17**	65.31±0.42**	76.55±0.42**

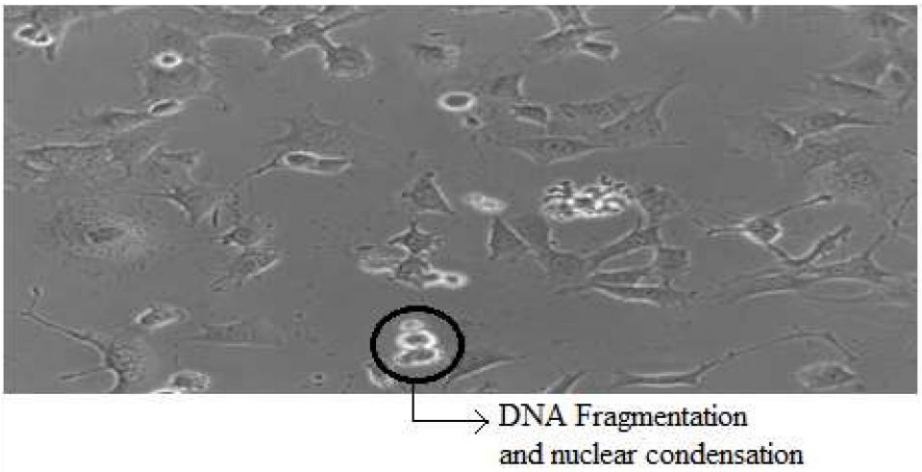
Values are expressed as mean ± SEM; n=6; *P<0.01, **P<0.001; one way ANOVA followed by Dunnett's multiple comparisons test.



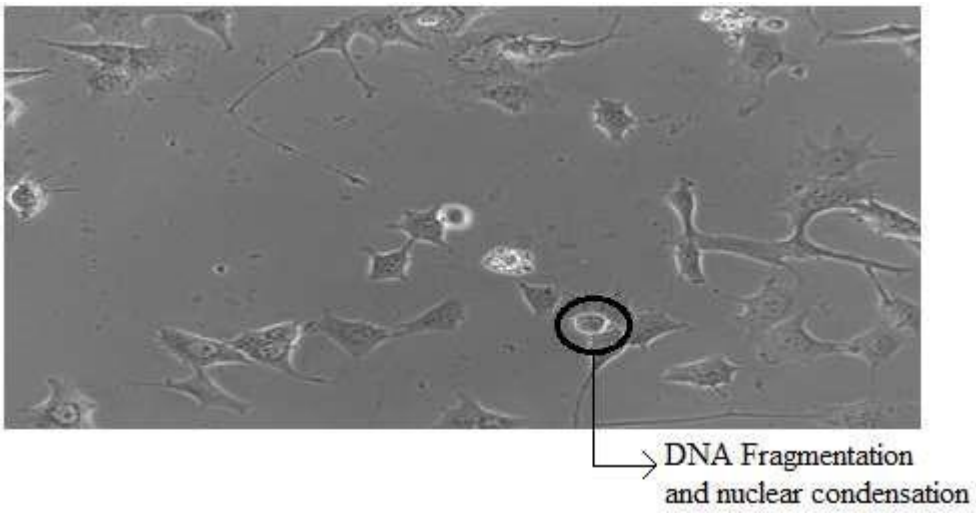
MCF7



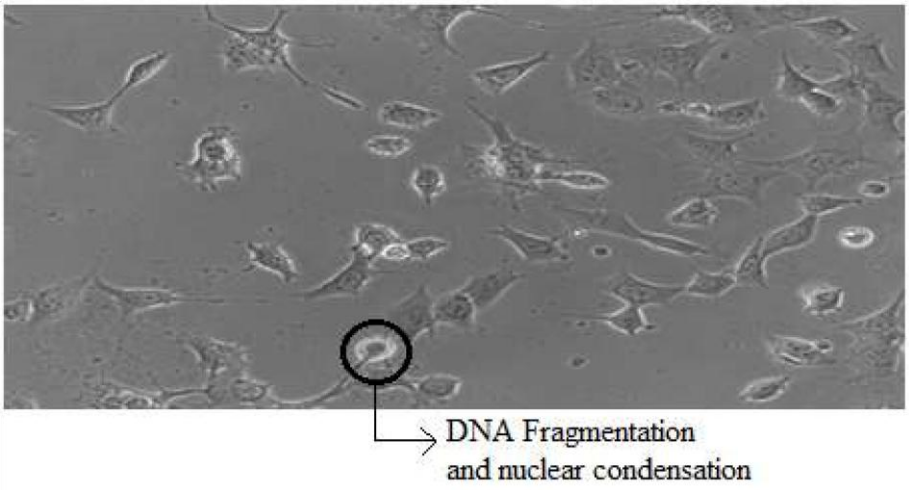
MCF-7 Positive control



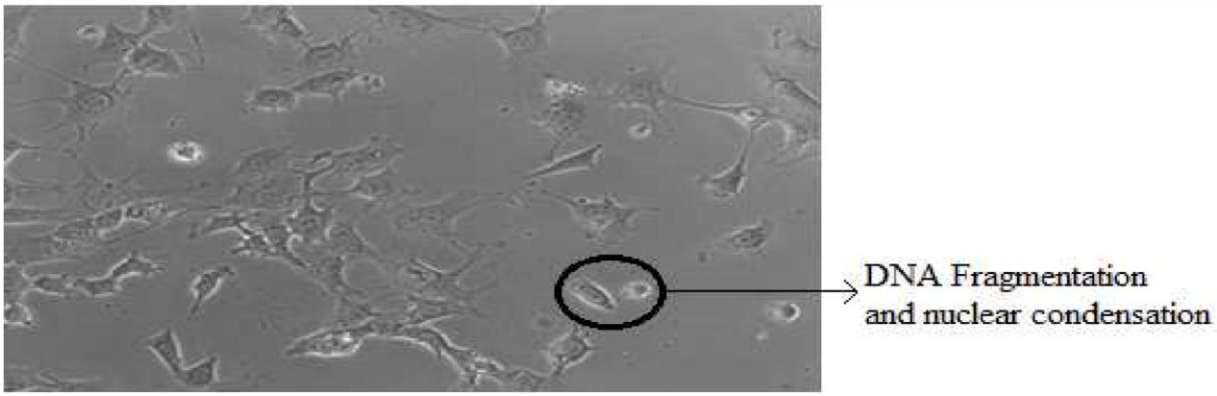
PS1 (Petrolium ether extract of Praecitrullus fistulosus)



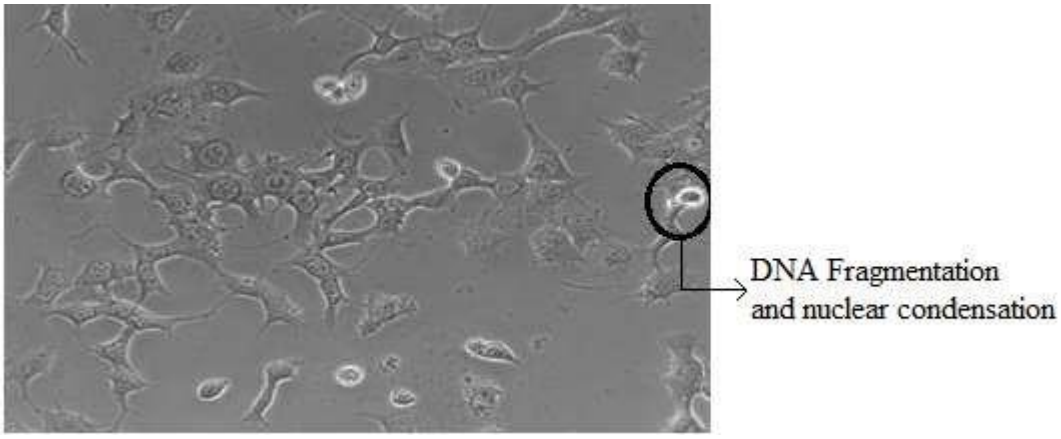
PS2(Chloroform extract of Praecitrullus fistulosus)



PS3 (Ethyl acetate extract of Praecitrullus fistulosus)



PS4 (Acetone extract of Praecitrullus fistulosus)



PS5 (Ethyl alcohol extract of Praecitrullus fistulosus)

The major components of various extracts of *Lagnaria sciceraria* and *praecitrullus fistulosus* are polyphenols, flavonoids, and saponins, which have anti-oxidant and anti-cancer activity. Plants with anti-oxidant activity show anti-cancer properties by inhibiting the proliferation of multiple human cancer cells. The present study suggests that the main compounds such as polyphenols, flavonoids and saponins could be responsible for its cytotoxic activity against MCF-7 breast cancer cell line. Significant activity has been observed in all extracts of P*raecitrullus fistulosus* against MCF Cell lines. However ethanolic *extract of* P*raecitrullus fistulosus* demonstrated higher activity against MCF-7 breast cancer cell..

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