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PHYTOCHEMICAL CHARACTERIZATION AND CYTOTOXIC EVALUATION OF METHANOLIC EXTRACT OF *GARCINIA INDICA* FRUIT RIND

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ABSTRACT: Natural products from plants are rich sources of chemical diversity and most of the pharmacologically active principles currently used as drugs, including anticancer agents are plant products. The present study aims at phytochemical screening and evaluation of anticancer potential of methanolic extract of *Garcinia indica* fruit rinds belonging to Family Clusiaceae. The methanolic extract of dried Fruit rinds of *Garcinia indica* were subjected to the phytochemical tests to confirm the presence of Carbohydrate, alkaloids, steroids, Glycosides, flavonoids, Tannic acid and phenolic compounds, proteins and fixed oils. *In vitro* antioxidant activity of the extract was assayed using DPPH radical scavenging assay, Hydrogen peroxide scavenging activity, reducing power activity. Total phenol and flavonoid contents were determined and expressed in gallic acid and catechin equivalent respectively. The results of the study indicates that the methanolic extracts of the *Garcinia indica* fruit rind possess significant scavenging activity against DPPH (78.1µg/ml), reducing power activity (0.387 absorbance at 100 µg/ml) free radical scavenging activity (44.64 µg/ml). The free radical scavenging and promising antioxidant activities of the extract may be attributed to the presence of adequate phenolic (0.34mg/gm) and flavonoid compounds (134.32 µg/g). Cytotoxic activity was assayed *in vitro* against HCT-116 by using MTT colorimetric procedure. From the analysis it was found that methanolic fruit rind extract of *Garcinia indica* showed nearly 50% HCT-116 cell line inhibition at 53.8µg/ml. Therefore it can be concluded that present study could be extended for the formulation and evaluation of herbal anticancer dosage forms.

INTRODUCTION: Cancer is a leading cause of mortality and it strikes more than one-third of the world's population and it's the cause of more than 20% of all deaths. Cancer known medically as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body.

Many cancer patients have received chemotherapeutic agents during the course of illness, treatment based on cell destruction potential by growth interruption. Surgery, chemo therapy and radiotherapy are the main conventional cancer treatment often supplemented by other complementary and alternative therapies¹.

Plants has been used as an age old remedy of cancer history of use in the treatment of cancer Several reports describe that the anticancer activity of medicinal plants is due to the presence of antioxidants present in them. In fact, the medicinal plants are easily available, cheaper and possess no toxicity as compared to the modern (allopathic) drugs².

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Garcinia indica Linn. belonging to family Clusiaceae commonly recognized as 'Kokum' found in Maharashtra and particular in Konkan, Goa and the western region of India.

In the Indian system of medicine, the fruits of *Garcinia indica* have been suggested for a number of diseases such as an appetizer, on skin rashes caused by allergies, treatment of burns, to relieve sunstroke, remedy for dysentery and mucous diarrhea, antiulcer, liver tonic, to allay thirst and as a cardiogenic.

The main constituent present in the fruit are especially from its rind, are rich in polyisoprenylated benzophenone derivatives such as Garcinol and its colourless isomer, isogarcinol that has antioxidative, chelating, free radical scavenging, antiglycation, anticancer, anti-inflammatory, and antiulcer activities. Garcinol shows strong antioxidant activity since it contains both phenolic hydroxyl groups as well as a β -diketone moiety. The rind also contains hydroxy citric acid (HCA), has been patented for use as hypocholesterolaemic agent³⁻⁴. Kokum contains other compounds with potential antioxidant properties include citric acid, malic acid, polyphenols, carbohydrates, anthocyanin flavonoids and ascorbic acid⁵⁻⁷. Based on reported traditional and chemical constituents we selected *Garcinia indica* fruits for the present study.

MATERIAL AND METHODS:

Plant Material: The fruits of *Garcinia indica* were collected from the Konkan region of Maharashtra and authenticated by A. Benniamin, Scientist, Botanical Survey of India, Pune, Maharashtra, India.

Preparation and Extraction of Plant Material:

The fruit rinds of *Garcinia indica* were cut into pieces and shade dried at room temperature. They were subjected to size reduction to coarse powder by using mixer grinder. The powder was extracted with methanol using soxhlet apparatus⁸⁻⁹. The methanolic extract was filtered and evaporated under reduced pressure using rotary vacuum evaporator. The sweet scented, chocolate coloured semisolid residue formed after complete dryness was kept in an airtight and waterproof container which is stored in the refrigerator. The extract was

used for determination of total phenol and flavonoid content and also for assessment of antioxidant capacity.

Phytochemical Screening: The phytochemical investigation of the methanolic extract of dried fruit rinds of *Garcinia indica* was carried out using standard protocol. The phytochemical tests were performed on the extract using standard methods¹⁰.

Estimation of total phenolic and Flavonoid content:

Folin-Ciocalteu assay: The total phenolic content of *Garcinia indica* was estimated according to the method of Makkar *et al.*, (1997)¹¹. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. After vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. Using gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 $\mu\text{g/ml}$. Using the standard curve, the total phenolic content was calculated and expressed as mg of gallic acid per gram of extract.

Estimation of Total flavonoid content:

Aluminum Chloride Assay: Total flavonoid was determined using the aluminum chloride colorimetric method¹². 1ml extract was added to 4ml distilled water in a flask. Then, 5% NaNO_2 (0.3 mL) was added. 10% AlCl_3 (0.3 mL) was added after 5 min and 1 M NaOH (2 mL) was added after 6 min. The mixture was diluted to 10 mL with distilled water. The absorbance of the solution was measured at 510 nm using a spectrophotometer. The results were expressed as mg catechin equivalents (CE)/g samples.

Evaluation of *in vitro* Antioxidant Activity¹³⁻¹⁸

2.5.1 DPPH (2,2 Diphenyl-1 picrylhydrazyl) assay: The DPPH assay was performed by the method given by Brand-Williams *et al.*,¹³. Test samples were prepared by adding 0.1ml of ethanolic DPPH solution in 0.1ml methanolic plant extract solution of varying concentrations (10, 25, 50, 75, 100 $\mu\text{g/ml}$). Blank sample were prepared and L-Ascorbic acid was used as reference

standard. DPPH and methanol (1:1) solution was used as control. The decrease in absorbance (A) was measured at 517 nm after 30 minutes in dark using UV-Visible spectrophotometer. The inhibition % was calculated using the following formula:

$$\text{Inhibition \%} = \frac{A_{\text{Control}} - A_{\text{Test}}}{A_{\text{Control}}} \times 100$$

A_{control} : Absorbance of the control sample.

A_{test} : Absorbance of the test sample.

Hydrogen Peroxide-Scavenging Activity: The Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch *et al.*,¹⁴. The extract was dissolved in 3.4 ml of 0.1M phosphate buffer (pH 7.4) and mixed with 600 μ l solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min. for each concentration, a separate blank sample was used for background subtraction.

Reducing Power Activity: The reducing power of extract was determined by the method of Yen and Duh¹⁵. Different concentrations of extracts were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min. After incubation, 2.5 ml of 10% trichloro acetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm.

In vitro Cytotoxicity Studies:^{19, 20}

Cell lines and cultural conditions: Colon cancer (HCT-116) cell lines were cultured in RPMI-1640 medium with 20% FBS, 2mM L-glutamine, 1% penicillin/streptomycin under a fully humidified atmosphere 5% CO₂ at 37 °C.

Method: MTT Assay: The effect of extract on the viability of HCT-116 were determined by MTT (3-[4,5-dimethyl thiozole-2-yl] -2-5-diphenyl tetrazolium bromide) assay. 100 μ l of cell suspensions in growth medium were plated in 96-well microtitre plate at concentrations of 1x10⁴ cells/well and incubated for 48h at 37 °C in a humidified incubator. After 48 hours incubation the cell

reaches the confluence. Then, cells were incubated in the presence of various concentrations of the extracts in 0.1% DMSO for 72 h at 37 °C. After removal of the sample solution and washing with phosphate buffered saline (pH 7.4), 20 μ L of MTT (5mg/mL) was added to each well of the plate. The plate was incubated for 4h at 37 °C. The solution in each well including MTT was aspirated and 100 μ L of buffered DMSO was added to dissolve formazone. The plates were shaken for 5min. Optical density was measured on a microplate ELISA reader at 540nm with DMSO as control. The cytotoxicity was obtained by comparing the absorbance between the samples and control. The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULT AND DISCUSSION:

Phytochemical Evaluation: Phytochemical analysis of the methanolic extract of *Garcinia indica* revealed the presence of following phytoconstituents:

TABLE 1: EVALUATION OF PHYTOCHEMICAL CONSTITUENT

S. no.	Constituent	Result
1	Carbohydrate	+ve
2	Alkaloids	+ve
3	Steroids	+ve
4	Glycosides	+ve
5	Saponins	-ve
6	Favonoids	+ve
7	Tannic and phenolic compound	+ve
8	Proteins and amino acid	+ve
9	fixed oils.	+ve
10	Anthraquinone	-ve

+ve shows presence of constituent

-ve shows absence of constituent

Estimation of Total Phenolic and Flavonoid Content: Flavonoids protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxy nitrite. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and the incidence of heart attacks. Total flavonoid content of methanolic extract of *Garcinia indica* was found to be 134.32 μ g/g by aluminium chloride colorimetric assay. The total phenolic and total flavonoid content results were enlisted in **Table 2**.

TABLE 2: TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT

Type of extract	Total phenolics Equivalent to gallic acid (mg/gm)	Total flavonoids Conc. Equivalent to CE(µg/g)
Methanolic extract of <i>Garcinia indica</i>	0.34	134.32

Evaluation of *in vitro* Antioxidant Activity:

DPPH (2,2 Diphenyl-1 picrylhydrazyl) assay: DPPH easily accepts an electron or hydride radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses color stoichiometrically depending on the number of electrons taken up. Anti oxidant activity of methanolic extract of *Garcinia indica* was performed by DPPH (2,2 Diphenyl-1 picrylhydrazyl) assay using ascorbic acid as standard. The amount of extract needed for 50% inhibition (IC₅₀).

The % Inhibition of DPPH radical was highest at the concentration of 100µg/ml and was found to be 68%, compared with the ascorbic acid showing % inhibition of 70% at the same concentration. IC₅₀ value of *Garcinia indica* fruit rind extract was found to be 78.1µg/ml. where as the ascorbic acid showed the value of 72.1µg/ml.

TABLE 3: EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY BY DPPH METHOD

Concentration (µg/ml)	Ascorbic acid Inhibition %	<i>Garcinia indica</i> Inhibition %
10	15	12
25	21	16
50	32	27
75	52	48
100	70	68

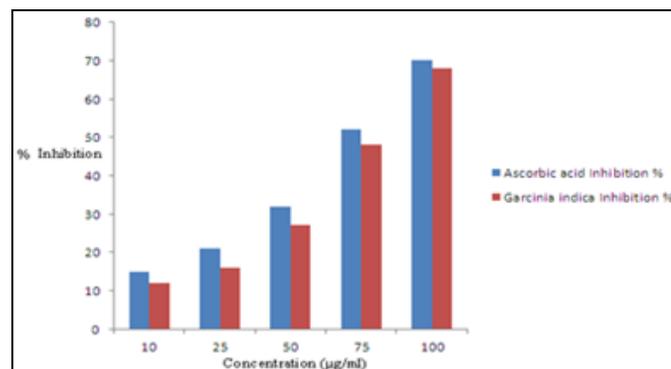


FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACT OF *GARCINIA INDICA* FRUIT RIND

Hydrogen Peroxide-Scavenging Activity: Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H₂O₂ can probably react with Fe²⁺, and possibly Cu²⁺ to form hydroxyl radical and this may be the origin of many of its toxic effects. Hydrogen peroxide scavenging activity of the extract is presented in **Fig. 2**; the extract exerted a concentration dependent scavenging of *Garcinia indica* fruit rind extract. The % Inhibition of hydrogen peroxide scavenging activity was highest at the concentration of 100µg/ml and was found to be 73.27%, compared with the ascorbic acid showing % inhibition of 94.78 % at the same concentration. IC₅₀ Value of the extract for hydrogen peroxide scavenging activity was found to be 44.64µg/ml, whereas the standard exerted an IC₅₀ of 31.95µg/ml.

TABLE 4: SHOWS HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF *GARCINIA INDICA*

Concentration µg/ml	Ascorbic acid Inhibition %	<i>Garcinia indica</i> Inhibition %
10	62.34	36.82
25	71.67	45.38
50	78.23	56.64
75	86.73	68.12
100	94.78	73.27

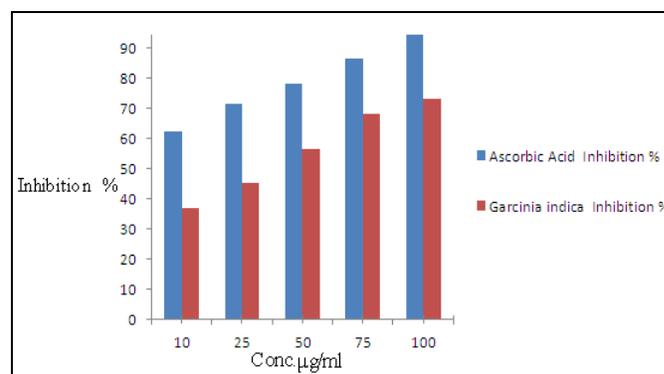


FIG. 2: HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF METHANOLIC EXTRACT OF *GARCINIA INDICA* FRUIT RIND

Reducing Power Activity: The antioxidant can donate an electron to free radicals, that leads to the neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of Fe³⁺(CN)₆⁻-Fe²⁺(CN)₆⁻. The product was visualized by forming the intense Prussian blue color complex and then measured at 700nm.

As shown in **Fig. 3**, a higher absorbance value indicates a stronger reducing power of the samples. *Garcinia* fruit rind extract showed concentration-dependent reducing power. However, its reducing power was weaker than that of ascorbic acid, which exhibited the strongest reducing power.

At concentration 10µg/ml and 100µg/ml Ascorbic Acid (Reference) and *Garcinia indica* showed absorbance with about 0.157-0.411 and 0.034-0.387 respectively at 700nm Thus *Garcinia indica* exhibited reducing activity might be due to hydrogen donating ability.

TABLE 5: SHOWS REDUCING POWER ACTIVITY OF METHANOLIC FRUIT EXTRACT OF *GARCINIA INDICA*

Concentration (µg/ml)	Absorbance at 700 nm	
	Ascorbic acid	<i>Garcinia indica</i>
10	0.157	0.034
25	0.211	0.134
50	0.298	0.203
75	0.358	0.278
100	0.411	0.387

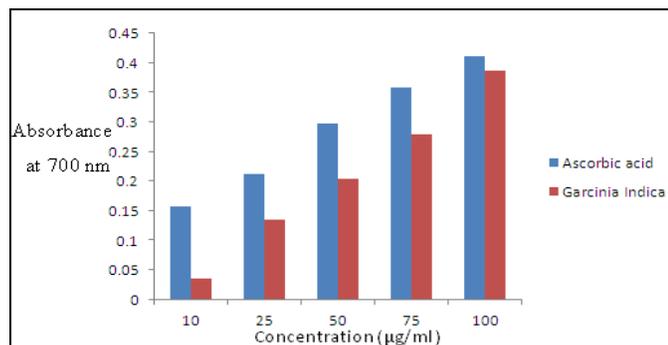


FIG. 3: REDUCING POWER ASSAY OF METHANOLIC EXTRACT OF *GARCINIA INDICA* FRUIT RIND

TABLE 6: EFFECT OF *GARCINIA INDICA* METHANOLIC EXTRACT ON HCT -116 CELL LINE

Concentration (µg/ml)	% Inhibition
12.5	27.24
25	37.15
50	46.42
100	52.54
200	63.21

In vitro Cytotoxicity Studies: In MTT assay, methanolic extract of *Garcinia indica* showed significant growth inhibitory effect. It was found that the % growth inhibition increases with the increasing concentration and IC₅₀ value of this assay was 53.8µg/ml. *Garcinia indica* has potential activity on HCT 116 that indicates extract possess relative selective cytotoxicity to colonic cancer

cells. Result of antitumor activity was depicted in **Table 6**.

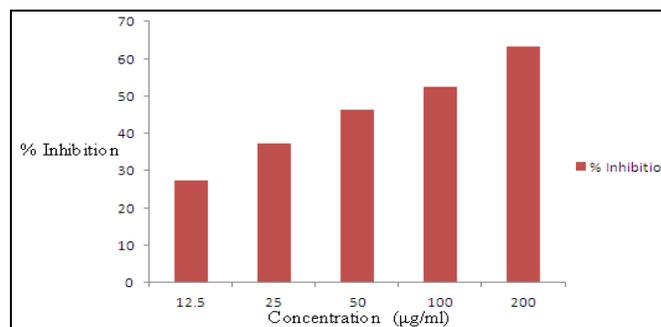


FIG. 4: CYTOTOXIC ACTIVITY OF *GARCINIA INDICA* EXTRACT ON HCT -116 CELL LINE

CONCLUSION: Thus the present study concluded that the methanolic extract of *Garcinia indica* fruit rind exhibited significant antioxidant and potential cytotoxic activity. Further phytochemical and pharmacological studies are also required to be extended for the development of novel herbal anticancer dosage forms.

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CONFLICT OF INTEREST: Nil.

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