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In vitro antioxidant and anticancer activity of *Ficus racemosa* leaf and fruit extract on MCF7 human breast cancer cell line

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Abstract

Humans are constantly exposed to high levels of radiation, which damages proteins, lipids, and DNA oxidatively and results in chromosomal mutations. As an illustration, oxidative stressors can cause diabetes, cancer, and even neurological and cardiovascular conditions. Nowadays, the majority of anticancer medications have a variety of adverse effects. As a result, current attention is being focused on studying plants in order to find the active ingredients that may be useful. Many medicinal plants contains the variable amounts of different phytochemicals such as saponins, triterpenoids, anthracyanins, alkaloids, phenols, flavanoids, resins, fatty acids and tannins. Many of these phytochemical agents have been found to possess anticancer activity. One of the biggest genera of medicinal plants, Ficus is mostly found in tropical and subtropical areas of the world and is used for a wide variety of different illnesses. It is well recognized that several plant components, including the bark, leaves, young shoots, fruits, seeds, and latex, have therapeutic use. The present study encompasses the ascertainment of *in vitro* antioxidant activity and anticancer activity of methanolic extract of *Ficus racemosa* leaf and fruit by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay and MTT assay. From the results, *F. racemosa* has been found to have the significant antioxidant activity in a dose-dependent manner and IC50 value of leaf and fruit extract were 89 µg/ml and 92 µg/ml for DPPH. Further, the cytotoxicity analysis was determined against MCF-7 (Human breast cancer) cell line. Methanolic leaf extract of *Ficus racemosa* showed the most cytotoxic activity at highest concentration (100µg/ml) with significant inhibition of cell growth. Hence, the study confirms that *F. racemosa* is enriched in phytochemicals which are the agents responsible for the natural antioxidant and anticancer property.

Key words: Antioxidant activity, therapeutic. cytotoxic, lignans, triterpenoids.

Introduction

Nature has always served as a gold mine of medicinal products for thousands of years and an attractive number of present-day drugs have been discovered from natural resources especially from the plant origin (1). Ancient knowledge served as the foundation for contemporary medicine and will continue to be a significant source for future treatments and medical care (2). Plant components in the form of powders, semi-solid preparations, decoctions, elixirs, and distillates make up the majority of Ayurvedic medicinal preparations. Minerals,

animal products, and inorganic chemicals are also present in many of them. Ayurvedic remedies also commonly use alcoholic extracts and alcoholic solutions of the constituents, tinctures, and elixirs. Antioxidants are compounds that have the ability to disrupt the chain reaction of free radicals. Antioxidants help reduce oxidative stress brought on by free radicals, which have a role in the development of many chronic illnesses such as diabetes, cancer, heart disease, and neurological diseases (3,4,5). Research has indicated that certain plant components are abundant in phytochemicals, such as flavonoids and phenolic acids, which have antibacterial, anti-carcinogenic, and antioxidant properties (6,7). Carotenoids, phenolics (flavonoids, lignans, and phenolic acids), and vitamins are the primary plant constituents that have antioxidant properties (8).

Gular, or *Ficus racemosa* Linn, is a plant that is grown extensively in India. To treat a variety of ailments, several chemical components, such as tannins, phenols, flavonoids, alkaloids, etc., have been extracted from the root, bark, and stem of the plant. It is one of the earliest medicinal herbs that is listed in all ancient Ayurvedic texts. Tetra-triterpene, glauanol acetate, and racemosic acid are all found in *F. racemosa* leaves. Several of these phytochemical substances have been found to possess anticancer effects. Numerous therapeutic qualities, including hepatoprotective, antioxidant, radioprotective, hypoglycemic, antidiuretic, and antimitotic actions, are well-known. *F. racemosa* has antioxidant components such as polyphenols and flavanoids, which are used to treat a variety of illnesses linked to oxidative stress (9). *Ficus racemosa* Linn plays a significant role as an antioxidant, with antibacterial, wound-healing, and cytotoxic characteristics (10,11,12). Therefore, the present study aims to investigate the In vitro antioxidant potential and anticancer effect of *F. racemosa* leaves and fruit on human breast cancer cell line MCF-7, by MTT assay.

Materials and Methods

Preparation of Extracts

The collected fresh leaf and fruits of *Ficus racemosa* was thoroughly washed in tap water, shade dried, powdered and was stored in air tight container. The powdered each plant material (100gm) was grounded successively with methanol solution using motor and pestle. Then the extracts were centrifugeed at 5000rpm for 15 minutes and filtered through Whatman Number 1 filter paper stored at 0-4^{0}C until further use.

In vitro antioxidant analysis

DPPH radical scavenging assay

Different aliquots of *Ficus racemosa* leaf and fruit methanolic extracts are taken in different concentration (12.5µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL and 200µg/mL) from stock solution were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. Standard ascorbic acid was taken at varying concentrations. An comparable volume of distilled water was used as a control. For twenty minutes, the reaction mixture was allowed to sit at room temperature in a dark environment.The mixture's absorbance was measured at 517 nm after 20 minutes. 3ml of DPPH was taken as control. The percentage inhibition activity was calculated as: ((Abs. of control -Abs. of sample)/Abs. of control) x 100%. The IC50 value was calculated, which is the effective concentration at which the antioxidant activity is 50%.

In vitro anticancer analysis

Anticancer effect determination by MTT assay

MCF-7 (Human breast cancer) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's modified Eagles medium (DMEM). In a 25 cm^2 tissue culture flask, the

cell line was cultivated using DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and an antibiotic solution that contained Amphoteracin B (2.5µg/ml), Streptomycin (100µg/ml), and Penicillin (100U/ml). The cultured cell lines were maintained in a humidified incubator with 5% CO2 at 37ºC. The MTT assay method was used after direct cell observation using an inverted phase contrast microscope to assess the vitality of the cells.

Cells seeding in 96 well plate

A 96-well tissue culture plate was seeded with 100µl of the cell suspension (5x103 cells/well) after a two-day-old confluent monolayer of cells had been trypsinized and suspended in 10% growth media. The cells were then kept at 37ºC in a humidified 5% CO2 incubator.

Preparation of plant extracts and compound stock:

1mg of sample was weighed and dissolved in 1ml 0.1% DMSO using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Anticancer Evaluation

The growth medium was taken out after 24 hours. Each compound that had been freshly prepared in DMEM was serially diluted five times using a two-fold dilution (100µg, 50µg, 25µg, 12.5µg, and 6.25µg in 500µl of DMEM). Each concentration was then added in triplicate to the corresponding wells, and the wells were then incubated at 37ºC in a humidified 5% CO2 incubator. Control cells that were not treated were also maintained.

Anticancer Assay by Direct Microscopic observation

Following a 24-hour treatment period, the entire plate was examined under an Olympus CKX41 inverted phase contrast tissue culture microscope equipped with an Optika Pro5 CCD camera. Microscopic observations were captured as photographs. Any discernible alterations in the cells' morphology, such as rounding or shrinking, granulation, or vacuolization in the cytoplasm, were regarded as markers of cytotoxicity.

Anticancer Assay by MTT Method

After being thoroughly diluted in 3 ml of PBS, a total of 15 mg of MTT (Sigma, M-5655) was reconstituted and filter sterilized. The sample content in the wells was removed after the 24-hour incubation period, and 30 µl of reconstituted MTT solution was added to each test and cell control well. The plate was then gently shaken thoroughly, and it was incubated for four hours at 37 °C in a humidified 5% CO_2 incubator. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004). The LC50 value was calculated using ED50 PLUS V1.0 Software.

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x } 100}{\text{Mean OD of control group}}$$

Results and Discussion

In vitro antioxidant effect

F. racemosa contains antioxidant compounds such as poly-phenols and flavonoids, which can be used in the treatment of many diseases related to oxidative stress (9). 1,1-diphenyl-2trinitrophenyl hydrazine free radical (DPPH) has been widely used to evaluate the ability of antioxidants to scavenging free radicals. The free radical DPPH reacts with a hydrogen donor to form a hydrazine equivalent. Stable free radicals can be generated by DPPH in methanol or aqueous solutions. The absorbance decreased and the color changed from purple to yellow when an antioxidant scavenged

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the DPPH by giving hydrogen to form a stable DPPH molecule. The scavenging effectiveness of the extracts was assessed by detecting the decolorization of DPPH at 517 nm. The results of the DPPH radical scavenging experiments performed on fruit, plant leaf, and ascorbic acid (standard) extracts are shown in Table 1. Figure 1 illustrates how DPPH radicals were scavenged by the methanolic leaf and fruit extract of *F. racemosa*. In contrast to ascorbic acid, the extract demonstrated a potent DPPH scavenging action. The substrate concentration that results in a 50% reduction in DPPH activity is known as the IC50 value. The IC50 values of the *F. racemosa* methanolic fruit and leaf extract in this investigation were 92 *µg*/ml and 89 *µg*/ml, respectively. At a 200 *µg*/ml concentration, the methanolic leaf and fruit extracts of *Ficus racemosa* showed 56.43% and 62.56% activity, respectively, while the ascorbic acid extract showed 96.30% activity. As a result, free radicals can be significantly reduced by *Ficus racemosa*. In the present study, it was observed that the leaf and fruit extracts of Ficus racemosa showed significant effects. According to the results of antioxidant studies, the leaves and stem bark of *F. racemosa* have high antioxidant activity (13).

Table 1: Shows the DPPH free radical scavenging activity of methanolic extracts of standard Ascorbic acid, *Ficus racemosa* leaf and fruit at various concentrations. Percentage of scavenging of each sample was taken in triplicate indicated as A, B and C.

Samples	Conc. mg/ml	% of scavenging			Mean % of scavenging ± STD	IC 50 (mg/ml)
		A	B	C		
AA	25	40.29	39.60	41.50	40.46 ± 1.04	38.56
	50	60.11	60.80	61.20	60.70 ± 0.5	
	100	78.77	76.53	77.81	77.70 ± 1.07	
	200	96.19	95.81	96.92	96.30 ± 0.62	
FL	25	31.67	32.11	30.52	31.42 ± 0.69	89.01
	50	41.25	43.33	41.10	41.89 ± 1.44	
	100	50.22	50.91	51.80	50.97 ± 0.83	
	200	57.39	56.81	55.11	56.43 ± 0.99	
FF	25	36.00	33.54	34.96	34.83 ± 1.17	92.11
	50	46.14	45.12	46.87	46.04 ± 0.83	
	100	54.91	53.27	55.13	54.43 ± 0.7	
	200	63.37	62.34	61.98	62.56 ± 0.81	

(AA: Ascorbic acid, FL: Ficus racemosa leaf, FF: Ficus racemosa fruit)

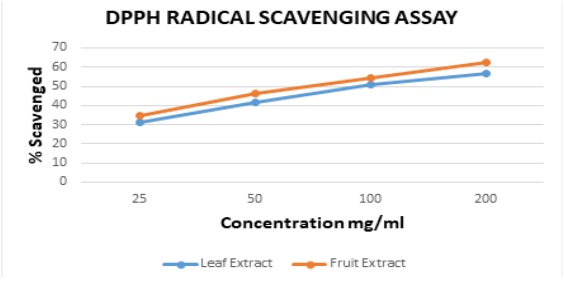

Figure 1: Shows the DPPH radical scavenging activity of methanolic extracts of *Ficus racemosa* leaf and fruit at various concentrations.

In the present study, it was observed that the leaf and fruit extracts of *Ficus racemosa* showed significant effects. The methanol extract of *F. racemosa* showed significant free radical scavenging activity in the dose range. However, many studies have been conducted on the anti- oxidant properties of different plants of *F. racemosa* (14,15). Sultana et al. (2013) examined the antioxidant activity of *Ficus racemosa* leaves by estimating the DPPH radical scavenging activity and reporting a higher hydrogen donating capacity for the DPPH assay.

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Our findings regarding the hydrogen peroxide scavenging activity of the methanolic extract of the leaves (56.43±0.99) were similar to those of the standard ascorbic acid (16). Atoui et al. also investigated the phenolic composition and antioxidant activity of tea and herbal infusions and discovered that they were more capable of donating hydrogen in the DPPH experiment. The results of the present investigation also aligned with previous research (17,18,19).

In vitro Anticancer effect

The in vitro cytotoxic activity of methanol extract of *Ficus racemosa* leaves and fruits against MCF7 (human breast cancer) cell lines was determined by the MTT method as indicated in figure 2. Following a 24-hour incubation period, the cells were treated to 6.25–100µg of extracts, and LC values were calculated. *Ficus racemosa* leaf and fruit extract inhibited MCF-7 cell growth in a dose and time dependent manner, according to cell viability analyses. The maximum dose of *F. racemosa* leaf extract (100 µg/ml) demonstrated considerable cytotoxic action after 24 hours of incubation, preventing 52% of cell growth. As the incubation period increased, cell viability declined. Cell viability in methanol leaf and fruit extracts was found to decrease with concentration, with 70% and 76% of cells persisting at a concentration of 25 µg/ml and there was a decline to 52% and 58% at a high concentration of 100 µg/ml.

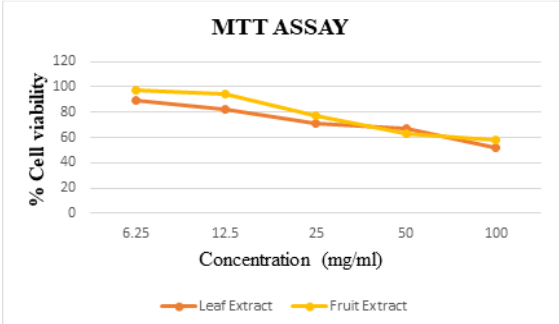

Figure 2: Shows the MTT assay of methanolic extracts of *Ficus racemosa* leaf and fruit at various concentrations.

In vitro research have demonstrated the anticancer effects of *Ficus racemosa* against specific cancer cell lines. These studies suggest that this plant may have anticancer effects. The growth of ehrlich ascites carcinoma cells was shown to be 76% inhibited by the root extract of *Ficus racemosa* (20). Furthermore, it has been demonstrated that *Ficus racemosa* bark extracts exhibit cytotoxic and anticancer effects against the Calu 6 cell line of lung anaplastic carcinoma (21). According to a study, the methanolic extract of *Ficus racemosa* was cytotoxic against many liver malignant cell lines such as HL60, HepG2, NCI-H23 and HEK293T. The results of the studies showed that, despite having relatively low IC50 values (85% inhibitory concentration), the methanol extract had stronger cytotoxic effects on HL-60 and HepG2 cells than other cell lines (22,23,24). The methanolic leaf and fruit extract of *Ficus racemosa* exhibited notable cytotoxic activity in the current investigation. The MTT assay was used after direct cell observation using an inverted phase contrast microscope to assess the vitality of the cells. At the highest concentration (100µg/ml), the methanolic leaf extract of *Ficus racemosa* exhibited the greatest cytotoxic action and growth inhibition. Akhila et al. examined the anticancer potential of *Tabernaemontana divaricata* hydroalcoholic extract against Hela cell lines at different doses. They discovered that the IC50 value surpassed 100 µg/ml and that the inhibition of cell growth increased with concentration (25). There was a correlation between the current study's findings and those of other studies. The current investigation suggests that the fruit and leaf extract of *Ficus racemosa* may have anticancer effects. It may therefore have anticancer properties that function as a therapeutic agent to halt or slow the proliferation of cancerous cells.

Conclusion

Natural antioxidants have become popular because they have fewer side effects and are more effective. Ficus is a well-known plant in this subcontinent and has many beneficial properties. The antioxidants in *F. racemosa* may

be responsible for one or more phytochemical anti-activities such as alkaloids, phenolic compounds and other components. The results showed that methanol extract of *F. racemosa* is a powerful natural antioxidant and has anticancer potential. These findings will also support the use of health-related foods in food products and the selection of antioxidant foods. Their products may play a role in preventing oxidative damage to cellular macromolecules because they eliminate free radicals. *Ficus racemosa* may be used as an adjuvant therapy in addition to other chemotherapy medications to discover any extra beneficial advantages.

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