ANTIPROLIFERATIVE EFFICACY OF *Curcubita pepo* L FLOWERS ON MCF 7 BREAST CELL LINE

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ABSTRACT

Nanotechnology is a rapidly developing discipline that is used extensively in nanomedicine and biomedical engineering. Since the beginning of time, people all over the world have used plants for basic preventive and therapeutic health care. The human race has looked for a number of plants to control sickness. For thousands of years, people have employed plants to heal a variety of illnesses. More than 80% of the world's population, or 4.3 billion people, are believed to rely on conventional plant-based medical systems for their primary healthcare. In this work, the *Curcubita pepo* L blooms, and UV-VIS spectroscopy is used to identify the chemicals present in the flower. Peaks at 297.55, 300.25, 346.11, and 467.21 nm with absorption values of 2.2307, 2.1279, 1.8237, and 1.0017 were visible in the UV-VIS profile. The results of the phytochemical examination reveal the presence of glycosides, sugars, saponins, tannins, phenol, coumarin, and volatile oils. The strongest antiproliferative activity against the MCF-7 cell line was demonstrated by the cytotoxic effects of *Curcubita pepo* L flower extracts, with an IC_{50} value of 16.50 g/mL. According to the study's findings, *Curcubita pepo* L flower extracts offer a variety of pharmacological qualities that could be employed to create novel medicines for the upkeep of health.

Key words: *Curcubita pepo* L, Phytochemicals, UV-VIS, MCF-7.

INTRODUCTION

For the diagnosis, treatment, mitigation, and prevention of sickness, natural-based pharmaceuticals are those with pharmacologically active ingredients derived from biological or mineral sources. Natural substances have proven to be the most productive sources of leads for the development of innovative therapeutics, especially anticancer drugs. More than

half of all newly approved drugs still have a natural product's structural ancestry to thank for the presence of many of today's therapeutic agents. **(Shynggys Sergazy *et al*., 2022)**

Some cancer types are treated using anticancer medications as the first line of defence. To treat specific types of malignancies, there are many anticancer drugs with different sources and mechanisms of action; nevertheless, the bulk of these drugs have negative side effects. For example, therapies that directly target DNA replication and cell division in cancer and normal cells, such as alkylating agents, topoisomerase inhibitors, and anti-microtubule medications, have unfavourable side effects. Therefore, it is essential to develop new anticancer drugs with fewer side effects. It has long been known that medicinal plants contain therapeutic ingredients and pharmacological leads, including anticancer drugs. According to estimations, vincristine, vinblastine, and taxol are among the chemotherapeutic medications with a natural or organic source that have been approved by the FDA. **(Alkhamaisah and Aljofan, 2020)**

A prospective source of medications for a number of diseases is plants and chemicals derived from plants. Pharmaceutical drug discovery continues to focus heavily on the use of natural items, such as medicinal plants and marine species. Today, more than 60% of the anti-cancer medications on the market are made from chemicals derived from plants. A recent analysis found that 64.9% of authorised anticancer medicines between 1940 and 2020 were either natural product mimics or derivations. The pathogenesis of cancer has been related to a number of intrinsic and extrinsic variables. However, carcinogenesis depends on cells being protected from normal apoptotic death and changing so that they have a low apoptosis rate or are resistant to apoptotic mechanisms. (**Newman and Cragg, 2020**)

Since ancient times, medical regimens have typically been a part of the human diet. Studies and knowledge on the phytochemical, anticancer potential of many medicinal plants are still inadequate at the present time. In order to evaluate the potential therapeutic applications of *Curcubita pepo* flower extract, this study concentrates on the phytochemical screening, antiproliferative activity, and UV-VIS analysis of the extract.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The *Curcubita pepo* L flower portion of the plant source chosen for the current study was gathered from the Tiruchirappalli district and nearby areas.

PREPARATION OF PLANT EXTRACT (Jeong *et al*., 2007)

200 mL of distilled water were used to extract 10 g of finely ground *Curcubita pepo* L flower powder over the course of 10 minutes at 60 °C. For later usage, the filtrates were kept in storage at 4 °C.

PHYTOCHEMICAL STUDIES

To identify the phytoconstituents present in the aqueous flower extracts from *Curcubita pepo* L, several phytochemical analyses were conducted. **(Robinson, 1964).**

UV-VIS Spectrum analysis

The extract was filtered using Whatmann No. 1 filter paper after being centrifuged at 3000 rpm for 10 minutes. With the same solvent, the sample was diluted to a ratio of 1:10 The typical peaks were found after the extract was scanned using a Perkin Elmer Spectrophotometer at wavelengths between 200 and 1100 nm. The UV-VIS peak values were captured. **(Dhivya and Kalaichelvi, 2017)**

MTT ASSAY

The method of Mosmann 1983 was used to test the cytotoxicity of a herbal preparation on MCF-7 cells.

Principle

The mitochondrial dehydrogenase of live cells reduces the yellow 3-4, 5-dimethylthiozol-2-Yl)-2,5diphenyltetrazoliumbromide (MTT), producing a quantifiable purple formation product. NAD (P) H-dependent reductase, which turns the MTT reagent into formazon, a substance with a deep purple hue, is present in viable cells. The solubilizing solution is then used to dissolve the formazon crystals, and a plate reader is used to detect the absorbance at 500–600 nm.

Reagents

MTT stock solution:

10 mL of PBS were used to dissolve 50 mg of MTT dye. It was filtered using 0.45 micro filters after 1 min of vortexing. Since MTT was light-sensitive, the bottle was wrapped in aluminium foil to block off light. At 4oC, the preparation was kept.

Procedure

MCF-7 cells were used for the cell viability assay. Viable cells were extracted and counted using a hemocytometer. They were planted in 96-well plates at a density of 1 104 cells/ml and allowed to attach for 24 hours. After MCF-7 cells were given the control treatment and the various concentrations (5 to 30 g/ml), they were added to each well. For 24 hours, MCF-7 cells were incubated at 37°C in an incubator with humidified 95% air and 5% CO2. The drug-containing cells were then treated for a further 4 hours at 37°C after which they were washed with fresh culture medium and the MTT (5 mg/ml in PBS) dye was added to each well. The cell viability was evaluated by absorbance at 540 nm using a multi-well plate reader, and the purple precipitated formazan was dissolved in 100 l of concentrated DMSO. The percentage of stable cells compared to the control was used to express the results. Calculating the half maximal inhibitory concentrations (IC50) values and analysing the best doses at various times were done.

$$= \frac{\text{Mean absorbence of the control} - \text{Mean absorbence of the sample}}{\text{Mean absorbence of the control}} \text{ X } 10$$

The IC_{50} values were calculated using the different extract dose responsive curve where suppression of 50% cytotoxicity relative to vehicle control cells. Each experiment was run in duplicate at least three times.

RESULTS AND DISCUSSION

Table 1: Preliminary Phytochemical screening of aqueous extract from *Curcubita pepo* L flowers

S.No	Phytochemical	*Aqueous extract*
1	Flavanoids	+
2	Phenol	++
3	Tannins	-

4	Saponins	+
5	Coumarins	+
6	Quinone	++
7	Alkaloids	-
8	Glycosides	+
9	Sugar	-
10	Volatile Oils	+

'+' - indicates the presence of phytochemical compounds

'-' - indicates the absence of phytochemical compounds

Figure 2: UV-VIS analysis of *Curcubita pepo* L flower extract

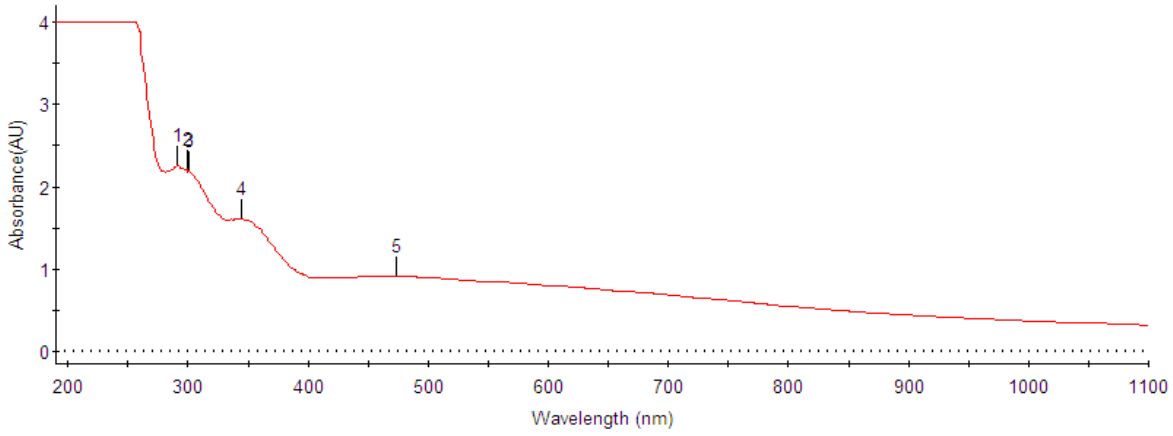

Table 2: UV-VIS analysis of *Curcubita pepo* L flower extract

S.no	Wave Length (nm)	Absorbance (AU)
1	297.55	2.2310
2	300.25	2.1254

3	346.11	1.8251
4	467.21	1.0017

Figure 3: Anti-proliferative effects of *Curcubita pepo* L flower extract on the activity of cytotoxicity in breast cancer cells MCF-7.

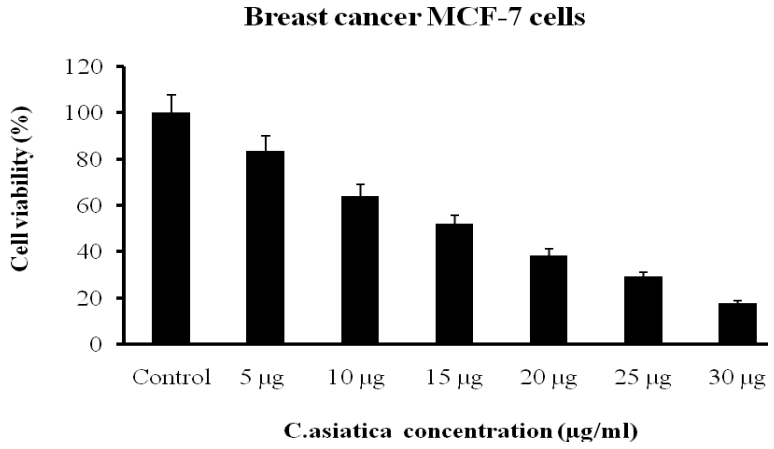

Table 1 displays the findings of a preliminary phytochemical analysis of Curcubita pepo L flower extract. The present study reveals the presence of Flavanoids, Phenol, saponin,Coumarin, quinone, Glycosides and Voltaile oils in aqueous extract.

Flavonoids are a key type of plant phenol and a water-soluble phytochemical. They exhibit antioxidant properties and have the ability to stop carcinogenesis and oxidative cell damage. They have significant effects on lower digestive tract and cardiac problems, as well as anti-cancer and anti-inflammatory properties. This plant contains flavanoids, which may

contribute to its antioxidant properties and shield cells from oxidative damage. Cardiac glycosides are utilised in the treatment of ulcers and diabetes. **(Karunyadevi *et al*., 2009)**

The aqueous flower extract contains phenols, which may be used as anti-microbial agents because to their presence. Phenols and phenolic compounds are often used extensively in treating skin infections, other wounds, and for healing when compared to other bactericides. Due to their capacity to transfer electrons, phenolic compounds are readily oxidised to yield the electron-accepting phenolate ion or quinone. Phenols and phenolic substances influence the prostaglandin pathways, inhibiting particular inflammatory enzymes and preventing platelet clumping. These substances also act as hormone regulators, immune system enhancers, anti-clotting agents, and antioxidants. **(Okwu *et al*., 2001)**

The use of these plants' extracts in wound healing was justified by the discovery of saponins in water. Red blood cells can be precipitated and coagulated by saponins, which also have the ability to bind cholesterol and cause foam to develop in aqueous solutions. **(Sodipo *et al*, 2000).**

The *Curcubita pepo* L flower extract contains coumarins, which have a number of biological effects, including anti-inflammatory, anti-clotting, anti-bacterial, anti-fungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, anti-adipogenic, Cytochrome P450 inhibition, estrogenic, dermal photosensitizing, antihelmentic, hypnotic, analgesic, hypotherm. **(Venugopala, 2013)**

Plants that contain carbohydrates are important as dietary supplements since they are known to strengthen the body and strengthen the immune system. **(Theis & Lerdau ,2003)**

The phytochemical study makes it abundantly evident that *Curcubita pepo* L. has a wide variety of phytochemicals. These phytochemicals looked to have the potential to act as a source of useful pharmaceuticals and to improve the health of the consumers due to the presence of several compounds that are necessary for good health. Due to the clarity of the peaks and appropriate baseline, the qualitative UV-VIS profile of *Curbita pepo L* flower extract was measured at wavelengths ranging from 200 nm to 1100 nm. Peaks at 297.55, 300.25, 346.11, and 467.21 nm were visible in the profile, with corresponding absorption values of 2.2307, 2.1279, 1.8237, and 1.0017. (Figure 2)

Figure 3 shows the observed absorption bands for *Curbita pepo* L flower extract. The development of one or more peaks in the range of 200 to 1100 nm in the UV-VIS spectrum is a certain sign that unsaturated groups and heteroatoms like S, N, and O are present. The

spectrum demonstrates that the from *Curbita pepo* L. contains organic chromophores. The challenges in assigning the absorption peaks to any specific system elements limit the applications of UV-visible spectrophotometery in the investigation of complex media. **(Patel *et al*., 2005)**

The presence of flavonoids and their derivatives is responsible for the formation of these absorption bands. The spectra of flavonoids typically has two absorption maxima in the wavelength ranges of 230–285 nm (band I) and 300–350 nm (band II). These maxima's precise location and relative intensities reveal important details about the flavonoids' makeup. The collected results are evaluated in light of earlier research on Acoruscalamus. **(NehaSahu, JyotiSaxena 2013)**

After being treated to various concentrations of *Curbita pepo* L (5–30 g/ml) for 24 hours, the MTT test was performed to calculate the cell cytotoxicity ratio for MCF-7 cells. The proportion of the control value used to express the results. Asterisks highlight statistically different experiments from the control in the data, which were provided as mean SD.

With an in vitro cytotoxicity experiment against MCF-7 cell lines, the cytotoxic effects of *Curbita pepo* L flower extracts at various doses (5-30 g/ml) were determined (Figure 10,11,12 & 13). By using the MTT assay, the percentage of cell viability was calculated. The highest antiproliferative action against the MCF-7 cell line was demonstrated by the methanol extract of *Curbita pepo* L flower extract, with an IC50 value of 16.50 g/ml (inhibiting 50% of cell growth). During a 24-hour incubation period, the MCF-7 cell viability varied between 89.86% and 25.09% at doses of 5 g/ml to 30 g/ml of leaves extract. The extracts demonstrated action against MCF-7 cancer cells that was concentration dependant.

With 23% of all female cancer cases, breast cancer is the second most prevalent malignancy among women. Patients with breast cancer are increasingly displaying severe toxicity and medication resistance. Anti-cancer medications are proven to be largely sourced from plants (Ramya et al., 2017). The results demonstrated that *Curcubita pepo* L at a dose of 30 g/ml was more cytotoxic. Significant apoptotic indicators, such as shrinkage, detachment, membrane blebbing, and deformed shape, were visible in the methanolic extract of *Curcubita pepo* L. Our findings from this study indicate that *Curcubita pepo* L's methanolic extract has strong anticancer activity against MCF-7 cell lines and may have therapeutic relevance for further research to produce natural chemicals as an anti tumour.

CONCLUSION

The current investigation supports the improvement of dietary plant substance-mediated drugs for the management of breast cancer. However, additional data are needed to sequence and identify specific molecules present in the *Curcubita pepo* L extract, which is responsible for its anticancer effect.

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