

## Characterization Of The Phytochemicals In Chamomile And Evaluation Of Their Biological Activity

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### ABSTRACT

Chamomile (*Matricaria chamomilla* L.) is an eminent medicinal herb used for centuries for its therapeutic properties. It is an annual plant that is native to Europe. It was introduced in India 300 years ago and naturalized in Uttar Pradesh and Punjab. Although Chamomile is popular for its calming and anti-inflammatory effects, studies on its phytochemicals in India are lacking. The present study explores the presence of active phytochemicals in the hydroalcoholic extract of chamomile flowers and found it rich in flavonoids, polyphenols, glycosides, carbohydrates, proteins, amino acids, and triglycerides. Maximum concentration was found for total polyphenol content (11.24%) followed by flavonoid content (4.16%), respectively. Further analysis of chamomile extract by HPTLC and HPLC methods revealed the occurrence of 0.81% w/w of apigenin-7-O-glucoside, a flavonoid as the major constituent of chamomile. We also evaluated the DPPH free radical scavenging of extract and found it 64.9% effective compared to 76.5% effectiveness of Apigenin-7-O-glucoside activity. Inhibition assay confirmed that the extract shows 76% COX-2 inhibitory activity making it suitable for anti-inflammatory treatments. We also checked the safety and toxicity of the extract against Hek293T cells and found it suitable for human use. So, the diverse pharmacological applications of Chamomile make it invaluable for advancing innovative drugs in the future.

**KEYWORDS:** anti-oxidant activity, anti-inflammatory activity, apigenin-7-O-glucoside, biological activity, Hydroalcoholic extract

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### I. INTRODUCTION

The herb chamomile, also known by its common name *Matricaria chamomilla* L. is well-identified for its medical benefits and used for thousands of years to treat a variety of illnesses (Singh et al., 2011). While it is originally from Europe and North Africa, it has since naturalized in numerous other regions of the globe including India Uttar Pradesh and Punjab are the main states where it came first (McKay & Blumberg, 2006). The chamomile plant's blossoms are used to make chamomile extract and are widely applied in conventional medicine and complementary therapies. It is frequently utilized to treat different health issues, including anxiety, insomnia, and digestive disorders. It is renowned for its anti-inflammatory, antioxidant, and calming qualities (Srivastava & Gupta, 2007).

Various active substances, such as flavonoids, terpenoids, and other phytochemicals, can be found in chamomile flower extract. The most researched active ingredients in chamomile flower extract are flavonoids such as quercetin, patuletin as well as apigenin, and bisabolol (Tantowi and Limanan, 2023). Chamazulene is a sesquiterpene with anti-inflammatory and antibacterial activities, whereas apigenin is a flavonoid with anti-inflammatory and anticancer characteristics (Sah et al., 2022).

Flavonoids apigenin-7-O-glucoside has been proven in studies to have anti-inflammatory, pro-apoptotic and anti-proliferative agents in both *in vitro* and *in vivo* settings (Farhat, 2022). It is shown to limit the activation of the inflammation regulator, nuclear factor-kappa B (NF-kB), and also prohibit the release of tumor necrosis factor-alpha and pro-inflammatory factor interleukin-6 (Ozcan et al., 2020). Antioxidant qualities have also allegedly been connected to apigenin 7-O-glucoside. In numerous cellular and animal models, it has been proven to scavenge free radicals and lessen oxidative stress. Apoptosis in cancer cells, inhibition of tumor growth, and prevention of metastasis are further ways that apigenin 7-O-glucoside has been demonstrated to have anticancer effects (Zheng et al., 2014). A neuroprotective effect of apigenin 7-O-glucoside has also been noted. In animal models, it has been demonstrated to enhance learning and memory while shielding neurons

from oxidative damage. Apigenin 7-O-glucoside has a lesser bioavailability than apigenin, which is probably because it is a glycoside. The standardization of this compound's content in herbal treatments is vital to ensure its efficacy and reproducibility, while it is still thought to be a significant component of chamomile flower extract (Paredes et al., 2015).

The objective of the current study is to assess the effectiveness and biological advantages of chamomile flowers found in India. To accomplish this, chamomile flowers were extracted in hydro alcohol, and phytochemical screening was performed to find the existence of a bioactive group of marker substances, such as flavonoids and polyphenols. The phytochemical characterization was performed by evaluating the presence and concentration of Apigenin 7-O-glucoside using HPTLC and HPLC methods. The Study could provide insight into the biological efficacy of the chamomile flower hydroalcoholic extract and its potential health benefits by comparing the activity of the extract to the Apigenin 7-O-glucoside standard for free radical scavenging and inflammation.

## **II. MATERIALS AND METHODS**

The Chamomile flowers were collected from Uttar Pradesh, India. Crude Extract was prepared by adding flower powder in a mixture of Ethanol: Water (1:1). Phytochemical screening was performed using hydroalcoholic extract.

### ***Qualitative Phytochemical Assay of hydroalcoholic extract of Chamomile Flower***

Qualitative phytochemical screening of the hydro-alcoholic extract was performed to analyse the group of marker compounds. The preliminary phytochemical screening of the Chamomile flower extract was conducted at Bangalore Institute of Technology, VTU, Belagavi. The hydroalcoholic extract of Chamomile flower was analyzed by standard chemical tests as described by Patil and Murthy, (2020), Harborne and Harborne, (1973), and Farnsworth, (1966) to determine steroids and triterpenoids, alkaloids, tannins, polyphenols, flavonoids, glycosides, carbohydrates, proteins, amino acids and Triglycerides.

### ***HPTLC Profiling of Chamomile flower extract***

Plant phytochemical profiling and compound quantification using the HPTLC technique are both possible. One milliliter of methanol was added to 100 mg of the sample and sonicated for 15 minutes. Using a CAMAG Linomate IV applicator, 20 µl of test sample solution was loaded to the Merck TLC Al Silica gel F254 Tracks 1, 2, and 3. The plate was developed in a 95:5 toluene: ethylacetate solvent mixture before being dried. Through the CAMAG TLC Visualizer, the plate was examined under UV light at 254 and 366 nm, and pictures were taken. The TLC plate was then coated with the anisaldehyde-sulfuric acid solution, and heated at 105°C using a hot plate until the spots' colors emerged, and a photo was taken under a white light to record the results. Prior to derivatization, the TLC was scanned with the CAMAG TLC Scanner and WINCATS 4.05 version software at UV wavelengths of 254 and 366 nm and Rf value was noted (Sethi, 1996; Saraswathy, 2003).

### ***Quantitative Phytochemical Assay of Polyphenols and Flavonoids in hydroalcoholic extract***

Total Polyphenols were extracted using the standard Folin-Dennis Method. The absorbance of the supernatant solution from the sample and the standard preparations was taken using a suitable UV Spectrophotometer which is set to 730nm. The calibration curve for standards of Catechin (in ppm) was plotted to get the linear curve equation and the coefficient of correlation value (r<sup>2</sup>). From the equation  $Y = mX + C$  of the linear curve, sample concentration was calculated as follows:  $X$  (Unknown Concentration in ppm) = (Sample Absorbance (Y) - Constant (C)) / (Slope value (m)) Percent Polyphenols in the sample was calculated as  $(X * \text{Standard Strength in \%}) / \text{Weight of the sample in ppm}$ .

The content of total flavonoids in the hydroalcoholic extract of Chamomile was identified by the following UV method. Absorbance was measured using UV Spectrophotometer at 435nm. Total Flavonoids content was calculated using below formula: Total Flavonoids content = (Sample absorbance x Conc. of standard in mg/ml x Potency of standard) / (Standard absorbance x Conc. of sample in mg/ml).

### ***HPLC Analysis for Quantitative estimation of Apigenin 7-O-glucoside***

Apigenin 7-O-glucoside (98.4%) was purchased from Sigma Aldrich. The Standard was prepared by dissolving 5 mg of Apigenin 7-O-glucoside reference standard in 50 ml of Methanol: water (1:1). Working standard concentration of 0.01mg/ml was used in the study. The sample was prepared by dissolving 250 mg of hydroalcoholic extract of Chamomile flower into 25 ml of Methanol: water (1:1 ratio). Shimadzu HPLC instrument with a photodiode array detector or UV detector was used as Chromatographic conditions. Column C18100A (5 µ): 250mm × 4.60mm. Acetic acid, acetonitrile, methanol, and water of HPLC grade were used in the study. Percent of Apigenin 7- glucosidase in the assay was calculated using the formula = (Avg. area of test solution × Weight of standard × purity) / (Avg. area of standard solution × weight of sample taken)

**Biological efficacy studies**

**Anti-oxidant activity of hydroalcoholic extract of Chamomile flower:** It was determined by using a well-liked technique, DPPH test (Chaves et al. 2020). The working solution of DPPH was individually supplemented with the Chamomile flower extract and apigenin-7-O-glucoside, and the blend is given a certain time period to incubate in the dark. Ascorbic acid (1 mg/ 100µl) was employed as the reference standard. The different concentrations tested for hydroalcoholic extract of Chamomile flower and apigenin-7-O-glucoside include 1, 2.5, 5 and 10 µg/µl. Spectrophotometer was used to measure the absorbance. The percentage of DPPH free radical scavenging activity was calculated as given by Chaves et al., (2020). 50% scavenger activity was determined by calculating Half maximal Inhibitory Concentration (IC<sub>50</sub>).

**In vitro anti-inflammatory Activity of hydroalcoholic extract from Chamomile flower**

A COX-2 Inhibition Assay kit can be used to assess the anti-inflammatory properties of Chamomile flower extract since it offers a quick, easy, sensitive test for COX-2 inhibitor screening. Using a COX Probe (Ex = 535 nm/Em = 587 nm) and a fluorometer, prostaglandin G2, an intermediate made by the COX enzyme, is detected in this assay. The amount of prostaglandin G2 was determined by the fluorescent signal generated. To perform the assay, the manufacturer's protocol was followed. The percentage of COX-2 inhibition can be calculated for each concentration of the extract, and a graph of percentage inhibition versus concentration can be plotted. The IC<sub>50</sub> value was calculated to find inhibit 50% COX-2 inhibition activity.

**Safety Evaluation of hydroalcoholic extract of Chamomile Flower**

The MTT test was used to compare the hydroalcoholic extract of chamomile flowers to the reference standard apigenin-7-O-glucoside to assess their safety. Kidney epithelial cells (Hek293T) were planted into a 96-well plate and left to grow for the night. The culture medium was changed to fresh media prior to extract treatment. Tests were performed using different extract and reference standard concentrations. A positive control was Doxorubicin (50 mM, 25 mM, 10 mM, and 1 mM/l), while a negative control was DMSO. The MTT assay was performed with the addition of Thiazolyl Blue Tetrazolium Bromide and incubated for about 4 hours after addition to check for the formation of insoluble purple crystals. Cells were incubated for 48 and 72 hours along with the chamomile flower extract. To dissolve the generated purple-colored formazan crystals, 100µl of DMSO was transferred to each well. The spectrophotometric absorbance of the multi-well plate was measured at 570 nm using a 96-well Tecan Microplate Reader and Megalan software was employed to retrieve the data, which was further exported in Microsoft Excel format for additional analysis, as described by Ghasemi et al. (2021).

**III. RESULTS AND DISCUSSION**

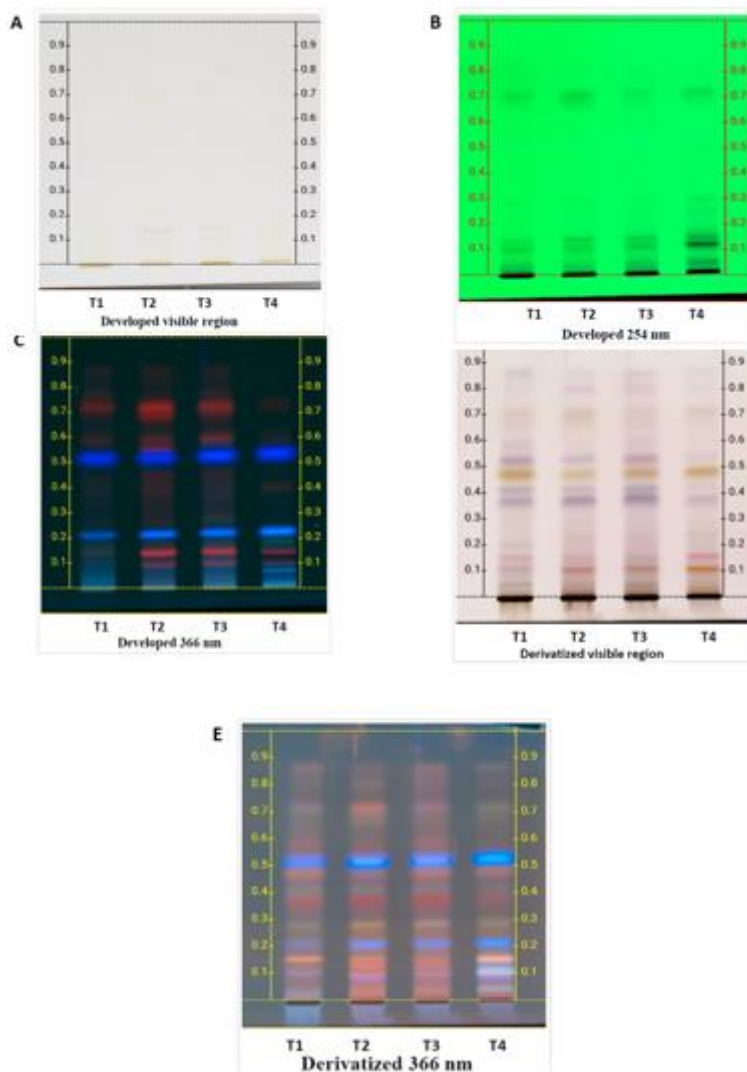
**Qualitative analysis of a hydroalcoholic extract of Chamomile flower**

The hydroalcoholic extract of flowers such as Moringa and citrus has drawn a lot of interest as a potential medical treatment due to its various pharmacological properties (Menichini et al., 2011; Mahajan et al., 2009). The qualitative analysis of phytochemicals of hydroalcoholic Chamomile flower extract signed positive for Flavonoids, Polyphenols, Glycosides, Carbohydrates, Proteins, Amino acids and Triglycerides whereas negative for Steroids, Triterpenoids, Alkaloids and Tannins (Table 1). Quantitative analysis of flavonoids and phenols has been conducted by Kashchenko et al., (2017) and Tsivelika et al., (2021) using HPLC methods. Studies by Guzelmeric et al (2015, 2017) assessed the presence of apigenin 7-O-glucoside using HPTLC method. In the present study, we used these two analytical methods HPTLC and HPLC to identify the presence of apigenin 7-O-glucoside.

**Table 1: Qualitative Phytochemical assay of the Chamomile hydroalcoholic flower extract**

SL. No	Phytochemical compound	Hydroalcoholic extract
1	Steroids and Triterpenoids	-
2	Alkaloids	-
3	Tannins	-
4	Polyphenols	+
5	Flavonoids	+
6	Glycosides	+
7	Carbohydrates	+
8	Proteins	+
9	Amino acids	+
10	Triglycerides	+

In order to control the quality of raw drugs, HPTLC profiling is helpful in authenticating the substances and locating any adulterants or substitutes (Guzelmeric et al., 2015). Raw pharmaceuticals or plant parts used to make compound formulation drugs require a high-performance thin-layer chromatography profile (HPTLC) in order to be effective. The outcome of qualitative analysis of HPTLC fingerprint profiles may be helpful in the authenticity and quality assurance of the raw medication (Guzelmeric et al., 2017). Different ratios of hydroalcoholic extracts of Chamomile flower were employed in HPTLC fingerprint studies at viewed the normal and derivatized plates at the visible region, 256nm, and 366 nm. We found the same band of different widths for different concentrations of hydroalcoholic extract (Figure 1a-e).



**Figure 1: HPTLC Images representing TLC plate of Chamomile flower extract.**

**T1** - Chamomile 70% Hydroalcoholic extract; **T2** - Chamomile 90% Hydroalcoholic extract; **T3** - Chamomile 80% Hydroalcoholic extract; **T4** - Chamomile 50% Hydroalcoholic extract.

#### ***Quantitative analysis of Chamomile hydroalcoholic flower extract***

The presence of polyphenols and flavonoids was quantitatively determined by employing the UV method. Total polyphenol content and the estimated flavonoid content were noted at 11.24% w/w and 4.16%w/w, respectively. The active marker ingredient in chamomile flower hydroalcoholic extract was measured in relation to the reference standard apigenin-7-O-glucoside using HPLC analysis. The current investigation focuses on the effectiveness of HPLC analysis in quantitative analysis of apigenin-7-O-glucoside from the hydroalcoholic extract of Chamomile flowers in comparison with apigenin-7-O-glucoside reference standard (Figure 2a and 2b). The crude extract was observed to contain 0.81% w/w of apigenin-7-O-glucoside. A similar study was conducted by Kashchenko and Olennikov in 2017 in another species *Matricaria chamomilla* flowers for quantitative analysis of flavonoids and was found effective.

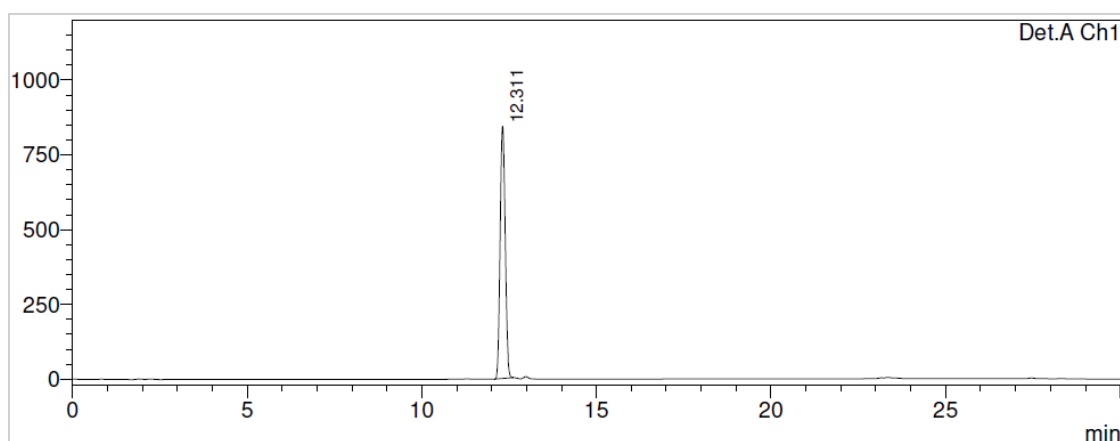


Figure 2a: HPLC chromatogram of reference standard

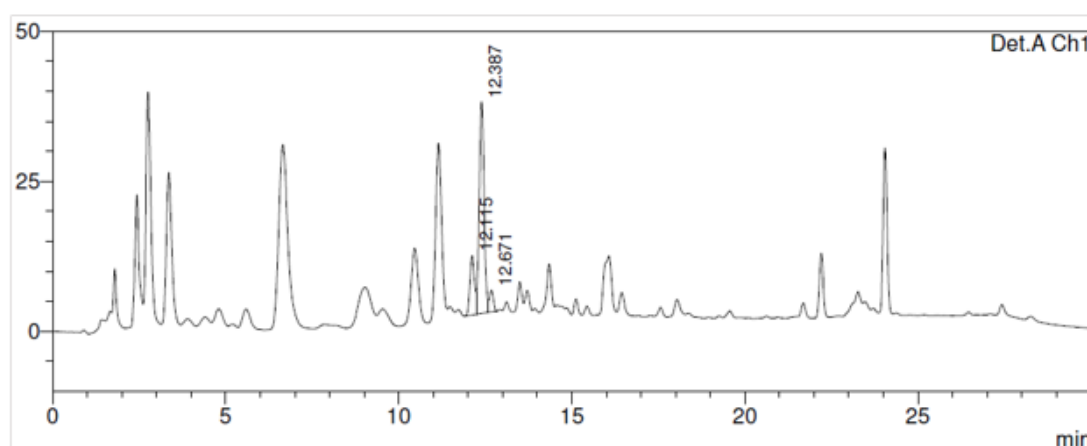


Figure 2b: HPLC chromatogram of hydroalcoholic extract of Chamomile flower extract

### Biological Efficacy of Chamomile Flower Extract

#### Free radical scavenging activity:

Free radicals are compounds with instability that can damage cells and have a role in the development of diseases like cancer, heart disease, and Alzheimer's. Antioxidants are chemicals that can assist in defending cells from this harm. Numerous studies have demonstrated the antioxidant efficacy of chamomile flower extract (Wang et al, 2020). The DPPH assay was employed to evaluate the hydroalcoholic chamomile extract's antioxidant capacity. The extract's IC<sub>50</sub> value was determined to be 31.23 µg/ml, which points to a considerable amount of scavenging activity (Table 2). According to our findings (Figure 3), chamomile flower hydroalcoholic extract would have a 64.9% free radical scavenging activity that is slightly less than Apigenin-7-O-glucoside activity (76.5%). So, the antioxidant effects of hydroalcoholic chamomile extract can be associated with the presence of Apigenin 7-O-glucoside. Menghini et al (2016) noted a similar effect in human adenocarcinoma HT29 cells lines and a model of a rat colon. Recent studies by Wang et al, (2020) and Sah et al., (2022) verified that apigenin can strongly inhibit the production of free radicals and associated damage in mice models.

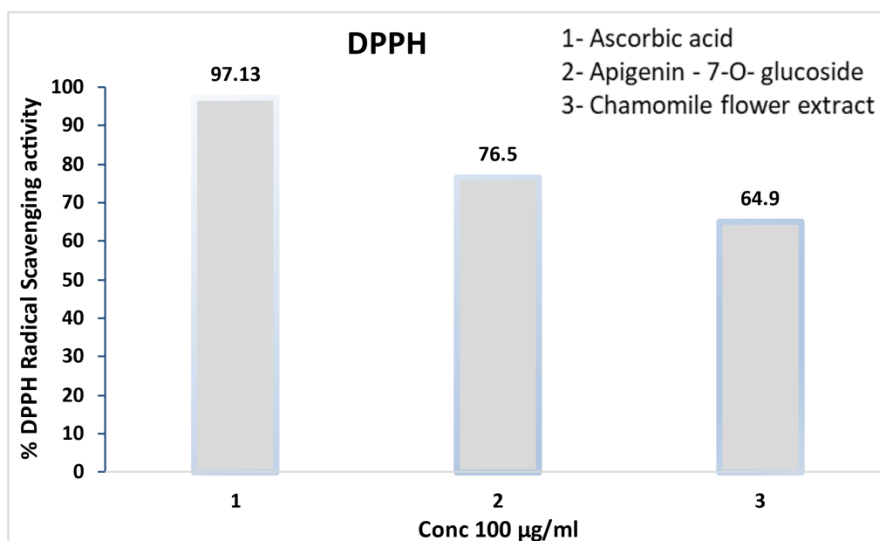


Figure 3: Graph representing the percentage free Radical scavenging activity of Chamomile flower

S. No	Name of the compound	DPPH IC <sub>50</sub> µg/ml
1.	Ascorbic acid	~ 7.12
2.	Apigenin-7-O-glucoside	~ 19.56
3.	Chamomile flower hydroalcoholic extract	~ 31.23

Table 2: Free radical scavenging activity of chamomile flower

*Anti-inflammatory Activity of Chamomile flower hydroalcoholic extract under In vitro condition*

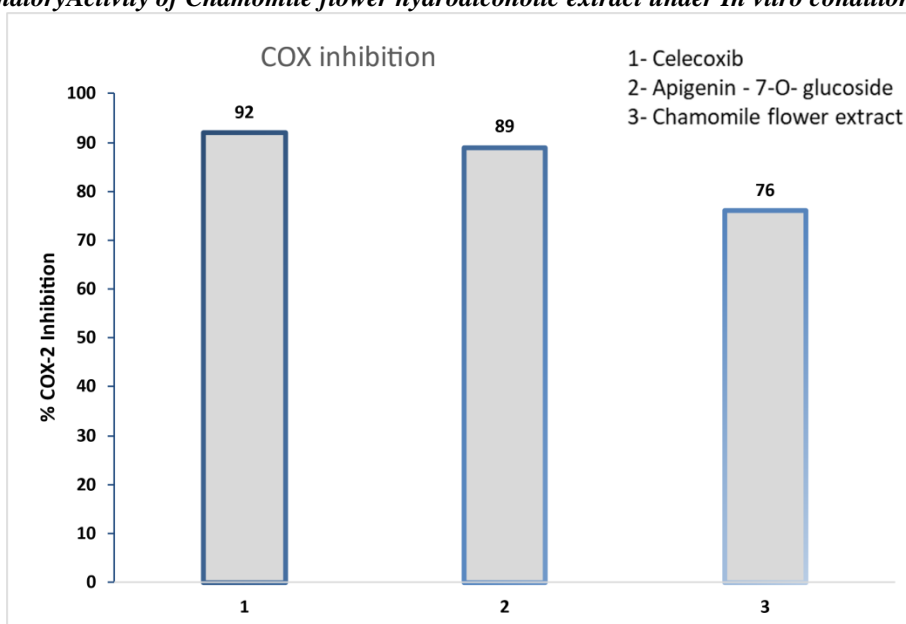


Figure 4: Graph representing the percentage of COX-2 Inhibition by hydroalcoholic extract of Chamomile flower

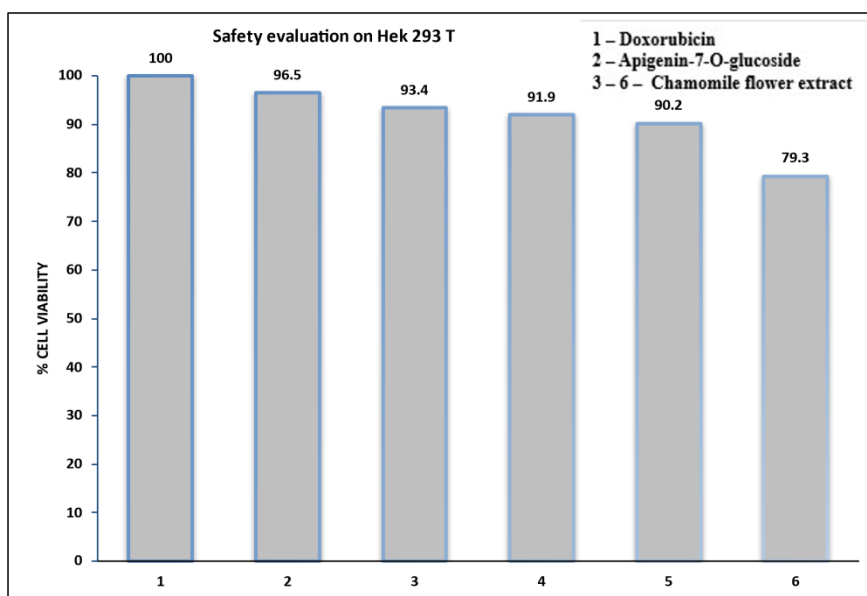
According to the present study's findings, the hydroalcoholic chamomile flower extract significantly reduces inflammation, by 76% COX-2 inhibition at 1 mg/ml. This implies that chamomile flower extract may have application as an anti-inflammatory. Although the COX-2 inhibitory activity of the Apigenin-7-O-glucoside reference standard and positive standard, celecoxib was higher at 89% and 92% at the same concentration (Figure 4). It is significant to remember that chamomile flower extract is a complex mixture of compounds and not just one like the reference standard. In light of this, the chamomile flower extract may have a more all-encompassing anti-inflammatory impact that may be beneficial in the treatment of a variety of inflammatory disorders. Tubaro et al., (1984) evaluated the anti-inflammatory activity of a chamomile extract



and found it useful in topical application. Later Srivastava et al (2009) found it as a selective inhibitor of COX-2. Many studies have revealed that it can lower inflammation in animal models of arthritis and inhibit the production of cytokines, which are pro-inflammatory (Weber et al., 2020). Furthermore, it has been discovered that chamomile flower extract has strong antioxidant activity, which could also donate to its anti-inflammatory properties (Talebi et al., 2022).

#### **Safety Evaluation of Hydroalcoholic Extract**

The safety evaluation of hydroalcoholic chamomile flower extract indicates that it is generally safe for use in cosmetic, personal care, and pharmaceutical products. We determined it as safe and non-toxic against Hek293T cells cytotoxicity assay when correlated to Apigenin-7-O-glucoside reference standard, with a concentration of >1 mg/ml IC50 value. The apigenin-7-O-glucoside reference standard was also found to be safe and non-toxic against Hek293T cells with a concentration of 0.51 mg/ml IC50 value (Figure 5). Srivastava et al (2009) also supported our study by finding 40µg/ml of chamomile flower methanol extract as the half maximum inhibitory dose for epidermoid carcinoma KB cells and melanoma SK-MEL-2 cells. Additionally, the chamomile flower showed cytotoxic activity against a variety of cancerous cells at higher doses only (Mati et al., 2013). As a result, it's high concentration can have negative effects which restrict its use in clinical settings. This implies that the chamomile flower extract has a large safety margin and is not cytotoxic at the measured quantities.



**Figure 5: Graph illustrating the percentage inhibition of proliferation of Hek293T cells on treatment with Hydroalcoholic extract of Chamomile flower**

- (1) Doxorubicin (1mM/ml); (2) Apigenin-7-O-glucoside (10mg/ml); (3)-(6) hydroalcoholic chamomile flower extract in different concentrations (1, 2.5, 5, 10 mg/ml).

#### **IV. CONCLUSION**

The present study concludes that the hydroalcoholic extract of the Chamomile flower has high total polyphenol and flavonoid content which are responsible for the biological benefits and therapeutic effectiveness of chamomile flowers. The presence of a high quantity of Apigenin-7-O-glucoside makes chamomile extract a viable option for creating all-natural medicines because it has high anti-inflammatory, anti-oxidant activity as well as low cytotoxicity effect.

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