Journal of Neonatal Surgery
ISSN (Online): 2226 -0439
Vol. 14, Issue 07 (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Quantitative Estimation of Quercetin by HPTLC And Exploring Anti-Inflammatory and Anti-Cancer Potential of *Althaea Officinalis Linn.*

Annu Saini[1], Meenu Bhan[1*]

[1]Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana-124001
***Corresponding Author:**
Meenu Bhan

Cite this paper as Annu Saini, Meenu Bhan, (2025) Quantitative estimation of quercetin By HPTLC and exploring anti-inflammatory and anti-cancer potential of *Althaea officinalis Linn*. *Journal of Neonatal Surgery*, 14 (07), 263-275.

ABSTRACT

The objective of the study aims to explore the medicinal importance of *Althaea officinalis* Linn as anti-inflammatory and anti-cancer agent. Molecular docking, Preliminary Phytochemical screening, quantification of quercetin by High Performance Thin Layer Chromatography, in-vitro anti-inflammatory and anti-cancer activities of *Althaea officinalis* root extracts were done. Docking of ligands with different target proteins was performed by schrodinger software. Aqueous extract was prepared by different extraction techniques like maceration, UAE and MAE. High Performance Thin Layer Chromatography (HPTLC) was used for the quantification of quercetin. In-vitro anti-inflammatory screening was performed by Hyaluronidase inhibition assay and Human Red Blood Cell stabilization assay. The anti-cancer activity of extracts was evaluated by MTT assay using A431 cell line. MAPK, Braf, P53, PD1 and COX-2 anti-inflammatory and anti-cancer target proteins were used for molecular docking of quercetin and it showed best docking score -4.00564 against PD1. The minimum 26.732±0.5 %w/w and maximum 52.76±.03% w/w extraction yield was found in aqueous extract. Quercetin was quantitatively determined by HPTLC and found maximum quantity in MAE extract 2.513µg/ml. In-vitro anti-inflammatory screening was performed by Hyaluronidase inhibition assay and Human Red Blood Cell stabilization assay. The maximum percentage of inhibition (96.77+1.22%) in Hyaluronidase assay and (98.39+2.1%) in HRBC assay was observed in MAE extract at higher concentration. The anti-cancer activity of extracts was evaluated by MTT assay using A431 cell line. The IC50 value of UAE extract was found to be 88.12µg/ml and IC50 of maceration, MAE extracts were 92.46µg/ml and 98.94µg/ml.

Keywords: Althaea officinalis, HPTLC, Maceration, Ultrasound assisted extraction and Microwave assisted extraction, Anti-inflammatory, Anti-cancer activity.

1. INTRODUCTION

Althaea officinalis is a perennial herb belongs to family Malvaceae native to Europe, U.S.A. western Asia and Iraq [1]. It grows between 60 to120cm high. It has primary and secondary roots, short petiolate leaves with ovate and acute blades, and light pink-white flowers that usually appear in terminal or axillary clusters with five heart-shaped petal [2]. The chemical constituents isolated from *A. officinalis* are starch (25–35%), pectins (11%), saccharose (10%), mucilage (5%), flavonoids, caffeic acid, p-coumaric acid, iso-quercetin, coumarins, phytosterols, tannins, and amino acids [3].

Pharmacological activities of *A. officinalis* are antimicrobial, anti-inflammatory, immunomodulatory, demulcent, soothing, anti-tussive, anticancer etc. [4,5]. It has been historically used to treat a variety of diseases such as gastritis, burns, insect bites, inflammation, ulcers, abscesses, constipation, diarrhea, and irritation of oral and pharyngeal mucosa. Research on *A. officinalis* over the last few decades has focused primarily on its biological properties and crude extracts [6]. Our research focus on docking study, quantification of quercetin and *in-vitro* anti-inflammatory and anti-cancer activities.

2. MATERIAL AND METHODS

Chemicals and Reagent: ethanol, methanol, distilled water , o-toulidine, toulene, ethyl acetate, formic acid, acetic-acid DMSO, sodium Phosphate Buffer, calcium chloride, hyaluronic acid solution, albumin serum, sodium acetate, quercetin, Alsever solution, isosaline solution, sodium phosphate buffer, diclofenac sodium, Hyaluronidase injection IP 1500 IU (Shreya Life Sciences Pvt. Ltd.), procured from Central Drug House Pvt. Ltd and Fluka, fetal Bovine Serum(Gibco), MTT Reagent, D-PBS, doxorubicin were procured from Sigma aldrich.

2.1 Plant collection and authentication

The roots of *Althea officinalis* were collected from Khari Baoli district of Delhi. The roots were identified and authenticated by Dr. Sunita Garg, former Chief Scientist and R. S. Jayasomu Chief Scientist, Raw Material Herbarium and Museum, CSIR-NIScPR, (National Institute of Science Communication and Policy Research), Delhi with authentication no. 4238-39-2.

2.2 Molecular docking

Protein preparation: 3-D crystal structure of molecular target proteins for anti-inflammatory and anti-cancer activities MAPK (PDB ID: 17GS), Braf (PDB:5ITA), P53(PDB:4A9Z), PD1(PDB:6KOY), COX2(PDB:5GMN) were downloaded from Protein Data Bank (www.rcsb.org). Hydrogen molecules were added after deleting pre-existing ligands and water molecule [7].

Ligand Preparation: The chemical constituents found in *A. officinalis* were used as ligands. The 2D structure of quercetin was obtained from Pubchem database. The ligands were obtained from Protein Data Bank (PDB) and saved in SDF Files format [8].

Docking: The receptor grid was generated between the prepared protein and ligand and run for docking**.** Docking was performed by using software schrodinger.

2.3 Extraction

Maceration: Fifty gram of powdered roots of *A. officinalis* were taken and added 500 mL of water in conical flask with occasional stirring for 7 days. Then, extract was filtered by filter paper and removed the solvent by rotary vacuum evaporator IKA with the water bath temperature of 45˚. Finally, the extract (residue) was collected [9].

Ultrasound Assisted Extraction: The UAE was carried out in Probe sonicator as shown in **"Fig. 1".** The powdered root was kept in direct contact with ultrasonic waves, solid to solvent ratio 1:8 gm/ml for 90 min at 25°C. The obtained extract was filtered through the Whatman filter paper and the filtrate was concentrated using rotary evaporator. Finally, the residues were collected [10]

Fig. 1 Ultrasound Assisted Extraction

Microwave Assisted Extraction: The extraction was performed in a closed vessel by using CEM microwave as shown in **"Fig. 2"**. The extraction of *A. officinalis* was done with temperature 90°C, microwave power 300W and solid to solvent ratio 1:7 for 10min. at 90°C and the mixture obtained was filtered with filter paper. The filtrate was dried at 60 °C using rotary evaporator [11].

Fig 2: Microwave Assisted Extraction

2.4 Preliminary phytochemical screening:

The phytochemical screening of aqueous root extracts was performed after extraction by three different extraction methods (Maceration, UAE and MAE). The aqueous extracts of *A. officinalis* were examined to determine the presence of various secondary metabolites including alkaloids, tannins, glycosides, flavonoids, and saponins [13,14]. The positive tests were noted as present (+) and absent (-).

2.5 HPTLC Finger printing profile for various extracts:

Instrumentation: The HPTLC was performed on 200x100mm pre-coated silica gel 60F 254 HPTLC plate (E. MERCK KGaA). The instrument used was CAMAG HPTLC system with a LINOMAT 5 applicator with a 100µl syringe, a CAMAG TLC scanner and vision Cats software.

Preparation of standard stock solution of quercetin

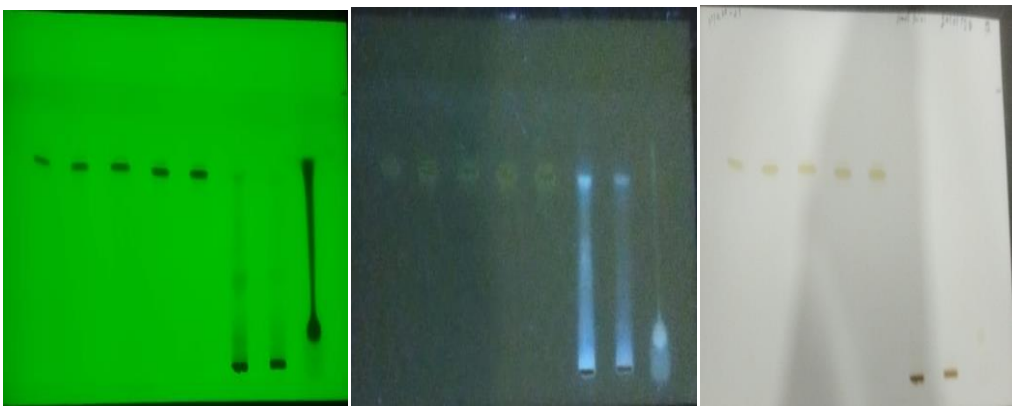
5 mg of quercetin was dissolved in 50ml methanol to get a concentration of 100µg/ml. Subsequently, the mixture was passed using a 0.45µm microfilter.

Preparation of samples

All the three extracts of *A. officinalis* prepared by maceration, UAE and MAE were dissolved in 10mg extract and 10ml HPTLC grade ethanol and filtered through a 0.45µm microfilter.

Condition of HPTLC and Quantification procedure: The sample solution was placed on plate as bands using a CAMAG Linomat applicator equipped with a 100µl syringe. The chromatogram was developed using a mobile phase Toluene: ethyl-acetate: methanol: formic acid (4.9:4.1:2:0.5 V/V/V) in a development chamber(20x10mm). The chamber was saturated with the mobile phase for 20 min. before development and dried the plate for 5min. at room temperature [14].

The CAMAG TLC densitometer with vision CATs software was used to do densitometric scanning. The analysis was conducted with slit dimensions of 6 x 0.45 mm, at a scanning rate of 100 mm/sec. The images were taken in white light, at 254 nm (short UV) and 366 nm (long UV) as shown in **"Fig. 3".** The CAMAG visualizer was used to see the bands. The bands were visualized in CAMAG visualizer. The concentration of the quercetin was calculated using a linear regression equation [15].



A **B** **C**

Fig. 3: HPTLC chromatograms visualized under A UV 254 nm, B UV 366 nm and white light

2.6 Anti-inflammatory activity:

Hyaluronidase inhibition assay: 5mg of plant extract was dissolved in 250µL DMSO. Samples were dissolved in sodium phosphate buffer (200 mM, pH 7) to prepare different concentrations. Hyaluronidase (1500IU, 100 µL) has been added to the sample solution (25 µL), and incubated at 37°C for 10 minutes. calcium chloride 12.5 m, 50 µL was added for the enzyme activation and incubate it for 20 min. at 37°C [16,17]. Subsequently, a hyaluronic acid solution (0.03% in 300 mM sodium phosphate, pH 5.4, 100 µL), was added as substrate to start the reaction, and incubated for 45 min. at 37 °C. The undigested hyaluronic acid was precipitated by using Acid albumin solution (0.1%) in sodium acetate (24 mM), pH 3.8, 1 mL). After incubation at room temperature for 10min. absorbance was measured at 575 nm. The absorbance measurement without the enzyme was taken as control for maximum inhibition. Quercetin was used as control [18]. The percentage inhibition was calculated by the formula:

Percentage inhibition = Abs sample/ Abs control X100

Human red blood cell (HRBC) membrane stabilization assay: The blood of a healthy human volunteer was mixed with the same quantity of Alsever solution then centrifuged at 3,000 rpm. The 10% isosaline solution was used to wash the packed cells. Different concentrations (200,400,600,800 and 1000µg/ml) of extracts were prepared with the help of distilled water. Two ml of hyposaline, one ml of phosphate buffer, and half ml of HRBC suspension were added in one ml of plant extract and incubate it at 37℃, for 30 minutes, centrifuged for at 3,000 rpm for 20 minutes. A control was made without the plant extracts and diclofenac (1mg/ml) was taken as reference standard [19]**.** The percentage stabilization of membrane was determined by the given formula:

% membrane stabilization = 100- O.D of sample /O.D of controlx100

2.7 Anti-cancer activity:

MTT cell viability assay:

Culture of cell lines: The NCCS in Pune, India, provided the human skin cancer cell line A431. The A431 cells were cultured every 2-3 days in a CO2 incubator at 37°C in DMEM with high glucose medium supplemented with 10% FBS and 1% antibiotic-antimycotic solution in an atmosphere of 5% CO_2, 18–20% O_2.

200µl cell solution was added to each well of 96-well plates at a density of 10,000 cells per well without the test agent and kept it for 24hrs. Prepare 1mg/ml conc. of extracts (20, 40, 60, 80, 1000µg/ml) by dissolving in 1ml of culture media and incubate plate at 37°C for 72hr in a 5% CO2 environment. After 72hrs discard the culture media and add 0.5mg/mL MTT reagent and again incubate it for 3hrs. The crystals of formazan were mixed in 100 µl of DMSO after MTT reagent transformed off and determined the absorbance at 570nm using a spectrophotometer or an ELISA reader [20]. The formula used to determine the % cell viability:

% cell viability= OD of treated cells/OD of Untreated cell

3. RESULTS AND DISCUSSION:

3.1 Molecular docking:

The docking study of Quercetin was performed against P53 (4A9Z) Cyclooxygenase enzyme 2 (COX-2) (PDB ID: 5GMN), MAPK (17GS) Braf (5ITA) and PD1. The 2D interaction of quercetin with 4A9Z, 5GMN, MAPK, 5ITA, 6KOY is given in **"Fig. 4"**. Quercetin showed best docking score against 6KOY among 17GS, 5ITA, 4A9Z and 5GMN in **Table 1.**

Table 1: Docking results of Quercetin with MAPK (17GS), Braf(5ITA), P53(4A9Z), PD1(6KOY), Cox-2(5GMN)

Compound name	CID	PDB ID	Docking Score
Quercetin	**5280343**	17GS	-3.892151033
		5ITA	-3.655371916
		4A9Z	-2.5915
		6KOY	**-4.00564**
		5GMN	-3.265730811

3.2 Percentage yield of *A. officinalis*

The Percentage yield of aqueous extract prepared by Maceration, UAE and MAE are shown in **Table 2.** The minimum 0.07574% w/w (UAE) and maximum yield 33.77% w/w (MAE) for aqueous extract was obtained.

Table 2: Percentage yield of *A. officinalis* root extract

Sr. No.	Extraction	Aqueous extract
1.	Maceration	26.732±0.5%w/w
2.	UAE	7.574±1.0%w/w
.	MAE	52.76±.03%w/w

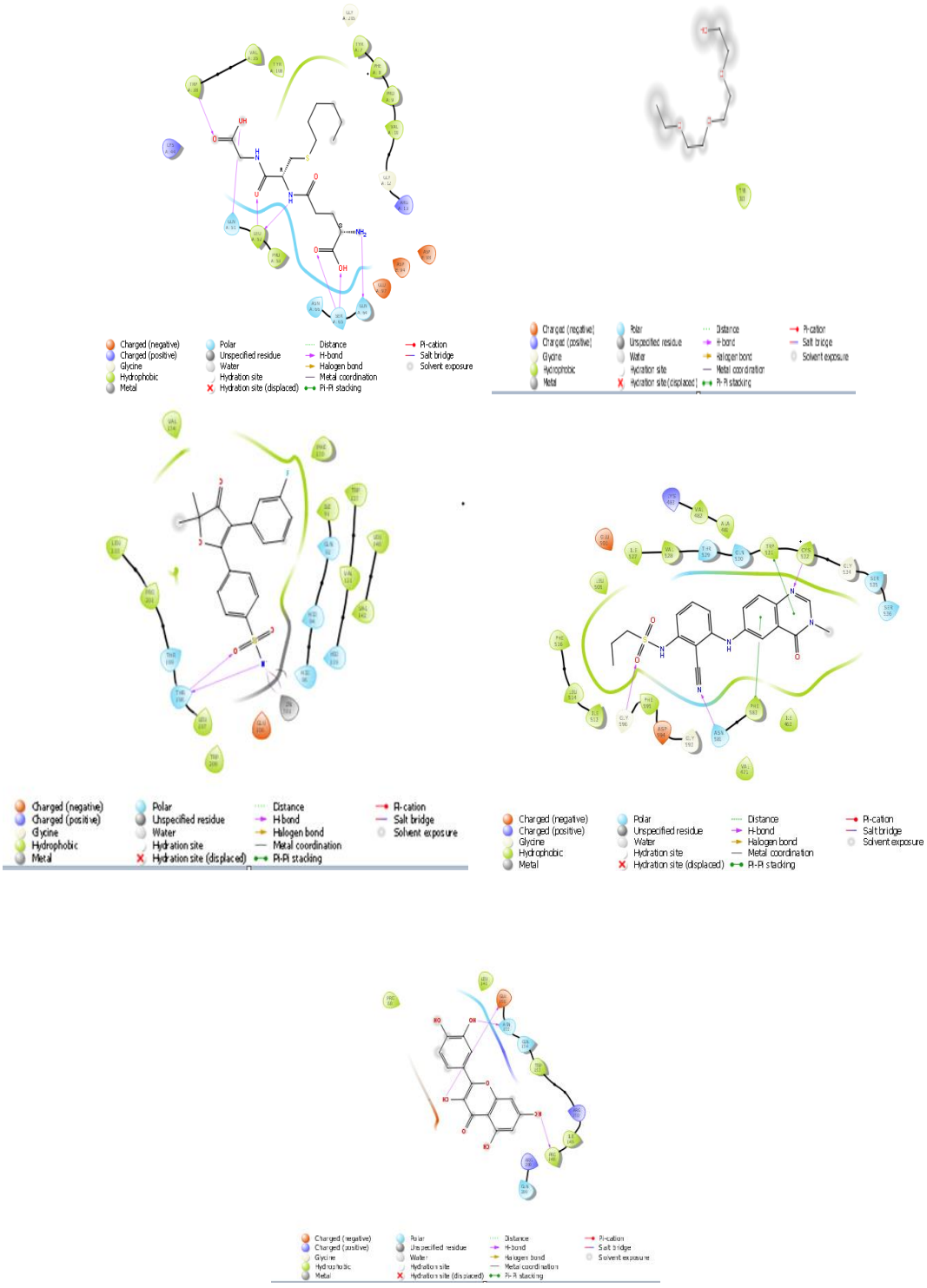


Fig: 4. 2D interaction of quercetin with 4A9Z, 5GMN, MAPK, 5ITA, 6KOY

3.3 Preliminary phytochemical screening of *A. officinalis* extract

The preliminary qualitative phytochemical screening of the prepared extracts by Maceration, UAE and MAE of *A. officinalis* extracts were performed to determine the presence of active constituents. The screening results showed the presence of alkaloids, flavonoids, carbohydrates, proteins, tannins and phenolic acid in the aqueous extract as shown in **Table 3.**

Table 3: Preliminary Phytochemical screening of *A. officinalis* extract

	Constituents	**Tests**	**Maceration**	**UAE**	**MAE**
1.	Alkaloids	Dragendroff's Test	+	+	+
		Hager's Test	+	+	+
2.	Flavonoids	Alkaline Reagent Test	+	+	+
3.	Tannins and Phenolic compounds	Ferric chloride Test	+	+	+
		Lead Tetra acetic acid Test	+	+	+
4.	Proteins	Biuret Test	+	+	+
5.	Carbohydrates	Molisch Test	+	+	+
.		Benedicts Test	-	-	-
6.	Steroids	Salkowski Test	-	-	-
7.	Starch	Iodine Test	-	-	-

+ = present, - = absent

3.4 HPTLC fingerprinting profile of quercetin

The component quercetin in the standard **"Fig. 4"** and extracts **"Fig. 5 (A, B, C)**" was determined to have an Rf value of (0.7). Plotting the peak area graph and the quercetin concentration in A. officinalis root extracts revealed a linear relationship; from the **"Fig. 6"** an equation of linear regression was obtained. Amount of β-sitosterol was calculated by the equation in three extracts that is maceration, UAE and MAE [21]. HPTLC plate and 3D chromatogram of Quercetin and extracts at 254nm are given in "**Fig. 7".**

Quantification of Quercetin: Quantification of quercetin in *A. officinalis* was done by developed and validated HPTLC method. The maximum concentration of quercetin was found in MAE extract (2.513 µg/ml) followed by UAE (1.925 µg/ml) and Maceration (0.75 µg/ml). Therefore, it can be concluded that *A. officinalis* roots are major source of quercetin and highest concentration was obtained in MAE extract as given in **"Table 4".**

Table 4: Conc. of quercetin in *A.officinalis* extract

Sr. No.	**Extraction**	**Conc. of quercetin**
1.	Maceration	0.75µg/ml
2.	UAE	1.925µg/ml
3.	MAE	2.513µg/ml

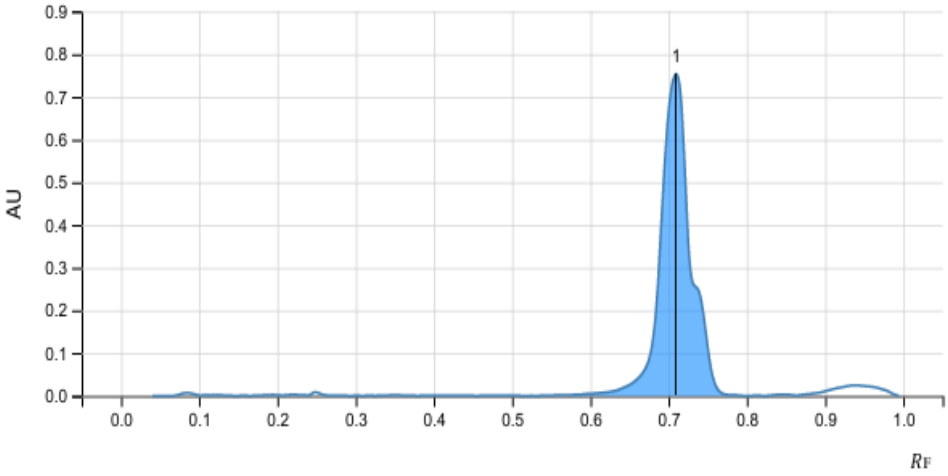
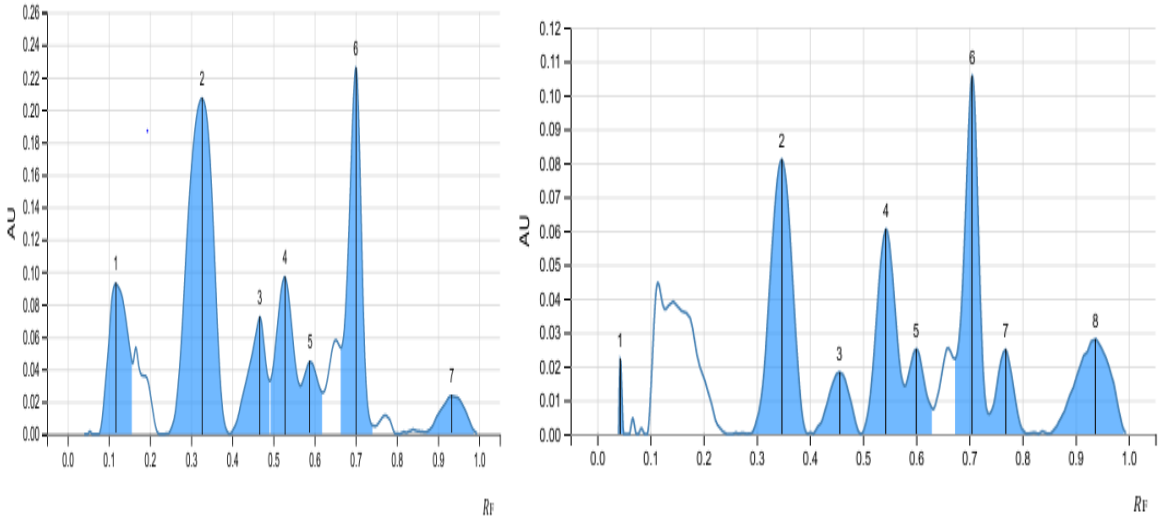
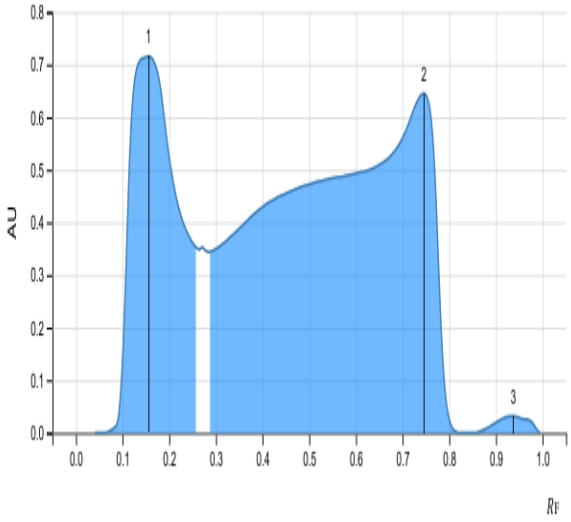


Fig 4. HPTLC chromatogram of standard Quercetin at 254nm



A **B**



C

Fig 5. A, B, C HPTLC chromatogram of aqueous extract of *A.officinalis* prepared by maceration, UAE, MAE at 254nm

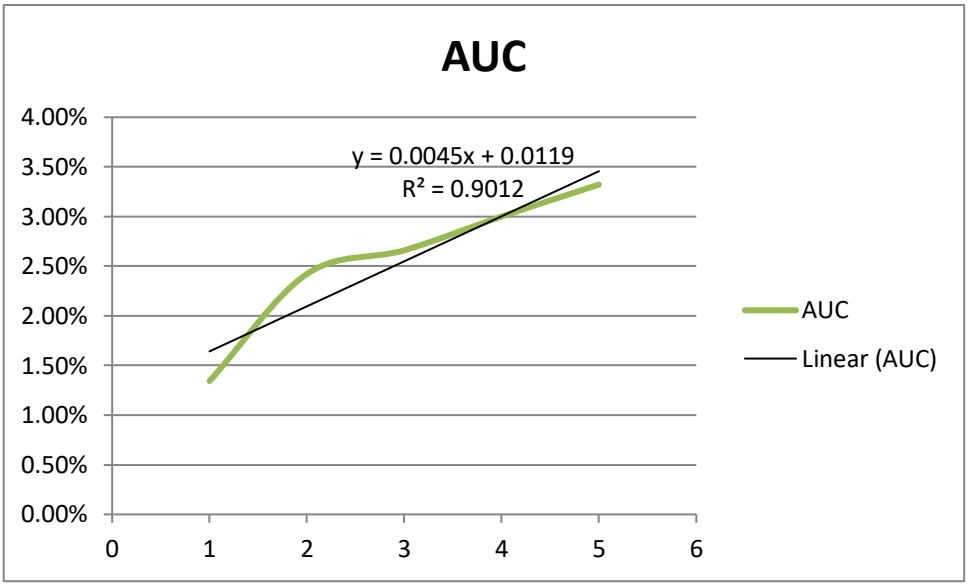


Fig 6. Calibration curve of Quercetin

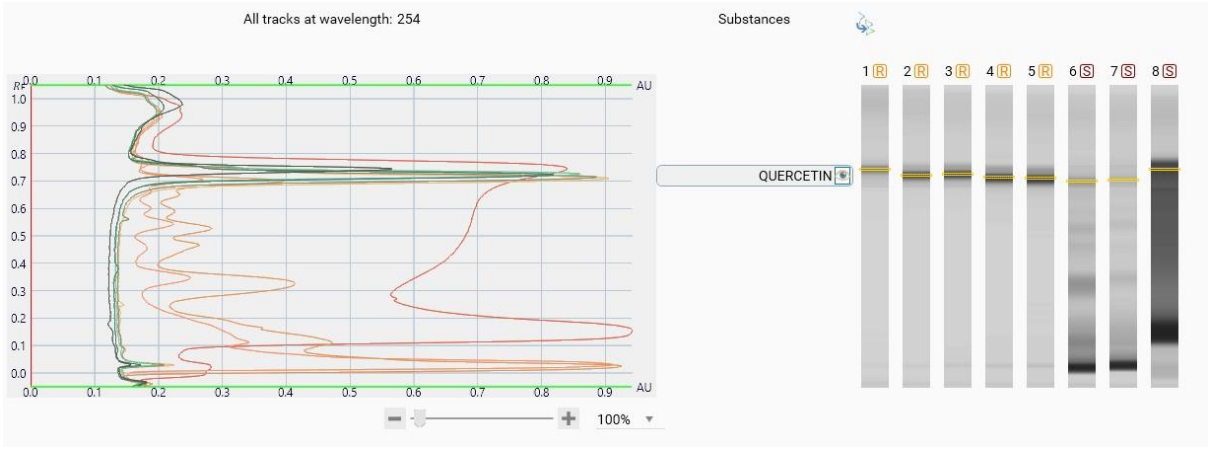


Fig 7. HPTLC plate and 3D chromatogram of Quercetin and extracts at 254nm

3.5 Hyaluronidse inhibition assay:

The anti-inflammatory effect of extracts was evaluated using Hyaluronidase inhibition assay. The percentage inhibition of standard and extracts at concentration range 200-1000 µg/ml are given in **Table 5**. The UAE extract possessed maximum percentage of hyaluronidase inhibition 48.52±1.44% as compared to standard 37.40±1.88%. Effect of *A. officinalis* extracts on %age inhibition is given in **"Fig. 8"**

Table: 5. *In vitro* anti-inflammatory activity of *A. officinalis* extract by Hyaluronidase inhibition assay

Conc.	Standard	Maceration	UAE	MAE
200µg	20.14±1.43%	18.84±7.88%	12.41±1.56%	19.39±1.01%
400µg	30.13±2.48%	26.04±0.89%	32.47±4.02%	19.73±1.70%
600µg	34.52±2.71%	26.33±1.22%	35.64±1.57%	19.83±1.12%
800µg	36.17±1.13%	30.20±1.50%	37.08±2.30%	19.92±1.12%
1000µg	37.40±1.88%	37.29±1.03%	48.52±1.44%	27.83±1.05%

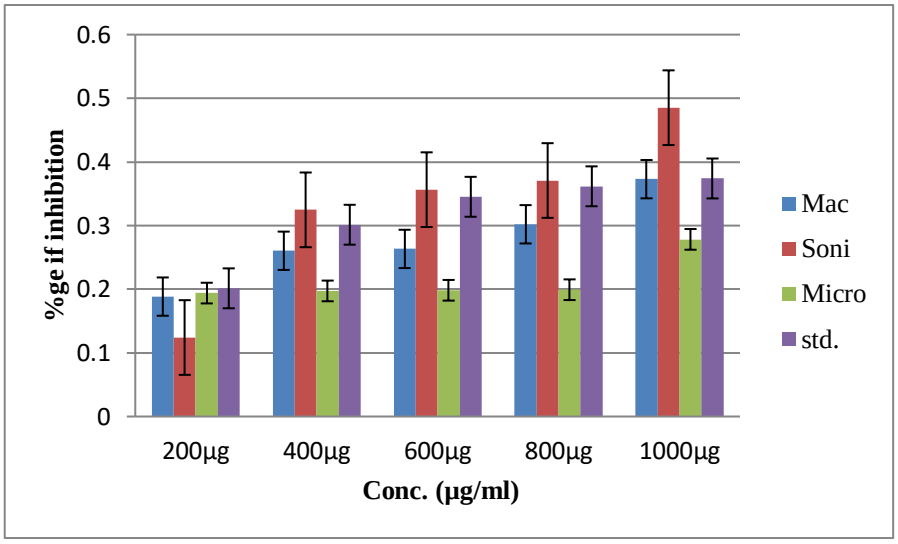


Fig: 8. Effect of *A. officinalis* extracts on %age inhibition

In-vitro anti-inflammatory activity of *A. officinalis* using hyaluronidase inhibition assay representing the % inhibition of Mac, UAE and MAE extracts and standard.

3.6 HRBC assay

The HRBC membrane stabilization test was used to assess the anti-inflammatory effect of extracts in comparison to standard diclofenac. The extract (MAE) exhibited maximum percentage inhibition of haemolysis/stabilization of blood cell 99.16±0.132% compared to diclofenac 64.12±1.05% at concentration of 800µg/ml as given in **"Table 6".** Effect of *G. diversifolia* extracts on %age inhibition of haemolysis is given in **"Fig. 9".**

Table: 6. *In-vitro* anti-inflammatory activity of *A. officinalis* extract by HRBC

Conc.	Standard%	Maceration%	UAE %	MAE%
200µg	43.14±0.14	20.69±0.26	72.02±0.86	76.22±0.55
400µg	52.44±0.65	37.74±0.456	76.22±0.122	92.93±0.102
600µg	55.13±0.72	42.16±0.86	81.46±0.132	96.38±0.144
800µg	64.12±1.05	43.40±0.103	81.74±0.144	99.16±0.132

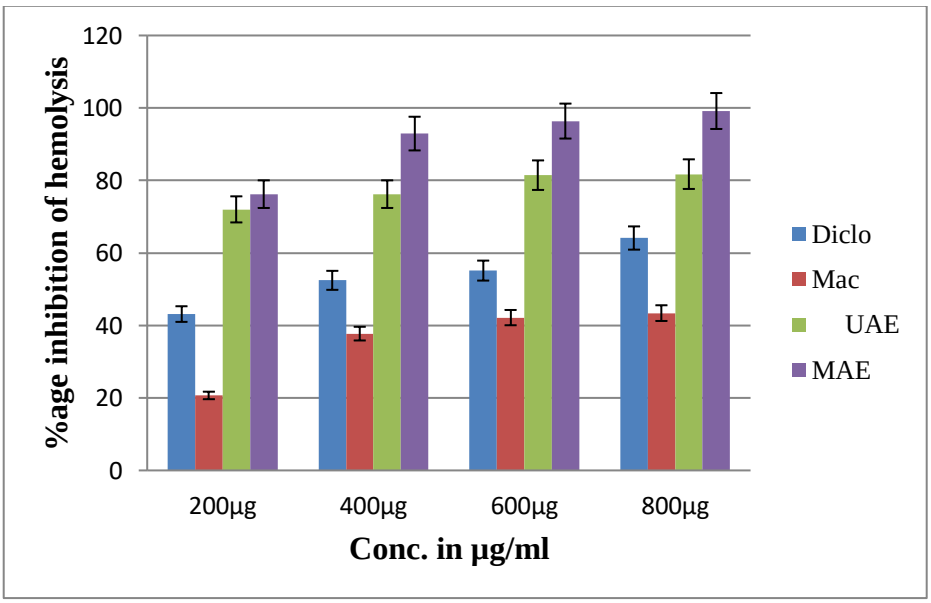


Fig: 9. Effect of *A. officinalis* extracts on %age inhibition of hemolysis

In-vitro anti-inflammatory activity of *A. officinalis* using a human red blood cell membrane stabilization model, representing the % inhibition of Mac, UAE and MAE extracts and the standard drug (diclofenac).

3.7 Anti-cancer activity:

The MTT assay demonstrated that the aqueous extract of *A. officinalis* had anti-cancer activity on A431 cell line [22]. As indicated in **Table 7**, the results showed a concentration-dependent decline in the extracts' percentage cell viability (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml). The extract at 100µg/ml was the most efficient of the five concentrations in causing a 50% inhibition of cell viability in **"Fig. 10"**. The morphological alterations in the cell exposed to the drug at different doses are illustrated in **"Fig. 11".**

Table 7: Effect of conc. on %age cell viability

A. officinalis conc.(µg/ml)	%age cell viability
Doxorubacin (1 µg/ml)	47.72
20	92.26
40	83.33
60	65.81
80	55.80
100	44.25

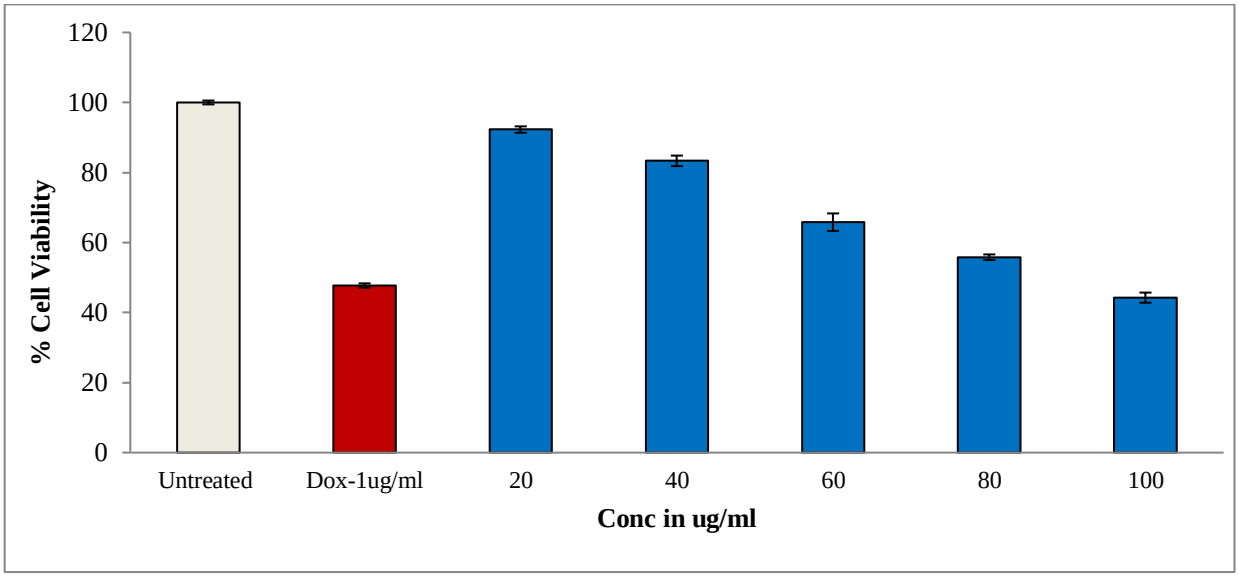


Fig.10: %age cell viability of A431 cells treated by different conc. (Maceration)

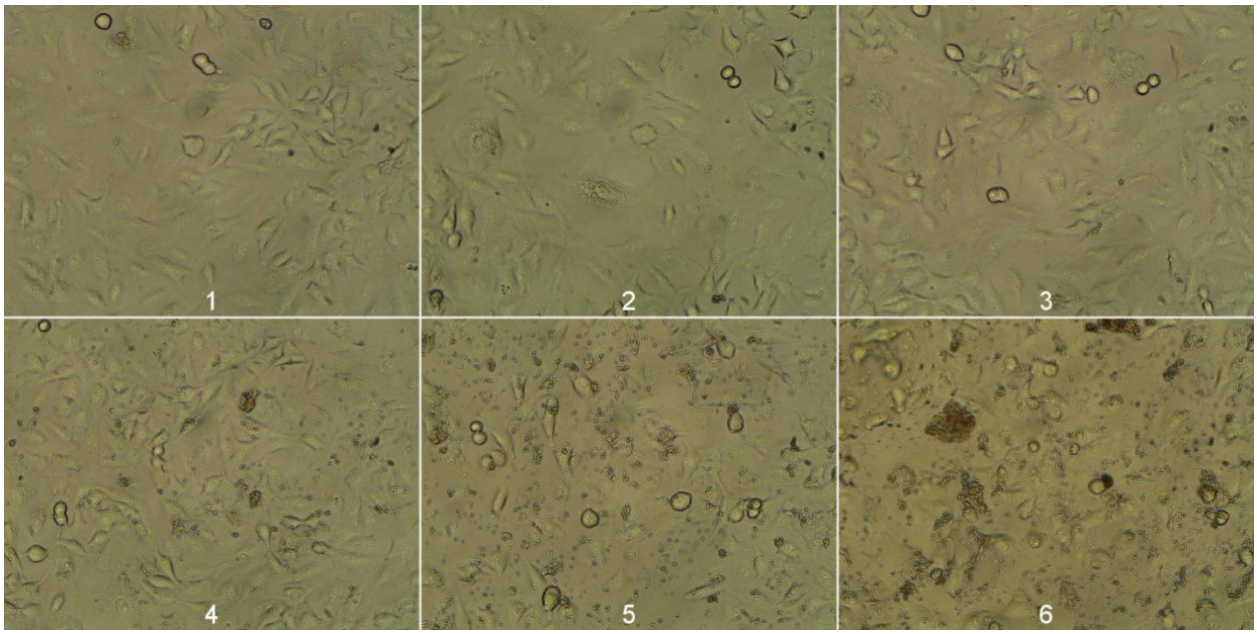


Fig. 11: The morphology of A431 cells treated by different conc. of extract Maceration (incubation period 72hrs)

4. Relationship between quercetin and observed activities:

Quercetin exhibits a number of pharmacological actions, such as anti-oxidant, anti-inflammatory, vasodilatory, and *in-vitro* anticancer properties. It's crucial to remember that it has no adverse health effects [23] It is well known that quercetin has anti-inflammatory effects. liver-resident macrophages (Kupffer cells) and invading macrophages (inflammatory infiltrate) both produces the inflammatory response. Tumor necrosis factor A (TNF-α) and interleukin-6 (IL-6) are two profibrogenic and proinflammatory cytokines that stimulate hepatic stellate cells (HSCs), the main producers of the thick extracellular matrix.

Effects of quercetin and its derivatives made from various plant extracts were compared by Carullo [24]. By decreasing ROS, inhibiting NF-κB, reducing levels of proinflammatory mediators, these substances demonstrated anti-inflammatory effects. Based on experimental models, quercetin can lower their gene expression by inhibiting the main transcriptional factor NF-KB for these cytokines [25].

Quercetin interacts with some of the primary intracellular signaling pathways related in cancer, it may have a carcinostatic effects. These pathways are identified as possible therapeutic targets as they promote the transcription of proteins necessary for the cell cycle to progress: Presently on the market the degree of C2–C3 instauration and the number of -OH groups in the B-ring appear to be important factors for assessing quercetin's unique biological activity [26].

The protein kinase C (PKC) pathway is another pathway that quercetin can affect. It is also downregulated [27]. This pathway can be blocked, by inhibition of DAG precursor which causes the entry of extracellular calcium and prevents the production of phosphatidylinositol (3,4,5) trisphosphate [28].

CONCLUSION

A.officinalis extract exhibited good anti-inflammatory activity against cellular inflammation. The aqueous extracts were also quantitatively analyzed by HPTLC for the estimation of quercetin. The MAE and maceration extracts demonstrated significant anti-inflammatory and anti-cancer properties and also have an excellent binding affinity towards the target receptors PD1 and MAPK in docking study. This information is helpful for extending our knowledge of the natural substance quercetin and exploring its potential uses in the production of new anti-inflammatory and anti-cancer therapies.

Acknowledgement: The author is grateful for Dr. Meenu, Assistant Professor, for her valuable guidance.

REFERENCES

1) Kadhum HH, Abdulkareem H. Abd, Al-Shammari AM. HPLC Analysis and Chemical Composition Identification of Isolated Flavonoid Fraction of Althaea Officinalis from Iraq. Technologies and Materials for Renewable Energy, Environment and Sustainability. 2019:1-6.

2) Farhat C, Younes H, Alyamani OA, El-Makhour Y, Dbaibo G, Mrad M, Hourani N, Hage-Sleiman R., Chemical characterization and in vitro biological evaluation of aqueous extract of Althaea officinalis L. flower grown in Lebanon. Journal of Herbal Medicine 2022;34:100575.
3) Bonaterra GA, Bronischewski K, Hunold P, Schwarzbach H , Heinrich EU, Fink C , Aziz-Kalbhenn H , Müller J. and Kinscherf R. Anti-inflammatory and anti-oxidative effects of phytohustil and root extract of Althaea officinalis L. on macrophages in-vitro. 2020;11: 1-14.
4) Al-Snafi AE. The pharmaceutical importance of Althaea officinalis and Althaea rosea: A review. Int J Pharm Tech Res, 2013;5(3):1387-1385.
5) Zhang , kong F, Zhang LLC., and Zhang R, Modulatory effect of Althaea officinalis L root extract on cisplatin-induced cytotoxicity and cell proliferation in A549 human lung cancer cell line. Tropical Journal of Pharmaceutical Research. 2016;15:2647-2652.
6) Xue TT, Yang YG , Tang Z, Duan J, Song ZX , Hu X, Hao-Dong Yang, Xu HB, Evaluation of antioxidant, enzyme inhibition, nitric oxide production inhibitory activities and chemical profiles of the active extracts from the medicinal and edible plant: Althaea officinalis. Food Research International. 2022;156:1-22.
7) Apeh VO., Asogwa E, Chukwuma FI, Okonkwo OF, Nwora F, Uke R. Chemical analysis and in silico anticancer and anti-infammatory potentials of bioactive compounds from Moringaoleifera seed oil. Advances in Traditional Medicine. 2022; 22:59–74.
8) Thamaraiselvi L, Selvankumar T, Wesely EG, Kumar NV. In-silico molecular docking analysis of some plant derived molecules for anti-inflammatory inhibitory activity. Current Botany 2021;12: 22-27.
9) Tripathi N, Kumar S, Singh R, Singh P and Varshney VK., Phytochemicals Isolation from Leaves of Girardinia Heterophylla. Journal of Biomedical and Pharmaceutical Research, 2013;2 (1) :71-77.
10) Benbassat N, Kostova B, Nikolova I and Rachev D. Development and evaluation of novel lozenges containing marshmallow root extract. ak. J. Pharm. Sci., 2013; 26(6):1103-1107.
11) Alara OR, Abdurahman NH, Olalere OA., Optimization of microwave-assisted extraction of flavonoids and antioxidants from Vernonia amygdalina leaf using response surface methodology. Food and Bioproducts Processing 2018;107:36–48.
12) Zohra SF, Meriem B, Samira S, Alsayadi M.M.S. Phytochemical Screening and identification of some compounds from Mallow. J. Nat. Prod. Plant Resour., 2012;2 (4):512-516.
13) Mishra PK, Gautam V, Sharma RK, Jain S, Mishra A, Dubey A, Tiwari A, Tripathi M, Tiwari A, Shrman K and Gond VK. Preliminary phytochemical evaluation of Emblica officinalis and quantification of gallic acid by HPTLC. International Journal of Advanced Biochemistry Research, 2024;8(6): 44-50.
14) Paul A, Chetia D and Zamanp Md. HPTLC quantification of garcinol in three endemic Garcinia species of Assam, estimation of secondary metabolites and evaluation of in vitro antioxidant activity. Acta Chromatographica 2024, 1-9.
15) Jeevalatha A, Kalaimathi RV, Basha AN, Kandeepan C, Ramya S, Loganathan T. Profile of bioactive compounds in Rosmarinus officinalis. Journal of Drug Delivery and Therapeutics, 2022;12(1):114-122.
16) Fariza IN. Anti-inflammatory activity of major compound of methanol extract of Phaleria macrocarpa leaves. Journal of Applied Sciences, 2012; 12(11): 1195-1198. 40. Cross ref.
17) Salvamani S et al. Anti-HMG-CoA Reductase, Antioxidant and Anti-Inflammatory Activities of Amaranthus viridis Leaf Extract as a Potential Treatment for Hypercholesterolemia. Evidence-Based Complementary and Alternative Medicine, 2016; 1-10.cross ref.
18) Sarveswaran R., Jayasuriya W.J.A.B.N, Suresh T.S. In vitro assays to investigate the anti-inflammatory activity of herbal extracts: a review. World Journal of Pharmaceutical Research. 2017; 6(17):131-148.
19) Sangeetha G., Vidhya R. In-vitro anti-inflammatory activity of different parts of Pedalium murex (L.) International Journal of Herbal Medicine 2016;4(3): 31-36.
20) Kamalanathan J, Venkatesh R, Swetha S, Sampath S, Ahmed MZ, Alqahtani AS, Suresh MS & Asaithambi P, Microwave-assisted green synthesis of silver nanoparticles using Cynoglossum furcatum extract: biomedical evaluation against A431 skin cancer cell line. Materials Technology. 2024;39(1):1-15
21) Lal M, Kadam A, Padole AS. HPTLC method development and validation of stigmasterol from different extracts of Tagetes erecta and Capsicum annuum. Journal of Drug Delivery & Therapeutics. 2019; 9(6):169-72.
22) Nizar A, Ravindran R, Palani J, Nripan T, Asha S. D, Meera K. Pynadath. Anticancer effects of ethanolic extracts of macaranga peltata leaves on human oral squamous carcinoma cell lines: An In Vitro Study. Journal of pharmacy and bioallied science. 2024;16(2):1-5.
23) Silva EP, Hermino VLD., Freitas FA, Souza C, Rodrigues L, Barbosa J, Piccolotto R., Maduro CIPNN, Macambira SG, Soares M, Biological activities of quercetin and other flavonols: A mini-review. Food Science and Technology. 2024;1678-457:1-6.
24) Carullo, G., Cappello, A. R., Frattaruolo, L., Badolato, M., Armentano, B., Aiello, F. Quercetin and derivatives: useful tools in inflammation and pain management. Future Medicinal Chemistry, 2017; 9(1): 79–93.
25) Wielgus M, Zaniewic N. Selected biological properties of quercetin, curcumin, and kaempferol. Folia Biologica et Oecologica 2024;18: 48–65.

26) Joyner, P.M. Protein Adducts and Protein Oxidation as Molecular Mechanisms of Flavonoid Bioactivity. Molecules 2021;26, 5102. [CrossRef].
27) Shah, S.; Narang, R.; Singh, V.J.; Pilli, G.; Nayak, S.K. A Review on Anticancer Profile of Flavonoids: Sources, Chemistry, Mechanisms, Structure-activity Relationship and Anticancer Activity. Curr. Drug Res. Rev. Former. Curr. Drug Abus. Rev. 2023;15, 122–148. [CrossRef]
28) Zhu,S.; Yu, W.; Bi, L.; Qin, F.; Li, J.; Zeng, H.; Lu, L. Quercetin induces apoptosis of human breast cancer cells by activiting PTEN and inhibiting PI3K/AKT and JNK signaling pathways. 2022, 38, 714–720. [PubMed]