ISSN:2229-6107

INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY

E-mail :
editor.ijpast@gmail.com
editor@ijpast.in

www.ijpast.in

INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY
IJPAST

ISSN 2229-6107 www.ijpast.in
Vol 13,Issue 1.March 2023

ISOLATION, CHARACTERIZATION, DOCKING AND ANTI-CANCER ACTIVITY OF QUERCETINFROMLEAVES OF*EUPHORBIAHETEROPHYLLA* LINN.

Ms. Nayela Ghazal[1], Dr S. Satyanandam[2], Mr. Mohammed Kafeel Urrahman Khan[3]

ABSTRACT: We conducted this work to identify and describe the bioactive component of Euphorbia heterophylla. Additionally, we tested the isolated molecule against the MCF-7 cell line, a breast cancer cell line, to determine its anticancer potential. Using column chromatography and ultra sonicator by gradient elution, the chemical was isolated from the ethanol extract. The foundation for establishing the structure of the isolated chemical wasanalytical procedures, spectroscopic evidence, and thin-layer chromatography. (infrared, 1H nuclear magnetic resonance, 13C nuclear magnetic resonance, liquid chromatography/tandem mass spectrometry, data base library search for identification). Using a solvent solution of n-butanol, acetic acid, and water (2:2:6) with an Rf value of 0.7, the yellow molecule exhibits a positive test for flavonoids in thin layer chromatography. Quercetin was determined to be the isolated chemical by the use of LCMS spectral library search system and TLC. Isolated quercetin was docked against 5, 10 methenyltetrahydrofolatesynthetase (MTHFS). You can see the results here. The development of breast cancer cells in human MCF 7 cells was demonstrated to be arrested by inhibiting (MTHFS), as shown by a docking score of -10.23. Therefore, quercetin was tested for its anti-cancer effects on the MCF-7 cell line in a controlled laboratory setting. The mean percentage of cytotoxicity was determined to be 89.77 and the percentage of viability to be 10.22 after 48 hours in a solution of 100 µg/ml.

Keywords:*Euphorbiaheterophylla*,MTT assay,Quercetin,Molecular Docking, 5,10-Methenyltetrahydrofolatesynthetase(MTHFS)

INTRODUCTION:

The branching shrub Euphorbia heterophylla L. is found all over the world, including in India, South Asian nations, Africa, Mexico, and Thailand. It is a member of the Euphorbiaceae family. Common names for this decorative plant include poinsettia, Mexican fire plant, Tamil Paalperuki, and many more. According to reports, this plant has anti-inflammatory, antibacterial, wound-healing, and anticancer properties. Constipation, bronchitis, asthma, laxative, and lactogenic agent are some of its traditional medical uses. 4.

The pigment porcetin gives the leaves their crimson hue. Results included protein, starch, coumarin, terpenoids, quinones, alkaloids, and sterol. Sugars, carbs, saponins, steroids, terpenoids, tannins, flavonoids, and alkaloids were collected from fresh leaves. In addition to being somewhat rich in protein and fiber, plants are excellent sources of water and energy. Anthraquinones, tannins, alkaloids, flavonoids, and phenols were the phytochemical components derived from the leaves. Alkaloids, flavonoids, tannins, sterols, quinones, lignin, and coumarin were some of the secondary metabolites extracted from the plant in an aqueous solution. Proteins (7.43 mg/g) and carbohydrates (10.29 mg/g) were detectable. It contained 3.26 mg/g of phenol and 1.14 percent ascorbic acid. 5.

Associate professor[1,2] Assistant professor[3]
Department of Pharmaceutical Chemistry,
Global College of Pharmacy, Hyderabad. Chilkur (V), Moinabad (M), Telangana- 501504

INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY
IJPAST

ISSN 2229-6107 www.ijpast.in
Vol 13,Issue 1.March 2023

Traditional African medicine and other tropical nations make extensive use of Euphorbia heterophylla. Traditional African medicine uses a mixture of the plant's stems and either fresh or dried leaves to alleviate constipation, ease stomachaches, and flush out worms from the digestive tract. Washing with a leaf infusion helps with a variety of skin issues, including as fungal infections and abscesses. 6. In Nigeria, skin tumors are treated with latex and concoctions made from the plant's leaves and roots. The East Africans utilize the roots to cure gonorrhea and encourage more milk supply in nursing mothers. Even though it may be used as a rubefacient and to eradicate warts, latex is irritating to the eyes and skin. On the other hand, if you're allergic to the latex of other species of Euphorbia, you may use this latex as an antidote. To alleviate aches and pains, people in peninsular Malaysia ingest an extract from a leaf. Arrow poison and fish poison are both made using latex. There have been reports that the leaves of E. heterophylla contain quercertin. There have been reports of diterpenoids in E. heterophylla root as well. 8. E. heterophylla leaf linn 9. There have been reports of the skin-irritant, tumor-promoting, anti-tumour/cancer, and, more recently, anti-HIV, properties of Euphorbia species.

A study was conducted in Chennai, Tamil Nadu, India, using materials and methods that included collecting the leaves of Euphorbia heterophylla and preparing an extract. Dr. SasikalaEthirajulu, a botanist and research official at the Central Plant Research Authority of Tamil Nadu (CCRAS), Chennai, India, verified the identity of the plants and their authenticity. To prepare the leaves for grinding, they were dried in the shade, separated, and then put through a 40-mesh sieve. The soxhlet apparatus, which uses ethanol as a solvent, was used to extract the dried powder of the leaves. A rotary evaporator was used to concentrate the extract.

Separation and Analysis: The ethanol extract was separated using the wet packing process and then submitted to column chromatography. At the beginning, hexane fills three quarters of the column.

proceed to gradually include silica gel (100 - 200 mesh size) in order to get consistent packing. A gradient elution was used to chromatograph 12 gm of ethanol extract over a 240 gm silica gel column. For the column's development, we eluted it with 100% hexane, then with various ratios of hexane to chloroform (80:20, 70:30, 50:50, 30:70), and finally with 100% chloroform. Mixed with methanol in the following ratios: 80:20, 70:30, and 50:50).

Purification and Extraction by Ultrasonication: There are many health advantages associated with quercetin and quercetin glycosides, which are molecules of flavonols that are physiologically active. The polarity of the solvent used for extraction is one of the variables that affect the success of removing these compounds from plant matrices. The majority of the quercetin and glycosides were recovered by methanol, even though it was one of many solvents tested with different dielectric constants. The ideal conditions for extracting quercetin and its glycosides when using ultrasonication were 15 minutes of exposure to certain ultrasound wavelengths. 10.

Action to take: The ratio of chloroform to methanol is 70:30. Using a ratio of 2:2:6, the fraction containing flavonoids (n-butanol, acetic acid, water; toluene, ethyl acetate, formic acid, methanol; 5.5:3:1:0.5) demonstrates a positive reaction in thin-layer chromatography. At an Rf value of 0.85 for UV and 0.7 for iodine, it reveals the presence of blue and reddish-brown fluorescence spots, respectively. In order to extract flavonols linked to quercetin, the aforementioned fraction was chosen.

The fraction (Chloroform: methanol 70:30) was subjected to 15 minutes of ultrasonic

INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY
IJPAST
ISSN 2229-6107 www.ijpast.in
Vol 13,Issue 1.March 2023

wavelengths in order to assist the extraction process. A total of 30 ml was used, with various fractions collected, using various chloroform: methanol ratios, including: 28:2, 26:4, 24:6, 22:8, 20:10, 18:12, 16:14, 14:16, 12:18, and 10:20. The results showed that the ratios of chloroform to methanol were as follows: 20:10, 18:12, 16:14, and 14:16. As a result, the yellow fraction was obtained by combining all of these fractions.

Recrystallization: The portion with the yellow tint is reconstituted by heating it in ethanol, letting it cool, and then filtering it with whatman filter paper. To get yellow crystal quercetin, the filtrate is placed in a desiccator that is filled with calcium chloride. Chemical analysis and thin-layer chromatography (Table 1) both indicate that the isolated molecule contains flavonoids.

TABLE 1: TLC SOLVENT SYSTEM FOR ISOLATEDCOMPOUND

Solventsystem	No.of Spot	$R_f$value
n-butanol:aceticacid: water(2:2:6)	1	0.80
Tolueneethylacetate(9:1),(8:2),(5:5)	1	0.85 0.80 0.70

ChemicalIdentificationofConstituents: Littleam ount of the isolated constituent are dissolve inalcoholand perform thefollowingtests

ShinodaTest(MagnesiumHydrochlorideReduc tionTest): TothetestSolution,addfewfragmentsof Magnesiumribbonandaddconcentrated Hydrochlo ricaciddropwiseandobservethecolor.

Zinc Hydrochloride Reduction Test: To the testsolutionaddamixtureofZincdustandconc.Hydr ochloric acid. Heat the solution and observethe color [11,12].

MolecularDockingStudy: Thecomputationalstud ieswereperformedbyperformedbymastero 9.3 (Maestro" v-9.3.515 (Schrodinger, LLC, NewYork,NY),**Table2**runningonIntelCorei53230 M with Radeon (tm) HD Graphics 1.90 GHz,RAM Memory 4GB under Windows 8 system. Thetargetenzyme5,10-Methenyltetrahydrofolatesynthetase(MTHFS)retr ievedfromtheProteinData Bank (PDBid–3HY3[13].

TABLE2:MOLECULARDOCKINGSTUDYOFISOLA TEDCOMPOUND

Task	
Ligand moleculeske tched	ChemDraw® Ultra, Version 8.0,CambridgeSoftCorporation,US A
LigandPreparation	"LigPrep"v-2.5(Schrodinger®)
ProteinPreparation	ProteinPreparationWizard"fromt heWorkflowsof"Maestro"v-9.3.515(Schrodinger®)platform
BindingSiteAnalysis	"SiteMap"v-2.6(Schrodinger®)
MolecularDocking	"Glide"v-5.8(Schrodinger®)

MTT Assay: *In vitro* cytotoxic effect of quercetinisolatedfrom*Euphorbiaheterophyllla*w asevaluated by MTT assay against MCF -7 cell line.(Breast Cancer Cell line).In brief, the trypsinizedcells from T-25 flask were seeded in each well of96-wellflat-bottomedtissueculture plateatadensity of 5 × 103 cells/well in growth medium andcultured at 37 °C in 5% CO_2 to adhere. After 48 hrincubation, the supernatant was discarded and thecellswerepretreatedwithgrowthmediumandw eresubsequentlymixedwithdifferentconcentratio ns of quercetin (3.125, 6.25, 12.5, 25,50,100µg/ml)intriplicatestoachieveafinalvol umeof100µland thenculturedfor24and48hr.

Thequercetinwaspreparedas1.0mg/mlconcentra tionstocksolutionsinPBS.Culturemedium and solvent DMSO are used as controls.Eachwellthenreceived5µloffreshMTT(0.5mg/mlinPBS)followedbyincubationfor2hrat 37°C.Thesupernatantgrowthmediumwasre-moved from the wells and replaced with 100 µl ofDMSO to solubilize the colored formazan product.After30minincubation,theabsorbance(OD)ofthe culture plate was read at a wavelength of 650nmonanELISAreader,Anthos2020spectroph otometer was used. The mean percentagecytotoxicity and percentage viability is determinedatvarious concentrationsused[14,15].

RESULTSANDDISCUSSION:

Infra-RedSpectrophotometer:3290.58,O-Hstretching vibration of phenol, 1668.24, C=O Arylketonicstretch,1612.16,C--- CAromaticringstretch,1516.26,C=Oaromaticstr

ISSN 2229-6107 www.ijpast.in
Vol 13,Issuse 1.March 2023

etc h,1429.54,C=Caromaticstretch,1359.37,O-Hbendingofphenols,1315.58,C-HbondinAromatichydrocarbon, 1240.55, C-O stretch of Aryl ether,1210.97, C-O stretch of phenol, 1163.60, C-CO-Cstretchandbendinginketone932.70,815.46,705.65, 596.88.

SpectralCharacterization:

^{1}HNMR:δ6.18(s,1H,Ar–H),δ6.40(s,1H,Ar-H),δ6.87-6.89(d,1H,Ar-H),δ7.53–7.55(m,1H, Ar-H),δ7.67-7.68(d,1H,Ar-H),δ9.37-9.58(m, 3H,OH),δ10.77(brH,1H,OH),δ12.49(s,1H,OH).

^{13}C–NMR: Ar-C=O–176.79,Ali–C-OH–93.80,AliC-O–98.63,ArC-(103.46–147.25),

Ar–C-OH-(148.15–164.45).C-OH–4(Ar),C-OH-1(Ali),C-O -2(Ali), ArC(8).

LiquidChromatography/TandemMassSpectrometry (LC/MS/ MS Identification): Theisolated compound was analyzed by LC-MS-MS. Ithas been successfully applied for a quick separationand identification of the isolated compound fromleavesof*Euphorbiaheterophylla*.Thefragmentpattern m/z 303.0493 $(M^{+}H)^{+}$ was found in massspectrum,anditiscorrespondtothemolecularweight of quercetin (302.2). From the use of massspectral library search system, it is confirmed thattheisolated compoundis found tobequercetin.

Docking: MTHFS catalyzes the initial irreversibleconversion of 5-formyltetrahydrofolate to 5, 10-ethenyltetrahydrofolate**Fig. 1**. The reaction is ATPdependent and is subject to feedback inhibition bytheproduct.5,10-Methenyltetrahydrofolatesynthetase (MTHFS) regulates the flow of carbonthrough the one-carbon metabolic network, whichsuppliesessentialcomponentsforthegrowthandproliferationofcells.InhibitionofMTHFSinhuman MCF-7 breast cancer cells has been shownto arrest the growth of cells. The reaction proceedsviatheformation of twointermediates[16].

TheaminoacidresidueTyrosine83formshydrogenbondingwithhydroxylgroupofquercetin.Theelectrostaticinteractionsofactivesites of aminoacid residues and hydrogen bondingleadsto thegood dockingscoreof-10.22**Fig.2**.

***Invitro*Cytotoxicity:**Quercetinshowsgoodcytotoxic activity against MCF -7 cell line. **Table 3**depicts, at 50 and 100 µg/ml the mean percentagecytotoxicitywasfoundtobe83.58and89.77respectively.Thepercentageviabilityat50and100µg/mlwasfoundtobe16.41and10.22respectively.Itclearlyindicatesthatquercetinpossessgoodanticanceractivityagainst Breast Cancer cells. At 48 hrs the GI_{50} value of quercetinagainst MCF – 7 cell line was found to be 29.20**Fig.3**.

FIG.1:GLIDE DOCKINGVIEW

FIG.2:LIGANDRECEPTORINTERACTION

INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCE & TECHNOLOGY
IJPAST

ISSN 2229-6107 www.ijpast.in
Vol 13,Issue 1.March 2023

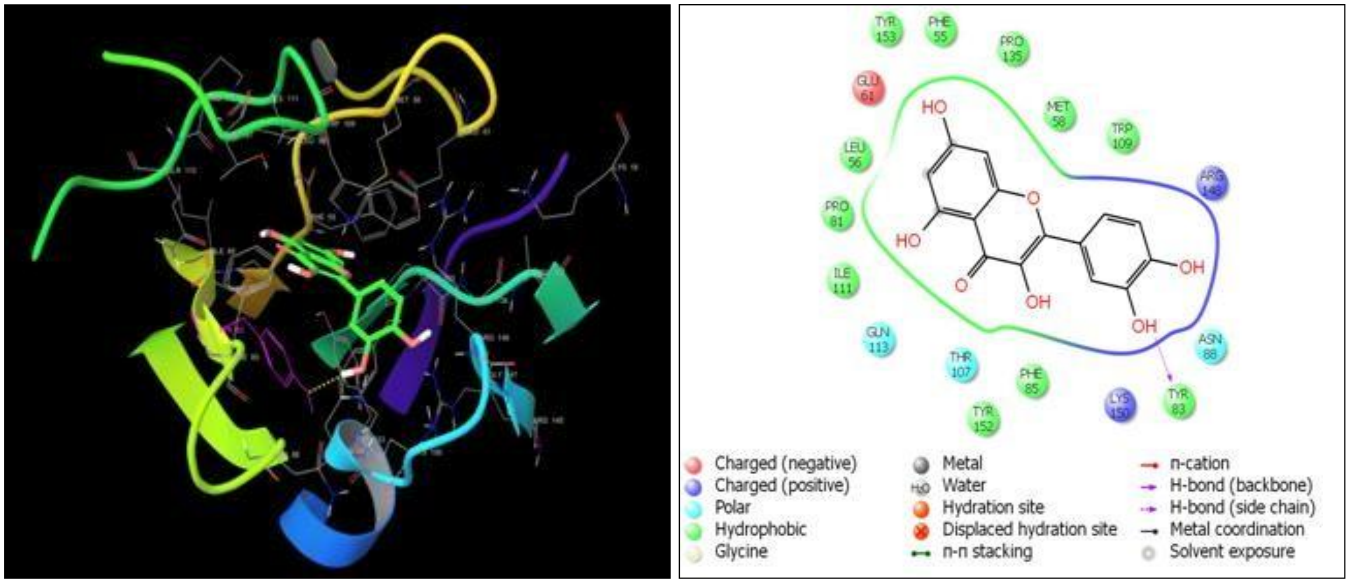

TABLE3: EFFECTOFQUERCETINAGAINSTMCF-7CELLLINEATDIFFERENTCONCENTRATIONS

Concentration(µg/ml)	Singlet	Duplicate	Triplicate	Mean	SD	SEM	%Viability100-toxicity
DMSO	0.143	0.161	0.159	81.9817	1.168308	0.674523	18.0183
3.125	0.8294	0.8472	0.8020	3.500097	2.659445	1.535431	96.4999
6.25	0.7858	0.8012	0.6889	11.39186	7.110735	4.105385	88.60814
12.5	0.6762	0.6364	0.7411	20.04283	6.172754	3.563841	79.95717
25	0.4303	0.5488	0.4687	43.63247	7.061766	4.077112	56.36753
50	0.1585	0.1336	0.1296	83.58186	1.828963	1.055952	16.41814
100	0.0892	0.0809	0.0926	89.77224	0.702969	0.405859	10.22776

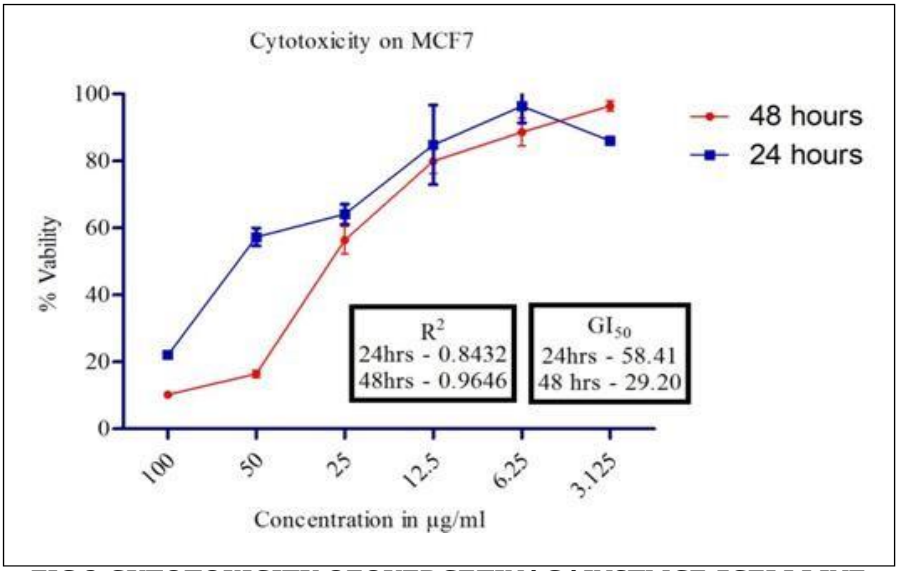

FIG.3:CYTOTOXICITY OFQUERCETINAGAINSTMCF-7CELLLINE

CONCLUSION: From the above study, quercetinwasisolatedandcharacterizedfromethanolicextractof*Euphorbiaheterophylla*Linn.andthisis a flavonoid constituent. Quercetin has anticancer,anti-inflammatory,antiviral,fibromyalgia,metabolicsyndrome*etc*.Quercetinisfrequentlyusedtherapeutically inallergicconditions,including asthma and hay fever, eczema, and hives.In this study, the molecular docking was applied toexplore the binding mechanism and to correlate itsdockingscorewiththeactivityofQuercetincompound.However,itsusesalsomaybeimportantincancertherapeutics.Quercetinisarecognized antioxidant and has been studied for itsgastro-

ISSN 2229-6107 www.ijpast.in
Vol 13,Issue 1.March 2023

prote ctiveeffects,inhibitionofcarcino-genicityeitheraloneorincombinationwithchemotherapeutic agents, reducing risk of cataract.Again,theabilityofquercetintoinhibitinflammatory leukotriene production may be a keytoits beneficial impact.

REFERENCES:

1. The impact of an aqueous Euphorbia heterophylla extract on the blood glucose levels of rats induced with diabetes by alloxan (Annapurna A and Ketan H, 1). Page numbers 669–672 in the 2014 edition of the International Journal of Research in Pharmacy and Chemistry.
2. The antimicrobial effects of Euphorbia heterophylla leaf extracts on many intestinal bacteria were investigated in both laboratory and animal models (Mary OU, n.d.). Published in 2017 by the World Journal of Microbiology, volume 4, issue 1, pages 072–078, full text.
3. Thirdly, Oso BA and Ogunnusi TA tested methanolic extracts of Euphorbia heterophylla and Tithoniadiversifolia for their antibacterial activity against certain microbes. Article published in 2017 in the European Journal of Medicinal Plants, volume 20, issue 3, pages 1-8.
4. 4. A study was conducted by Keerthana K, Deepa A, Shobana G, Jothi G, and Sridharan G to assess the antioxidant capacity of Euphorbia heterophylla and to conduct preliminary phytochemical screening. Print version of this article: International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6, 8, 549–553.
5. 5. Okeniyi SO: Euphorbia heterophylla stem and leaf extracts tested for phytochemical screening, cytotoxicity, antioxidant, and antimicrobial properties. Biology and Life Science Journal, 2014, 4(1), 24-31.
6. Environmental Pharmacology and Life Sciences, 2012, 1(8), 87–91; Okeniyi SO, Adedoyin BJ, and Garba S. Phytochemical Screening, Cytotoxicity, Antioxidant, and Antimicrobial Activities of Stem and Leave Extracts of Euphorbia heterophylla Bull.
7. Journal of Ethnopharmacology, 2000, 70: 281-300, Hamill FA, Apio S, Mubiru NK, Mosango M, Bukenya- Ziraba R, and Maganyi OW. Traditional herbal medications of southern Uganda.
8. 8. Rowan NG and Onwukaeme DN: EpphorbiaceaeDiterpenoid Esters in Euphorbia Hyles. Journal of Pharmacology in Nigeria, 2001, 32, 60–64.
9. Phytochemistry 1995; 38(1): 267–70; Williams CA, Hoult JR, Harborne JB, Greenham J, and Eagles J. A lipophilic flavonol from Tanacetumparthenium with biological activity.
10. 10. The Impact of Different Extraction Methods on the Antioxidant Activity, Total Flavonoids, and Phenol Content of various Plant Extracts (Bharti and Ray, 2010). Applied Science and Engineering Technology Research: An International Journal, 2014, 2(1), 41-48.
11. 11. Bargah RK: A water and crude ethanolic extract of MoringapterygospermaGaertn was subjected to preliminary phytochemical screening. Published in the 2015 January issue of the Journal of Pharmacognosy and Phytochemistry, volume 4, pages 7–09.
12. 12. Gupta J and Gupta A: Rauwolfia serpentine flavonoid rutin isolation and identification. Journal of Chemical Studies: International, 2015, 3(2), 113–115.
13. The identification of new inhibitors of methionyl-tRNAsynthetase by pharmacophore-based virtual screening (Kim et al., 2013). Published in Bioorg. Med. Chem. Lett. 2006, 16(4898–4907).