IJPSR (2018), Volume 9, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 April, 2017; received in revised form, 05 October, 2017; accepted, 10 December, 2017; published 01 January, 2018

ISOLATION, CHARACTERIZATION, DOCKING AND ANTI-CANCER ACTIVITY OF QUERCETIN FROM LEAVES OF *EUPHORBIA HETEROPHYLLA* LINN.

P. A. Manikandarajan [1], M. Sathish[*1], R. Suresh [2] and A. Jerad Suresh [1]

Department of Pharmaceutical Chemistry [1], College of Pharmacy, Madras Medical College, Chennai - 600003, Tamil Nadu, India.
Greensmed Lab [2], Old Mahabalipuram Road, Thoraipakkam, Chennai - 600097, Tamil Nadu, India.

Keywords:

Euphorbia heterophylla, MTT assay, Quercetin, Molecular Docking, 5, 10-Methenyltetrahydro-folate synthetase (MTHFS)

Correspondence to Author:
Dr. M. Sathish

Assistant Professor,
Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai - 600003, Tamil Nadu, India.

E-mail: sathishpharmacy14@gmail.com

ABSTRACT: This study was carried out to isolate and characterize the bio active constituent from *Euphorbia heterophylla* and also to evaluate anticancer activity for the isolated compound against Breast cancer cell line (MCF -7 Cell line). The isolation of compound was performed on ethanol extract by using column chromatography and ultra sonicator by gradient elution. The structure of isolated compound was established on the basis of chemical test, thin layer chromatography and spectroscopic evidences. (IR, 1H NMR, ^{13}C NMR, Liquid chromatography / tandem mass spectrometry (LC/MS/MS identification with data base library search). The yellow colour compound shows positive test for flavonoids in Thin layer Chromatography for flavonoids in the solvent system n-butanol: acetic acid: water (2:2:6) with R_f value 0.7. From the use of LCMS spectral library search system and TLC it is confirmed that the isolated compound is found to be quercetin. The Docking study was performed for the isolated quercetin against 5, 10 methenyltetrahydrofolate synthetase (MTHFS). The inhibition of (MTHFS) in human MCF 7 breast cancer cells has been shown to arrest the growth of breast cancer cells with the docking score of -10.23. Hence quercetin was subjected to *in vitro* anti-cancer activity against MCF–7 cell line. At 48 hrs in 100 µg/ml, the mean percentage cytotoxicity was found to be 89.77 and the percentage viability was 10.22.

INTRODUCTION: *Euphorbia heterophylla* L. is branched shrub belonging to the family *Euphorbiaceae* and are widely distributed in India, South Asian countries, Africa, Mexico, Thailand. It is an ornamental plant and common names include Paalperuki in Tamil, Mexican fire plant, poinsettia *etc*., This plant is reported to have wound healing activity [1], anti-inflammatory [2], antimicrobial [3] and anticancer activity.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(1).197-02

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(1).197-02

In traditional medicine it is used to treat constipation, bronchitis, asthma, laxative and as lactogenic agent [4].

Leaves contain a red coloring matter, porcetin. Study yielded terpenoids, quinones, alkaloids, sterol, coumarin, starch, and protein. Fresh leaves yielded carbohydrates, reducing sugars, saponins, steroids, terpenoids, tannins, flavonoids, and alkaloids. Plant is a high source of energy and water, moderate in protein and fiber contents. Phytochemical analysis of leaves yielded tannins, anthraquinones, alkaloids, flavonoids, and phenols. Aqueous extract of plant yielded secondary metabolites such as alkaloid, flavonoid, tannin, sterol, quinone, lignin and coumarin.

Carbohydrate (10.29 mg/g) and proteins (7.43 mg/g) were present in measurable amount. Phenol yield was (3.26 mg/g) and ascorbic acid (1.14%) [5].

Euphorbia heterophylla is widely used in traditional African medicine and elsewhere in tropical countries. In Africa a decoction or infusion of the stems and fresh or dried leaves is taken as a purgative and laxative to treat stomach-ache and constipation, and to expel intestinal worms. A leaf infusion is used as a wash to treat skin problems, including fungal diseases, and abscesses [6]. In Nigeria the latex and preparations of the leaves and root are applied to treat skin tumours. In East Africa the roots are used in the treatment of gonorrhoea or to increase milk production in breast-feeding women. The latex is irritant to the skin and eyes and may be employed as a rubefacient and to remove warts. However, the latex is also used as an antidote against the irritation caused by the latex of other *Euphorbia* species. In peninsular Malaysia a leaf extract is taken to treat body pain. The latex is used in the preparation of arrow poison and fish poison [7].

The leaves of *E. heterophylla* have been reported to contain quercertin. Diterpenoids have also been reported in the root of *E. heterophylla* [8]. The skin irritant, tumour-promoting and anti-tumour /cancer and recently anti-HIV activities of Euphorbia species have also been reported in *E. heterophylla* leaf linn [9].

MATERIAL AND METHODS:

Collection of Plant and Preparation of Extract: The leaves of *Euphorbia heterophylla* were collected from Chennai, Tamil Nadu, India. The plant material was identified and authenticated by Botanist Dr. Sasikala Ethirajulu, Research officer, CCRAS, Government of India, Chennai. The leaves were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40- mesh sieve. The dried powder of the leaves was extracted by soxhlet apparatus using ethanol as solvent. The extract was concentrated by using a rotary evaporator.

Isolation and Charecterization: Ethanol extract was subjected for column chromatographic isolation and wet packing method was followed. Initially 3/4th of the column is filled with hexane and then silica gel (100 - 200 mesh size) is added slowly to ensure uniform packing. 12 gm of ethanol extract was chromatographed over a column of 240 gm silica gel by gradient elution. The column was developed by elution with hexane (100%) followed by Hexane: chloroform (80:20, 70:30, 50:50, 30:70), chloroform 100%. Chloroform: methanol (80:20, 70:30, 50:50).

Extraction and Purification by Ultrasonicator: Quercetin and quercetin glycosides are physiologically active flavonol molecules that have been attributed numerous health benefits. Recovery of such molecules from plant matrices depends on a variety of factors including polarity of the extraction solvent. Among the solvents of a wide range of dielectric constants, methanol recovered the most quercetin and its glycosides. When ultra-sonication was employed to facilitate the extraction, exposure of 15 min of ultrasound wavelengths for the optimum extraction conditions for quercetin and its glycosides [10].

Procedure: Chloroform: methanol (70:30) fraction shows positive test in TLC for flavonoids (n-butanol: acetic acid: water (2:2:6), Toluene: ethyl acetate: formic acid: methanol (5.5:3:1:0.5). It shows the presence of blue fluorescence spot in UV and reddish brown in Iodine at an R_f value of 0.85 and 0.7 respectively. Hence the above fraction was selected for extraction of quercetin related flavonols.

Ultra-sonication was employed to facilitate the extraction, exposure of 15 min of ultrasound wavelengths for the fraction (Chloroform: methanol 70:30) Different solvent compositions of chloroform and methanol was used and total of 30 ml is employed with different fractions collected such as: Chloroform: methanol (28:2), Chloroform: methanol (26:4), Chloroform: methanol (24:6), Chloroform: methanol (22:8), Chloroform: methanol (20:10), Chloroform: methanol (18:12), Chloroform: methanol (16:14), Chloroform: methanol (14:16), Chloroform: methanol (12:18), Chloroform: methanol (10:20). It was found that the fractions, Chloroform: methanol (20:10), Chloroform: methanol (18:12), Chloroform: methanol (16:14), Chloroform: methanol (14:16). Hence all these fractions were mixed to get yellow color fraction.

Recrystallization: The obtained yellow color fraction is recrystallized using ethanol as solvent, boiled and cooled and filtered by whatman filter paper. The obtained filtrate is kept in a desiccator filled with calcium chloride to get yellow crystal quercetin. The isolated compound shows positive test for flavonoids in thin layer chromatography **Table 1** and chemical test.

TABLE 1: TLC SOLVENT SYSTEM FOR ISOLATED COMPOUND

Solvent system	No. of Spot	R_f value
n-butanol : acetic acid : water (2:2:6)	1	0.80
Toluene ethyl acetate (9:1), (8:2), (5:5)	1	0.85 0.80 0.70

Chemical Identification of Constituents: Little amount of the isolated constituent are dissolve in alcohol and perform the following tests

Shinoda Test (Magnesium Hydrochloride Reduction Test): To the test Solution, add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid drop wise and observe the color.

Zinc Hydrochloride Reduction Test: To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. Heat the solution and observe the color [11, 12].

Molecular Docking Study: The computational studies were performed by performed by mastero 9.3 (Maestro" v-9.3.515 (Schrodinger, LLC, New York, NY), **Table 2** running on Intel Core i5 3230M with Radeon (tm) HD Graphics 1.90 GHz, RAM Memory 4GB under Windows 8 system. The target enzyme 5, 10-Methenyltetrahydrofolate synthetase (MTHFS) retrieved from the Protein Data Bank (PDB id –3HY3 [13].

TABLE 2: MOLECULAR DOCKING STUDY OF ISOLATED COMPOUND

Task	
Ligand molecule sketched	ChemDraw® Ultra, Version 8.0, Cambridge Soft Corporation, USA
Ligand Preparation	"LigPrep" v-2.5(Schrodinger®)
Protein Preparation	Protein Preparation Wizard" from the Workflows of "Maestro" v-9.3.515 (Schrodinger®) platform
Binding Site Analysis	"Site Map" v-2.6 (Schrodinger®)
Molecular Docking	"Glide" v-5.8 (Schrodinger®)

MTT Assay: *In vitro* cytotoxic effect of quercetin isolated from *Euphorbia heterophyllla* was evaluated by MTT assay against MCF -7 cell line. (Breast Cancer Cell line).In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of 5 × 103 cells/well in growth medium and cultured at 37 °C in 5% CO_2 to adhere. After 48 hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of quercetin (3.125, 6.25, 12.5, 25, 50, 100 µg/ml) in triplicates to achieve a final volume of 100µl and then cultured for 24 and 48 hr.

The quercetin was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent DMSO are used as controls. Each well then received 5µl of fresh MTT (0.5 mg/ml in PBS) followed by incubation for 2 hr at 37 °C. The supernatant growth medium was re-moved from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 650 nm on an ELISA reader, Anthos 2020 spectrophotometer was used. The mean percentage cytotoxicity and percentage viability is determined at various concentrations used [14, 15].

RESULTS AND DISCUSSION:

Infra-Red Spectrophotometer: 3290.58, O-H stretching vibration of phenol, 1668.24, C=O Aryl ketonic stretch, 1612.16, C---C Aromatic ring stretch, 1516.26, C=O aromatic stretch, 1429.54, C=C aromatic stretch, 1359.37, O-H bending of phenols, 1315.58, C-H bond in Aromatic hydrocarbon, 1240.55, C-O stretch of Aryl ether, 1210.97, C-O stretch of phenol, 1163.60, C-CO-C stretch and bending in ketone 932.70, 815.46, 705.65, 596.88.

Spectral Characterization:

^{1}H NMR: δ6.18 (s, 1H, Ar –H), δ 6.40 (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 (br H, 1H, OH), δ12.49 (s, 1H, OH).

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

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

Liquid Chromatography / Tandem Mass Spectrometry (LC/MS/ MS Identification): The isolated compound was analyzed by LC-MS-MS. It has been successfully applied for a quick separation and identification of the isolated compound from leaves of *Euphorbia heterophylla*. The fragment pattern m/z 303.0493 $(M^+H)^+$ was found in mass spectrum, and it is correspond to the molecular weight of quercetin (302.2). From the use of mass spectral library search system, it is confirmed that the isolated compound is found to be quercetin.

Docking: MTHFS catalyzes the initial irreversible conversion of 5- formyltetrahydrofolate to 5, 10-ethenyltetrahydrofolate **Fig. 1**. The reaction is ATP dependent and is subject to feedback inhibition by the product. 5, 10-Methenyltetrahydrofolate synthetase (MTHFS) regulates the flow of carbon through the one-carbon metabolic network, which supplies essential components for the growth and proliferation of cells. Inhibition of MTHFS in human MCF-7 breast cancer cells has been shown to arrest the growth of cells. The reaction proceeds via the formation of two intermediates [16].

The amino acid residue Tyrosine 83 forms hydrogen bonding with hydroxyl group of quercetin. The electrostatic interactions of active sites of aminoacid residues and hydrogen bonding leads to the good docking score of -10.22 **Fig. 2**.

***In vitro* Cytotoxicity:** Quercetin shows good cytotoxic activity against MCF -7 cell line. **Table 3** depicts, at 50 and 100 µg/ml the mean percentage cytotoxicity was found to be 83.58 and 89.77 respectively. The percentage viability at 50 and 100µg/ml was found to be 16.41 and 10.22 respectively. It clearly indicates that quercetin possess good anticancer activity against Breast Cancer cells. At 48 hrs the GI_{50} value of quercetin against MCF – 7 cell line was found to be 29.20 **Fig. 3**.

FIG. 1: GLIDE DOCKING VIEW

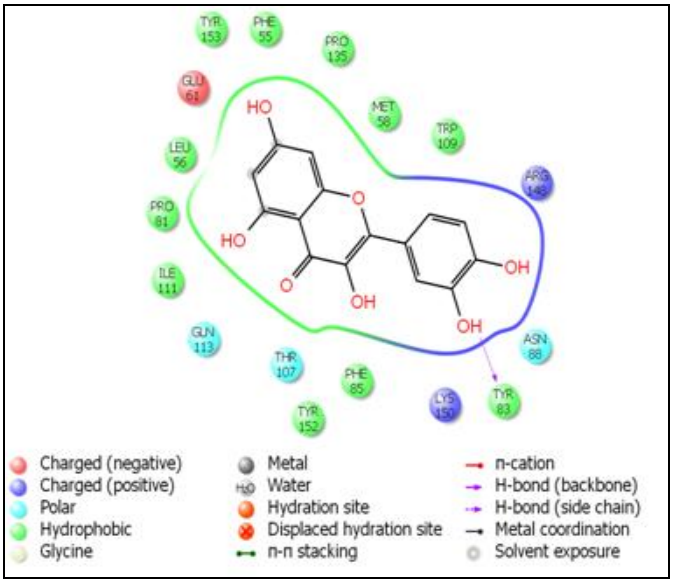

FIG. 2: LIGAND RECEPTOR INTERACTION

TABLE 3: EFFECT OF QUERCETIN AGAINST MCF -7 CELL LINE AT DIFFERENT CONCENTRATIONS

Concentration (µg/ml)	Singlet	Duplicate	Triplicate	Mean	SD	SEM	%Viability 100-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

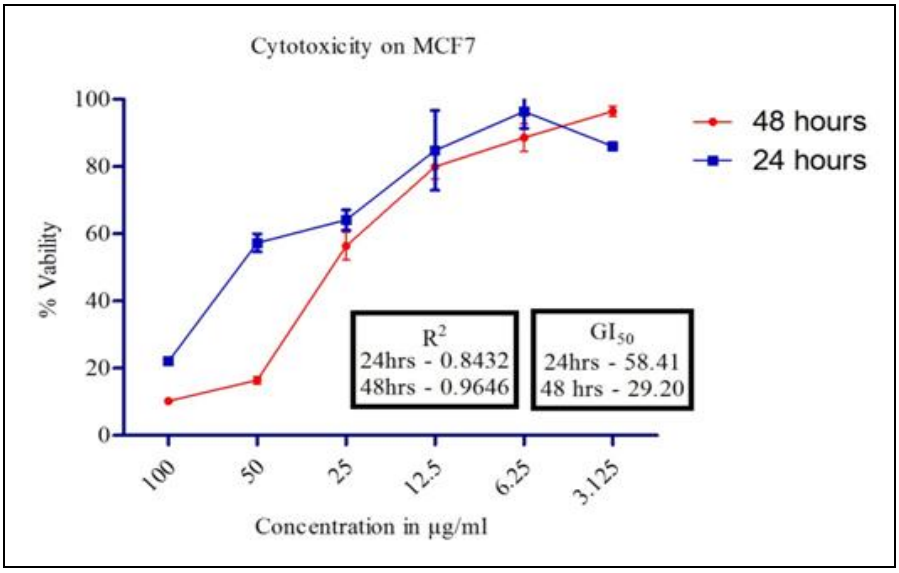

FIG. 3: CYTOTOXICITY OF QUERCETIN AGAINST MCF -7 CELL LINE

CONCLUSION: From the above study, quercetin was isolated and characterized from ethanolic extract of *Euphorbia heterophylla* Linn. and this is a flavonoid constituent. Quercetin has anticancer, anti - inflammatory, antiviral, fibromyalgia, metabolic syndrome *etc*. Quercetin is frequently used therapeutically in allergic conditions, including asthma and hay fever, eczema, and hives. In this study, the molecular docking was applied to explore the binding mechanism and to correlate its docking score with the activity of Quercetin compound. However, its uses also may be important in cancer therapeutics. Quercetin is a recognized antioxidant and has been studied for its gastro-protective effects, inhibition of carcino-genicity either alone or in combination with chemotherapeutic agents, reducing risk of cataract. Again, the ability of quercetin to inhibit inflammatory leukotriene production may be a key to its beneficial impact.

ACKNOWLEDGEMENT: We thank our colleagues from the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, who provided valuable insight and expertise that greatly assisted this research work. We also thank Greensmed Lab, Old Mahabalipuram Road, Thoraipakkam Chennai, for supporting us during the *in vitro* anticancer activity by MTT assay.

CONFLICT OF INTEREST: The authors are declared that there is no conflict of interest exists.

REFERENCES:

1. Annapurna A and Ketan H: Effect of aqueous extract of *Euphorbia heterophylla* on blood glucose levels of alloxan induced diabetic rats. International Journal of Research in Pharmacy and Chemistry 2014; 4(3): 669-672.
2. Mary OU: *In vitro* and *in vivo* antibacterial study of leaf extracts of *Euphorbia heterophylla* on some enteric bacteria. World Journal of Microbiology 2017; 4(1): 072 - 078.
3. Oso BA and Ogunnusi TA: Antibacterial activity of Methanolic extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* against some microorganisms. European Journal of Medicinal Plants 2017; 20(3): 1-8.
4. Keerthana K, Deepa A, Shobana G, Jothi G and Sridharan G: Preliminary Phytochemical screening and *in vitro* antioxidant potential of *Euphorbia heterophylla*. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(8): 549-553.
5. Okeniyi SO: Phytochemical Screening, Cytotoxicity, Antioxidant and Antimicrobial activities of stem and leave extracts of *Euphorbia heterophylla*. Journal of Biology and Life Science 2014; 4(1): 24-31
6. Okeniyi SO, Adedoyin, BJ and Garba S: Phytochemical Screening, Cytotoxicity, Antioxidant and Antimicrobial Activities of stem and leave extracts of *Euphorbia heterophylla* Bull. Environ Pharmacol Life Sci 2012; 1(8): 87-91.
7. Hamill FA, Apio S, Mubiru NK, Mosango M, Bukenya-Ziraba R and Maganyi OW: Traditional herbal drugs of southern Uganda Journal of Ethnopharmacology 2000; 70: 281-300.
8. Rowan NG and Onwukaeme DN: Diterpenoid Esters of Euphorbiaceae in *Euphorbia hyles*. Nigeria Journal of Pharmacology 2001; 32: 60-64.
9. Williams CA, Hoult JR, Harborne JB, Greenham J and Eagles J: A Biologically active lipophilic flavonol from *Tanacetum parthenium* Phytochemistry 1995; 38(1): 267-70.
10. Bharti I and Ray A: Effects of Extraction Techniques on Total flavonoids, phenols and antioxidant activity of different plants extract. International Journal for Research in Applied Science and Engineering Technology 2014; 2(1): 41-48.

11. Bargah RK: Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. Journal of Pharmacognosy and Phytochemistry 2015; 4(1): 07-09.
12. Gupta J and Gupta A: Isolation and identification of flavonoid rutin from *Rauwolfia serpentine*. International Journal of Chemical Studies 2015; 3(2): 113-115
13. Kim SY, Lee YS, Kang T, Kim S and Lee J: Pharmacophore-based virtual screening: The discovery of novel methionyl- tRNA synthetase inhibitors. Bioorg. Med. Chem. Lett 2006; 16: 4898-4907.
14. Sharif A, Akhtar MF, Akhtar B, Manan M and Shabbir M: Genotoxic and cytotoxic potential of whole plant extracts of *kalanchoe laciniata* by AMES and MTT assay, EXCLI Journal 2017; 16: 593-601
15. Vandana CD and Shanti KN: Cytotoxic activity of *Justicia wynaadensis* (nees) T. anderson leaf extract on human cancer cell lines 2017; 8(12): 5298-5302.
16. Wu D, Li Y, Song G, Cheng C, Zhang R and Joachimiak A: Structural Basis for the Inhibition of Human 5,10-Methenyltetrahydrofolate Synthetase by N10-Substituted Folate Analogues. Cancer Res 2009; 69(18): 1-8.

How to cite this article:
Manikandarajan PA, Sathish M, Suresh R and Suresh AJ: Isolation, characterization, docking and anti-cancer activity of quercetin from leaves of *Euphorbia heterophylla* linn. Int J Pharm Sci & Res 2018; 9(1): 197-02. doi: 10.13040/IJPSR.0975-8232.9(1).197-02.