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Anticancer Activity of Kaempferol-3-*O*-*β*-D-(6″-coumaroyl)-Glucopyronoside from *Euphorbia Hirta* Flowers

R. Benjamin[1], S. Senguttuvan[1], T. Vadivel[2*]

[1]PG & Research Department of Chemistry, Thiru. Vi. Ka. Government Arts College, Thiruvarur, (Affiliated to Bharathidasan University, Thiruchirappalli-24), Tamilnadu-610 003, India

[2]Department of Chemistry, Dr. Kalaignar M. Karunanidhi Government Institute for Post Graduate Studies and Research, Karaikal, (Affiliated to Pondicherry University), Union Territory of Puducherry-609605, India

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Abstract

Kaempferol-3-O-β-D- (6″-coumaroyl)-glucopyronoside flavonoid was extracted from the flower of *Euphorbia Hirta*. This compound was characterized by UV, ^{13}C, and ^{1}H NMR spectroscopy. The in-vitro anticancer study was performed using this flavonoid compound. *Euphorbia Hirta* flower showed good anticancer activity due to its higher content of flavonoids compound.

Keywords: *Euphorbia Hirta* flowers; Flavonoids; Anticancer activity.

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1. Introduction

Our ancestral have lived very allegro because of using natural medicines made up of many indigenous plants of our country. Unfortunately, some rare medicinal plants have been extinct due to several man made activities such as urbanization, deforestation, forest fires etc. [1]. In India, Tamilnadu has uniqueness in having flourished natural resources including medicinal plants. The ancient people of Tamilnadu have been documented numerous valuable palm leaf manuscript for medicinally important plants. All the natural resources, now-a-days are exploiting in the name of development in which the human are harvesting many incredible living organisms including plants [2,3]. It has been proved that the orthodox people of Tamilnadu were living healthy without diseases such as diabetics, blood pressure etc. because they have been living unitedly with nature and practiced with natural food.

The development of artificial drugs related research has flourished to the active constituent of a natural product as drug [4,5]. The purpose of such investigation has been typically producing a drug having some advisable therapeutic action [6]. Natural products play a vital role as the crucial sources for new drugs designing which are unique structural

* *Corresponding author*: vadivel.tvsr80@gmail.com

diversity, healing action, non-toxicity etc. [7-9]. Although, some effective phytochemicals have been recommended for dietary supplementary [10-12], conventional treatment [13], sidha medicines [14,15] etc., some researchers are interested to explore the chemical constituents which are present in many indigenous medicinal plant. These active chemical constituents are giving plausible health benefits to people who are interested in natural medicines. These beneficial phytochemicals of indigenous plants have been giving additional health support by consumption of natural foods [16].

The phytochemical investigation of medicinal plants comprised extraction of plant materials, preliminary phytochemical screening, separation and isolation of the phyto components [17]. The isolated phyto-constituents have been characterized with suitable analytical tools [18-20]. This phytochemical examination of medicinal plants is achieving better outcome for bioactive constituents, which have been applied to the natural treatment [21,22]. The bioactive constituents such as alkaloids, saponins, tannins, flavonoids and anthraquinones have extracted from medicinal plants which are focused for crucial role in the designing of new drug models [23]. Moreover, these phytomedicine are non-toxic, less expensive, moreover safe for human beings [24,25].

Additionally, alkaloids can be well-defined as naturally occurring herbal element which consist of a pyridine ring [26,27]. It has constituted least one nitrogen atom in a heterocyclic ring in naturally taking area alkaloids. Some alkaloids are used for therapeutic remedy with very small quantity [28]. Besides, flavonoids, placed as a predominant energetic constituent which show massive feature and it have been applied for antibacterial, antiviral, antifungal, anti-allergic, anticancer, antioxidant and anti-inflammatory agents [29,30].

2. Materials and Methods

2.1. *Chemicals*

All chemicals used in this research work were purchased as analytical grade from Sigma-Aldrich Chemical Co., Bangalore, India.

2.2. *Extraction and fractionation of Kaempferol-3-O-β-D- (6″-coumaroyl)-glucopyronoside from Euphorbia hirta flowers Extraction and fractionation*

Fresh flowers of *Euphorbia hirta* (2 kg) were collected from Kovilacherry, Thanjavur-District, Tamil Nadu, India in the month of December. The dried flowers were extracted with 85 % of methanol (6 x 500 mL) and refluxed the alcoholic extract. This extract was concentrated *in vacuo* and the aqueous concentrate successively fractionated with benzene (4 x 500 mL) and peroxide free ether Et_2O (4 x 250 mL) and EtOAc (8 x 250 mL). The ether fraction was concentrated *in vacuo* and left in an ice-chest for a week. Yellow solid was separated and filtered for analysis. On crystallization from MeOH, yellow needles were obtained [m.p. 278–280 °C]. It was used to be readily soluble in organic solvents and

sparingly soluble in warm water. It was developed as reddish – orange coloration with Mg-HCl and yellow coloration with NaOH. It was responded to Wilson's boric acid, Horhammer-Hansel and Gibb's test however did no longer answer Molisch's tests.

2.3. *Ethyl acetate fraction Kaempferol-3-O-β-D- (6″-coumaroyl)- glucopyronoside*

The ethyl acetate fraction was concentrated *in vacuo* and left in an ice-chest for a few days. A faded yellow solid [m.p. 268-270 °C] that separated was once filtered and studied. It was developed a green coloration with alc. Fe^{3+}, purple color with Mg-HCl. It was regarded crimson under UV that grew to become yellow on exposure to NH_3 with responded to Wilson's boric acid test. It answered Gibb's test and Molisch's test. It did now not reply Horhammer-Hansel test. Pale yellow crystal, m.p. 268-270 °C, λ_{max}^{MeOH} 255, 340 nm; IR (KBr, ν_{max}, cm^{-1}): 3440, 3082, 3058, 3025, 2922, 2849, 1972, 1947, 1802, 1724, 1656, 1617, 1603, 1585, 1559, 1495, 1443, 1409, 1363, 1339, 1233, 1199'; ^{1}H-NMR (400 MHz, DMSO-*d6*): δ 16.00 (br s, 1H, C_5-OH), 12.62 (br s, 1H, C_7-OH), 9.75 (br s, 1H, phenyl C_4-OH), 9.16 (br s, 1H, hydroxyphenyl acrylate C_4-OH), 7.93 (d, *J* = 6.2 Phenyl C_2-H), δ 7.84 (d, *J* =6.0 Phenyl C_6-H), 7.76 (d, *J* =2 Phenyl acrylate -HC=CH), 7.73 (d, *J* =2 Phenyl acrylate -HC=CH), 7.58-7.55 (dd, *J* = 3.2, Phenyl C_2 & C_6-H), 7.50, 7.48 (dd, 2H, phrnyl C_3-H,C_5-H), 6.93-6.89 (dd, 2H, acrylate C_3,C_5-H), δ 8.03 (br s, 1H, Chromone-C_6-H), 8.01 (br s, 1H, Chromone-C_8-H), 4.93 (br s, 1H, pyranose-C_3-OH), 4.40 (br s, 1H, pyranose C_4-OH), 4.22 (br s, 1H, pyranose C_5-H), 3.80 to 3.08 (unresolved pyranose and ethylene proton). ^{13}C-NMR (100 MHz, DMSO-*d6*): δ 177.42, 177.40, 164.14, 164.10, 161.21, 156.37, 156.30, 156.26, 149.39, 148.43, 146.88, 144.78, 133.31, 130.86, 122.03, 121.57, 121.15, 121.07, 116.19, 115.19, 115.09, 113.48, 104.02, 103.96, 100.86, 100.77, 98.70, 93.63, 93.66, 77.54, 74.32, 74.08, 69.92, 69.89, 60.96, 60.59, 55.67; GC-MS: m/z [M+1] 595.

Fig. 1. Picture of *Euphorbia hirta* flowers.

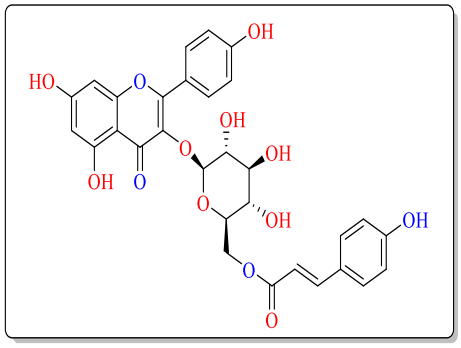

Fig. 2. Structure of Kaempferol-3-O-β-D- (6″-coumaroyl)-glucopyronoside

3. Results

The clean flowers of *Euphorbia hirta* have been discovered as Kaempferol-3-O-β-D- (6″-coumaroyl)-glucopyronoside (Fig. 2). Pale yellow crystal; m.p. 242-244 °C.

3.1. *UV Spectroscopy*

The UV spectrum (Fig. 3) of the glycoside exhibited two important absorption peaks at 340 nm (band I) and 255 nm (band II). The band I absorption of the glycoside is reminiscent of a flavonol skeleton. An evaluation of band I absorption of the glycoside and that of the aglycone published that there can also be 3-glycosylation in the flavonol.

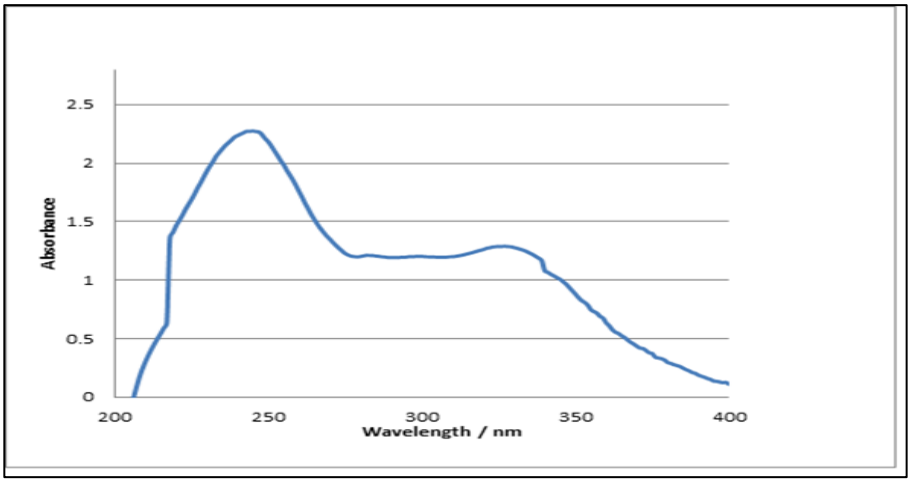

Fig. 3. UV spectrum of Kaempferol-3-*O-β*-D- (6″-coumaroyl)-glucopyronoside.

3.2. *^{1}H NMR spectroscopy*

The ^{1}H-NMR spectrum (Fig. 4) indicates that the flavonoid skeleton in C5 hydroxy protons appeared as a broad singlet at δ 12.62 ppm. Chromone-2-substituted phenyl ring-4-hydroxy was presented at δ 9.75 and 4-hydroxyphenyl acrylate group proton was exhibited as broad singlet at δ 9.16 ppm. Moreover, the doublet phenyl ring in C2-proton and C6-H at δ 7.93, δ 7.84 ppm respectively. In addition, the 4-hydroxyphenyl acrylate ethene -HC=HC- proton was two doublets for δ 7.76 and 7.73 ppm respectively and Chromone-2-substituted phenyl ring C2 and C6 proton more than one coupled doublet in the range at δ 7.58-7.55 ppm. Further, chromone-2-substituted phenyl ring and two meta C3 and C5 coupled doublet were displayed at δ 7.50 and 7.48 ppm respectively and 4-hydroxyphenyl acrylate two meta C3 and C5 coupled peaks were shown in the range at δ 6.92-6.89 ppm. The chromone building C6-H and C8-H proton singlet peaks were exhibited at δ 8.03 and 8.01 ppm respectively. The pyranose C2-linkage proton was shown at δ 5.56 ppm for doublet. Furthermore, in the pyranose ring, three C3, C4 and C5

hydroxy proton broad singlet were assigned in the order of δ 4.93, 4.40 and 4.22 ppm respectively. Moreover, the glucose moiety proton indicates at unresolved peak was existing at δ 3.83-3.08 ppm.

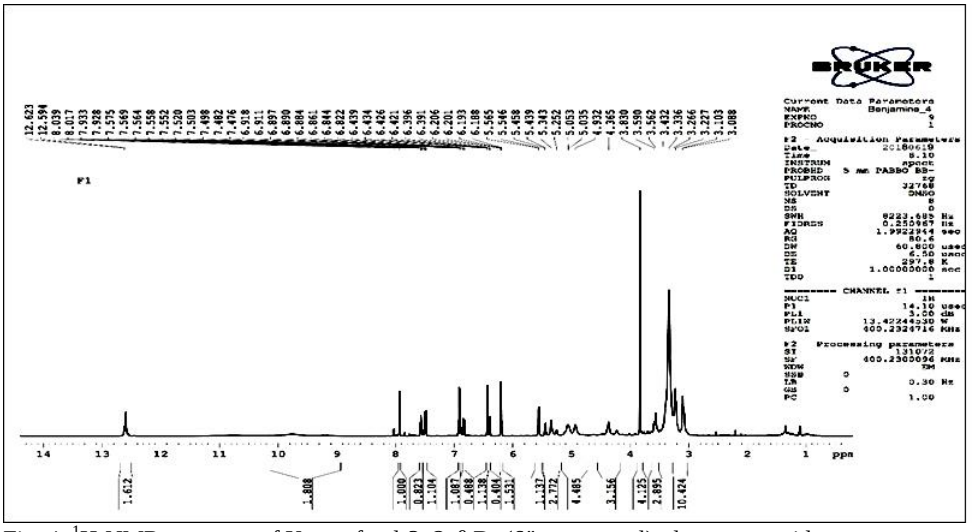

Fig. 4. ^{1}H-NMR spectrum of Kaempferol-3-*O*-*β*-D- (6″-coumaroyl)-glucopyronoside.

3.3. *^{13}C NMR spectroscopy*

In ^{13}C-NMR spectrum of Kaempferol-3-O-β-D- (6″-coumaroyl)-glucopyronoside (Fig. 5), the chemical shift of the carbon signals was obtained at δ 177.42 ppm which is confirmed the presence of C=O group. In addition, the (C-2′, 6′) at δ 104.02 ppm, (C-3′, 5′) δ 100.86 ppm were shown in the spectrum which are corresponding to the hydrogen bearing carbons of *p*-cresol δ 116.19, 115.19 115.09, 113.48 ppm respectively and oxygen bonded ethylenic carbon (C-3) was presented at δ 69.89 ppm. The hydroxyphenyl acrylate ethene carbon exhibited in the range of δ 146.88 and δ 121.07 ppm respectively. All this evidence has revealed the structure of Kaempferol-3-*O*-*β*-D-(6″-coumaroyl)-glucopyronoside. The mass spectrum of Kaempferol-3-*O*-*β*-D-(6″-coumaroyl)-glucopyronoside was given the molecular formula $C_{30}H_{26}O_{13}$ m/z (%): 595 [M+1] (38 %). Based on the above spectral evidence, glycoside acquired from *Euphorbia hirta* flowers has been elucidated as Kaempferol-3-*O*-*β*-D- (6″-coumaroyl)-glucopyronoside.

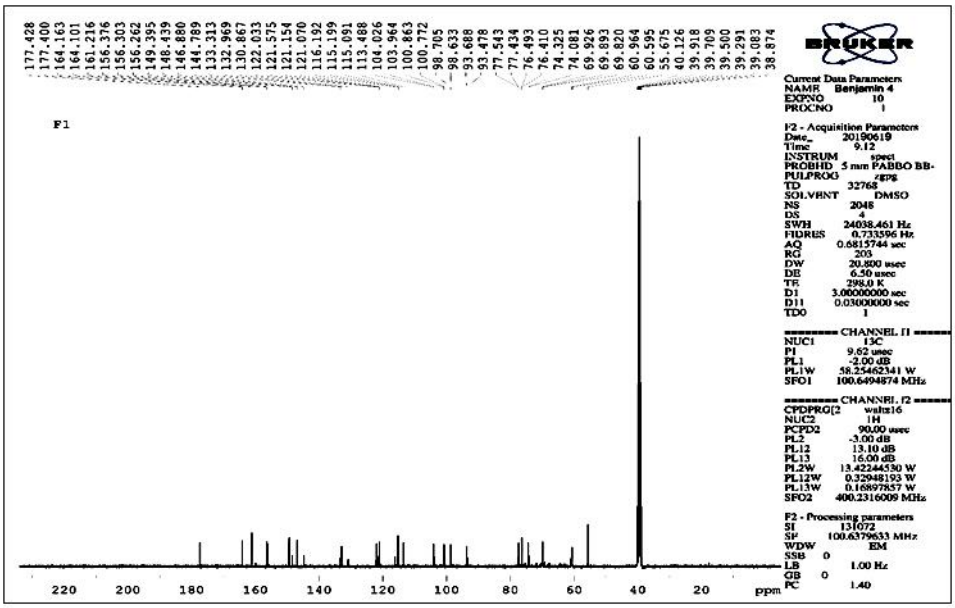

Fig. 5. ^{13}C-NMR spectrum of Kaempferol-3-*O-β*-D- (6″-coumaroyl)-glucopyronoside.

4. Discussion

4.1. *Anti-cancer activity*

Among a few cancers, hepatocellular carcinoma (HCC) is one of the most frequent and lethal cancers worldwide. It debts for about 90 % of all liver cancers and it represents greater than 4 % of all most cancers instances worldwide [31]. The isolated Kaempferol-3-*O-β*-D-(6″-coumaroyl)-glucopyronoside was exhibited average inhibition in HeLa cell lines with GI_{50} of 100 µg, TGl of >100 and LC_{50} of >100 respectively, which has illustrated in Fig. 6. This study is mainly focused on to determine the inhibition activities of the flavonoid glycosides of Kaempferol-3-*O-β*-D-(6″-coumaroyl)-glucopyronoside in HeLa human cancers cell lines. The Percentage of growth of HeLa towards the flavonoid glycoside consequences and raw facts has been given in Table 1.

Table 1. Percentage growth of HeLa against the flavonoid glycoside.

Name of the compound	Percentage growth (µg)					Growth inhibition (µg)		
Kaempferol-3-*O-β*-D- (6″-coumaroyl)-glucopyronoside	100	10	1	0.1	0.01	GI_{50}	TGl	LC_{50}
	99	95	104	99	100	>100	>100	>100

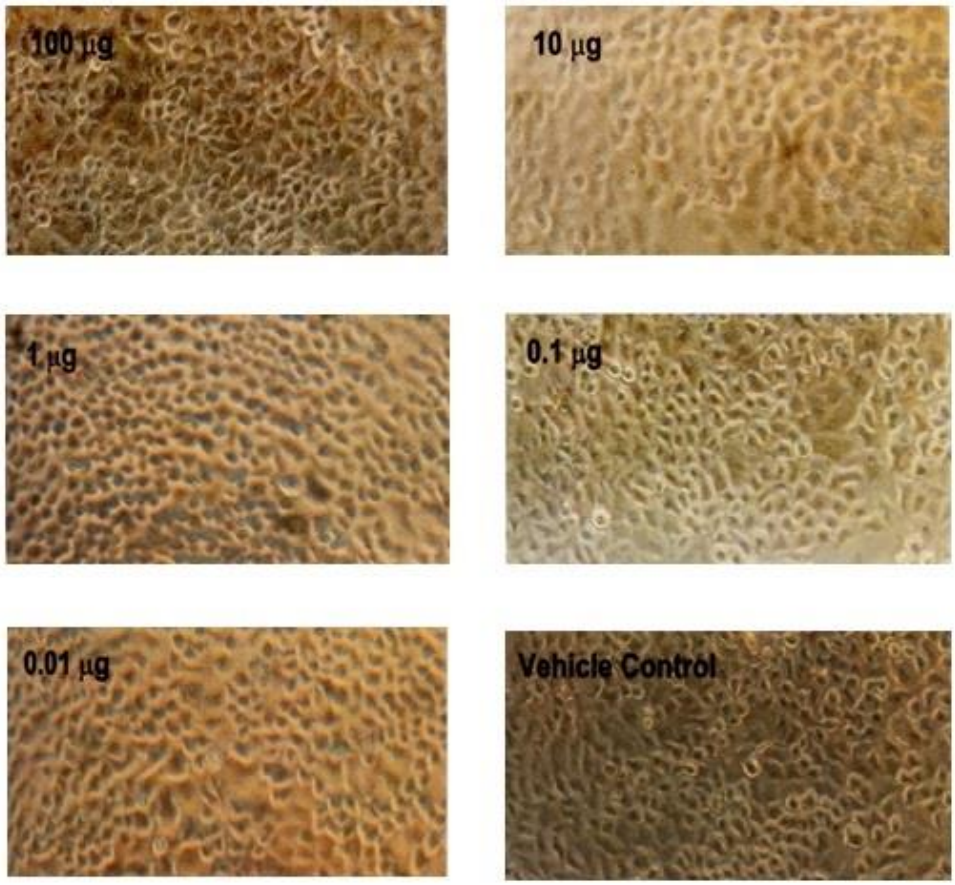

Fig. 6. HeLa cells treated with the Kaempferol-3-*O*-*β*-D- (6″-coumaroyl)-glucopyronoside for forty eight hours.

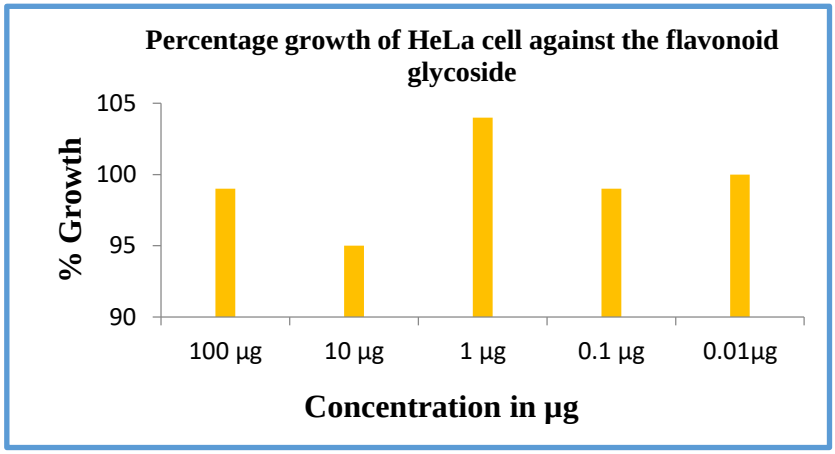

Fig. 7. Pictorial representation of percentage growth of HeLa cell against the flavonoid glycoside.

4.2. *In silico molecular docking analysis of natural flavonoids as anti-cancer agents*

The molecular docking intentions have been carried out on Auto Dock-Vina software and as pronounced in literature [32]. The docking protocol expected the same conformation as used to be present in the crystal structure with RMSD value nicely inside the dependable vary of 2A°. Amongst the docked conformations, one which binds properly at the active site was once analyzed for unique interactions in Discovery Studio Visualizer 4.0 software. Molecular docking is a precious method computational chemistry and medicinal chemistry to acutely analyses ligand recognition and it has led to vital breakthroughs in drug discovery and design. Molecular docking methodology explores the binding mode and affinity of a small molecule inside the binding site of the receptor target protein [33, 34]. The docked ligands have been ranked in accordance to their binding affinity in a ligand–receptor complex (Fig. 2). Based on the binding affinities as exhibited by way of the docking research supported with the aid of the *in-vitro* assays (Table 2.), the contemporary data was given conclusion that the Kaempferol-3-*O*-β-D-(6″-coumaroyl)-glucopyronoside compound has more affinity with cancer cells [35,36].

Table 2. The binding affinity values of different doses of Kaempferol-3-*O*-β-D-(6″-coumaroyl)-glucopyronoside ligands on breast cancer target protein 1DI8 predicted by autodock-Vina Protein.

Kaempferol-3-*O*-β-D-(6″-coumaroyl)-glucopyronoside		
Affinity (kcal/mol)	Distance from the best mode	
	rmsdl.b.	rmsdub.
-7.6	0.000	0.000
-7.2	4.001	10.592
-6.9	4.425	11.501
-6.7	4.604	7.867
-6.7	4.384	10.388
-6.6	1.747	2.400
-6.6	2.150	8.060
-6.5	3.108	5.811
-6.5	4.381	10.392

4.3. *Structure of target proteins*

The most important therapeutic targets of breast cancer taken for the study had been ERα, PR, EGFR, and mTOR. The three-dimensional structures of the following breast cancer target proteins have been availed from protein data bank with the PDB ids: 1DI8, 1XO2 and 2OJ9 respectively. The ligand binds at the active site of the substrate by weak non-covalent interactions and these interactions are depicted in Fig. 8. In the ligand protein

docking calculations, the most positive conformation for each and every ligand is chosen from 10 conformations.

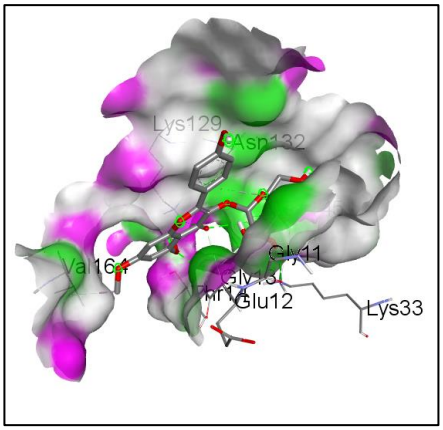

Fig. 8. 2D interaction of Kaempferol-3-O-β-D-(6″-coumaroyl)-glucopyronoside ligand with the H-bond surfaces of the 1DI8 breast cancer target protease.

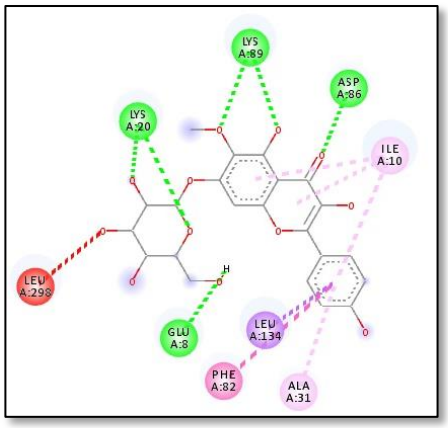

Fig. 9. 2D interaction of Kaempferol-3-O-β-D-(6″-coumaroyl)-glucopyronoside with H-bond surfaces of the receptor breast cancer target inhibitor.

Hydrogen bonds are invented to construct fundamental aid to the connections between the ligand and protein. There are seven hydrogen bonds in Kaempferol-3-*O*-β-D-(6″-coumaroyl)-glucopyronoside (Fig. 9) and 1DI8 protein -7.9 kcal/mol binding energy with five conventional, four carbon hydrogen bonds Thr14, Lys129, Gly11, Asp145, Unl1, Gly13, Glu12 and Asn132 with bond lengths of 2.69, 2.86, 2.82, 2.54, 2.39, 3.65, 3.57, 3.62 and 3.79 Å respectively. One hydrophilic interaction of alkyl was observed in the ligand (4) Val164 residues having bond distance of 4.59 Å.

5. Conclusion

Now-a-days, many carcinogenic products are growing rapidly from various artificial sources which lead to produce cancer diseases in human beings. Owing to give more importance to control such kind of epidemic diseases, many indigenous medicinal plants and its derivatives are attracted towards people to live a healthy life. For the sake of performing to control the growth of cancer cell, Kaempferol-3-*O-β*-D-(6″-coumaroyl)-glucopyronoside flavonoid compound was isolated from important indigenous flower of *Euphorbia hirta*. From the anticancer results, it was concluded that this compound have been shown good anticancer activity against HeLa cells. Thus, it has been proved that this flavonoid compound taken from *Euphorbia hirta* flower acts more efficiently against the cancer cells without any toxic effects.

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Availability of data and materials

"The data supporting the findings of the article is available in the Mendeley data" DOI: 10.17632/jjxmv35jhp.1" under CC BY 4.0

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