Academic Journal of Current Research
Vol.7, No.8; August-2020;
ISSN (2343 – 403X);
p –ISSN 3244 - 5621
Impact factor: 6.37

THEORETICAL STUDY, ANTIOXIDANT ACTIVITY AND ANTI-CANCER STUDIES OF GALANGAL (*ALPINIA GALANGAL*)

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Abstract: The implication of oxidative stress in the promotion of carcinogenesis suggests that antioxidant therapy represents a promising opportunity for cancer prevention. Additionally, a therapeutic strategy to increase the antioxidant capacity of cells may be used to fortify the long term effective treatment [1]. Herbs and spices are recommended for prevention and care of various diseases including cancer. *Alpinia galanga* a member of the ginger family, previous studies have demonstrated an anticancer effect of the *Alpinia galanga*. Polyphenols and flavonoids components were determined. Results indicated *Alpinia galanga* contained high content of total phenol (34.21 ± 17.45 mg/g) and flavonoids (28.79 ± 12.23 mg/g). Measurement of potential cytotoxicity activity of ethanol extract of *Alpinia galanga* against the liver carcinoma cell line (HEPG-2H), breast carcinoma cell line (MCF-7) and blood carcinoma cell line (HCT) were tested by SRB assay. Data was recorded (IC50) on liver (HEPG-2H) cells, when the concentration was 117 µg/ml. While the effective concentration on breast (MCF-7) cells was 64 µg/ml on the same trend, in blood (HCT) cells effective concentration was to 85 µg/ml. Conclusively, the effective concentration ranged from 64 to 117 µg/ml. These effects may be due to the concentration of phenol and flavonoids contents in galangal. Density functional theory calculations (DFT) under the level of B3LYP/6-311G** have been utilized to explore the structure, molecular properties and antioxidant abilities of the main active compounds in *Alpinia galangal* (quercetin, Trans-p-coumaryl alcohol, Trans-p-hydroxycinnamaldehyde, 3,5,7-trihydroxy-2-phenylchrom an-4-one and Galangi). The calculated E_{HOMO} and E_{LUMO} energies of the studied compounds can be used to calculate the global properties; chemical hardness (η), softness (S) and electronegativity (χ). In this work, we try to explain theoretically which of the studied components in *Alpinia galangal* is more effective as antioxidant. No theoretical studies in the literature explained this point.

Keywords*: antioxidants and DFT. Galangal (Alpinia galangal), liver and Blood Carcinoma*

➢ ***No conflict of interest statement in the article file.***

presence of the cell's defense system, oxidative damage accumulates during the life period. Moreover, this radical-related damage to DNA, to proteins and to lipids could play a crucial role in the development of age-dependent diseases such as cancer, arteriosclerosis, arthritis, neurodegenerative disorders and other conditions [4]. The roles of antioxidants are to act as "free radical scavengers" to prevent and/or repair damages

INTRODUCTION:

Cancer is one of the major causes of mortality worldwide. Despite tremendous efforts to create effective chemotherapy drugs, there are still a huge toxicity and selectivity issue. The toxicity of modern chemotherapy and cancer cell resistance to anticancer agents lead us to seek new treatments and prevention methods of this insidious disease [2,3]. Despite the

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Methods:

1. Microcomponents analyses

1.1. Determination of Carotenoids:

β-Carotene and lycopene were determined according to the method of Nagata et al., [47]. The dried ethanolic extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whitman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of β-carotene was calculated according to the following equations:

β-Carotene (mg/100 ml) = 0.216 A663– 1,22 A645– 0.304 A505+ 0.452 A453

1.2. Determination of Tannins (as tannic acid)

Total tannins were determined calorimetrically as described by A.O.A.C., [40]**.** Sample was mixed with 50ml methanol in a closed conical flask and left for 20h at room temperature (25±2°C). The mixture was centrifuged for 30min at 3000 rpm. The tannins in the supernatant were estimated using Folic-Denis reagent. The formed color was determined at 760nm. Tannic acid was used to prepare the standard curve.

1.3. Determination of Saponins

Saponins in dried galangal were determined by gravimetrically methods as described by Anhawange et al., [18]**.**

1.4. Determination of phenols

The content of total phenols in the ethanol extract was determined according to the procedure described by Amarowicz et al., [19], using the Folic- Ciocalteu's phenol reagent. Gallic acid was used in the standard curve.

1.5. Determination of Total Flavonoids:

Flavonoids were determined according to Chang et al., [20]**.** A solution of ethanol extract (5ml) was mixed with 3ml ($AlCl_3$, 2.4%) and potassium acetate (9.8%) was added. Then the mixture was allowed to stand for 10 min at room temperature. The absorbance of the reaction mixture was measured at 420 nm with an ultraviolet visible spectrophotometer (UV-Vis Jenway 6405, UK). The total flavonoids content was determined using the Quercitine calibration curve, at concentrations from

caused by ROS and RNS, and ultimately to lower the cancer risk [5, 6]. The importance of plant substances in medicine and pharmacy is well known from ancient times; herbal substances are often used as the basic structure in the development of new anticancer drugs [7]**.** Cancer cells usually attack and destroy normal cells; these cells are born due to imbalance in the body [8]. Every year, millions of people are diagnosed with cancer loading to death [9]. The SRB analysis carried out in the cancer of liver (HEPG-2H), breast cancer cells (MCF-7) and blood cancer cells (HCT) revealed that the ethanolic extract of *Alpinia galanga* could effectively reduce the growth and multiplication of the tumor cells. *Alpinia galanga* (Family-Zingiberaceae) known as greater galangal in English and Barakulanjan in Hindi [10], have been widely cultivated in India, Malaysia, Indonesia, Egypt and Saudi Arabia [11]. *Alpinia galanga* is rich in phenolic compounds such as flavonoids and phenolic acids [12]. Ethanolic extract of *Alpinia galanga* showed the potent scavenging activity by DPPH [13]. The ant-carcinogenic effect of the ethanolic may be due to rich antioxidant content (phenolic and flavonoids) [14]. In addition, it was Concluded that using cell culture model anticancer showed effect of extract of *Alpinia galanga* on human liver cancer cells (HEPG-2H), breast cancer cells (MCF-7) and blood cancer cells (HCT). This effect ranged from 64 to 117 µg/ml [15]**.**

DFT calculations were used in studying the antioxidant activities of many phenolic compounds such as flavonoid [16, 17].

To our knowledge, there is no computational study on antioxidant activity of phenolic or flavonoid compounds in *Alpinia galanga*.

Materials and Methods

Materials:

1. Identification of plant materials:

Plants: Samples of Galangal *(Alpinia galanga)* were collected from El-Fayoum city (Egypt) in April 2014 during the flowering period. Those samples were purchased from Egyptian organic products company.

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3. Computational methods:

All computations were carried out using Gaussian 09W hyperchem 8.0, chemcraft 1.6, Gaussview 9.0 and isis draw software package. Molecular geometries of all the studied compounds were fully optimized using B3LYP/6-311G** [24-26]. No symmetry constrains were applied during the geometry optimization [27,28]. The choice of this basis set was due to its flexibility and the fact that the diffused p functions on the H-atom tend to compensate the inharmonic effects of the CH and NH stretches. By using HOMO and LUMO energy values for a molecule, using RHF/6-311G**, electronegativity and chemical hardness can be calculated as follows: X=(I+A)/2 (electronegativity), η=(I-A)/2 (chemical hardness), S=1/2η (chemical softness) where I and A are ionization potential and electron affinity, and I= $-E_{HOMO}$ and A= $-E_{LUMO}$, respectively [29]. Throughout this work; MOs were constructed using the Gauss-view 5.08 visualization program. The total static dipole moment (μ), the mean polarizability <α>, the anisotropy of the polarizability Δα and the mean first hyper polarizability <β> using the x, y, z components were calculated by using the following equations [30,31]:

$$\mu = (\mu_x^2 + \mu_y^2 + \mu_z^2)^{1/2}$$

$$\alpha = \frac{(\alpha xx + \alpha yy + \alpha zz)}{3}$$

$$\Delta\alpha = \sqrt{\left[\frac{(\alpha xx - \alpha yy)2 + (\alpha yy - \alpha zz)2 + (\alpha zz - \alpha xx)2}{2}\right]}$$

$$<\beta> = \beta_x^2 + \beta_y^2 + \beta_z^2$$

Where:

$$\beta_x = \beta_{xxx} + \beta_{xyy} + \beta_{xzz}$$

$$\beta_y = \beta_{yyy} + \beta_{xxy} + \beta_{yzz}$$

$$\beta_z = \beta_{zzz} + \beta_{xxz} + \beta_{yyz}$$

4. Anticancer activity:

Human Hepatoma (HepG2) Cells and Ehrlich Ascites Carcinoma (EAC) cell lines were prepared from National cancer institute, Cairo, Egypt. The cancer cells were cultured in HCT, HEPG2-H and MCF7-H medium and supplemented with10%FBS, 100U/ml penicillin, 100μg/ml streptomycin, and 2mML-glutamine incubated at 370C for 24hrs with 5%CO2 in air [32].

0.005 to 0.125 mg/ ml in methanol, and expressed as mg of Quercitine equivalents.

2. Fractionation of Polyphenols and Flavonoids by HPLC:

2.1. HPLC Determination of Phenolic compounds:

Phenolic compounds determined by HPLC according to Goupy et al., [21], as following: 5g of sample were mixed with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtrated through 0.2μm Millipore membrane filter then 1-3ml was collected in avail for injection in HPLC Hell wet Packared (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set as 289 nm and quarter HP pump (series 1050). The Colum temperature was maintained at 35 ^{0}C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1ml/min. phenolic acid standard from sigma co. were dissolved in mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis by Hell wet Packared software.

2.2. HPLC Determination of Flavonoid compounds:

Flavonoid compounds determined by HPLC according to Mattila et al., [22], as following: 5g of sample were mixed with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtrated through 0.2μm Millipore membrane filter then 1-3ml was collected in avail for injection in HPLC Hell wet Packared (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set as 330nm and quarter HP pump (series 1050). The Colum temperature was maintained at 35^{o}C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1ml/min. Flavonoid acid standard from Sigma Co. were dissolved in mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis by Hell wet Packared software.

Antioxidants content:

DPPH scavenging activity tests were carried out according to Brand-Williams et al., [23].

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al.,[37], who worked on similar compounds in ethanol extracts of *Alpinia galanga.*

Fortunately, rhizome of galangal tended to have high amount of polyphenols and flavonoids [38]**.** Flavonoids have been shown to be powerful antioxidants in vitro [39]**,** and the *Alpinia galanga* contain high amount of phenolic so it acts as an antioxidant.

2. Geometry optimization

Geometry optimization is carried out under the B3LYP/6-311G basis set of Gaussian 09. Figures (1,2) showed the structure and numbering system of quercetin, Trans-p-coumaryl alcohol, Trans-p-hydroxycinnamaldehyde, 3,5,7-trihydroxy-2-phenylchrom an-4-one and Galangi molecules while the optimized structures, the vector of the dipole moment, net charge, a part of calculating bond length and bond angles of these neutral components are shown in figures (3:7). In addition, the optimized structures, the vector of the dipole moment, net charge, a part of calculating bond length and bond angles of these radical components are shown in figures (8-12)**.** Ground state parameters of the studied neutral molecules and their radical at B3LYP/6-311G level of theory are tabulated in tables (5,6).

From the optimized molecular structure of the selected molecules, it can be seen that 2, 3-double bond exists only in the Quercetin (Qur), Galangi (Ga), and 3,5,7-trihydroxy-2-(3-hydroxyphenyl) chroman-4-one (THPhC). In addition, it ensures π-electron delocaliza-tion between the B- and C-rings, which contributes to the stabilization of MO· (it has been used to represent the antioxidant free radical) after H-abstraction. According to the theoretically study quercetin, 2, 3-double bond and the C3–OH group make it planar and C-ring expands the planar structure as the connect ring. It is beneficial for the H-atom transfer (HAT) and forms the more stable half-quinone free radical in scavenging the free radical. The optimized structures are planar because the hydroxyl groups on the rings A and B are oriented in the same direction. furthermore, Trans-p-coumaryl alcohol (TPCA), Trans-p-hydroxycin-namaldehyde (TPHC) are planer as shown in figures (3:8). However, the H atom of

RESULTS AND DISCUSSION

1. Antioxidants contents:

The beneficial effects of total phenols, carotenoids, flavonoids, tannins, and saponins are well known as antioxidants. They also have anti-carcinogenic agent [33]. Total phenolic compounds were 34.21 ± 17.45 mg/g, respectively as shown in Table (1). Results in the same Table (1) revealed that carotenoids in rhizome of *Alpinia galanga* were 10.4 ± 9.05 mg/g., while total flavonoids contents were 28.79 ± 12.23 mg/g. The content of tannins was 14.7 ± 12.23 mg/g, and contents of saponine was 11.9 ± 9.12 mg/g. Tannins and saponins are considered beneficial; since they act as antioxidants, anti-microbial, anti-hyperglycemic, anti-hyperchloestero-lemic, ant-carcinogenic and antidiarrheal agents. These studies agreed with the study of Mahae et al., [34].

DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. DPPH is a well-known radical and a trap (scavenging) for other radicals [35]. The galangal showed greater antioxidant activity. The results of this study confirmed that the ethanol extract of galangal had a higher amount of DPPH component than water extract of galangal (87.45±3.41, 48.60±5.12) respectively (see table 2)**.**

Data in tables (3 and 4) revealed that both polyphenols and flavonoids contents were relatively high in ethanol extracts (70%) from rhizome of *Alpinia galanga* which contains relatively high amount of polyphenols like Syringic (206.86 mg/100g), Pyrogallol (200.84 mg/100g), Benzoic (29.53 mg/100g), and Protocatchuic (27.66 mg/100g)**.** Flavonoids are the largest group of phytonutrients, with more than 6,000 types. Some of the best-known flavonoids are Hespertin, quercentin and kaempferol [36]**.** Results in Table (4) indicated that flavonoids rocket contained high amounts of Hisperdin (10.14 mg/100g), Rutin (5.04 mg/100g), Naringin (4.67 mg/100g), 7-OH flavones (1.14 mg/100g), Narengenin (1.09 mg/100g) and Kampherol (98.14 mg/100g). These data were in agreement with data obtained by An et

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Dipole moment of the studied compounds (in case of neutral) are in following order TPHC > Ga > Qur > THPhC > TPCA and (in case of free radical) is the same order, TPHC > Ga > Qur > THPhC > TPCA. It is mean that TPCA and THPhC are more reactive than the others especially if these have low energy gap. The ionization potential of the studied compounds and their free radicals are in following order; TPHC > Ga > THPhC > Qur > TPCA in case of neutral and in free radical is, TPHC > Ga > Qur > THPhC > TPCA

As very low ionization potential of neutral TPCA indicates its low antioxidant ability in the neutral form, as it can scavenge the free radicals through the single electron transfer- proton transfer (SET-PT) mechanism. The ionization potential of TPCA free radical is also very low as compared to others free radicals. This is probably due to the absence of intramolecular hydrogen bond which are present in galangi, quercetin and THPhC. On the other hand, O12 in case of Galangi and 3,5,7-trihydroxy-2-(3-hydroxyphenyl) chroman-4-one while O12, O16 and O18 in case of quercetin are engaged in intramolecular hydrogen bonding in C3`-OH and C4`-OH in free radicals, respectively. Therefore, electrons on O12**,** O16 and O18 cannot donate easily, so they have relatively higher ionization potential. The order of electron affinity of these compounds and its free radicals can be given as follow;

TPHC > THPhC > Ga > Qur > TPCA in neutral and
TPHC > Ga > Qur > THPhC > TPCA in free radical

The electron affinity of TPCA free radicals is very low as compared to its neutral. The electron affinity of TPHC and Ga radicals are very high, which indicates that these radicals have low electron accepting tendency but high electron donating ability.

Frontier molecular orbital theory

The frontier molecular orbital energies, E_{HOMO} and E_{LUMO} are also very crucial factors of molecular electronic structure. The lower of the E_{HOMO} implies that the molecule has the electron donating ability, while higher the E_{HOMO} implies that the molecule is a good

C3–OH group is relatively stable because the hydrogen bonds as shown in figure (11)**.** Compared with all phenol hydroxyl groups on Quercetin (Qur), Galangi (Ga), and 3,5,7-trihydroxy-2-(3-hydroxyphenyl) chroman-4-one (THPhC) molecules, O-H bond length of C3 is noticeably longer than the others. It may be related to the ketone group in C-ring which forms intramolecular hydrogen bonds (IHBs) with the C5–OH group, so that the O-H bond length of C3 is stretched. Many hydrogen bonds are also found as between the O-H of C3′, O-H of C4′ groups and between O-H of C3 and C=O ketone group in the Quercetin (Qur) while hydrogen bonds are found between the O-H of C3 and C=O ketone group in Galangi (Ga), and 3,5,7-trihydroxy-2-(3-hydroxyphenyl) chroman-4-one (THPhC) as shown in figures (3:12). However, in case of Trans-p-hydroxycinnamaldehyde and Trans-p-coumaryl alcohol there are not hydrogen bonds.

In these compounds, the behavior of the different OH groups is largely influenced by the neighboring groups as well as by the geometry. Hence, the conformation can be regarded as the important parameter of interest to analyze the antioxidant ability of phenolic compounds. By comparing the bond distance as shown in figures (3:12) shows that, in neutral and radical compounds the two intramolecular O—H--- O hydrogen bonds are present and the length of it is ranged between 1.940 - 2.380 Å. And nearly the same values in radical compounds. The intramolecular hydrogen bonds can stabilize the ground state molecule resulting in the slow abstraction of H atoms. On the other hand, intramolecular hydrogen bond present in the free radicals formed after the abstraction of H atom so it makes reaction very fast.

3.4 Electronic properties

The electronic properties of the studied compounds and its free radicals are presented in tables (5,6). The molecular dipole moment essentially constitutes an index of reactivity, which is very important to define the biological properties particularly related to the interaction with enzyme active sites.

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ISSN (2343 – 403X);
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have the same reactivity. The lower E_{gap} indicates that the easier the electrons inspire give vigorous evidence to the antioxidant action. The low magnitude of the band gap energy indicates that the compounds could be a highly reactive system. The different energy of E_{gap} in these compounds are representation of molecular planarity and quantum electronic feature of the frontier orbital [42]. The analysis of band gap values obtained for TPCA, TPHC, Ga, THPhC and Qur are in accord with the results of the frontier orbital theory.

Generally, from the above result, it is clear that, the galangal plant has antioxidant properties where it contains on many high reactive molecules as Qur, Ga and THPhC which act as powerful antioxidant. Where TPCA and TPHC have low antioxidant properties.

Anticancer activity: -

The anticancer activity of ethanolic extract of *Alpania galanga* has been examined against three cancer cell lines represents hepatic cancer, breast cancer and blood cancer (HEPG2-H, MCF7-H and HCT, respectively). The extract was applied [15] at concentrations ranged from 0.0 to 50µg/ml (table 8). The hepatic cancer in the form of HEPG2-H cell line exhibited more resistance for extract and this was appeared clearly in the value of LC50 which recorded 117µg/ms (figure 15) this result was agree with [43]. Breast cancer is cancer that develops from breast tissue [44]. Signs of breast cancer may include a lump in breast, a change in breast shape and dimpling of the skin [45]**.** Moreover, the different concentration of rhizome extracts showed sharply decrease in LC50, comparison to the other concentration (table 9). The best results were recorded in 64 µg/ml this was appeared clearly in the value of LC50 (figure 16). On the other hand, the blood cancer in the form of (HCT) cell line exhibited more resistance for extract and this was appeared clearly in the value of LC 50 which recorded 85 µg/ml (figure 17 and table 10), this agrees with Spector et al.[15]

Generally, the inhibition on different cancer cells increase gradient by increasing extract concentration [46]**.** Conclusively, the effective concentration was ranged

electron donor. The E_{LUMO} represents the ability of a molecule to accept electron.

The frontier orbital calculated for the studied neutral compounds at the level of B3LYP/6-311G in the gas phase is shown in figures (13,14) and the frontier orbital energy is also indicated in table (7). The active site can be demonstrated visually by the distribution of frontier orbital. From figures (13,14) we can find that the HOMO of the studied neutral compounds is mainly distributed in the B-ring, while the LUMO allocated in the C-ring. So the higher ability to respond to the functional site is mainly focused on B-ring and the conjugate part. The electron-donating capability of a molecule can be determined by the values of HOMO. A high HOMO corresponds with a strong capability to donate electrons [41]. It can be seen from table (7) that, TPHC possesses the highest HOMO among the studied compounds, while TPCA owns the lowest. Based on our calculations, the trend for computing HOMO is scarcely different from that of BDE, correspond to the arrangement: TPHC > Ga > THPhC > Qur > TPCA.

Another parameter to be given importance is the energy gap (*E*gap) of these three compounds. The energy gap order for neutral compounds is, TPCA > TPHC > Ga > THPhC > Qur. From the above the order of reactivity of neutral compounds is, TPCA < TPHC < Ga < THPhC < Qur, its mean that, Qur is the highest active than others and TPCA is the lowest active than the others also, TPHC has low reactivity where its dipole moment is 4.82 D with high value of E_g (4.134 eV). In case of free radicals, the energy gap order is, TPHC > Ga > Qur > TPCA > THPhC. From the above the order of reactivity of free radicals' compounds is, TPHC < Ga < Qur < TPCA < THPhC. Also, TPHC free radical has the lowest reactivity, while THPhC free radical has the highest reactivity. Generally, Qur and THPhC are more reactive than others. The band gap energy difference between quercetin and Ga is 4 eV, and the difference in the band gap energies of quercetin and THPhC is found to be 3.4 eV. While the band gap energy difference between THPhC and Ga is 0.62 eV. So the THPhC and Ga nearly

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Conclusion

Polyphenols and flavonoids components in *Alpinia galanga* were determined. Results indicated Alpinia galanga was content of total phenol (34.21 ± 17.45 mg/g) and flavonoids (28.79 ± 12.23 mg/g). Measurement of potential cytotoxicity activity of ethanol extract of Alpinia galanga against the liver carcinoma cell line (HEPG-2H), breast carcinoma cell line (MCF-7) and blood carcinoma cell line (HCT) was tested by SRB assay. Conclusively, the effective concentration was ranged from 64 to 117 µg/ml, these effect may be due to the concentration of phenol and flavonoids contents in galangal.

Density functional theory calculations under the level of B3LYP/6-311G** was used to explore the structural features and molecular properties of some flavonoid compounds of *Alpinia galanga* including (quercetin, Trans-p-coumaryl alcohol, Trans-p-hydroxycinnamaldehyde, 3,5,7-trihydroxy-2-phenylchrom an-4-one and Galangi). The study has concerned the geometrical properties and various molecular descriptors such as the HOMO, LUMO and *E*gap in order to identify the order of flavonoids compounds as powerful antioxidant and so, the most compounds act as anti-cancer. We find quercetin and 3,5,7-trihydroxy-2-(3-hydroxyphenyl) chroman-4-one are more reactive than others compounds so they are responsible for antioxidant activity of *Alpinia galanga plant*. ***So quercetin and 3,5,7-trihydroxy-2-(3-hydroxyphenyl) chroman-4-one can be used as antioxidant, anti-cancer prevention and treatment.***

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Tables

Table (1): Antioxidants content in rhizome of galangal (mg/g on dry weight basis).

Constituents	mg/g *
Total phenols	34.21±17.45
Carotenoids	10.4±9.05
Total flavonoids	28.79±12.23
Tannins	14.7±9.67
Saponins	11.9±9.12

***Means of triplicate samples ± standard division**

Table (2): Free radical-scavenging activity of galangal (water and ethanol) extracts.

Samples	DPPH (µg/ml) *
Water extract	48.60±5.12
Ethanol extract	87.45±3.41

***Means of triplicate sample ± Standard Division**

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Table (3): Polyphenolic fractions from Rhizome by HPLC (mg/100g) on dry weight basis

Phenolic compounds	**(mg/100g)**
Syringic acid	206.86
Gallic acid	2.26
Pyrogallol	200.84
4-Amino-benzoic acid	1.19
Protocatchuic acid	27.66
Catechein	2.92
Chlorogenic acid	5.94
Catechol	4.38
Epicatechein	9.36
Caffeine	0.57
P-OH-benzoic acid	7.16
Caffeic acid	6.07
Vanillic acid	2.92
Ferulic acid	4.64
Vanillic acid	10.78
Ellagic acid	3.10
Alpha– counmaric acid	0.71
Benzoic acid	29.53
Salycilic acid	52.61

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Coumarin	2.19

Table (4): Flavonoids fraction of extracts from Rhizome by HPLC (mg/100g on dry weight basis)

Flavonoids	**mg/100g**
Naringin	4.67
Rutin	5.04
Hisperdin	10.14
Rosmarinic acid	0.29
Quercetrin	0.40
Quercetinin	0.14
Narengenin	1.09
Kampherol	0.98
Hespertin	0.46
Apegenin	0.46
7-OH flavones	1.14

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Table (5): The ionization potential (I), electron affinity (A), chemical hardness (η), softness (S), chemical potential (Ц) and electronegativity (χ), total energy (ET) and dipole moment (μ) of neutral Ga, THPhC, TPHC, Qur and TPCA using B3LYP/6-311G.

Component	***Ga***	***THPhC***	***TPHC***	***Qur***	***TPCA***
Charge	***neutral***	***neutral***	***neutral***	***neutral***	***neutral***
I (eV)	5.9724	5.9593	6.4568	5.7411	5.7313
A (eV)	2.1211	2.1309	2.3231	2.0398	0.8732
η (eV)	1.92565	1.9142	2.06685	1.85065	2.42905
χ (eV)	4.04675	4.0451	4.38995	3.89045	3.30225
Ц (eV)	-4.04675	-4.0451	-4.38995	-3.89045	-3.30225
S (eV^{-1})	0.962825	0.9571	1.033425	0.925325	1.214525
E_T (eV)	-25749.54	-27780.33	-13450.94	-29811.22	-13483.23
μ (D)	4.7723	3.3687	4.8247	1.7754	1.3821

Table (6): The ionization potential (I), electron affinity (A), chemical hardness (η), softness (S), chemical potential (Ц) and electronegativity (χ), total energy (ET) and dipole moment (μ) of free radical Ga, THPhC, TPHC, Qur and TPCA using B3LYP/6-311G.

Component	***Ga***	***THPhC***	***TPHC***	***Qur***	***TPCA***
Charge	***radical***	***Radical***	***radical***	***Radical***	***radical***
I (eV)	10.4403	6.4774	11.1576	9.95482	0.24151

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A (eV)	9.0868	6.2083	9.7012	8.7608	0.22275
η (eV)	0.67675	0.1345	0.7282	0.597023	0.00938
χ (eV)	9.76355	6.3429	10.4294	9.3578	0.23213
Ц (eV)	-9.76355	-6.3429	-10.4294	-9.3578	-0.23213
S (eV^{-1})	0.338375	0.0673	0.3641	0.2985	0.00469
E_T (eV)	-25742.45	-27773.23	-13442.91	-29804.11	-13475.89
μ (D)	2.5775	1.7643	5.582	2.2827	0.3644

Table (7): The frontier orbital energy and energy gap of neutral and free radical Ga, THPhC, TPHC, Qur and TPCA using B3LYP/6-311G.

Component	***Charge***	***E_{HOMO}***	***E_{LUMO}***	***E_{gap}***
Ga	Neutral	-5.9724	-2.1211	103.9851
	Free radical	-10.4403	-9.0868	36.5446
THPhC	Neutral	-5.9593	-2.1309	103.3668
	Free radical	-6.4774	-6.2083	7.2664
TPHC	Neutral	-6.4568	-2.3231	111.618
	Free radical	-11.1576	-9.7012	39.3228
Qur	Neutral	-5.7411	-2.0398	99.9351
	Free radical	-9.9548	-8.7608	32.238
TPCA	Neutral	-5.7313	-0.8732	131.1687

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	Free radical	-0.2415	-0.2227	13.7832

Drug Cytotoxicity	
Conc., µg/ml	**HEPG2**
00.000	1.000
62.500	0.759
125.000	0.450
250.000	0.420
500.000	0.410

Table (8): Effect of different extract concentration of Alpinia galanga on surviving fraction liver:

Drug Cytotoxicity	
Conc., µg/ml	**MCF7^{-}**
00.000	1.000
62.500	0.502
125.000	0.251
250.000	0.379
500.000	0.420

Table (9): Effect of different extract concentration of Alpinia galanga on surviving fraction breast:

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Drug Cytotoxicity	
Conc., µg/ml	**HCT**
00.000	1.000
62.500	0.520
125.000	0.475
250.000	0.471
500.000	0.615

Table (10): Effect of different extract concentration of Alpinia galanga on surviving fraction blood:

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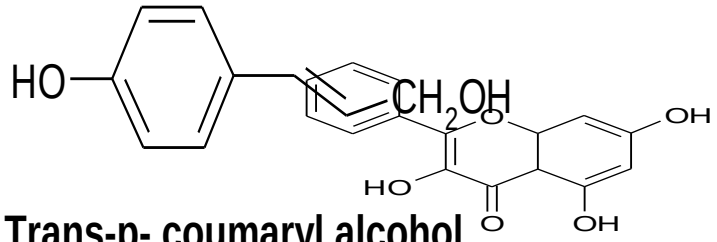
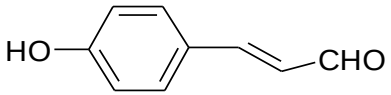


Trans-p- coumaryl alcohol

IUPAC : 4-((*E*)-3-hydroxyprop-1-enyl)phenol

Galangi

IUPAC : 3,5,7-trihydroxy-2-phenylchroman-4-one



Trans-p- hydroxycinnamaldehyde

IUPAC : (E)-3-(4-hydroxyphenyl)acrylaldehyde

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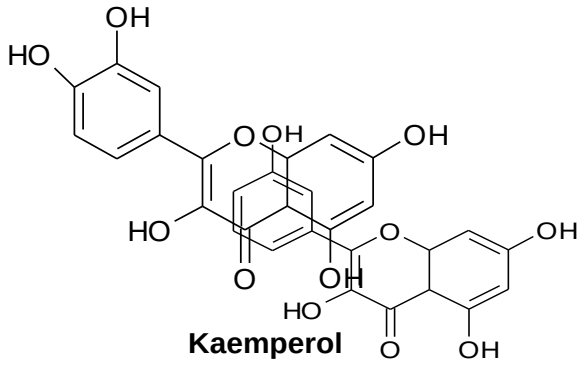

IUPAC : 3,5,7-tIUPAC : 3,5,7-trihydroxy-2-(p-hydroxyphenyl)chroman-4-one -2-(3,4-dihydroxyphenyl)chroman-4-one

Figure (1): The Structures of some selected components.

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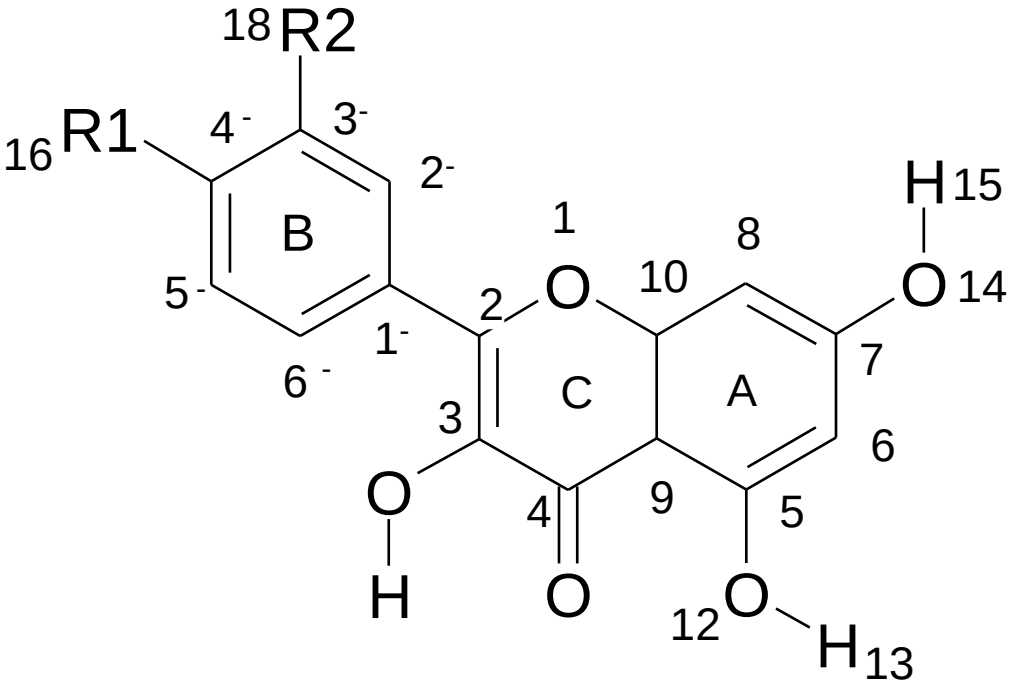

Where, R1 = H or OH and R2= H or OH

Figure (2): The Structures and numbering system of some selected components.

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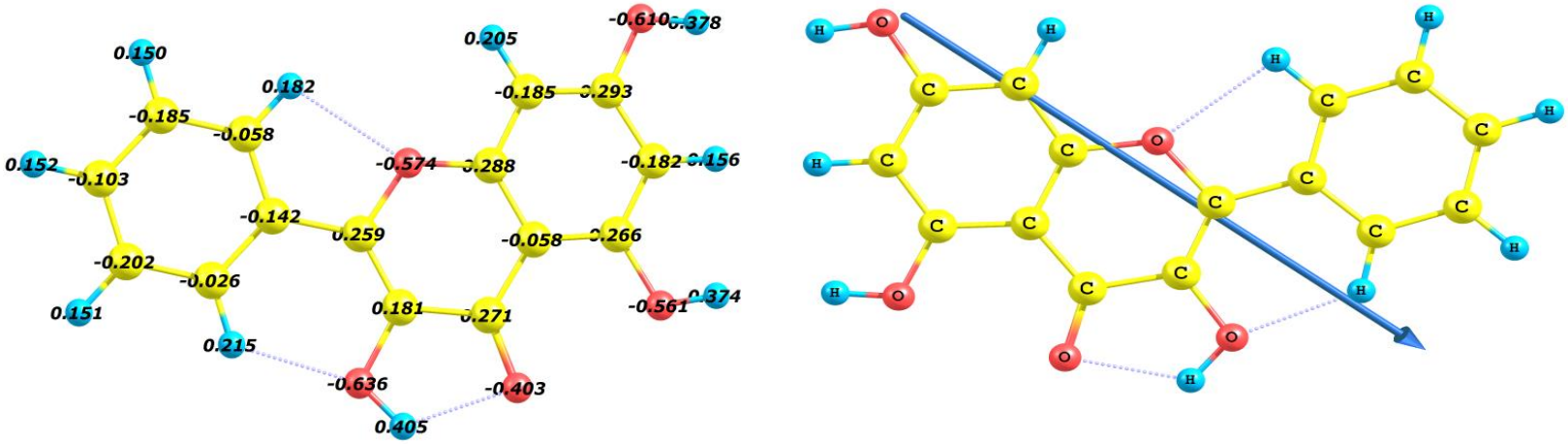

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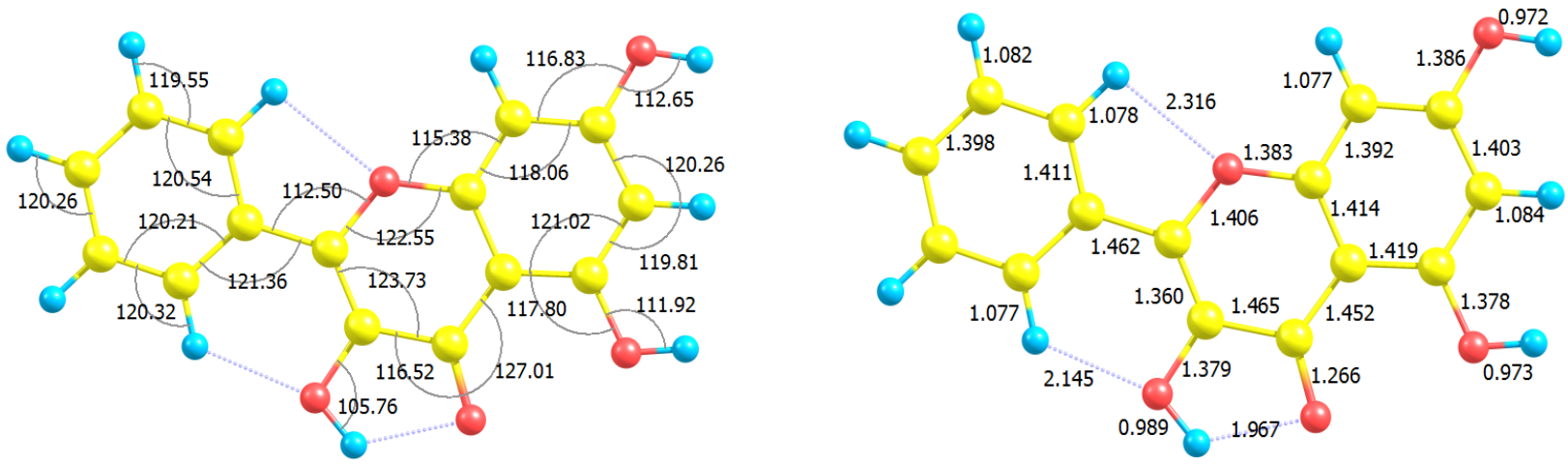

Figure (3): Geometry optimization, the vector of the dipole moment, net charge, bond lengths and bond angles of neutral Galangi

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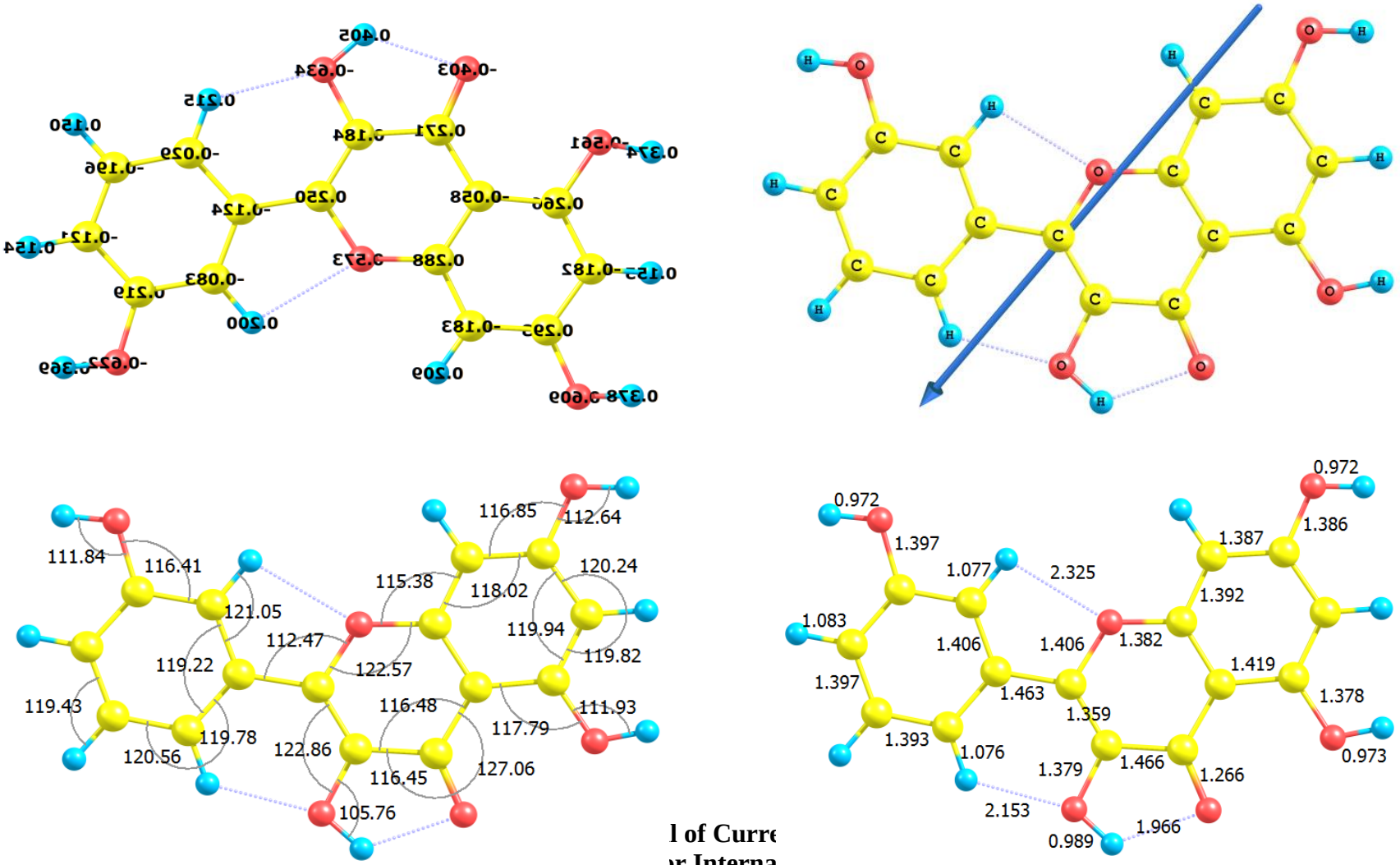

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Figure (4): Geometry optimization, the vector of the dipole moment, net charge, bond lengths and bond angles of neutral 3,5,7-trihydroxy-2-phenylchrom an-4-one.

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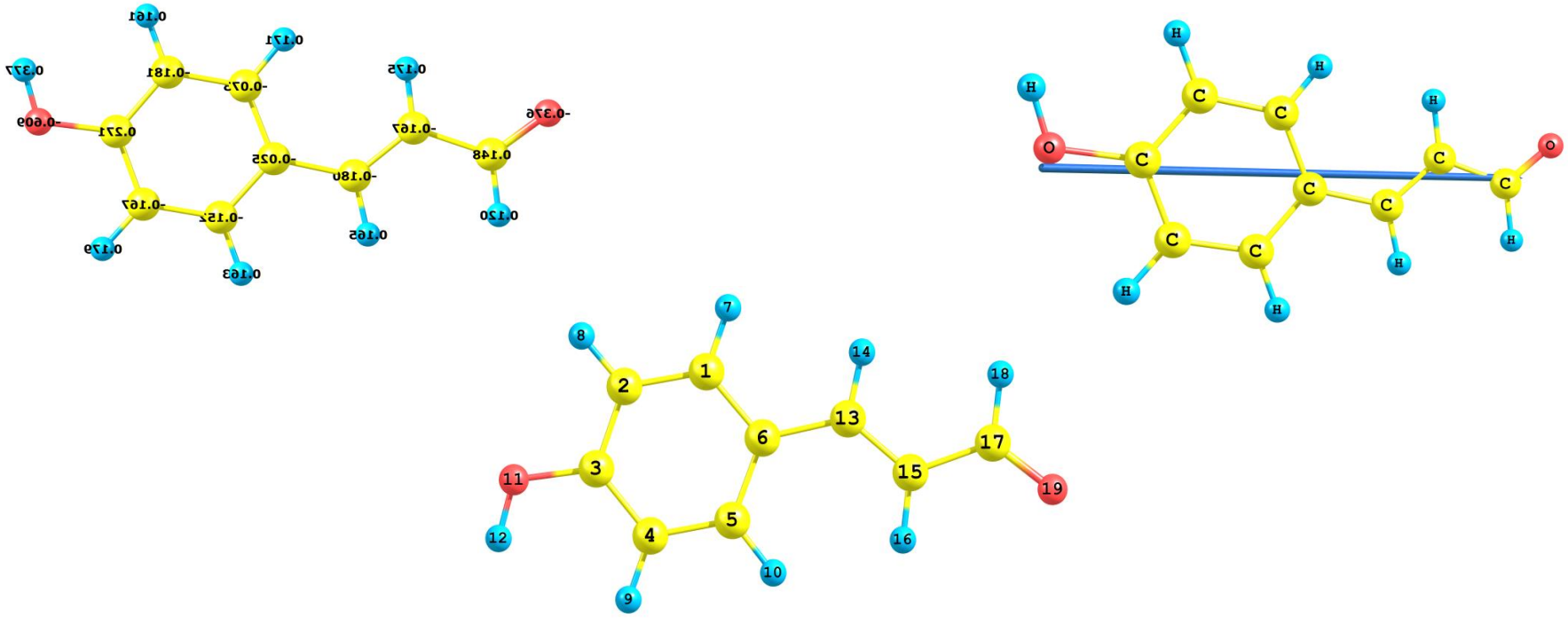

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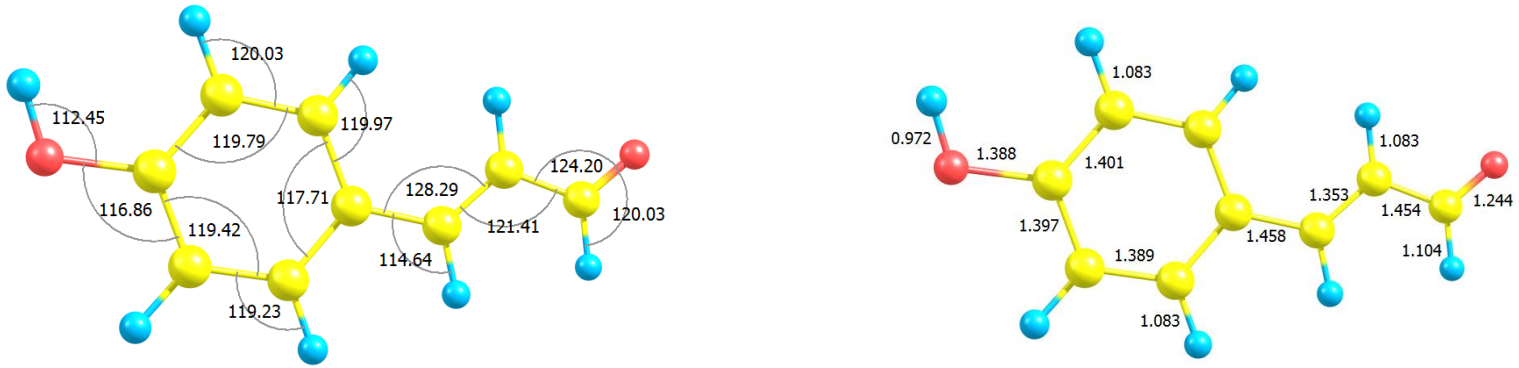

Figure (5): Geometry optimization, the vector of the dipole moment, numbering system, net charge, bond lengths and bond angles of neutral Trans-p-hydroxycinnamaldehyde.

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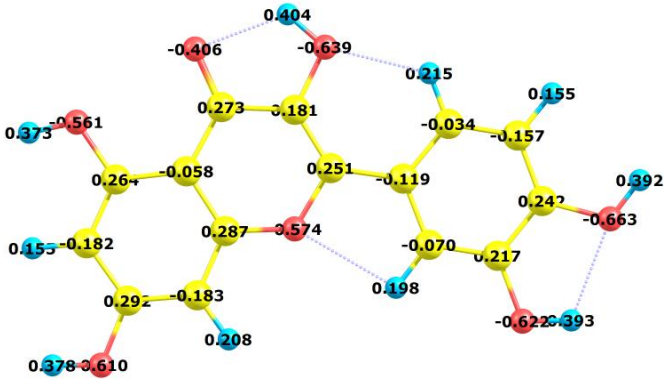

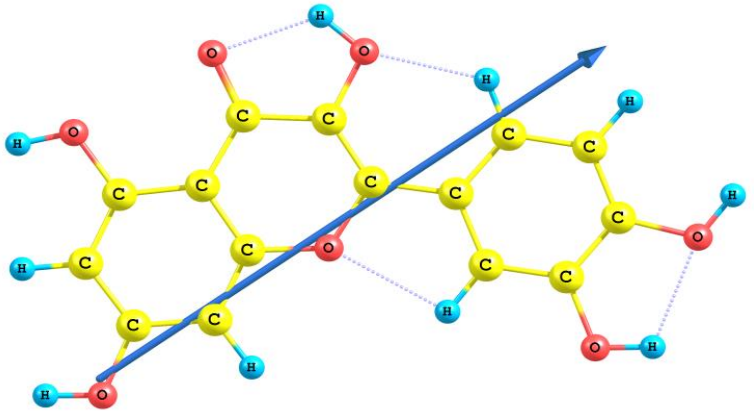

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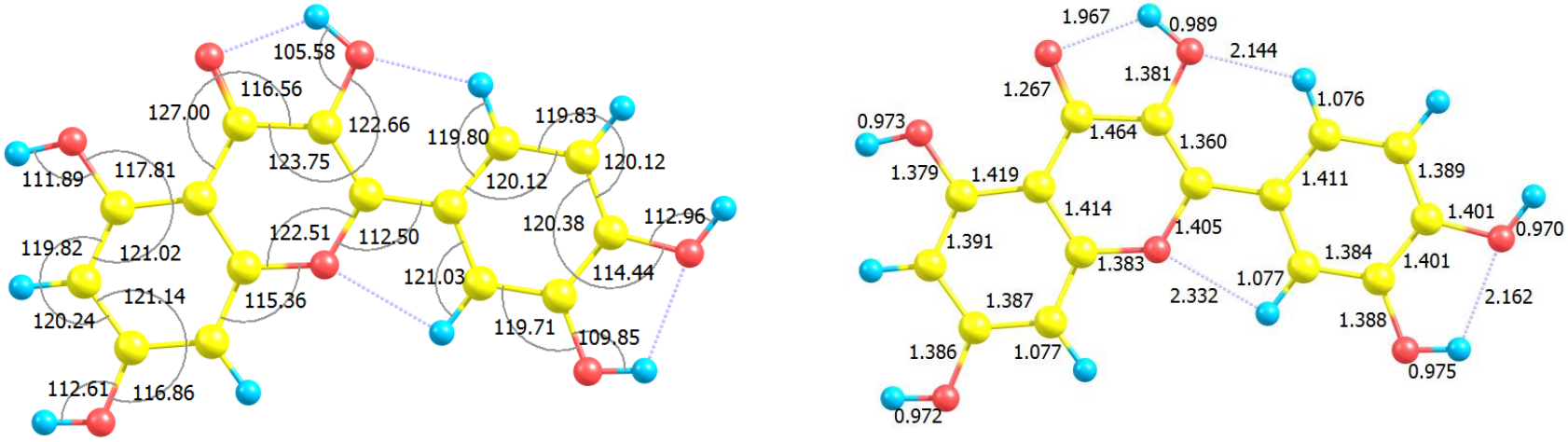

Figure (6): Geometry optimization, the vector of the dipole moment, net charge, bond lengths and bond angles of neutral quercetin.

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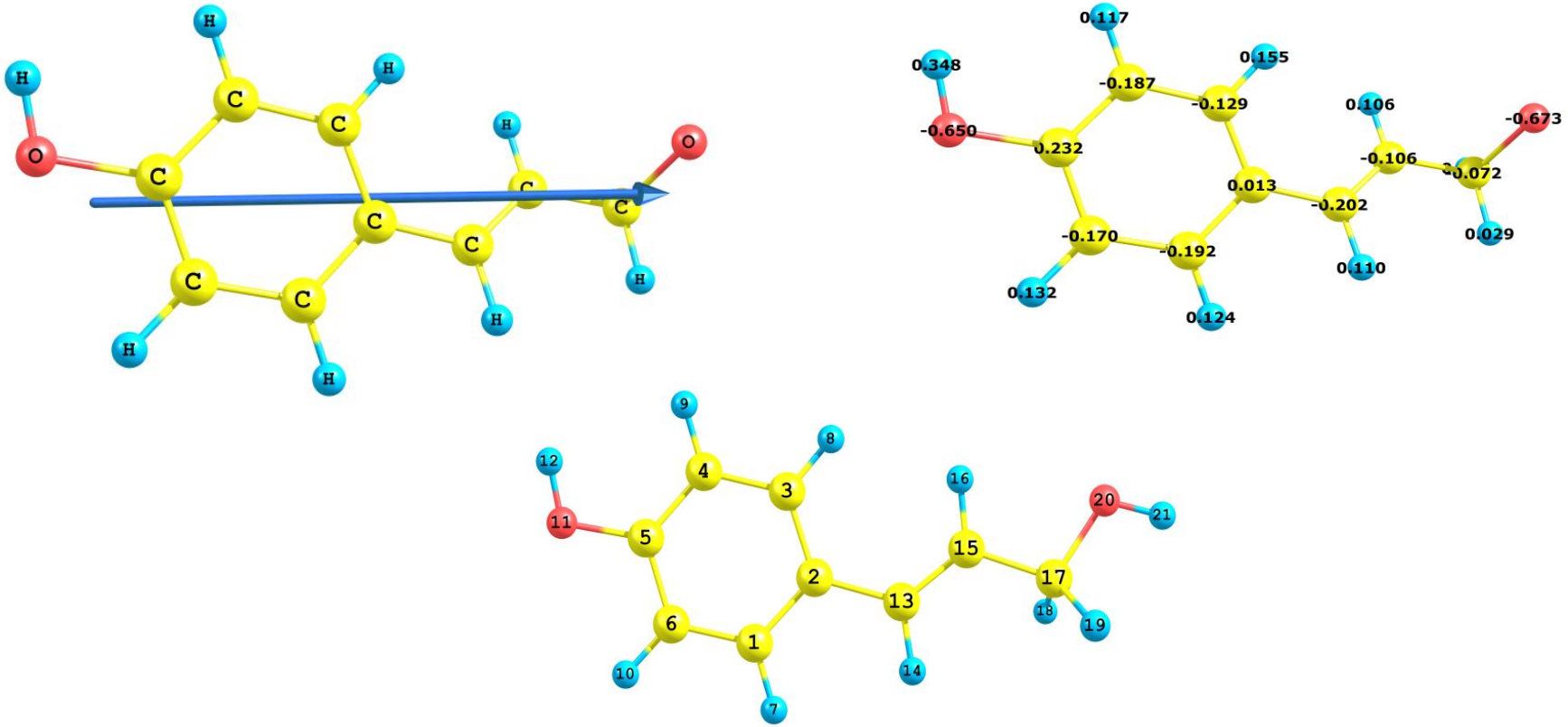

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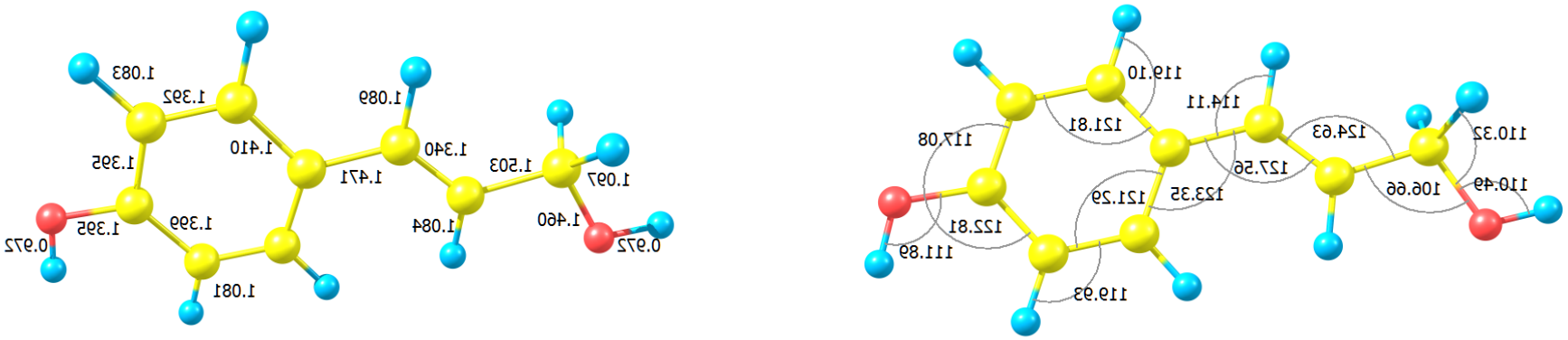

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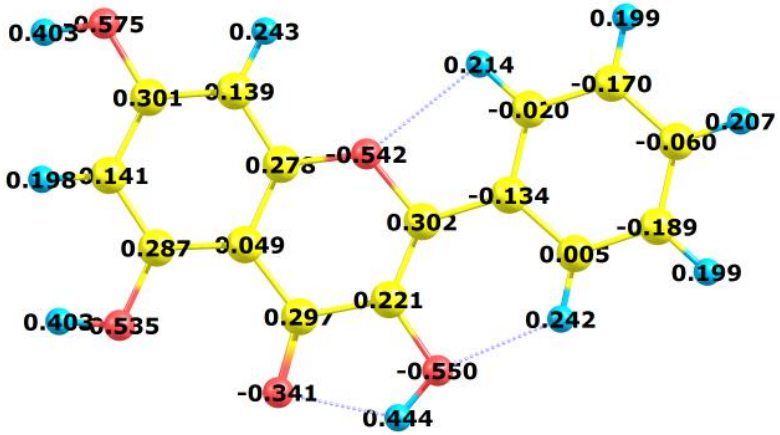

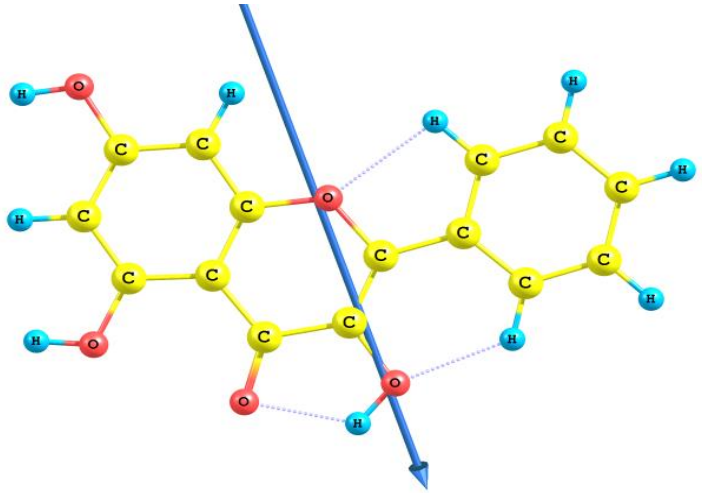

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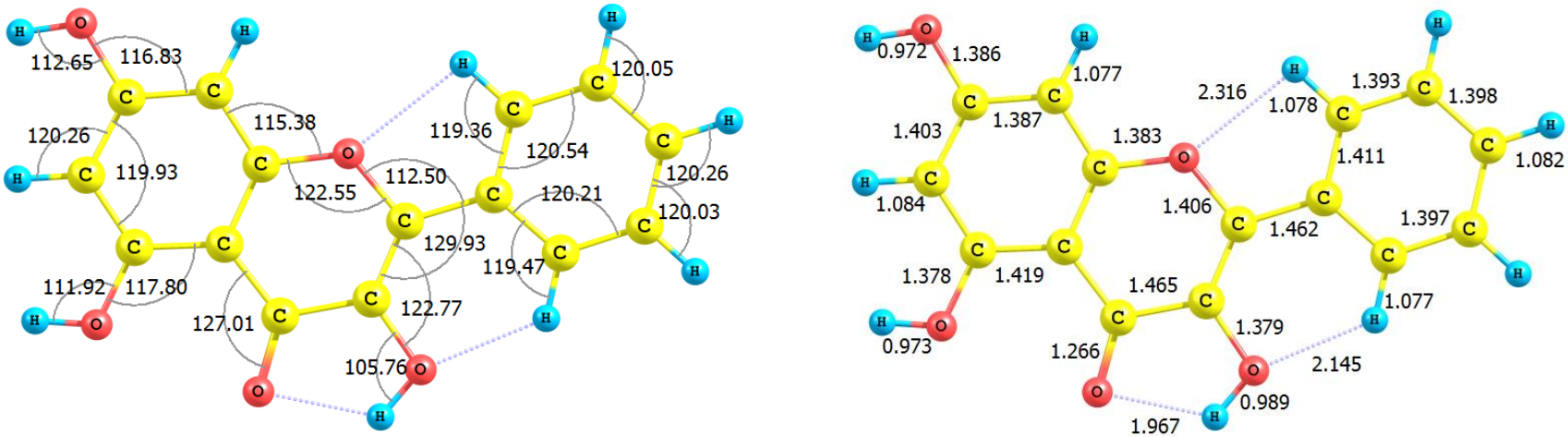

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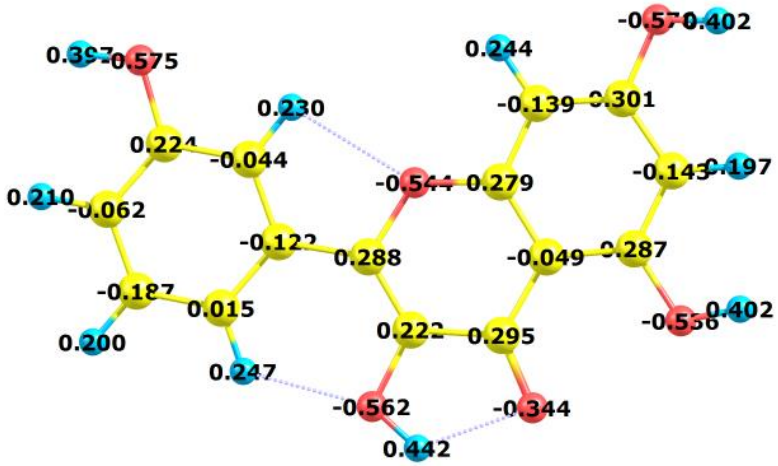

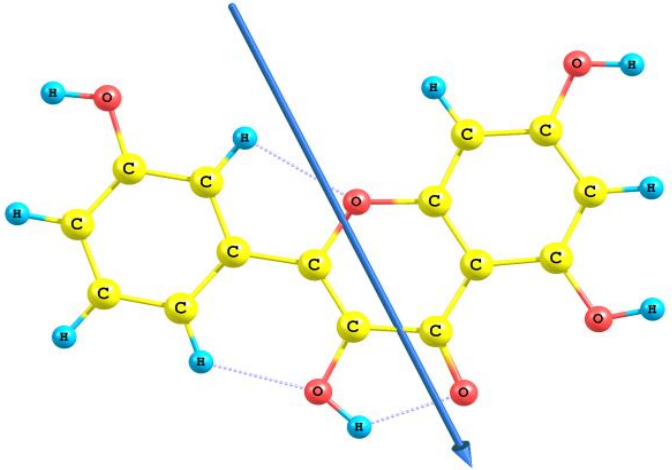

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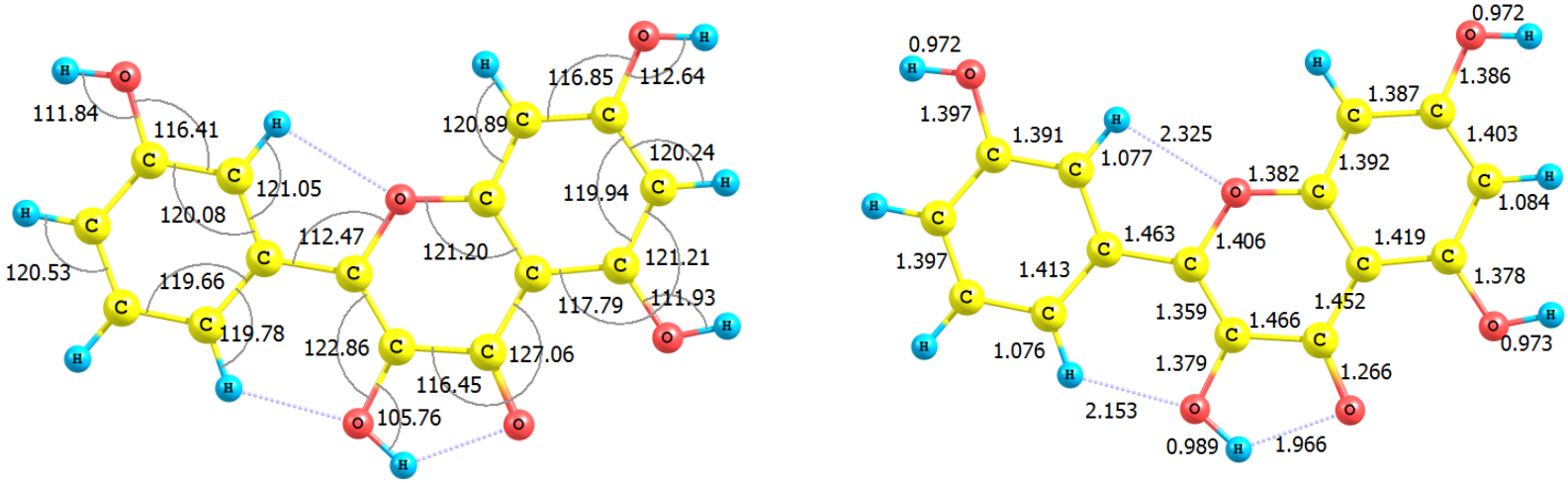

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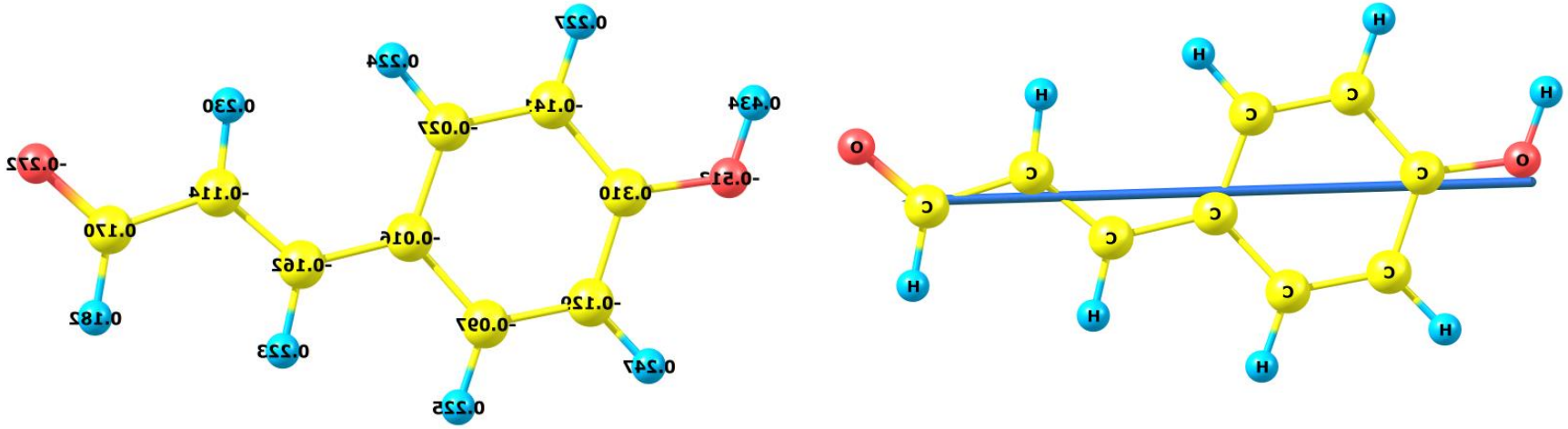

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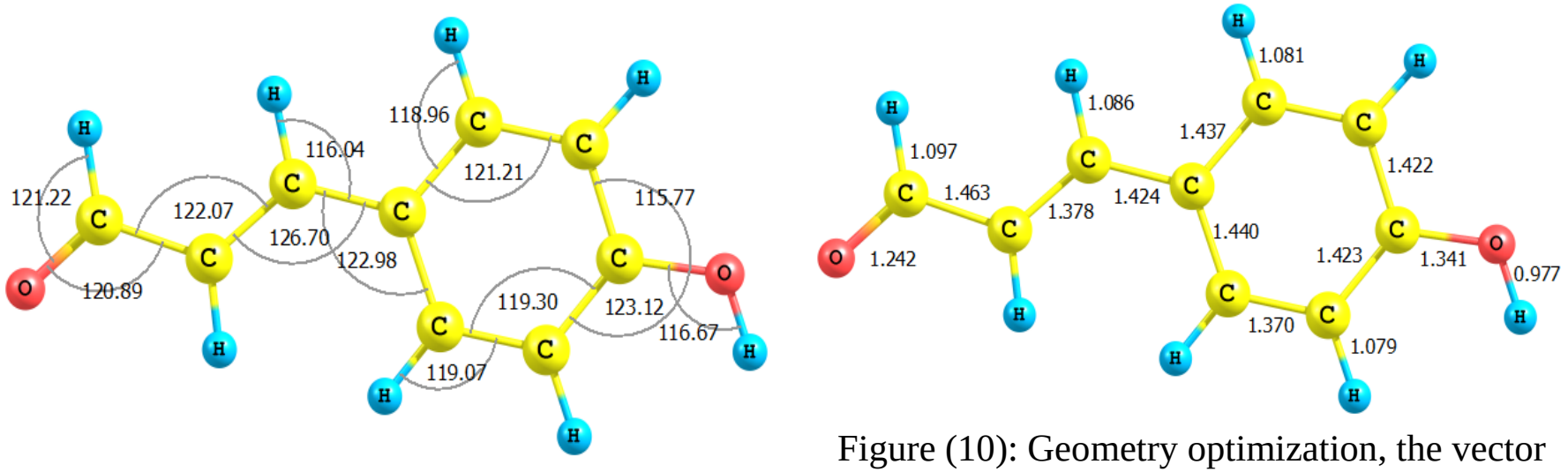

Figure (10): Geometry optimization, the vector of the dipole moment, net charge, bond lengths and bond angles of radical Trans-p-hydroxycinnamaldehyde.

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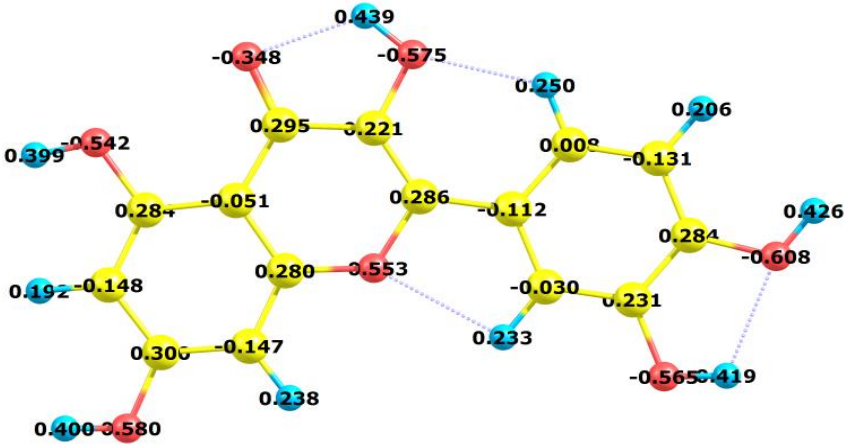

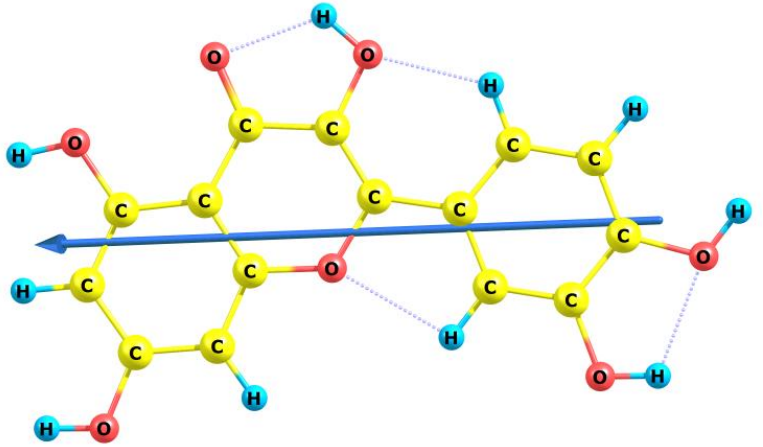

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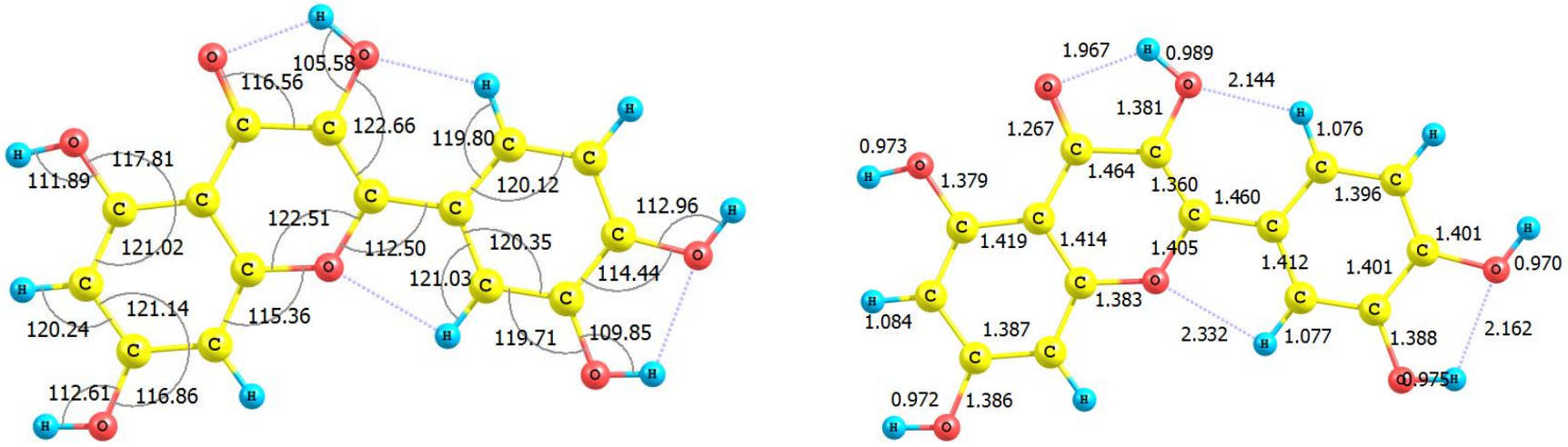

Figure (11): Geometry optimization, the vector of the dipole moment, net charge, bond lengths and bond angles of radical quercetin.

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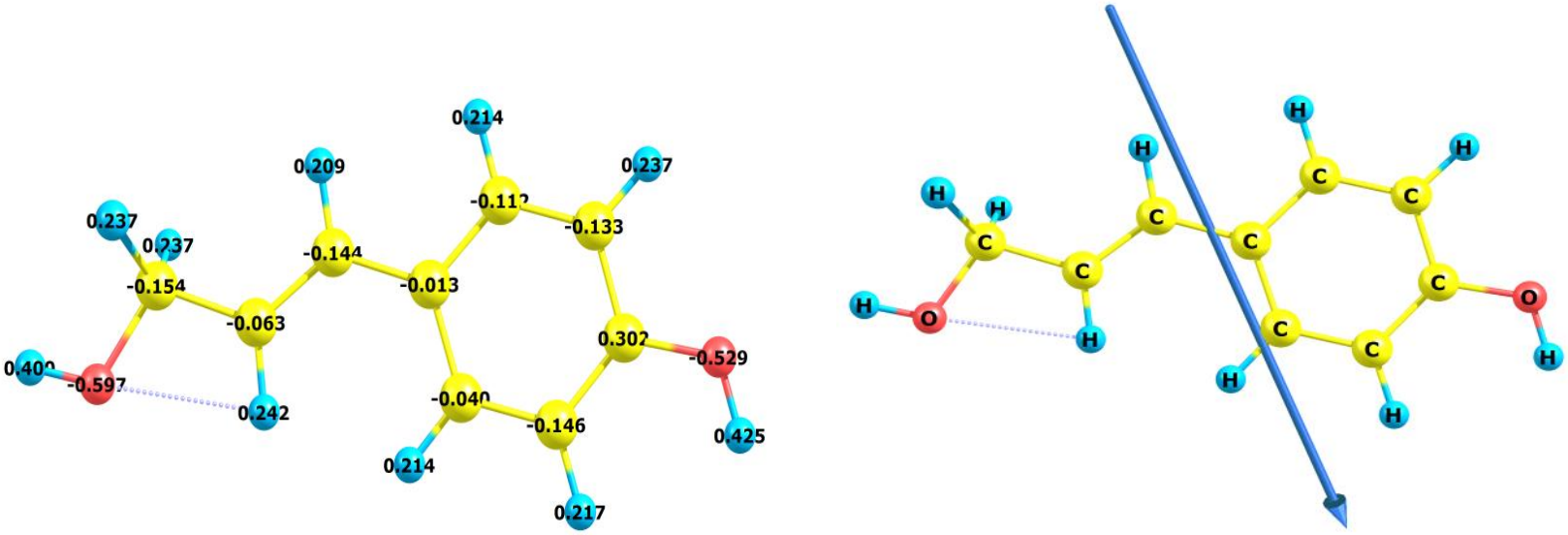

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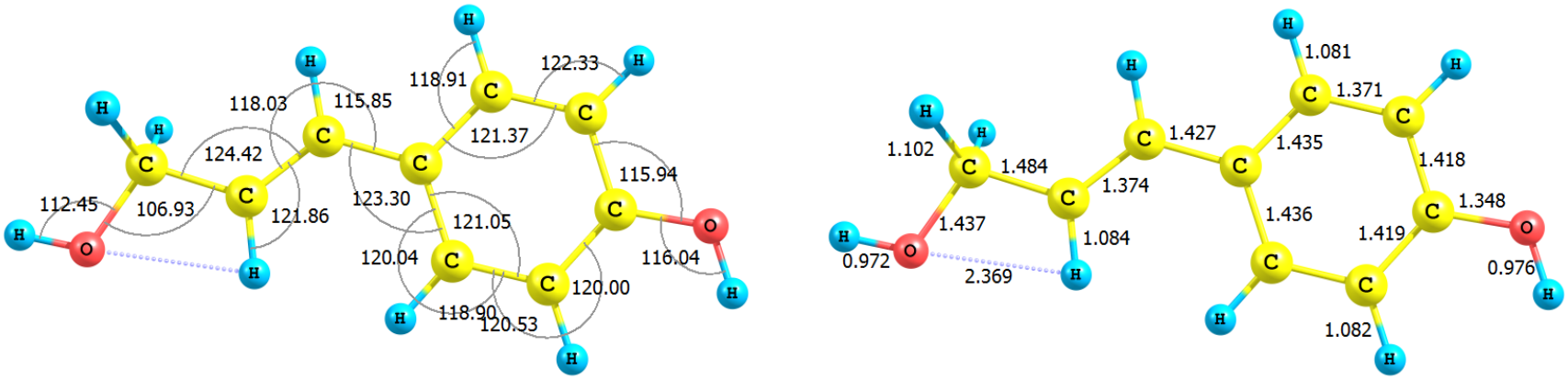

Figure (12): Geometry optimization, the vector of the dipole moment, net charge, bond lengths and bond angles of radical Trans-p-coumaryl alcohol.

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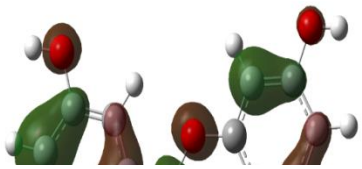
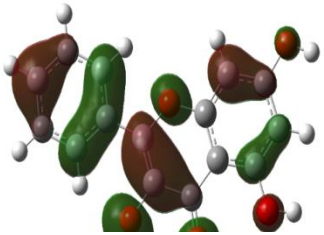
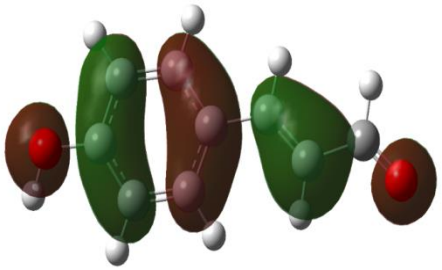
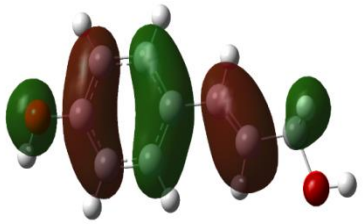






Trans-p-



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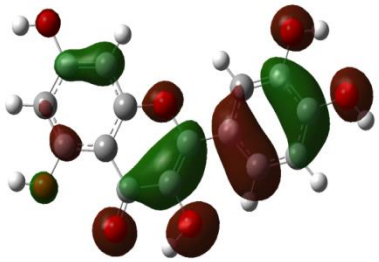

Quercet

Fig. (13): The charge density maps of the HOMO for optimized structure of neutral compents.

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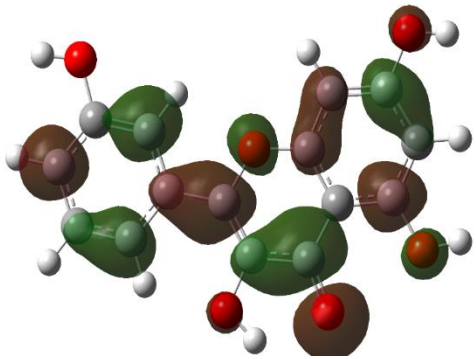
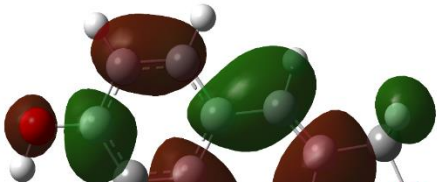
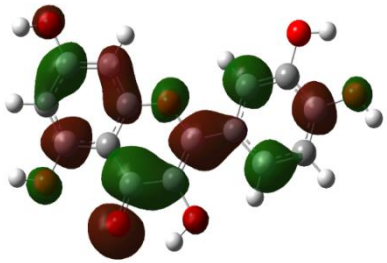


Galangi





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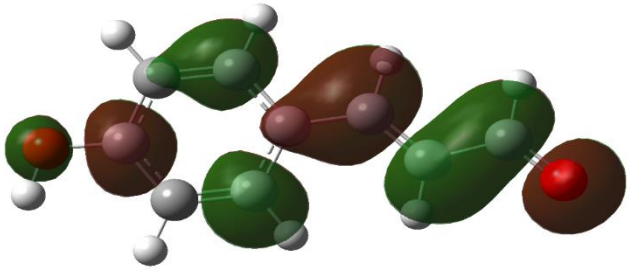

Trans-p-hydroxycinnamaldehyde

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Fig. (14): The charge density maps of the LUMO for optimized structure of neutral compents

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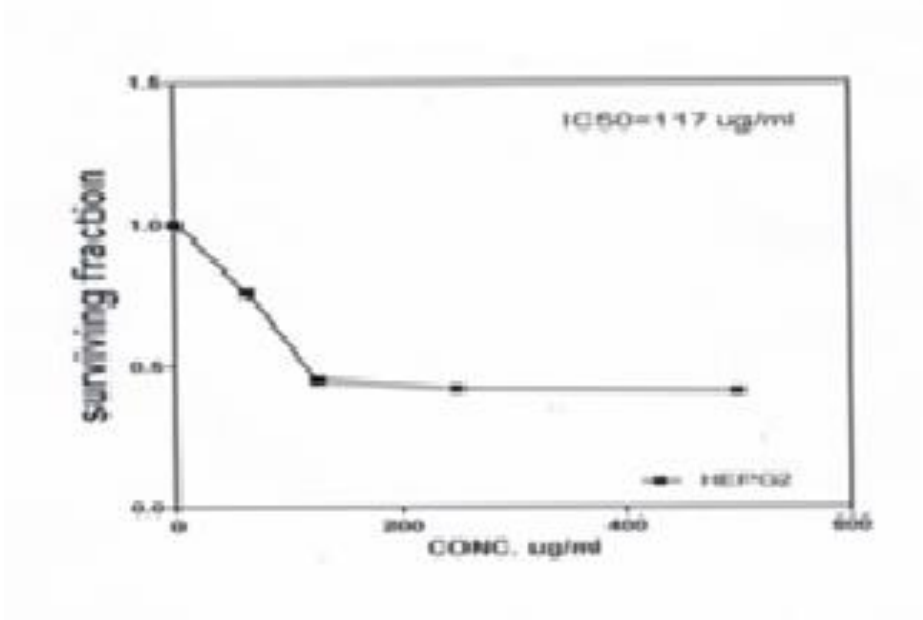

Figure (15): Effect of different extract concentration of Alpinia galanga on surviving fraction liver.

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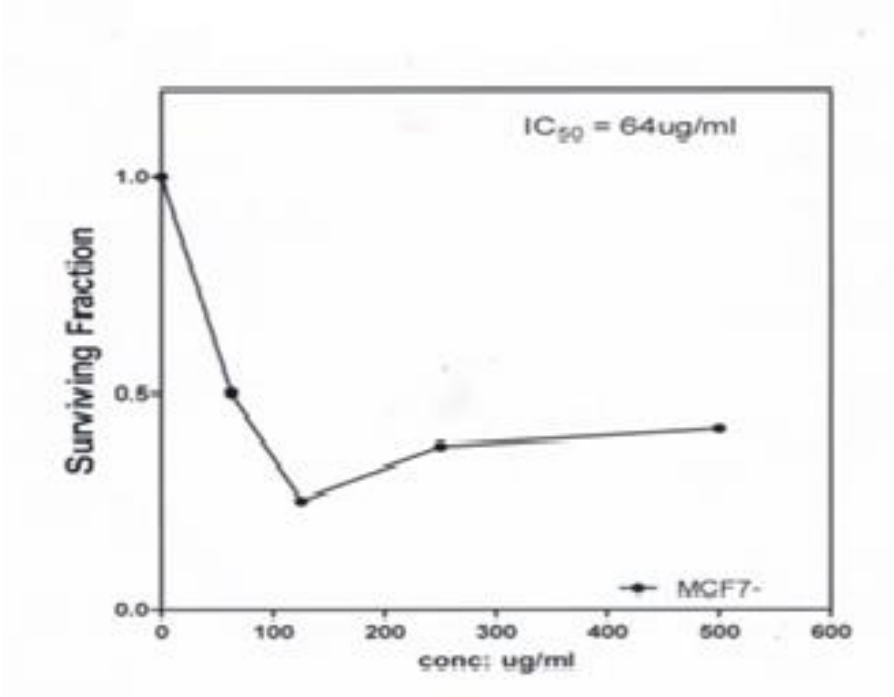

Figure (16): Effect of different extract concentration of Alpinia galanga on surviving fraction breast:

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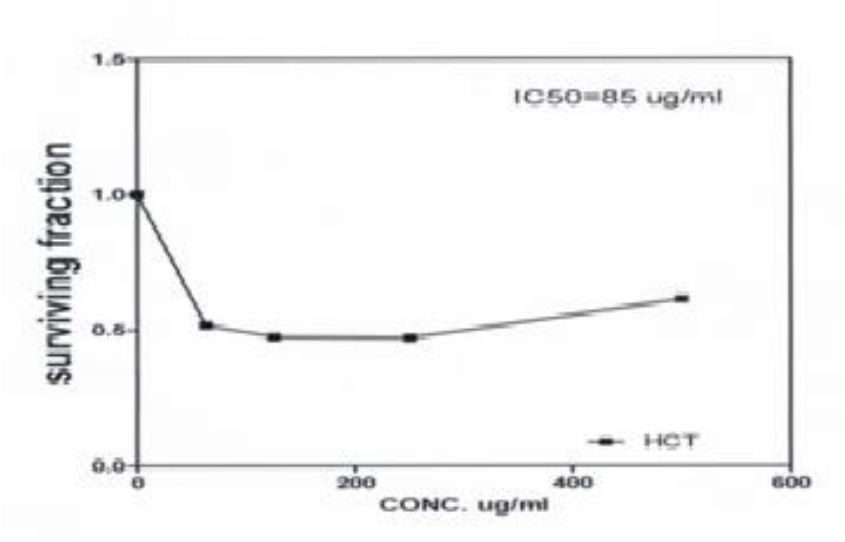

Figure (17): Effect of different extract concentration of Alpinia galanga on surviving fraction blood.

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