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ORIGINAL ARTICLE

Study of Gilan's environmental plants & natural products as anti-cancer drugs: S-NICS method

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As using the drug-plants around of the worlds such as Gilan in Iran (including plants compounds with the local names of Trshvash, Chuchaq, Cote D'Couto and Khlvash) are significantly important, naturally-derived compounds were used for developing of over 70% of clinically useful anti-cancer agents. In this work, important investigation of some of these agents are exhibited and classified to the identity of their significant role in the production of agents thought derived from plants or marine compounds. The mechanisms by which these agents exercise their antitumor action are calculated via analyzing of NMR and physical chemistry properties. The drugs which are discussed in this article, some of them are in pre-clinical trials or clinical development and some are available in market such as Matrine, Cytarabine, Gemcitabine and Vidarabine. In this work, we have optimized and discussed about several active compounds which are extracted from famous plants through NMR study. There are no works in theoretical of a statistical approach in NMR shielding and nucleus independent chemical shifts for study of antidepressant and antihistamine drugs, while the asymmetry (η) and skew (κ) parameters are fluctuated in small distances and are alternative in large distances in the center of heterocyclic rings for Matrine, Cytarabine, Gemcitabine and Vidarabine. In this work, we have investigated the statistical methods by computing of nucleus-independent chemical shifts-SNICS in point of Bq motions in the center of sphere in shielding and de-shielding spaces of antidepressant and antihistamine drugs.
Keywords: NMR shielding; S-NICS; Matrine; Cytarabine; Gemcitabine; Vidarabine

Introduction

Cancer is a main public health burden in developed or developing countries in all parts of the world. It is an abnormal growth of cells as a bad tumor that can lead to death. Cancer cells usually destroy normal cells. These cells are born due to imbalance in the body and by correcting this imbalance, the cancer may be treated. Cancer is a result of mutations in DNA, which leads the cells how to grow and divide more and more. Normal cells by themselves have the potential for repairing approximately all of the mutations in their DNA, but the mutation which is not repaired and causing the cells to grow becomes cancerous (Krishnamurthi 2000). Popular plants which are used for removing the cancer have extracted from 60% natural sources that so-called as anticancer agents. These natural products are divided in several groups such as (1) - Vincaalkaloids, vincristine, vinblastine, (2) - Taxens- paclitaxel, docetaxel, (3) - Podophyllotoxin, etoposide, teniposide, (4) - Camptothecin, topothecan, (5) - Anthracyclines, doxorubicin, daunorubicin.

Vinca alkaloid was a potent compound in prevention of leukemias and non-hodgkin's diseases. Podophyllum exhibit their activity in small cell lung carcinoma. Etoposide is a Topoisomerase 2 inhibitor; it is a stabilizing enzyme and it lead to break the DNA. Taxanes also show anti-tumor activity against ovarian and breast cancer. Camptothecin show anti-tumor activities against colorectal and ovarian cancer. Vincristine inhibits microtubule assembly. Following are the compounds that are still in clinical practice.

Environmental factor including diet, smoking, infectious diseases, chemicals and radiation radioactive materials due to pollutants of air, foods and drinking water could be increase the problem of cancer in the world. The cancer matter becomes widely increased when human receive an ionizing radiation. Natural products derived from vegetables, herbs and fruits, have prevented us against cancer as instant, induction of apoptosis and inhibition of cell proliferation. Terpenoids, Polysaccharide, Alkaloids and flavonoids obtained from natural products have biological's potential and effective properties as anti-tumor, anti-viral and analgesia. Although most of natural products and anti-neoplastic drugs often cannot remove tumor cells directly, but regulates the human immune function to achieve the purpose (Wang and Ren, 2010). A large number of natural products battle

cancers via cell cycle arrest, induction of apoptosis, inhibition tumorization and proliferation (Abubakar et al., 2012). The research for novel drugs is still a important target for cancer diseases and cancer therapy due to the fact that chemotherapeutic drugs resistance are becoming more and more frequent.

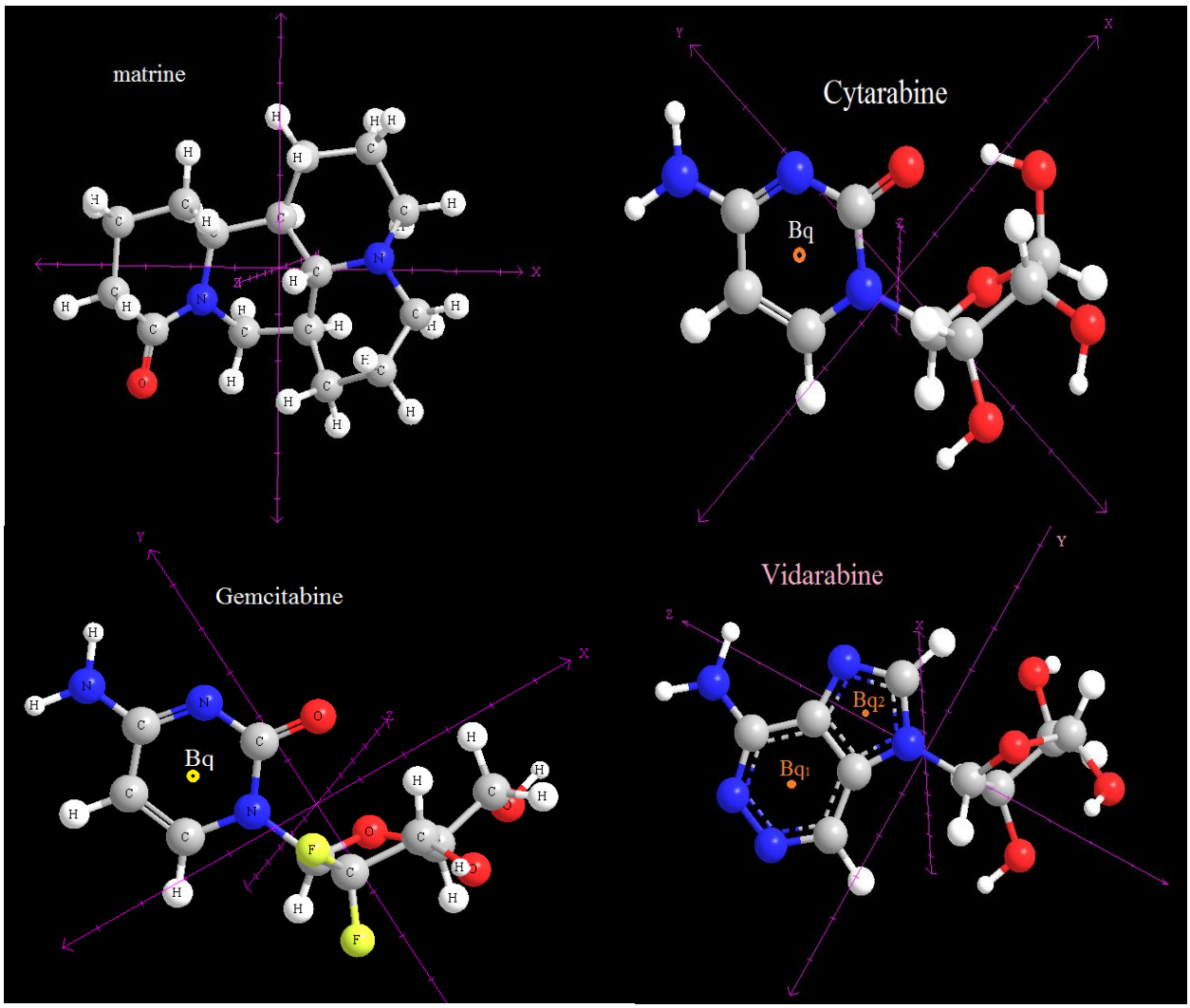

Fig. 1. Chemical optimized structures of Matrine, Cytarabine, Gemcitabine and Vidarabine with M06 and m06-L (DFT) functional /cc-pvdz & cc-pvtz basis sets including NMR=CSGT including Pop=ChelpG

Regards the Alkaloid compounds and molecules which are extracted from the natural products such as Mangosteen fruit (an equatorial plants), Kuramoto et al. (2006) in an experimental measurement exhibited that glucocorticoid expression was affected by 24 h variation in circulating glucocorticoid levels and it has been reported in 2010 (Zhang et al), that 10-methoxy-9-nitrocamptothecin possessed potent anti-tumor activity in A549 cells *in vitro* and *in vivo*. Matrine (Fig. 1), a major component which is extracted from *Sophora flavescens* plants, has been exhibited to exert anti-cancer effects and inhibit tumor cell line MNK45 in a number of cancer cells including, breast, gastric, lung, etc. (Li et al., 2013). Matrine has several potential as inhibiting of proliferation, influencing the mitochondrial membrane and inducing apoptosis in AML cell lines via increasing the release of cytochrome C (Zhang, 2012).

Marine natural products are the molecules which are derived from marine compounds. There are different numbers of molecules that are derived from marine organisms by aquaculture biotechnology. Cytarabine (Fig. 1), which isolated from *Cryptotheca crypta* is currently used in treatment of leukemia and lymphoma patients it is the first marine derived anticancer agent. Cytarabine is in the antimetabolite and nucleoside analog families of medication. It works by blocking the function of DNA polymerase. It is given by injection into a vein, under the skin, or into the cerebrospinal fluid (Fischer, 2006). Gemcitabine (Fig.1) which is a fluorinated derivative of Cytarabine is approved for exactly of breast cancer, pancreatic cancer and bladder cancer. Important side effects include bone marrow suppression, fever, rash, shortness of breath, and also hairs loss. It is in the nucleoside analog family of medication and works by blocking the creation of new DNA, which results in cell death (Myers, 2009).

Vidarabine (Fig. 1) or 9-β-D-arabino-furanosyl-adenine (ara-A), which isolated from *Cryptotheca crypta* is famous as adenine arabinoside, Ara-A which is converted into adenine arabinoside triphosphate as inhibitor for viral DNA polymerase and DNA synthesis of herpes.it is also an antiviral drug which is active against herpes simplex and varicella zoster viruses (Sneader, 2005). Vidarabine is more toxic and less metabolically stable than many of the other current antivirals such as acyclovir and

ganciclovir. Viral strains resistant to vidarabine show changes in DNA polymerase. Chemical synthesis of Vidarabine was first attained in 1960, as a part of studies on developing potential anticancer agents (Lee et al., 2010).
The Xanthones in the Mangosteen Fruit pericarp are the most abundant source of Antioxidants in the nature. A xanthone is an organic compound with the molecular formula $C_{13}H_8O_2$ (Jung, 2006).
Currently there are over 40 known xanthones in Mangosteen fruit. These xanthones work as super powered Antioxidants In addition to Free Radical neutralization, the xanthone has other anti-cancer behaviors including anti-inflammatories functions. Some kind of xanthones have even killed cancer cells in laboratory tests (Jung, 2006).

Methods

The main effective molecules in Mangosteen, Alpha mangosteen, Gama mangosteen and Deoxy gartanin that have Methyl functional group in their active sites selected. By using the Chemdraw software it is possible to show the chemical structure of the molecules that have main role in the anti-cancer effect of Mangosteen. Energy, NMR, Opt computational studies was done with using Gaussian 09 program at Hortree-Fock and DFT methods. NMR chemical shift measurement has been implemented to a vast range of problems in chemistry and biochemistry and has unraveled to be an invaluable microscopic probe which has played a significant role in the structural understanding.
The gauge including atomic orbitals (GIAO), or applying a continuous set of gauge transformations (CSGT) are adopted to solve the gauge problem in the calculation of nuclear magnetic shielding. The study of chemical shift reveals a serious drawback inherent in the classical–quantum hybrid approach. The quantum chemical calculations yield the CS tensors in principal axes system (pas) is:

$$(\sigma_{33} > \sigma_{22} > \sigma_{11}) \quad (1)$$

Therefore, eqs.2 and 3 are used to evaluate the isotropic chemical-shielding (CSI), anisotropic chemical-shielding (CSA) parameters, asymmetry parameter η (eq.3).

$$CSI\ (ppm) = (\sigma_{33} + \sigma_{22} + \sigma_{11})/3 \quad (2)$$

$$CSA\ (ppm) = \sigma_{33} - (\sigma_{22} + \sigma_{11})/2 \quad (3)$$

$$\eta = (\sigma_{22} - \sigma_{11})/(\sigma_{33} - CSI) \quad (4)$$

The polarized continuum model is the most frequently used method employed to Changes in magnetic properties. The Span factor According to the:

$$\Omega = \sigma_{33} - \sigma_{11} \quad (5)$$

$$\text{And } \kappa = 3(\sigma_{iso} - \sigma_{yy}) / \Omega \quad (6)$$

For any further discussion of statistical methods in S-NICS especially in short range of distances, we exhibited that the asymmetry (η) and skew (κ) fluctuate around the center of rings. The maximum fluctuations are visible around the extremums functions mathematically. The fundamental of this work is based on random motions of dummy atom in de-shielding spaces of heterocyclic rings for considering the most abundant of points.
Aromaticity in terms of nucleus-independent chemical shifts in long distances of NICS, around the ring center, NICS (0), at the center of ring plane and aromatic ring current shielding (ARCS) were compared in several studies. In short range of distances a few works have been done in theoretical and reports the statistical approach in our works (Monajjemi, 2010, 2015)
For further discussion of statistical approach in nucleus independent chemical shift calculations, especially in short range of distances, it has been focused in relaxations of CAS, dipole-dipole and contribution.
We have shown that the asymmetry (η) and skew (κ) parameters fluctuate in behavior around the center of rings due to minimum isotropy in the center. The most fluctuations are appearing around the minimum or maximum functions mathematically (Monajjemi, 2015).
In this study, the major components of Herzfeld (Herzfeld, 1980) and Haeberlen (Haeberlen, 1976) parameters erocyclic rings. The numerous random points around the center of those molecules have been produced by generation of pseudo-random numbers, which are distributed in a Gaussian function in the interval [0, 1). Our result has been compared by the energy decomposition analysis (EDA) method. The total π bonding energy and the "π" conjugation between heteroatoms "π" bonds in heterocyclic rings are significantly accurate.
We have optimized the geometries and calculated the carbon NMR for Buclizine, Cetirizine, loratacline, Promethazine molecules for understanding which members of rings are more stable our methods and physical chemistry approach have been done based on our previous works (Monajjemi, 2010-2015).

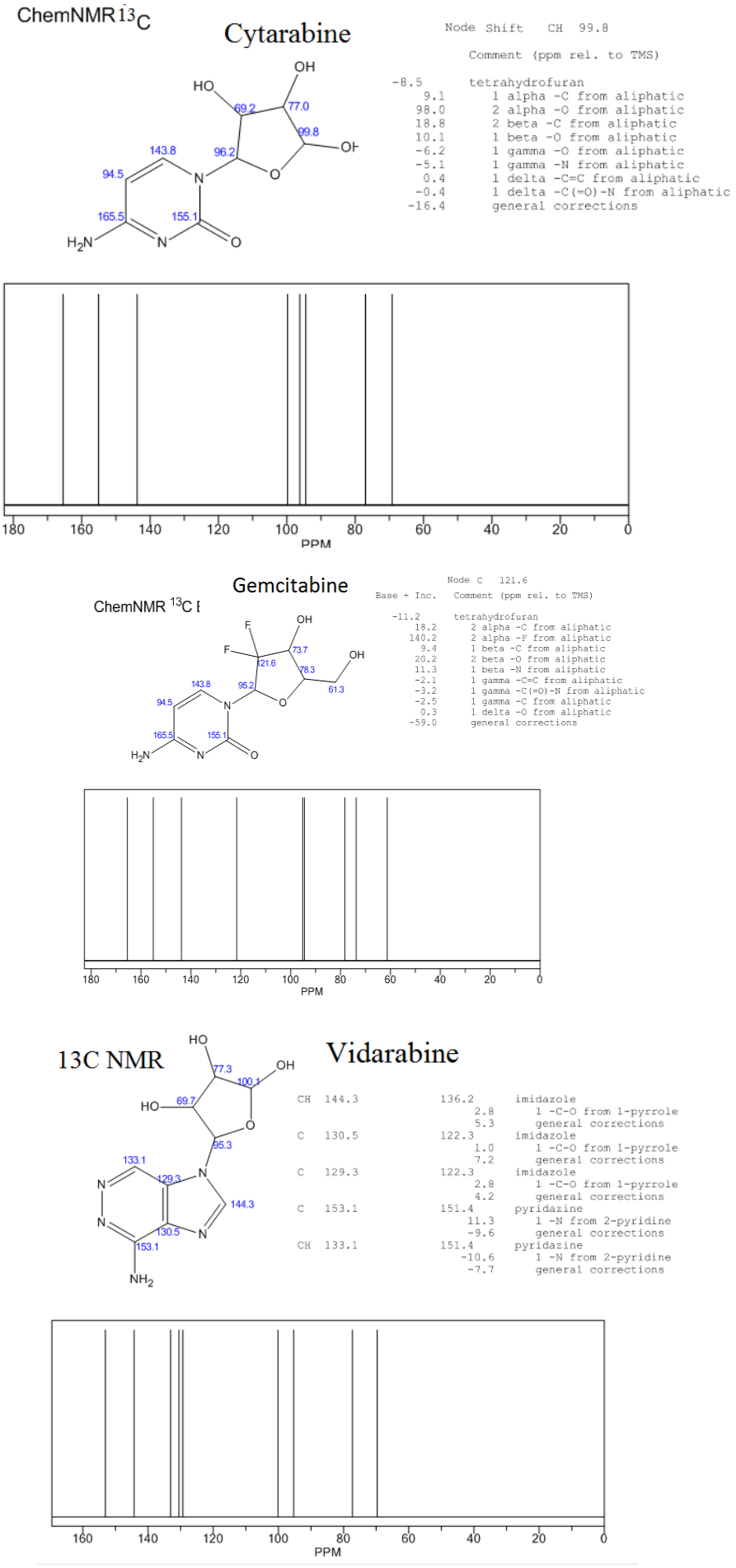

Fig. 2. ^{13}C NMR and Chemical shifts of Cytarabine, Gemcitabine, Vidarabine

Results and discussion

The NMR shielding tensors and S-NICS of aromaticity including Laplacian of electron density for each of the natural molecules have been calculated. The data are listed in 3 tables and 5 figures. We have used the spin polarization parameter function of spin density .ELF, Localized orbital locator (Lu, 2012) (LOL) and Local Entropy (Lu,T 2012), Gap energy, charges from ESP, electrostatic potential (Lu, 2012), Ionization energy, the charges of atoms in molecules and the stability energies have been calculated and listed. These data have been plotted in Fig. 5. The kinetic energy density, Lagrangian kinetic energy density, and the electrostatic potential from nuclear atomic charges which are calculated denote the stability and anticancer properties of those molecules. It is obvious that the difference between isotropies for NICS data can explain the quality of the aromaticity for natural product molecules. In the S-NICS method through the statistical calculations, the best point of the shielding space around the center of symmetric or non-symmetric the aromatic molecules in our can evaluated as an aromaticity criterion and in this method the expectation of the (η*) and (*κ**) (Monajjemi, 2010, 2015) have been estimated as the Gaussian curve functions versus one, two or three dimensional distances around the center of the aromatic rings. The isotropy (σ_{iso}^{*}) which is related to all functions are the best criterion for various aromatic molecules by the S-NICS method, which can express both qualitative and quantitative magnitudes for symmetric or non-symmetric aromatic molecules.

Optimization and NMR shielding constants including orientations of the principal data such as standard components, Haeberlen-Mehring (Mehring, 1978) and Herzfeld-Berger (Herzfeld, 1980) parameters for Buclizine, Cetirizine, loratacline, Promethazine compounds in various statistical situations have been calculated through DFT methods and the data are listed in Tables 1, 2. Similar to the NICS method, in S-NICS, negative nucleus-independent-chemical-shifts denote aromaticity. Therefor positive values denote anti-aromaticity.

The stability of the isotropy criterion is highly affected on the best places in the shielding area spaces and it is dependent on the structures of the aromatic rings. So, by using this method, a suitable and stable magnitude of isotropy can calculated as an aromaticity criterion. It is obvious that structural factors cause changes in the magnetic field experienced by the nuclei and change the resonant frequency. Therefore the chemical shielding and many other factors such as electronegativity, hydrogen bonding, and magnetic anisotropy of π-systems will be changed because of the electrons around the proton which produce a magnetic field, countering the applied field. This reduces the field experienced at the nucleus. The electrons are said to shield the proton, an effect that is exactly dependent on the distance of the center. In this work we have calculated the local Information entropy for each atom and the integrating of this function over whole space yields the information entropy. Weak interaction has significant influence on conformation of macromolecules; however, reproduction of electron density by *ab initio*.

Table 1. The Isotropy and SNICS as an aromaticity criterion

Matrine in gas phase							Matrine in water pH=7						
atom	charge	σiso	S-NICS	η	Δδ	Ω	atom	charge	σiso	S-NICS	η	Δδ	Ω
1 H	-0.33	160.1	169.8	0.334	-13.2	13.22	1 H	-0.340	159.0	158.7	0.291	-13.4	13.91
2 C	-0.294	164.0	163.5	0.2341	-4.497	4.858	2 C	-0.294	164.70	163.1	0.517	-5.98	5.2589
3 C	-0.109	144.6	142.7	0.5679	-20.60	17.52	3 C	-0.109	149.34	143.9	0.986	-25.56	-17.041
4 N	-0.217	146.9	144.9	0.2451	-10.16	10.88	4 N	-0.214	147.43	146.6	0.500	-11.64	10.345
5 C	0.156	47.00	46.7	0.7037	-134.3	105.1	5 C	0.167	44.451	44.8	0.647	-136.3	110.36
6 N	0.098	55.50	55.4	0.9760	-114.1	77.01	6 N	0.096	56.897	57.5	0.992	-115.3	-76.923
7 C	-0.149	60.68	62.1	0.9963	-114.3	-76.2	7 C	-0.147	60.482	61.8	0.984	-113.8	76.539
8 O	0.191	47.84	46.8	0.8919	-139.1	-92.77	8 O	0.191	48.078	49.7	0.884	-138.8	-92.595

Table 2. The Isotropy and SNICS as an aromaticity criterion

Cytarabine in gas phase							Cytarabine in water pH=7						
Atom	**charge**	**σiso**	**S-NICS**	**η**	**Δδ**	**Ω**	**atom**	**charge**	**σiso**	**S-NICS**	**η**	**Δδ**	**Ω**
1 O	-0.334	166.51	167.3	0.624	-10.8	8.936	**1 O**	-0.334	166.6	165.8	0.612	-10.87	8.993
2 C	-0.287	165.98	164.7	0.955	-6.175	-4.1172	**2 C**	-0.287	165.8	164.5	0.875	-6.230	-4.1534
3 O	-0.090	144.94	142.9	0.987	-23.8	-15.88	**3 O**	-0.091	145.0	143.7	0.961	-24.1	-16.087
4 N	-0.193	147.65	145.8	0.511	-12.45	10.989	**4 N**	-0.196	147.8	141.9	0.485	-11.06	9.934
5 O	0.1262	46.492	149.6	0.741	-143.8	110.15	**5 O**	0.122	47.31	46.5	0.757	-143.6	108.95
6 C	-0.061	72.119	74.9	0.747	-120.1	-80.08	**6 C**	-0.060	70.69	72.4	0.760	-121.3	-80.882
7 N	-0.178	64.33	65.8	0.370	-139.3	-92.91	**7 N**	-0.174	60.01	61.8	0.544	-139.7	-93.18
8 C	0.1905	57.44	58.9	0.982	154.3	103.80	**8 C**	0.143	56.22	57.6	0.997	-151.3	101.05
9 O	-0.353	160.51	164.0	0.495	-15.0	13.43	**9 O**	-0.35	163.4	164.5	0.348	-11.73	11.608
10 N	-0.33	161.4	163.3	0.483	-15.8	-10.59	**10 N**	-0.340	162.0	161.3	0.452	-16.14	-10.76

Conclusion

In conclusion, our calculations have been designed a way of study for the natural molecules and some of these agents which are exhibited and classified to the identity of their significant role in the production of agents thought derived from plants or marine compounds. The mechanisms by which these agents exercise their antitumor action are calculated via analyzing of NMR and physical chemistry properties.

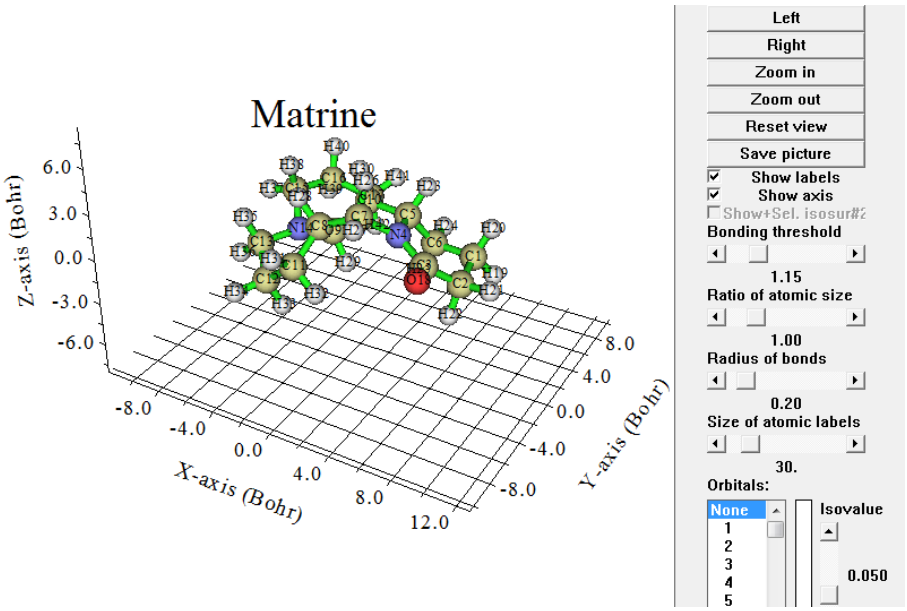

Fig. 3. Matrine's 3D structure in stable and optimized form

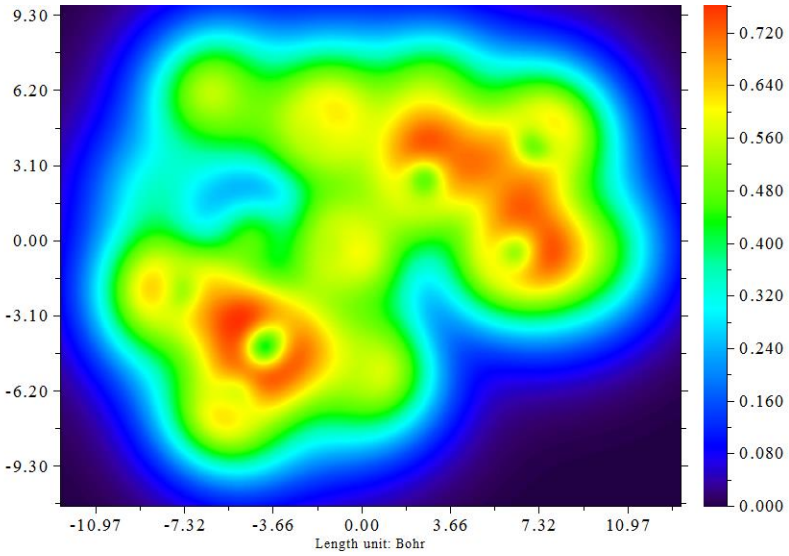

Fig. 4. ELF color filed map of Matrine in stable form

Table 3. Density, energy, Electron localization function (ELF), localized orbital locator (LOL) and Local Entropy for each C=O of the (1) Matrine, (2) Cytarabine, (3) Gemcitabine, (4) Vidarabine respectively

Molecules	Density of all electron × (10^{-4})	Density of α electron × (10^{-4})	Density of βelectron × (10^{-4})	Spin density of electron	Hamiltonian kinetic energy (×10^{-4}J)	LOL ×10^{-3}	Local entropy ×10^{-6}	Ellipticity	ELF ×10^{-7}	Eta index
^{1}C	0.78	0.39	0.39	0.00	-0.14	0.15	0.61	0.66	0.14	0.12
^{1}O	0.28	0.14	0.14	0.00	-0.48	0.31	0.16	1.02	0.28	0.05
^{2}C	0.86	0.43	0.43	0.00	-0.20	0.14	0.60	0.28	0.16	0.09
^{2}O	0.30	0.15	0.15	0.00	0.52	0.34	0.21	1.53	0.39	0.01
^{3}C	0.88	0.44	0.44	0.00	-0.22	0.19	0.64	0.19	0.14	0.12
^{3}O	0.22	0.11	0.11	0.00	-0.55	0.43	0.18	1.64	0.40	0.03
^{4}C	0.76	0.38	0.38	0.00	-0.24	0.13	0.59	0.14	0.19	0.17
^{4}O	0.32	0.16	0.16	0.00	-0.49	0.37	0.20	1.84	0.33	0.03

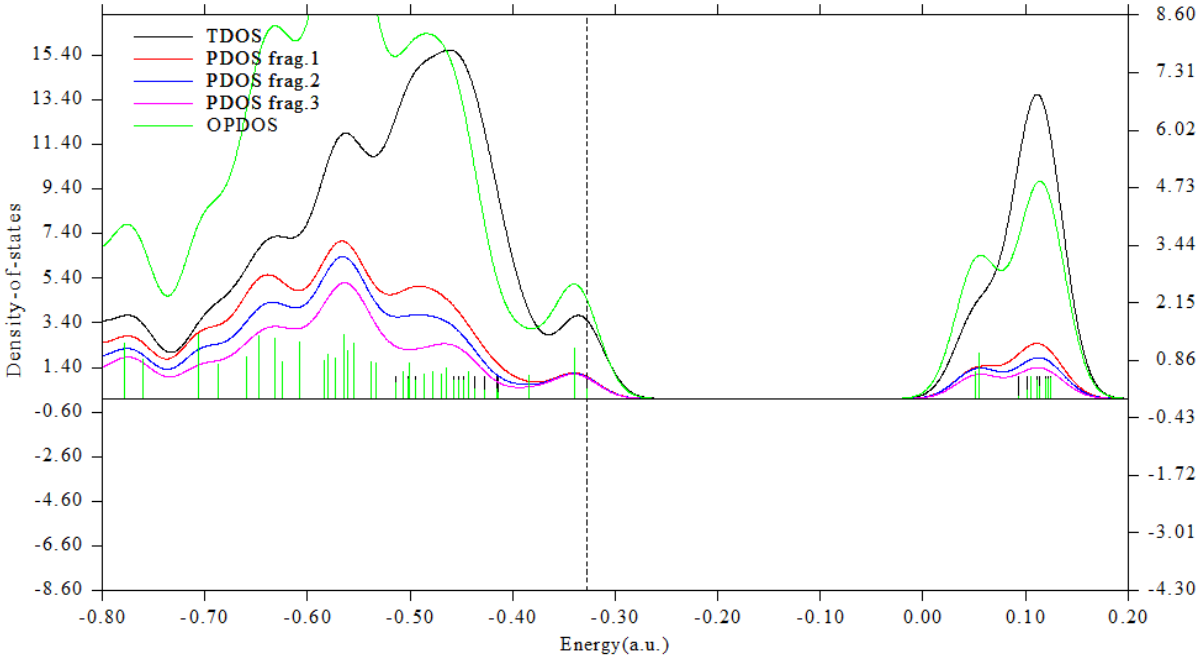

Fig. 5. Various Density of states (DOS) for Matrine

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