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Reactivity indexes of antioxidant molecules from *Psorospermum febrifugum*

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Abstract
Density functional theory (DFT) analysis was carried out to explore the antioxidant properties of some flavonoids and fatty acids previously isolated from *Psorospermum febrifugum* spach. The geometry optimization of the chemical structures was done following the B3LYP method and 6-311+G (d, p) basis set. DFT methods are fast and reliable theoretical models used for evaluating the reactivity and electronic properties of chemical compounds. Reactivity indexes such as the ionization potential energy (IP), chemical hardness (η), electrophilicity index (w), chemical potential (μ), electron acceptor index (w^+), and electron donor index (w^-) which are indispensable tools for explaining chemical reactivity and stability of molecules were evaluated. The results for the energy gap showed a decreasing trend in the studied compounds in the order Stearic acid>palmitic acid>Xanthone>Flavan with the values 6.458>6.013>4.236>3.042, indicating that flavan is the most reactive of the studied antioxidants while Stearic acid is the least reactive and by interpretation, the fatty acids, Stearic acid and palmitic acids are better antioxidants than the flavonoids.

Keywords: Antioxidants, DFT, fatty acids, flavonoids, FMO, NBO

Introduction
Nowadays, the world's greater population especially in developing countries depend on herbal medicines to avert their health problems. Although, there are varieties of innovations by natural product chemists towards drug discovery, its development remains a protracted process with little progress due to poor funding and investment. With the numerous plant species on earth, there is unlimited therapeutic phytochemicals requiring only but careful separation and purification. Therapeutic agents form plants have enormous benefits compared to synthetic especially with regards to cost, toxicity and availability [1]. Today, phytochemistry has become attractive with interest in understanding the chemical, and pharmacological properties of phyto-drugs. Phytocompounds are the major components of plant's support mechanism protecting it from microbial attack and proper response to changes in the environment. In humans, they are employed in treatment of various diseases [2-4]. They occur in leaves, fruits, root, back and flowers. Considering the chemistry and distribution, phytochemicals are broadly classified into alkaloids, terpenoids, polyphenols, lipids (fatty acids) etc. Phytochemicals show varied biological activities against oxidative stress, immune-related disorder, infective microbes, cancer and heart problems.
Xanthones are flavonoid compounds which comprises the biggest class of phytochemicals in natural product chemistry. Some of the biological activities of xanthones include but not limited to hepatoprotective, anti-carcinogenic, anti-leprosy, anti-malarial, anti-oxidant, radioprotective, immunomodulatory, anti-parasitic, anti-fungal, anti-inflammatory, anti-ulcer, and anti-diabetic activities which have been reported for naturally occurring xanthones [4]. Flavans are widely distributed in nature and a product of double reduction of a flavanone. They are abundant in unripe fruits and in leaves providing plants with resistant capacity against insects and fungi.
Fatty acids constitute the major components of fat-soluble lipids of both plant and animal origin. Hexadecanoic acid (palmitic acid) and 13-Octadecanoic acid (stearic acid) occur in abundance in plant and animal tissues. Computational models offer effective advantages to exploring both the kinetic and absorptive properties of therapeutic agents [5, 6] and more recently researches have shown the need for modelling of phyto-drugs for cancer, inflammatory and treatment of infections [7]. Again, it is expensive to develop a protocol for most synthetic drugs[8] compared to phyto-drugs.

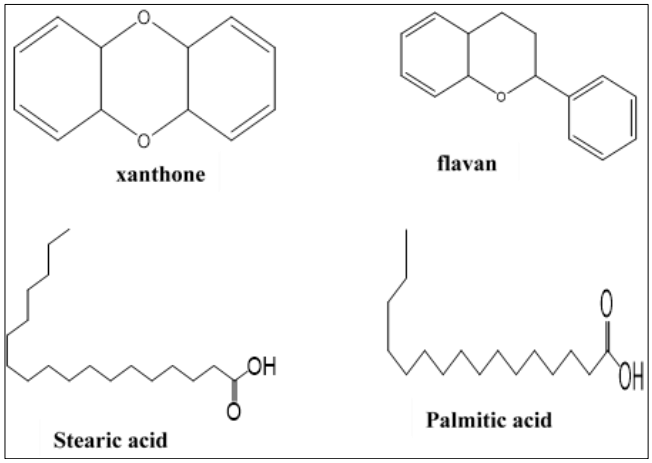

Fig 1: 3D structures of the studied compounds

A typical drug research, including patent takes up to 14 yrs with a huge cost implication. However, first principle theoretical chemistry is fast and in combination with screening and modelling technology provides a work space for easy and effective drug manufacture. New compounds are being developed and deposited in libraries for use in modelling [9-11]. Computer-assisted drug development (CADD) is fascinating and provides varied opportunities for quick and multiple objectives [11-13].

It is understood that oxygen-based radicals are the root cause of oxidative stress which in turn exert a negative influence on the defense mechanisms of the animal, causing DNA damage, cell aging, cardiovascular disease and cancer. Both synthetic and natural products are widely studied for their antioxidant potentials using chemical and biological approaches [14]. In this study, we employed computational chemical methods (B3LYP/6-311-G+ (d,p) functional/basic set) to explore the Natural bond orbital analysis (NBO), frontier molecular orbital (FMO) interactions and chemical quantum descriptors which were used to characterize the antioxidant activity of the studied compounds [15].

Computational Methods

Density Functional Theory (DFT) calculations were performed on xanthone and flavan, stearic acid and palmitic acid which were previously reported from *Psorospermum febrifugum* by Francesco and Fredrick and their co-workers [16, 17] respectively, using the Gaussian 09 computational software [18] in combination with Gauss View 6.0.16 [19]. The geometries of chemical structures were optimized by the B3LYP functional [20] at the 6-311+G (d, p) basis set without any symmetry constraints. The natural bond orbital (NBO) analysis was performed together with the frontier molecular orbital (FMO). Energy gap, and quantum chemical descriptors were as well calculated as previously described [20] using equations 1-8.

$$IP = -E_{HOMO} \quad (1)$$

$$EA = -E_{LUMO} \quad (2)$$

Applying koopman's approximation, the following electronic descriptors were computed.

$$-\mu = {}^{1}/_{2}\,(E_{HOMO} + E_{LUMO}) = \chi \quad (3)$$

$$\eta = {}^{1}/_{2}\,(IP - EA) = \frac{E_{LUMO} - E_{HOMO}}{2} \quad (4)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (5)$$

$$S = \frac{1}{2\eta} = \frac{1}{IP - EA} = \frac{1}{E_{LUMO} - E_{HOMO}} \quad (6)$$

$$w^{+} = (IP + 3Ea)^2/16(IP - Ea) \quad (7)$$

$$w^{-} = (3IP + Ea)^2/16(IP - Ea) \quad (8)$$

IP represents ionization potential, EA is electron affinity, Ƞ is chemical hardness, μ is chemical potential, σ is softness, ω is electrophilicity index and electronegativity (χ) while w^{+} and w^{-} are derivatives of electrophilicity(ω).

Results and Discussion

Frontier Molecular Orbital (FMO) Analysis

Table 1: Homo-Lumo analysis

Compound	HOMO	LUMO	Eg/eV
Xanthone	-6.729	-2.493	4.236
Flavan	-4.281	-1.239	3.042
Stearic acid	-6.635	-0.177	6.458
Palmitic acid	-7.084	-1.071	6.013

The HOMO and LUMO-orbitals energy and the difference (energy gap) for the compounds are shown in Table 1. In chemistry, the HOMO and LUMOs are types of molecular orbitals. Understanding the orbital energy is very important in determining the chemical stability, reactivity and other electronic properties of a compound [21]. The deviation in orbitals energy is very useful as it dwells on the investigation of compounds stability. By implication, small values of energy gap indicate greater reactivity and less stability [22, 23]. In this study the antioxidant properties of the molecules were analysed using the frontier molecular orbital (FMO). The density of electrons occupying the highest molecular orbital helps in visualizing the donor site. Consequently, the density of electrons in the orbitals indicate clearly the site of donation to the oxidative radicals by the antioxidant molecule. The energies (eV) and chemical reactivity descriptors which were generated using the Koopman's approximation equations (1-8) were shown in Table 1. However, the HOMO and LUMO orbitals energy may not entirely be applied in isolation to explain the antioxidant activity of molecules but in company with other reactivity indexes such as ionization potential energy (IP), hardness (η), electrophilicity index (w), chemical potential (μ), electron acceptor index (w^{+}), and electron donor index (w^{-}) which are indispensable for explaining chemical reactivity and stability of molecules [24]. This is due to the fact that HOMO-LUMO orbital energies depends on the reacting species. The results for the energy gap show a decreasing trend in the title compounds in the order Stearic acid> palmitic acid > Xanthone > Flavan with the values 6.458>6.013>4.236 and 3.042, Indicating that flavan is the most reactive of the studied antioxidant while Stearic acid is the least reactive and by interpretation, Stearic acid is the best antioxidant compared to the studied molecules.

Natural Bond Orbital (NBO) Analysis

The natural bond orbital (NBO) analysis was applied for investigating the donor- acceptor interactions in the studied structures. NBO analysis is an interesting option for evaluating quantum chemical equations following the principles of bonding. Table 2 shows the most important donor acceptor orbital interactions and their energies $E^{(2)}$ of

stabilization for the respective studied structures. The results obtained for Flavan showed that it has the highest perturbation $E^{(2)}$ which is directly correlated to the energy gap. This means that flavan is a poor antioxidant as compared to the other compounds studied. The most interesting and significant interaction of the donor-acceptor behaviour of the compounds were observed from the transition and excitation of electrons from the $\sigma C_1 - H_7 \rightarrow \sigma^* C_{18} - H_{22}$ (21901.90 kcal/mol) for flavan, $\pi^* C_8 - C_9 \rightarrow \pi^* C_{10} - C_{14}$ with (284.57 kcal/mol) for xanthone, $LPO_2 \rightarrow \sigma^* C_1 - O_3$ with (32.98 kcal/mol) for palmitic acid and $LPO_{32} \rightarrow \sigma^* C_1 - H_2$ with (16.53) for stearic acid. The transition was predominantly observed from $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$ and $Lp \rightarrow \sigma^*$. Generally, the $E^{(2)}$ analysis presented Stearic acid with least perpetuation energy compared to other studied structures which is also correlated with the FMO analysis where the HOMO-LUMO energy difference further proved that Stearic acid is an effective antioxidant.

Table 2: NBO Analysis

Flavan				
Donor	**Acceptor**	**$E^{(2)}$**	**E(j)- E(i)**	**F(i,j)**
$\sigma^* C_1 - H_7$	$\sigma^* C_{18} - H_{22}$	21901.90	1.61	7.520
$\pi^* C_{19} - H_{24}$	$\sigma^* C_{19} - H_{24}$	10535.74	0.05	2.156
$\sigma C_{23} - H_{26}$	$\pi C_{15} - C_{21}$	17761.64	4.51	12.299
$\sigma C_{23} - H_{26}$	$\sigma^* C_{18} - H_{22}$	7916.37	8.18	10.167
$\sigma C_{18} - H_{22}$	$\sigma^* C_{19} - H_{24}$	3634.23	0.89	2.270
Xanthone				
Donor	**Acceptor**	**$E^{(2)}$**	**E(j)- E(i)**	**F(i,j)**
$\pi^* C_8 - C_9$	$\pi^* C_{13} - C_{14}$	172.59	0.02	0.081
$\pi^* C_8 - C_9$	$\pi^* C_{10} - C_{14}$	284.57	0.01	0.083
$\pi^* C_9 - O_{22}$	$\pi^* C_3 - C_4$	98.08	0.028	0.073
LPO_{23}	$\sigma^* C_{14} - H_{21}$	183.20	0.68	0.333
$\pi C_3 - C_4$	$\sigma^* C_{14} - H_{21}$	70.92	0.61	0.204
Stearic acid				
Donor	**Acceptor**	**$E^{(2)}$**	**E(j)- E(i)**	**F(i,j)**
LPO_{32}	$\sigma^* C_1 - C_2$	16.26	0.63	0.091
LPO_{32}	$\sigma^* C_1 - H_2$	16.53	0.71	0.091
$\sigma C_2 - H_{31}$	$\sigma C_{27} - C_{28}$	4.62	1.03	0.062
$\sigma C_{22} - H_{26}$	$\pi^* C_{27} - C_{28}$	3.50	0.54	0.039
$\sigma C_{21} - H_{23}$	$\sigma^* C_{16} - H_{20}$	2.38	0.96	0.043
Palmitic acid				
Donor	**Acceptor**	**$E^{(2)}$**	**E(j)- E(i)**	**F(i,j)**
LPO_3	$\pi C_1 - C_2$	29.90	0.34	0.091
LPO_2	$\sigma^* C_1 - O_3$	31.98	0.52	0.116
LPO_2	$\sigma^* C_1 - C_5$	13.98	0.61	0.084
$\sigma C5 - H_8$	$\pi^* C_1 - O_2$	4.50	0.51	0.044
$\sigma O4 - H_4$	$\sigma^* C_1 - C_5$	3.14	1,11	0.053

Reactivity Indexes

First principle computational and molecular modelling approach was utilized in studying the properties of chemical compounds with respect to reactivity and effects of substituents[25]. Reactivity descriptors are very important not only in chemistry but in other areas of science like pharmaceutical, environmental and health sciences research. Descriptors such as chemical hardness (Ƞ) chemical potential (μ), softness (σ) electrophilicity index (ω), electronegativity (χ) was estimated using a relation which relate the HOMO and LUMO to Ionization energy (IE) and electron affinity (EA) respectively. Ionization energy and electron affinity is often used to estimate the electron-donor (ω^-) power and electron-acceptor (ω^+) power indexes.

Ionization energy of Antioxidants (IE)

IE is a versatile tool for evaluating the antioxidant property of chemical compounds [26]. It shows the electron donating capacity of the antioxidant molecule to the oxidant, thereby disrupting the oxidation process. Often, this property is compared with that of 2, 2-diphenyl-picrylhydrazyl (DPPH) as a reference molecule. We calculated the IE of DPPH which is usually the standard for antioxidant experiments. DPPH produces stable radicals whose properties are used in characterizing the antioxidant behaviour of molecules [27]. DPPH, showed IE of 8.1eV; hence, molecules having approximate values of IE close that of DPPH are considered effective donors. Apart from flavan which has the least IE, all the compounds studied are efficient electron donors as DPPH.

Electron Affinity (EA) of Antioxidants

The electron affinities for the studied molecules are as presented in Table 3. DPPH, has electron affinity of 2.0 eV, whereas the highest electron affinity value was observed for Xanthone with 2.493eV, palmitic acid has the lowest EA (0.177), Flavan had EA value of 1.239eV followed by stearic acid with EA value of 1.071eV. Considering EA, all the compounds showed good capacity to donate electrons. Hardness, softness, electronegativity index, chemical potential, electrophilicity and the energy gap are also presented in Table 3. Chemical hardness is the resistance of chemical compounds to change the number of its electron[28]. Flavan has the least hardness, therefore, the possibility that it will change its orientation. Other compounds showed hardness close to that of DPPH (3.04), palmitic acid (3.23), stearic acid (3.01), flavan (1.52) and xanthone (2.13), confirming that flavan has a poor antioxidant activity relative to DPPH and other compounds under investigation. Compounds having high values of electronegativity include DPPH, xanthone and Stearic acid. Consequently, electronegativity may not be an appropriate instrument of analyses for the structures. Similarly, electrophilicity showed the highest value was obtained for xanthone, with apparent difference to that of other compounds. However, relatively higher values of the electronegativity and electrophilicity is indicative of a good antioxidant.

Table 3: Quantum Chemical Descriptors

Molecule	IE	EA	Ƞ	μ	ω	Σ	χ
Xanthone	6.729	2.493	2.118	4.611	5.019	0.236	-4.611
Flavan	4.281	1.239	1.521	2.760	2.504	0.329	-2.760
Stearic acid	7.084	1.071	3.007	4.075	2.765	0.166	-4.075
Palmitic acid	6.635	0.177	3.229	3.406	1.796	0.155	-3.406
DPPH	8.07	2.00	3.04	5.03	4.17	0.164	-5.03

All the parameter used in this study showed good correlation with respect to antioxidant activity and class of compound. First, there was a strong correlation between 6.458 and 6.013 eV respectively obtained for stearic acid and palmitic acid versus 4.236 and 3.042 eV obtained for xanthone and flavan respectively. Again, the second order perturbation energy calculated from NBO analyses explains the stability of compounds and shows here that flavan is the most stable followed by xanthone and by implication, exhibit less ability to donate electron (antioxidant property). The energy of stabilization as shown by NBO were 2.38 to 16.53 eV (stearic acid), 3.14 to 31.98 eV (palmitic acid), and 70.92 eV to 284.57 eV for xanthone and 3634.23 to 21901.90 eV for flavan. This enormous amount of energy needed for orbital interaction makes flavan a less antioxidant molecule.

Besides, considering the reactivity descriptors and matching with the properties of DPPH which is an accepted antioxidant standard, we found that stearic acid shares a relatively close chemical properties with DPPH having IP (7.084 eV), Ƞ (3.01 eV), μ (4.075 eV), σ (0.166eV) and χ(-4.075eV) while DPPH in the same order were 8.07 eV, 3.04 eV, 5.03 eV, 0.164 eV and -5.03 eV (Table 3). The present study showed that the order of antioxidant capacity of the studied compounds was stearic acid>palmitic acid>xanthone>flavan.

Donor-Acceptor Characteristics of the compounds

The donor-acceptor map can be done using an approximation proposed by Martinez [29]. This concept using fluorine as a standard for a perfect acceptor and sodium as a standard for donor, provides the needed information on the donor-acceptor properties of chemical entities. This concept was therefore employed using the donor-acceptor properties of sodium and fluorine computed by the same method. Calculations were done as shown:

$$Ra = \omega^{+}_{L}/\omega^{+}_{F}$$

Where Ra is the electron acceptance index. If Ra = 1, L is a compound with acceptor efficiency similar to fluorine. If Ra is greater than 1, then, L is more an acceptor than Fluorine. But, if Ra < 1, then, L represent less an acceptor than fluorine.

$$Rd = \omega^{-}_{L}/^{-}_{Na}$$

Where Rd is the electron donor index. So that if Rd = 1, then L represents a compound with donor efficiency similar to sodium. if Rd is greater than 1, then, L is a less donor than sodium atom. Again, if Rd< 1, then, L is more an acceptor than sodium. A graph of Rd against Ra gives a clear explanation of the antioxidant activity according to Martinez.

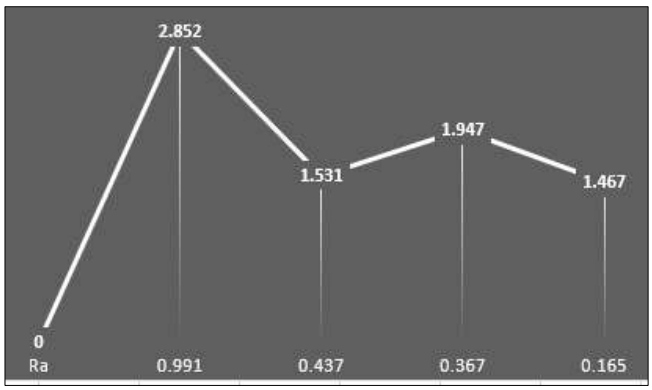

Fig 1: Donor-Acceptor Characteristics of the molecules

The values obtained for the compounds (Fig.1) indicates they have excellent antiradical properties with appropriate donor but poor acceptor capacity having all Ra values less than 1. The values for Rd which are little above 1 show the ability to donate electron similar to sodium [28].

Conclusion

Several scientific research have shown that phyto-drugs are effective in management of oxidative stress due to their antiradical properties. All the parameters used in this study showed good correlation with respect to antioxidant activity. There was a strong correlation between 6.458 and 6.013 eV respectively obtained for stearic acid and palmitic acid HOMO-LUMO energy gap versus 4.236 and 3.042 eV obtained for xanthone and flavan respectively, indicating that the fatty acids possessed higher antioxidant activity than the flavonoids. Again, the second order perturbation energy calculated from the natural bond orbital (NBO) explains the stability of compounds and shows here that flavan is the most stable followed by xanthone and by implication, exhibit less ability to donate electron (antioxidant activity). The energy of stabilization as shown by NBO were 2.38 to 16.53 eV (stearic acid), 3.14 to 31.98 eV (palmitic acid), and 70.92 eV to 284.57 eV for xanthone and 3634.23 to 21901.90 eV for flavan. This enormous amount of energy needed for orbital interaction makes flavan a less antioxidant molecule. The present study showed that the order of antioxidant capacity of the studied compounds was stearic acid>palmitic acid>xanthone>flavan.

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Conflict of Interest

There are no conflicts of interest among the authors

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