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Sterols from *Jatropha tanjorensis* leaves exhibit anti-inflammatory potential: in vitro and in silico studies

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Abstract

Background: Inflammation has continued to raise global challenges and *Jatropha tanrogenesis* (JT) is used traditionally for its management. In this study, the in silico and in vitro anti-inflammatory potential of bioactive sterols were investigated. The active compounds of ethanol extract of JT leaves were identified using Gas chromatography-mass spectrometry (GC.MS) followed by molecular docking against COX-1 and COX-2 using maestro Schrödinger and pharmacokinetic profile prediction using webserver tools. The in vitro anti-inflammatory and anti-oxidantive potentials were investigated using standard protocols.

Results: GC–MS analysis of ethanol extract of JT leaves revealed the presence of eight (8) compounds, the molecular docking analysis of these compounds demonstrated varying degrees of binding affinities against the target proteins. The extract exhibit concentration dependent anti-oxidant activity with IC_{50} of 106.383 and 6.00 Fe^{2+}E/g for DPPH and FRAP respectively. The extract showed significant ($P < 0.05$) reduction in percentage inhibition of hemolysis at 200 µg/ml while non-significant ($P > 0.05$) increase was observed at 600 and 1000 µg/ml compared to 200 µg/ml of diclofenac sodium. At lower concentration of 25 and 50 µg/ml, percentage inhibition of albumin denaturation was significantly ($P < 0.05$) higher compared to 200 µg/ml of diclofenac sodium. Drug likeness prediction and ADME/toxicity screening showed that the bioactive compounds possess no side effects.

Conclusion: The results obtained in this study suggested that, JT leaves possess anti-inflammatory activity and could be used as a source of new drug.

Keywords: Anti-inflammatory, Anti-oxidant, In silico, In vitro, Cyclooxygenase

Background

Inflammation is the natural response of body's immune system triggered by noxious stimulus and conditions such as pathogens, cellular damage and irritants that occurs in vascular tissues (Bouhlali et al. 2016). The major aim of inflammatory response is to localize and eliminate the harmful agents and remove damaged tissue components (Oguntibeju 2018). Acute inflammatory reactions reduce damage and infections, also involve in the restoration of homeostasis within the tissue (Nathan and Ding 2010). However, uncontrolled inflammatory responses may result in chronic inflammation which has been implicated in many inflammatory diseases (Akinloye et al. 2020). Inflammation and oxidation are related since oxidants that damage cells result into inflammation (Yesmin et al. 2020). Free radicals are natural by-products, which are constantly produced in vivo for specific metabolic purposes such as signal transduction, gene expression and activation of receptors. Excessive production of free radicals leads to inflammation (Yesmin et al. 2020).

The available non-steroidal anti-inflammatory drugs (NSAIDs) act by blocking the cyclooxygenase (COX) enzyme, reducing the release of prostaglandins thereby ameliorating pains and inflammation. Two COX isoforms

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have been studied with different pattern of expressions (Akinloye et al. 2020). NSAIDs selectively inhibits COX-1 and reduce inflammation with associated side effects such as gastrointerstinal tract (GIT) damage and ulcer while COX-2 induced by pro-inflammatory cytokines without GIT disorders (Williams et al. 1999). Hence, natural compounds of plant origin may serve as drugs that are COX-2 selective as anti-inflammatory agents.

The use of natural products as drug candidate has been attributed to their phytochemical constituents (Erukainure et al. 2018) and their anti-inflammatory efficacy has been observed to surpass synthetic drugs (Attiq et al. 2018). *Jatropha tanjorensis* (Ellis and Saroja) is a perennial herb that belongs to the family Euphrobiaceae, it is an exotic plant in Nigeria and native of America (Olayiwola et al. 2004). The plant is commonly known as catholic vegetable, the Yoruba speaking tribe of South-western Nigeria called the plant Lapalapa or iyana ipaja (Ochulor et al. 2018).

J. tanjorensis leaf has been reported to possess hematopoietic activity (Orhue et al. 2008), hypoglycemic and anti-diabetes activity (Olayiwola et al. 2004). The antioxidative potential of *J. tanjorensis* leaves against reactive oxygen species produced in protein energy malnutrition (PEM) has been reported by Omoregie and Osagie (2011). Its anti-arthritic property has also been documented by Arun and Brindha (2012). This study aimed to evaluate the in silico and in vitro anti-inflammatory activities of *J. tanjorensis* leaves and to identify the active compounds.

Methods

Collection and preparation of plant sample

Jatropha tanjorensis (JT) leaves were obtained within the metropolis of Okitipupa, Nigeria. The leaves were taxonomically identified and authenticated at the Department of Biological Science, Olusegun Agagu University of Science and Technology (OAUSTECH), Okitipupa, Ondo State, Nigeria. The (OAUSTECH-18008) voucher number was deposited at their herbarium. The leaves were washed and air-dried at room temperature in the laboratory for three weeks, the dried leaves were ground into powdery form.

Preparation of extract

Exactly 138 g of powdered JT leaves was soaked in 960 ml ethanol for 72 h and shaken intermittently. The slurry formed was filtered and the filtrate was dried under pressure with rotary evaporator. The concentrated sample was weighed and extract yield was calculated, the crude extract was stored in the refrigerator until use for GC–MS analysis, in vitro antioxidant and anti-inflammatory assays. The extract yield was calculated as:

$$\text{Extract yield} = \frac{\text{Crude extract}}{\text{Dried Sample}} \times 100$$

Gas chromatography-mass spectrometry (GC–MS) analysis

GC–MS analysis of the ethanol extract was performed using Gas chromatography coupled to a mass detector Turbo mass gold (Perkin- Elmer) spectrometer with an Elute- 5 ms (5% diphenyl/95% dimethyl poly siloxane, 30 m × 0.25 mm × 0.25 µg df) of capillary column as previously described by Okereke et al. (2017). The oven was set to an initial temperature of 110 °C for 2 min, further increased up to 200 °C at the rate of 10 °C/ min. finally temperature was raised up to 280 °C, at the rate of 5 °C/ min for 9 min. Helium gas was used as the carrier gas at constant flow rate of 1 ml/ min. An aliquot of 2 µl of sample was injected into the column with the injector temperature at 250 °C (spilt ratio of 10:1). The electron ionization system with ionizing energy of 70 eV was used. Mass spectral scan range was set at 45 to 450 m/z (Okereke et al. 2017). The National Institute of Standard and Technology (NIST) library database was used in the interpretation of the mass spectrum.

In silico *ADME/toxicity screening and drug likeness prediction*

The ADME (absorption, distribution, metabolism, and excretion) and toxicity screening and drug-likeness prediction of the compounds were carried out using admetSAR1.0 prediction online tool (http://lmmd.ecust.edu.cn) (Cheng et al. 2012) and swissADME server (http://www.swissadme.ch) (Daina et al. 2017) respectively to predict the pharmacokinetics and pharmacodynamics profiles of the compounds.

Protein crystal structure and ligands collection

Eight (8) chemical compounds obtained from GC–MS analysis were subjected to molecular docking with selected anti-inflammatory protein targets. These molecular targets include cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) with pdb ID: 5U6X and 3NTG respectively retrieved from the protein data bank (http://www.rcsb.org) in 3D format. The existing ligands and water molecules were removed and hydrogen molecules were added during protein preparation. The structure-data file (SDF) structures of the compounds and standard ligands were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) (Omoboyowa et al. 2021).

Molecular docking and docking validation

Molecular docking was performed between the 3D crystallography structure of COX-1 and 2, and the bioactive

compounds obtained from GC–MS analysis of *Jatropha tanrogenesis*. The docking protocol was conducted using the default parameters in the Schrodinger suite 2018. The 3D structure of the target proteins were preprocessed by employing Protein preparation Wizard module in the Schrodinger suite 2018 (Sastry et al. 2013). Herein, missing hydrogen atoms and loops were added to the protein while the native co-crystallized ligand and water molecules were deleted from the protein. The protein's electronic polarization and small backbone fluctuations were defined using the standard distance-dependent dielectric constant at 2.0 Å (Balogun et al. 2021). Conjugated gradient procedure was utilized for the subsequent optimization of the proteins and restrained refinement of hydrogen atoms with the maximum root-mean-square deviation (RMSD) at 0.3 Å under optimized potentials for liquid simulations (OPLS)-3 force field (Friesner et al. 2006; Roos et al. 2019).

Similarly, *J. tanrogenesis* ligands library were obtained from GC–MS analysis and prepared for molecular docking by utilizing the LigPrep tool under default parameters in the Maestro-Schrodinger suite 2018 (Maestro 2018). The binding site of the co-crystalized ligands of each protein was considered for molecular docking pocket of *J. tanrogenesis* ligands library under default parameters by extra precision (XP) protocol of Schrodinger GLIDE tool (Maestro 2018).

Furthermore, the co-crystallized ligand was extracted from the crystal complex, prepared using the above ligand preparation method, and re-docked in the same selective active site in order to validate the docking procedure. A RMSD value of 1.02 Å was obtained (< 2. Å).

Molecular mechanics/generalized born surface area (MM/GBSA) calculation

MM/GBSA calculation, an advanced quantum mechanics calculation that helps remove false-positive obtained from molecular docking analysis was performed to compute the relative binding free energy for each Cyclooxygenase-ligand complex. The Prime MM/GBSA module in the Maestro-Schrodinger suite under default parameters was used (Prime 2018). The mathematical representation of the step to calculate the binding free energy was represented by the equation below.

$$\Delta G^{\text{bind}} = G^{\text{complex}} - \left(G^{\text{protein}} + G^{\text{ligand}}\right)$$

In vitro *anti-inflammatory analysis*

The Hypotonicity-induced haemolysis and inhibition of albumin denaturation of ethanol extract of *Jatropha tanjorensis* leaves were carried out according to the methods described by Leelaprakash and Mohan-Dass (2011) and Azeem et al. (2010) respectively.

In vitro *antioxidant assays*

The 1,1 diphenyl-2-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant potential (FRAP) of ethanol extract of *Jatropha tanjorensis* leaves was evaluated according to the methods of Alam et al. (2013) and Oyaizu (1986) respectively.

Statistical analysis

Three analytical determinations were carried out on each independent replication for every parameter. Statistical analyses were performed by one-way analysis of variance, followed by turkey test. Significance value was set at $P < 0.05$ using graph pad prism 6.

Results

Extract yield

The yield of the crude ethanol extract of *Jatropha tanjorensis* leaves after concentration and drying was 10.1 g (7.3%).

GC–Ms analysis of ethanolic extract of Jatropha tanjorensis leaves

Table 1 and Fig. 1 show the result of GC–MS analysis of ethanol extract of *J. tanjorensis* leaves. The result revealed the presence of 8 compounds from 11 chromatogram peak. Compounds detected comprise some different classes of hydrocarbons, alcohols, steroidal compounds, fatty acids and their derivatives.

Table 1 List of compounds identified at various retention times (RT) from ethanolic extract of *Jatropha tanjorensis* leaves by GC–MS

S/N	Retention time (Min)	Compounds name	Peak area ratio (%)
1	12.363	3,4-dimethylcyclohexanol	3.68
2	12.741	Hexadecanoic acid	6.69
3	13.434	1-cyclohexylnonene	19.58
4	13.485	Phytol	13.24
5	13.662	2(1H)-Naphthalenone	4.22
6	13.685	cyclopropyloctanoic acid	5.55
7	14.275	Adipic acid	4.23
8	14.275	propyl(4-((propoxycarbonyl) oxy)butyl)carbamate	4.23

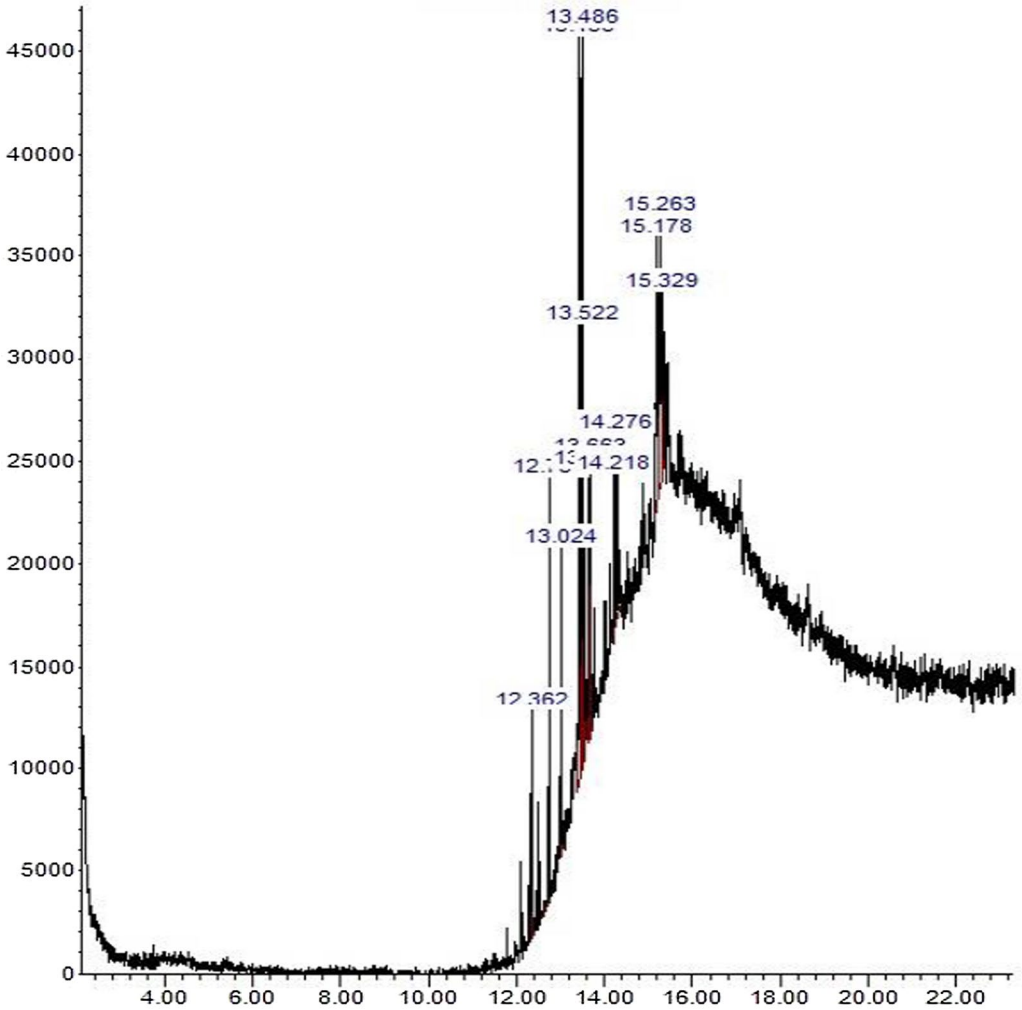

Fig. 1 Mass chromatogram of ethanolic extract of leaves of *Jatropha tanjorensis*

ADME/toxicity screening and drug likeness prediction of compounds from ethanolic extract of Jatropha tanjorensis leaves

From Table 2, all the eight compounds showed positive to blood brain barrier (BBB+), human intestinal absorption (HIA+) and Caco-2 permeability (CAco-2+) except adipic acid that was negative to Caco-2. The compounds revealed non-substrate and non-inhibitors of p-glycoprotein and CYP3A4. All the detected compounds were observed to be non-carcinogenic. It was observed that, the eight compounds have not violated more than one of the lipinski's rule of five (Table 3). The logkp reveals that adipic acid has the highest skin penetrating potential (− 5.95 cm/s).

Molecular docking analysis

As shown in Table 4. The standard drug (diclofenac) possesses binding affinity of − 8.81 kcal/mol with COX-1 and − 6.98 kcal/mol with COX-2. The binding affinities of all the compounds against COX-1 were observed to be lower compared to that of the reference drug. The interaction of hexadecanoic acid (− 7.66 kcal/mol), 1-cyclohexylnonene (− 7.37 kcal/mol) and cyclopropylactonoic acid (− 6.15 kcal/mol) showed the highest binding affinity against COX-1 compared to other compounds while phytol (− 7.21 kcal/mol), 1-cyclohexylnonene (− 6.97 kcal/mol) and 2(1H)-Naphthalenone revealed high binding affinities against COX-2. Phytol showed higher binding affinity compared to the standard drug.

Table 2 In silico ADME toxicity screening of the compounds

Compounds	BBB	HIA	Caco2 perm	p-gp substrate/inhibitor	CYP3A4 non-inhibitor	Carcinogenicity	AMES/ Toxicity
3,4-dimethylcyclohexanol	+	+	+	Non-substrate/non-inhibitor	Non-inhibitor	Non-carcinogens	Non-toxic
Hexadecanoic acid	+	+	+	Non-substrate/non-inhibitor	Non-substrate/non-inhibitor	Non-carcinogens	Non-toxic
1-cyclohexylnonene	+	+	+	Non-substrate/non-inhibitor	Non-substrate/non-inhibitor	Non-carcinogens	Non-toxic
Phytol	+	+	+	Non-substrate/non-inhibitor	Non-substrate/non-inhibitor	Non-carcinogens	Non-toxic
2(1H)-Naphthalenone	+	+	+	Non-substrate/non-inhibitor	Non-substrate/non-inhibitor	Non-carcinogens	Toxic
cyclopropyloctanoic acid	+	+	+	Non-substrate/non-inhibitor	Non-substrate/non-inhibitor	Non-carcinogens	Non-toxic
Adipic acid	+	+	−	Non-substrate/non-inhibitor	Non-substrate/non-inhibitor	Non-carcinogens	Non-toxic
propyl(4-((propoxycarbonyl)oxy) butyl)carbamate	+	+	+	Non-substrate/non-inhibitor	Non-substrate/non-inhibitor	Non-carcinogens	Non-toxic

BBB Blood Brain Barrier, *HIA* Human Intestinal Absorption, Caco-2 permeability P-glycoprotein substrate/ inhibitor; p-gp Substrate/Inhibitor, Cytochrome 3A4 Inhibition/Substrate, CYP3A4 Inhibition/Substrate

Table 3 In silico drug likeness prediction of the compounds

Compounds	MW	HBA	HBD	TPSA	XLOGP	Log Kp (cm/s)	Lipinski Violation
3,4-dimethylcyclohexanol	128.21	1	1	20.23	2.04	−5.63	0
Hexadecanoic acid	256.42	2	1	37.30	7.17	−2.77	1
1-cyclohexylnonene	208.38	0	0	0	6.98	−2.62	1
Phytol	296.53	1	1	20.23	8.19	−2.29	1
2(1H)-Naphthalenone	144.17	1	0	17.07	1.87	−5.85	0
cyclopropyloctanoic acid	184.28	1	01	37.30	4.00	−4.58	0
Adipic acid	261.31	5	1	73.86	2.74	−5.95	0
propyl(4-((propoxycarbonyl)oxy) butyl)carbamate	153.22	2	0	29.43	4.09	−4.33	0

MW molecular weight (< 500), *HBA* number of hydrogen bond acceptors (< 10), *HBD* number of hydrogen bond donor (< 5), *XlogP* the partition coefficient ≤ 5, *TPSA* topological polar surface area

Table 4 Docking score of compounds

Compounds Name	Binding Affinity (kcal/mol)	
	COX-1	COX-2
3,4-dimethylcyclohexanol	−4.90	−6.23
Hexadecanoic acid	−7.66	−5.64
1-cyclohexylnonene	−7.37	−6.97
phytol	−6.10	−7.21
2(1H)-Naphthalenone	−6.02	−6.33
cyclopropyloctanoic acid	−6.15	−3.61
Adipic acid	−4.48	−4.14
propyl(4-((propoxycarbonyl)oxy)butyl) carbamate	−3.77	−4.03
Diclofenac	−8.81	−6.98

COX-1 cyclooxygenase-1, *COX-2* cyclooxygenase-2

These compounds interacted with various amino acids by conventional hydrogen bonds, pi-pi stacking etc. at the same binding pocket of the target proteins as shown in Figs. 2a, b and 3a, b.

Molecular Mechanics/generalized born surface area (MM/GBSA) analysis

MM/GBSA of the protein–ligand complexes were calculated for reliability and accuracy in computing energy (Fig. 4). The result presented in Fig. 5a, b revealed that 2(1H)-naphthalenone, cyclopropylactanoic acid, adipic acid and propyl(4-(propoxycarbonyl)oxy)butylcarbamate posse higher MM/GBSA binding energy compared to the standard drug (diclofenac) against COX-1 while adipic acid and cyclopropylactanoic acid showed lower MM/GBSA binding energy against COX-2 compared to the standard drug.

97960: 3,4-dimethylcyclohexanol; 985: Hexadecanoic acid; 5364533: 1-cyclohexylnonene; 5280435: Phytol; 11400841: 2(1H)-Naphthalenone; 57346156: Cyclopropylactanoic acid; 196: Adipic acid; 91713661: propyl(4-((propoxycarbonyl)oxy)butylcarbamate; 3033: Diclofanac.

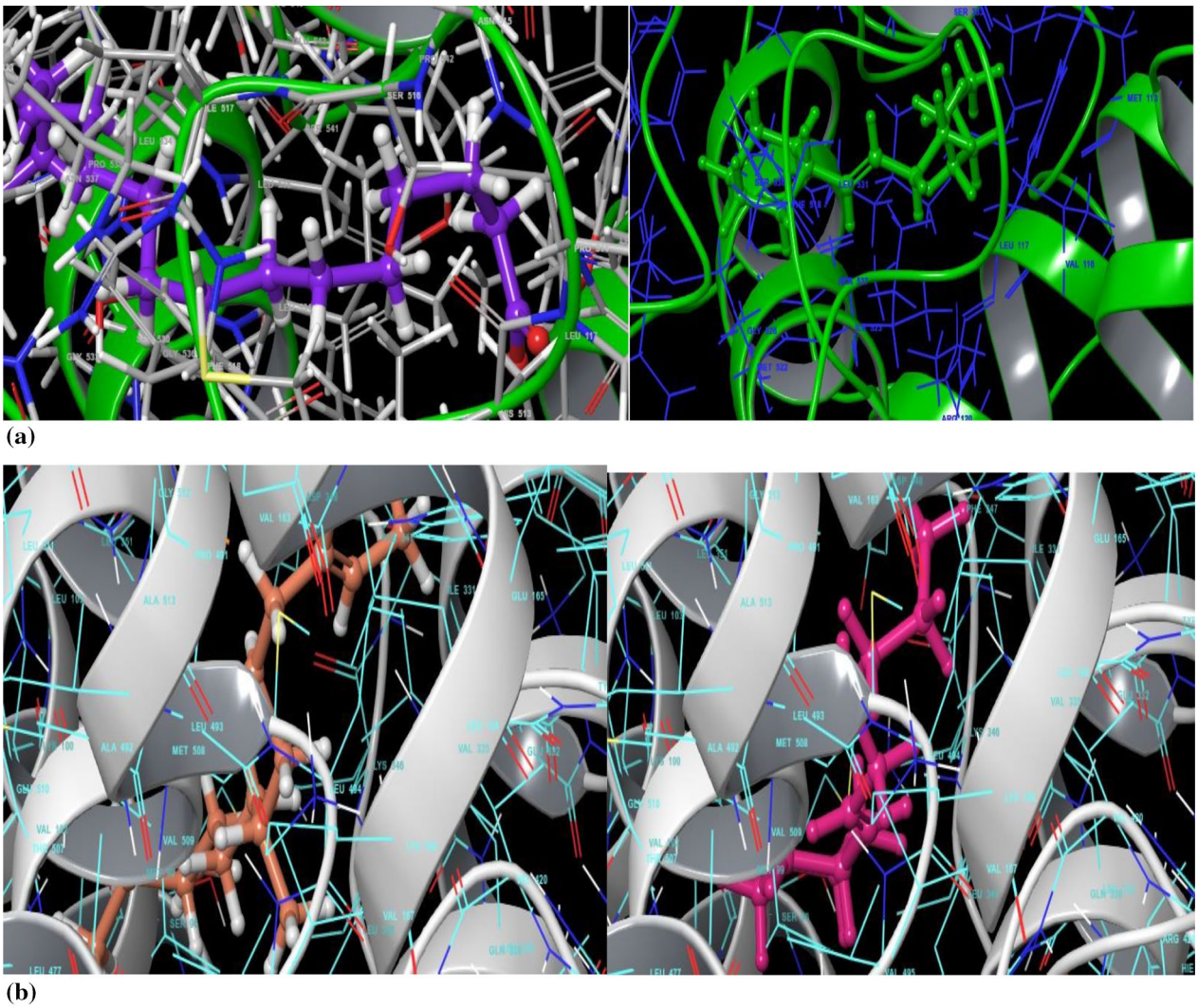

Fig. 2 (**a**) Interactions of compounds with the highest affinity with the active site amino acids of the target (a) COX 1-Hexadecanoic acid (b) COX1-1-cyclohexylnonene, **b** Interactions of compounds with the highest affinity with the active site amino acids of the target (a) COX 2-Pytol (b) COX 2–1-cyclohexylnonene

In vitro antioxidant effect of ethanol extract of *J. tanjorensis* leaf

The ethanol extract of *J. tanjorensis* leaf showed highest DPPH scavenging activity at 1000 µg/ml. The DPPH scavenging capacity of the extract across the concentration were observed to be lower compared to the standard (garlic acid) as shown in Fig. 6a. The ferric reducing antioxidant power (FRAP) of the extract was significantly ($P < 0.05$) higher at concentration of 50 µg/ml. the extract shows appreciable antioxidant power but at concentration of 100 µg/ml, the antioxidant power significantly ($P < 0.05$) reduced (Fig. 6b).

In vitro anti-inflammatory effect of ethanol extract of *J. tanjorensis* leaf

Figure 7a shows the percentage inhibition of hypotonicity induced haemolysis of ethanol extract of *J. tanjorensis* leaf. The extract at concentration of 200 µg/ml showed significant ($P < 0.05$) reduction in percentage inhibition of haemolysis compared to the standard drug (diclofenac sodium). The percentage inhibition of haemolysis observed at 600 and 1000 µg/ml concentration of the extract were non-significantly ($P > 0.05$) higher compared to the standard drug.

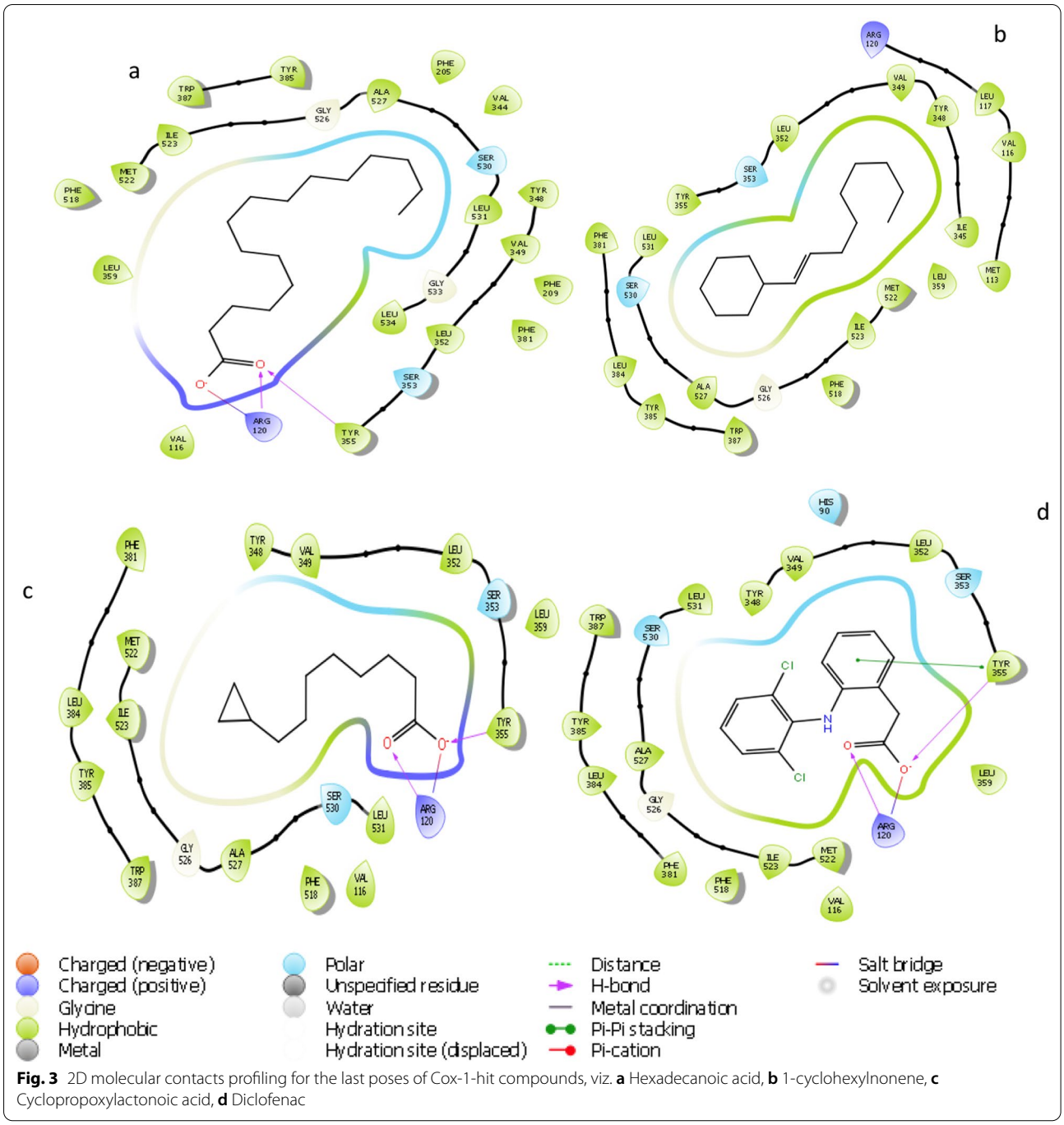

Fig. 3 2D molecular contacts profiling for the last poses of Cox-1-hit compounds, viz. **a** Hexadecanoic acid, **b** 1-cyclohexylnonene, **c** Cyclopropoxylactonoic acid, **d** Diclofenac

As shown in Fig. 7b, the percentage inhibition of albumin denaturation of ethanol extract of *J. tanjorensis* leaf at 25 and 50 µg/ml were significantly ($P < 0.05$) higher compared to diclofenac sodium. The percentage inhibition of the extract at 200 µg/ml was significantly ($P < 0.05$) reduced compared to diclofenac sodium.

Discussion

GC–MS is suitable for the identification and quantification of sterols moieties at ambient temperature due to its stable nature (Chen et al. 2015). Table 1 and Fig. 1 showed the result of GC–MS analysis of ethanol extract of *J. tanjorensis* leaf. The result revealed the presence of

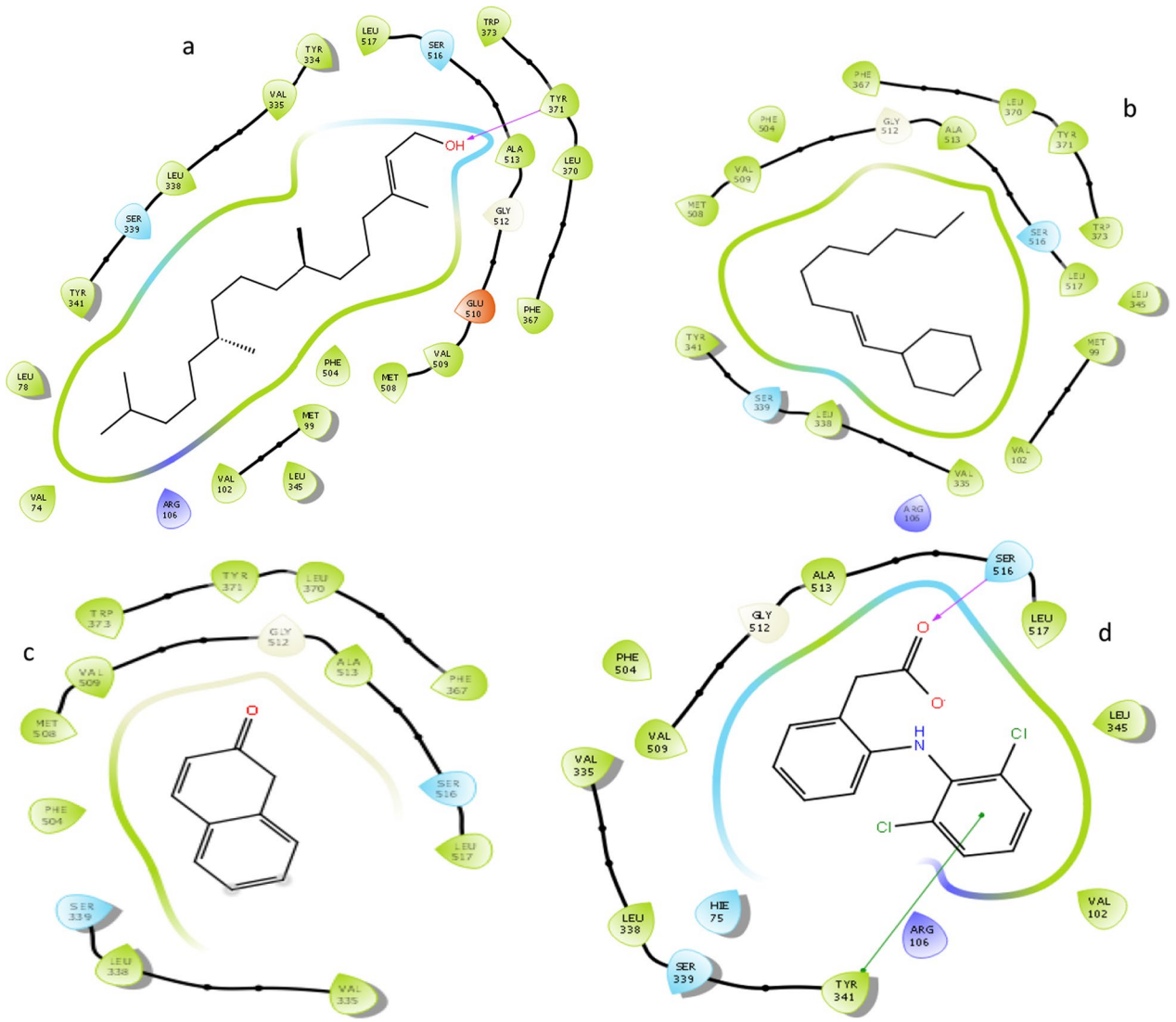

Fig. 4 2D molecular contacts profiling for the last poses of Cox-2-hit compounds, viz. **a** Phytol, **b** 1-cyclohexylone, **c** 2-(1H)-Naphthalenone, **d** Diclofenac

eight (8) compounds; these compounds detected comprise some different classes of hydrocarbons, alcohols, steroidal compounds, fatty acids and their derivatives. The presence of eicosanoic acid, methyl esters, heneicosanoic acid and fatty acid derivatives revealed by GC–MS possess many physiological and biochemical functions (Eseyin et al. 2018).

The pharmacokinetics and pharmacodynamics profiles of the eight compounds from GC–MS analysis of ethanol extract of *J. tanjorensis* leaf were predicted by absorption, distribution, metabolism, excretion, toxicity and drug likeness prediction, the results were presented in Tables 2 and 3.

From Table 2, all the compounds were positive to human intestinal absorption (HIA), which implies that they can be easily absorbed into the blood stream. All the compounds can also cross the blood brain barrier (BBB+). Blood brain barrier is the regulated interface between the peripheral circulation and the central nervous system (Hawkins and Davis 2005). The failure of many therapeutic molecules as drug candidates has been attributed to their inability to cross the barrier between the blood and the brain (Ballabh et al. 2004). Therefore, it is worthy to note that, all the compounds can cross the BBB. The result agreed with the findings of Akinloye et al. (2020) who reported that phytosterols from *Nicotiana*

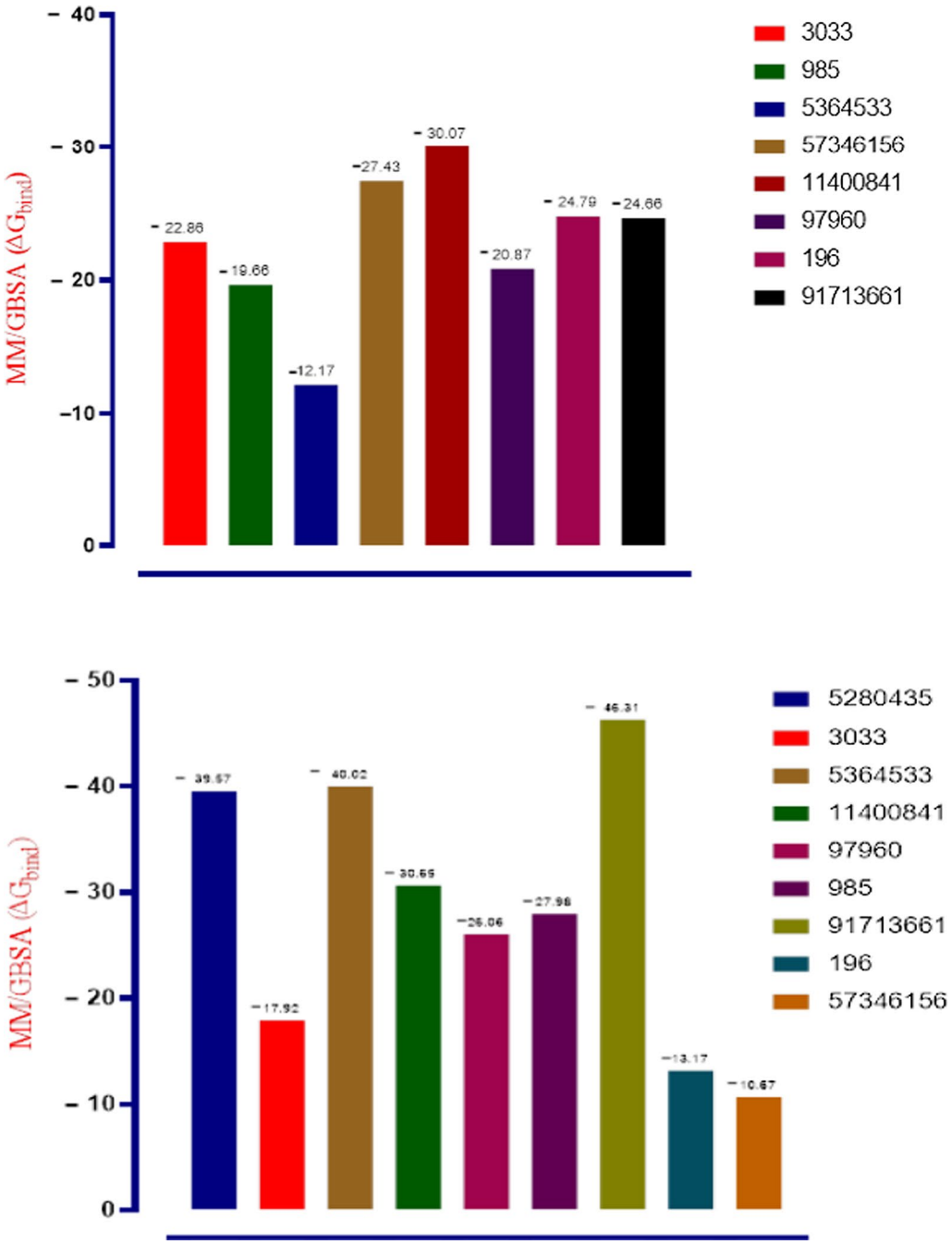

Fig. 5 **a** Graphical representation of the and Prime/MM-GBSA binding energy (ΔG_{bind}) of COX 1-Ligand Complex, **b** Graphical representation of the and Prime/MM-GBSA binding energy (ΔG_{bind}) of COX 2-Ligand Complex

tabacum can cross the BBB. All the compounds were positive to colon cancer carcinoma cell line (Caco2) except adipic acid, this indicates that most of the compounds can penetrate the intestine.

The compounds showed non-substrate and non-inhibitor of p-glycoprotein (p-gp). P-gp is an efflux membrane transporter; one of the first members of the ATP-binding cassette (ABC) transported acting as a physiological barrier by extruding drugs out of the cell (Amin 2013). All the compounds are good drug molecules showing non-substrate and non-inhibitor of p-gp. CYP 3A4 is often considered the most important drug-metabolizing

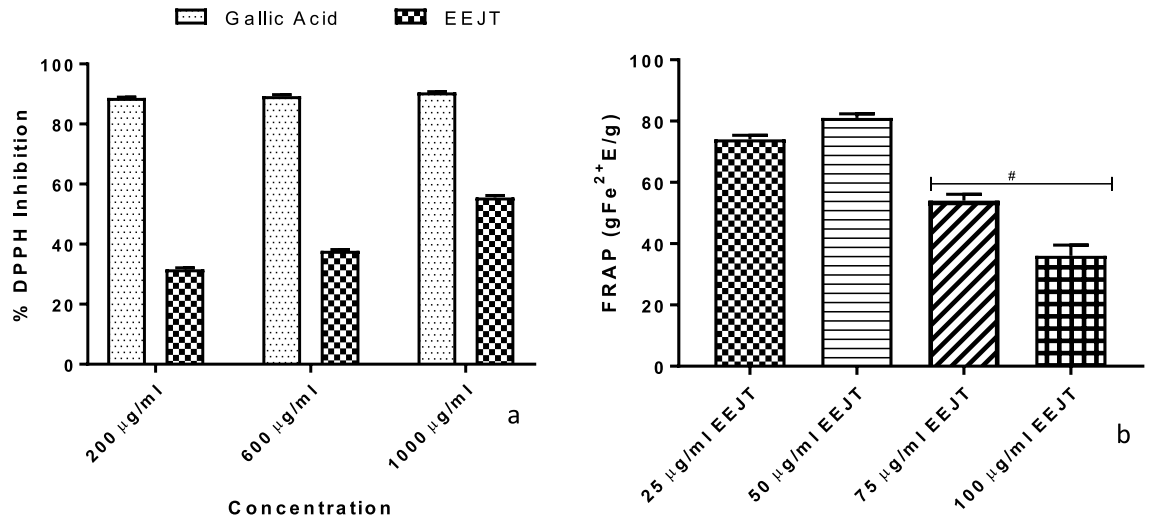

Fig. 6 In vitro antioxidant potential of ethanol extract of *Jatropha tanjerensis* leaves (**a**) DPPH scavenging ability (**b**) Ferric reducing antioxidant power (FRAP) #$p < 0.05$, considered significant compared with 25 µg/mg EEJT

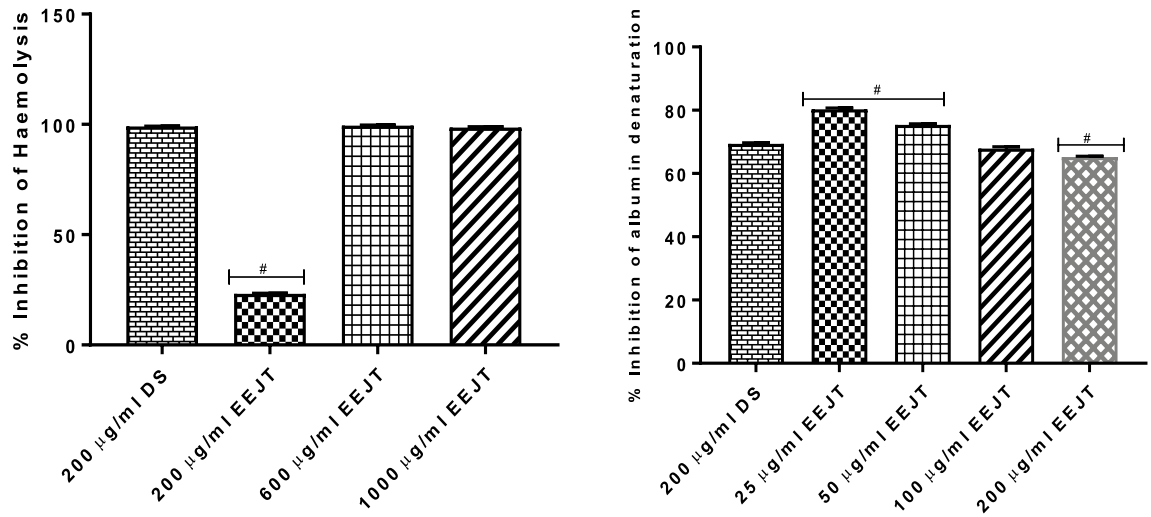

Fig. 7 In vitro anti-inflammatory potential of ethanol extract of *Jatropha tanjerensis* leaves (**a**) hypotonicity induced haemolysis of erythrocyte (**b**) Albumin denaturation. #$P < 0.05$, significant compared to 200 µg/ml of Diclofenac sodium (DS)

enzyme, due to its relative expression in the liver and intestine (Tirona and Kim 2017). It oxidizes small foreign organic molecules such as drug for removal from the body. The tendency of drug molecules to interact with CYP 3A4 is associated with adverse side effects (Zhou 2008). All the compounds are non-substrate and non-inhibitor of CYP 3A4 therefore, side effects or drug-drug interactions will be very low or absent. Increasing numbers of carcinogens and promoters of carcinogenesis are being found in natural plants products (Bednar and Linsmaier-Bednar 1989). Drug likeness is a complex balance of various molecular properties and structural features which determines a particular molecule as drug or non-drug (Turner and Agatonovic-Kustrin 2007). In doing so, Lipinsk's rule of five is followed according to Lipinski et al. (2001). The rule state that drug like compounds should not violate more than one of the rules. There is poor absorption or permeation when molecular weight (MW) is greater than 500 KD and calculated LogP is greater than 5, there are more than five hydrogen bond donors (HBD) and the number of hydrogen bond acceptor is greater than 10. It is interesting that, the

compounds (Table 3) have not violated more than one of the lipinsk's rule of five which predict the compounds as drug-like candidates. The in vitro method of prediction of skin permeability has been reported as human skin permeability coefficient (Logkp) (Scheler et al. 2015). The logkp values of the compounds (Table 3) show that adipic acid has the highest permeability coefficient (−5.95 cm/s) compared to other compounds.

The bioactive compounds from *J. tanjorensis* showed a favorable binding affinity and optimally saturated the active site of cyclooxygenase-1, ranging from hexadecanoic acid to 1-cyclohexylnonene with the binding energy of −7.66 and −7.37 kcal/mol respectively. The interaction of these compounds against cyclooxygenase-2 active site showed binding affinity of −7.21 and −6.97 kcal/mol for phytol and 2(1H)-Naphthalenone respectively.

Following the docking approach, the lead compounds (hexadecanoic acid) bind firmly within the active site of COX-1 forming three hydrogen bonds with the amino acid residues: ARG 120 and TYR 355. The lead compound (phytol) interacted with COX-2 by forming one hydrogen bond with TYR 371 at the binding site of the protein (Fig. 3).

The MM/GBSA (ΔG^{bind}) for COX1/COX2-hit ligand complexes were calculated using the MM/GBSA module integrated with the prime program of the Schrodinger suite. MM/GBSA has been widely involve in bio-molecular studies such as protein folding, protein–ligand binding, protein–protein interaction etc. it is arguably very popular method for free energy prediction since they are more accurate than most scoring functions of molecular docking and less computationally demanding (Wang et al. 2019). The ΔG^{bind} was utilized for advanced mechanics calculation of the binding energy for the screened compounds following the docking analysis. Based on the MM-GBSA output (Fig. 5), 2(1H)-Naphthalenone, Cyclopropylactanoic acid, Adipic acid, propyl(4-((propoxycarbonyl)oxy)butylcarbamate complexes with COX 1 demonstrated ΔG^{bind} of −30.07, −27.43, −24.79, and −24.66, kcal/mol, respectively (Fig. 5a). Diclofenac had the ΔG^{bind} of −22.88 kcal/mol. Hence, the ΔG^{bind} results further ascertain that *J. tanjorensis* compounds bind better compared to the positive control ligand (diclofenac).

The in vitro antioxidant activities of ethanol extract of *J. tanjorensis* leaf are presented in Fig. 6. The percentage inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity increase with increase in the extract concentration. The highest inhibitory activity was observed at 1000 µg/ml. The effect of antioxidant DPPH is due to their hydrogen donating ability (Ashafa et al. 2010). FRAP assay evaluates the reducing power of antioxidant interaction with ferric-tripyridyltriazine complex. Donation of hydrogen atom contributes to the breaking of the free radical chain (Rajurkar and Hande 2011). The ferric reducing antioxidant potential (FRAP) of ethanol extract of *J. tanjorensis* leaf revealed concentration dependent decrease in FRAP activity. The highest FRAP activity was observed at low concentration of 25 µg/ml. the IC_{50} of DPPH and FRAP were 136.129 and 4.43gFe^{2+}E/g respectively. Oxidative stress enhances pro-inflammatory cytokines production (Karigidi et al. 2020). Hence, the free radical scavenging potential of *J. tanjorensis* leaf through antioxidant production might enhance their anti-inflammatory property.

Red blood cell (RBC) membrane stabilization was employed to study anti-inflammatory potential of *J. tanjorensis* because RBC resembles lysosomes in terms of the membrane similarity (Chou 1997). Hypotonicity induced haemolysis could result from shrinkage of the cells due to osmotic loss of electrolytes (Parvin et al. 2015). The ethanol extract of *J. tanjorensis* leaf at 600 and 1000 µg/ml effectively inhibit hypotonicity induced hemolysis. The percentage inhibition of hemolysis of the extract at 200 µg/ml was significantly ($P<0.05$) reduced compared to diclofenac sodium at 200 µg/ml. At concentration of 600 and 1000 µg/ml of the extract, the percentage albumin denaturation inhibition was non-significantly ($P>0.05$) higher compared to diclofenac at 200 µg/ml as shown in Fig. 7a. This result demonstrated that *J. tanjorensis* could possess anti-inflammatory potential. Previous studies have reported that, plants with anti-inflammatory potential have the capacity to stabilize cell membrane against hemolysis (Anyasor et al. 2019; Chamlagai and Singh 2016). The result obtained in this study is consistent with the findings of Arun and Brindha (2012) who reported effective membrane stabilization of *J. tanjorensis* leaf extract at 100 and 250 µg/ml.

Denaturation of protein is a biochemical reaction that occurs as a result chronic inflammatory reaction resulting into loss of tissue functions (Sangeetha and Vidhya 2016). It is well documented as one of the causes of inflammation (Govindappa et al. 2011). One of the mechanisms of NSAIDs action is inhibition of protein denaturation (Gunathilake et al. 2018). Figure 7b shows that, ethanol extract of *J. tanjorensis* significantly ($P<0.05$) increase percentage inhibition of albumin denaturation at 25 and 50 µg/ml compared to 200 µg/ml of diclofenac sodium. The extract shows significant ($P<0.05$) reduction in percentage albumin denaturation inhibition at 200 µg/ml compared to diclofenac sodium at 200 µg/ml. The percentage inhibition of albumin denaturation observed in this study is consistent with the findings of Arun and Brindha (2012) who reported high percentage inhibition at 100 and 250 µg/ml of *J. tanjorensis* leaf.

Conclusions

This study was conducted to identify the bioactive compounds present in *J. tanjorensis* leaves using GC–MS as potent inhibitors of COX-1 and COX-2 by employing structure-based drug design techniques. After the computational analysis via molecular docking and MM/GBSA quantum chemical calculations, *J. tanjorensis* ligands exhibited higher binding energy and favorable intermolecular interactions in the binding pocket of COX-1 and COX-2 in comparison to the reference ligand (diclofenac).

Furthermore, in vitro antioxidant and anti-inflammatory assay revealed *J. tanjorensis* leaves possess good percentage DPPH inhibition and ferric reducing antioxidant power. The percentage inhibition of heamolysis and albumin denaturation was comparable to the reference drug (diclofenac) which shows that *J. tanjoresis* leaves might be a good source of antioxidant and anti-inflammatory agent, however further in vivo investigation is required to validate the result from this study.

Abbreviations

JT: *Jatropha tanrogenesis*; GC-MS: Gas chromatography-mass spectrometry; COX-1: Cyclooxygenase-1; COX-2: Cyclooxyganse-2; DPPH: 1,1 Diphenyl-2-picrylhydrazyl; FRAP: Ferric reducing antioxidant potential; ADME: Absorption, distribution, metabolism, and excretion; NSAID: Non-steroidal anti-inflammatory drugs; GIT: Gastro-intestinal tract; PDB: Protein data bank; SDF: Structure-data file; 3D: 3-Dimenssional; RMSD: Root mean square deviation; OPLS: Optimized potentials for liquid simulations; XP: Extra precision; MM/GBSA: Molecular mechanics/generalized born surface area; BBB: Blood Brain Barrier; HIA: Human Intestinal Absorption; MW: Molecular weight; HBA: Hydrogen bond acceptors; HBD: Hydrogen bond donor; XlogP3: Partition coefficient; TPSA: Topological polar surface area.

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Authors' contributions

DAO performed all the computational analysis and wrote the manuscript.

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