

PHYTOCHEMICAL EVALUATION AND MOLECULAR DOCKING OF BIOACTIVE COMPOUNDS IN *Citrus sinensis* STEM BARK WITH ANTI-INFLAMMATORY ACTIVITY

ABSTRACT

Sweet orange (*Citrus sinensis*) is one of the most abundant citrus species consumed. Sweet orange has been claimed to possess anti-carcinogenic properties, anti-inflammatory, and antioxidant, and as a relief agent to many health conditions. This study has been carried out to validate the claim of the anti-inflammatory activity of the plant stem bark. The Chloroform extract of *Citrus sinensis* stem bark was screened for its phytochemical composition and anti-inflammatory activity using gas chromatography-mass spectrometry (GCMS), and in silico molecular docking. Twenty-four (26) phytochemical compounds were identified in the extract. The major phytochemical with the highest percentage area was 9-Octadecenoic acid. The compounds had good binding affinity against the target protein cyclooxygenase-2 (COX-2) active site from molecular docking analysis. The best binding affinity was observed in 9-Octadecenoic acid (-8.3 kcal/mol), and Cyclododecanol, 1-aminomethyl- (-7.2 kcal/mol). The binding affinities were better than the non-steroidal anti-inflammatory drug (NSAIDs), Ibuprofen (-6.8 kcal/mol). These findings provide more evidence to support the traditional use of sweet orange stem bark for anti-inflammatory treatment. Structural models of its interactions at the (COX-2 active site) active site are plausibly useful for the future design of anti-inflammatory agent.

Key words: *Citrus sineensis*, inflammation, cyclooxygenase, non-steroidal.

INTRODUCTION

One of the most significant medical discoveries of the past two decades, has been the realization that inflammatory processes and the immune system play a major role in a broad range of mental and physical health issues that causes the majority of morbidity and death in the world today [1]. Inflammatory diseases have been attributable to diseases such as ischemic heart disease, stroke, cancer, diabetes mellitus, chronic kidney disease, non-alcoholic fatty liver disease (NAFLD) and autoimmune and neurodegenerative conditions [2].

Non-steroidal anti-inflammatory drugs (NSAIDs) are clinically used for relieving inflammation by suppressing cyclooxygenase (COX) enzymes [3,4].

Cyclooxygenases (COXs) are key an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins and that has two isoforms of which one is involved in the creation of prostaglandins which mediate inflammation and pain [5,6]. The two major cyclooxygenase isoforms are COX-1 and 2 [7]. COX-1 is the isoform of cyclooxygenase that is expressed in most tissues of the body and is not involved in producing the pain and inflammation of arthritis [8, 9]. However, the COX-2 is cyclooxygenase accountable for synthesizing of pain and inflammatory mediating prostaglandins [10,11]. The administration of conventional NSAIDs non-selectively cyclooxygenase inhibitor is associated with gastrointestinal side effects [12–14]. Therefore, selective COX-2 inhibitors is needed to achieve enhanced safety profile on gastric mucosa. However, some anti-inflammatory drugs, including valdecoxib and rofecoxib, have also been linked to hypertensive effects and an elevated risk of myocardial infarction incidents [15]. In this regard, discovery of selective COX-2 blockers for management of pain and inflammation with diminished side actions emerged as an urgent medical need.

With the primary aim to explore novel, leading compounds for the treatment of inflammation, this research plan is to focus on natural products and phytochemicals. Many pharmacological classes of drugs include a natural product prototype [16, 17]. Aspirin, atropine, artimesinin, colchicine, ephedrine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, vincristine, and vinblastine are a few examples of important molecules that medicinal plants have given us in the past. Also, there are many historical examples in which the natural product has not just been the medicinal product, but has also helped in revealing novel aspects of pharmacology and physiology [18]. Some of the clinical used drugs for treating inflammation and pains like ibuprofen (Motrin), aspirin, and naproxen (Naprosyn) are celocoxibs which are good COX-2 inhibitors [19].

Orange being a rich source of nutrients has also been known for a number of health and nutritional benefits. Many phytonutrients and flavonoids found in oranges have been demonstrated to be active against a variety of cancer cells. These phytochemicals have also been reported to prevent bone loss and have potent anti-

inflammatory and antioxidant qualities [20, 21, 22, 23]. Amongst the constituents of the essential oils in orange juice (*Citrus sinensis*) are the monoterpenes and sesquiterpenes with d-limonene as a major constituent [24] which has been reported as responsible for its cholesterol lowering effect in animal [25] models as well as humans [26]. Its anti-inflammatory role in different disease is also well documented. Narirutin or Naringenin 7-Orutinoside is another important flavonoid present abundantly in orange juice [27, 28, 29]. It is also shown to possess anti-inflammatory, [30, 31] anti-allergic and anti-asthmatic activities [32, 33]. While there is vast research on the orange juice, information on the leaves is scanty while it has been used as a major ingredient in many medicinal concoctions in Nigeria.

The use of Orange bark in medicinal formulations for treatment of chronic inflammations has not been reported scientifically although it is consistently used by many local medicine practitioners. There is need to identify and study the mechanism of action of the phyto-compounds responsible for the anti-inflammatory activities of the plant bark.

In this research, molecular docking analysis is used to predict the novel leads compounds present in the plant bark. The present study uses a molecular docking analysis in an attempt to set a logical validation of the medicinal use of the plant bark. To assist in determining the potential mechanisms of action of the phytochemical compounds from the plant leaves, docking analysis of structurally diverse phytochemicals identified from the *C. sinensis* chloroform bark extract was done against the enzymes COX-2 which play a crucial role in chronic inflammation and its subsequent modulation.

This study will expose the phytochemicals in orange tree stem barks, identify possible anti-inflammatory bioactive compounds and also validate the medicinal use of the leaves.

Materials and Methods

Plant material collection

The stem bark *Citrus sinensis* were collected in November 2022 from Umuezeala Nsu in Ehime Mbano LGA in Imo state, Nigeria. The leaves were shade dried at

room temperature and powdered with mortar and pestle kept in an amber colour container.[34]

Preparation of the crude extract

The powdered plant material (40 g) was soaked in 500 ml of 80 percent chloroform (Carlo ERBA reagents SAS, France) for three days, and intermittently shaken at 130 rpm on an orbital shaker. The extract was filtered after 72 hours, and the residue was macerated twice in the same way. The filtrates were mixed together, concentrated using a rotary evaporator (Stuart, SO1, UK), and dried at 40°C in a Genlab oven [34].

Gas chromatography-mass spectrometry (GC–MS) analysis

The sample was dissolved in ethanol and injected in an Agilent (Agilent 19091-433HP, USA) GC-MS coupled to a mass spectrophotometer MS (Agilent technologies) by author injection at the Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria in Kaduna State, Nigeria. The following were the GCMS operating conditions for the analysis: Temperature in the oven: 50°C for 2 minutes, then 100°C at 10°C/min, then 200°C and held isothermally for 10minutes. The sample injection volume was 2µliters, and the carrier gas was helium at a rate of 1 mL per minute. The sample components were ionized at a voltage of 70 eV. The GC ran for a total of 24.50 minutes. The structures of the identified compounds were then compared to those in the NIST database using NIST14.Library (2018). The retention durations and mass spectra of the compounds were then compared to those of already known compounds in the NIST library (C:\Database\NIST14.L) [35].

Molecular docking

Ligand preparation:

The three dimensional (3D) structure of the identified compounds was downloaded from PubChem online server. Hydrogen Bonds were added and the energy minimization was done using the CHARMM force field in open Babel software.

Protein target preparation:

The 3D structure of cyclooxygenase-2 (COX-2) receptor was retrieved from Protein Databank (PDB ID:3LN1). The 3D structure has been prepared by removing water molecules, cofactor and substrate and determination of the active sites using the pymol software. Further preparation includes the addition of Kollman charges and polar hydrogen using autodock tools.

Docking studies:

Autodock Vina software was used to do docking analysis on the prepared ligand and protein. Based on several scoring functions, the software allows us to virtually screen a library of compounds and anticipate the strongest binders. The docking result was visualized using the accelrys discovery studio software [35].

Results and discussion

Extraction:

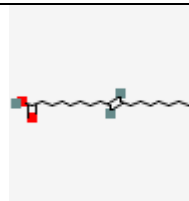
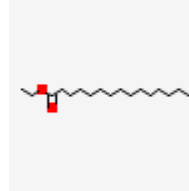
The dark brown chloroform extract of *Citrus sineensis* stem bark (0.61%) gave twenty six (26) compounds on GC-MS. The extraction was carried out in chloroform. Chloroform is emerging as one of the more popular non-polar solvents because of the ease of recovery. Moreover, solvents used during extraction process influence the nature and the number of secondary metabolites extracted from medicinal plants. Thus, the need to choose proper extraction solvent for the desired pharmacological activity of these extracts [36]. The extract was dark brown in colour, and 0.61% extract yield was collected after recovery. The lower percentage yield can be attributed to the type of solvent and also the extraction technique [37].

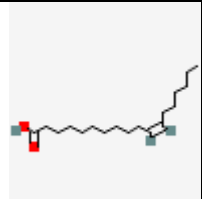
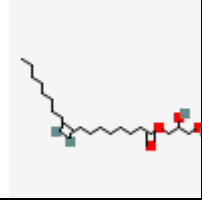
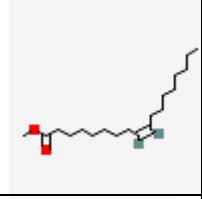
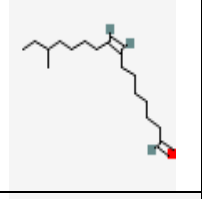
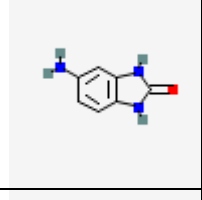
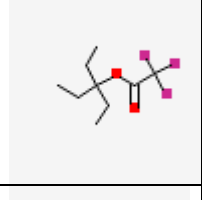
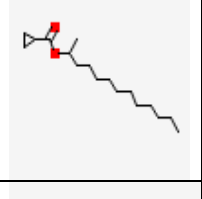
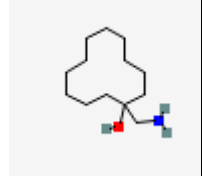
Gas chromatography-mass spectroscopy (GC-MS):

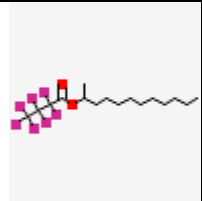
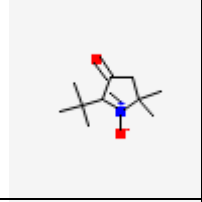
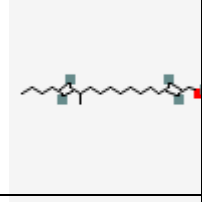
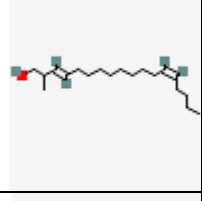
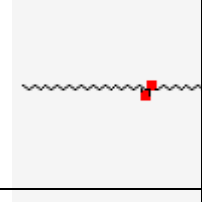
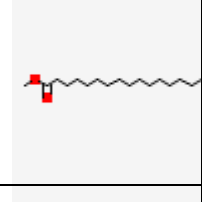
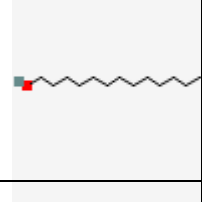
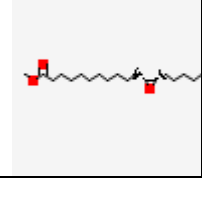
The Gas chromatography/mass spectrometer analysis identified ten (26) phytochemicals from the stem bark extract. The selected compounds were chosen based on their percentage peak area which is described as their concentration (Table 1). The bioactive compounds are mostly unsaturated fatty aldehydes, fatty acid, Phthalate ester, pheromones, perfluoroalkyl ethers, organic peroxide,

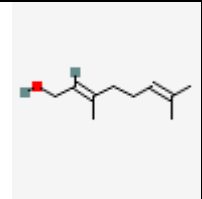
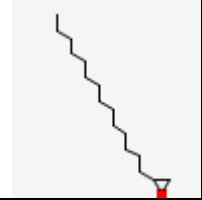
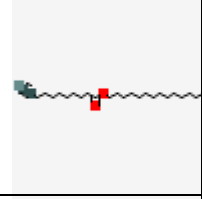
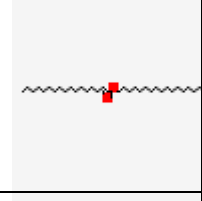
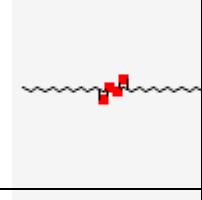
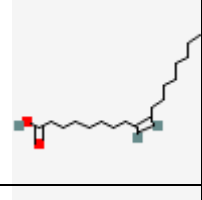
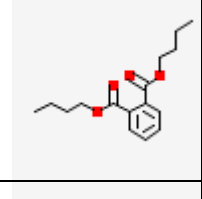
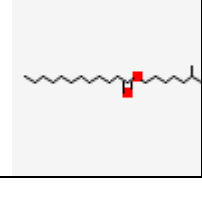
monounsaturated fatty acid, terpenoids, fatty alcohol, fatty acid ester, fatty acid derivative, epoxide, benzimidazoles, alkylpyrrolidine among other compounds. These compounds are responsible for the medicinal potentials of the plant bark for example; Oleic acid has been mentioned to be an arachidonic acid inhibitor, urinary-acidulant and urine acidifier [38]. Arachidonic acid inhibitors hinder the 5-lipoxygenase catalyzed conversion of arachidonic acid to 5-hydroxyeicosatetraenoic acid (5-HETE), a survival and proliferative factor for prostate cancer cells, thereby inducing apoptosis in these cells. Urine acidifiers, in combination with proper diet, help maintain low urine pH, thereby dissolving alkaline bladder stones, eliminating urinary tract infections and enhancing renal health [39]. Hexadecanoic acid, ethyl ester has been reported to possess Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, insectifuge, antihistaminic, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary potentials [40]. Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis- possess Antimicrobial potentials, while 2-Cyclopropylcarbonyloxytetradecane possess aphrodisiac, anti-inflammatory, and antihypertensive potentials. Squalene is an anti-cancer compound, and benefits cholesterol levels. Dibutyl phthalate showed remarkable antibacterial activity. Geraniol has demonstrated a wide spectrum of pharmacological activities including antimicrobial, anti-inflammatory, antioxidant, anti-cancer, and neuroprotective amongst others [41].

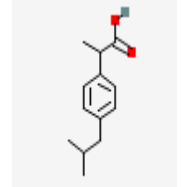
TABLE 1: Phytochemicals in the chloroform extract of *C. sineensis*

s/n	% area	R.t	Compound	Phytochemical class	Pubchem id	Mol. Weight (g/mol)	Mol. Formular	Structure
1	15.70	21.891	9-Octadecenoic acid	Monounsaturated fatty acid	13767140	373.6	C ₂₄ H ₃₉ NO ₂	
2	10.43	30.865	Hexadecanoic acid, ethyl ester	Saturated fatty acid	12366	284.5	C ₁₈ H ₃₆ O ₂	

3	6.919	25.029	cis-Vaccenic acid	Unsaturated fatty acid	46235519	296.5	C ₁₉ H ₃₆ O ₂	
4	1.80	22.155	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Unsaturated fatty acid ester	101627	372.5	C ₂₁ H ₄₀ O ₅	
5	1.48	23.741	9-Octadecenoic acid (Z)-, methyl ester	Unsaturated fatty acid methyl ester	8122	312.5	C ₂₀ H ₄₀ O ₂	
6	1.35	21.128	8-Hexadecenal, 14-methyl-, (Z)-	Unsaturated fatty aldehydes	6450379	326.6	C ₂₁ H ₄₂ O ₂	
7	0.87	20.526	2H-1,3-Benzimidazol-2-one, 5-amino-1,3-dihydro-	Benzimidazoles	66765	149.15	C ₇ H ₇ N ₃ O	
8	0.37	20.979	3-Ethylpentan-3-yl trifluoroacetate	Fatty ester	85569591	212.21	C ₉ H ₁₅ F ₃ O ₂	
9	0.30	19.458	2-Cyclopropylcarboxyloxytetradecan	Ester	549493	268.4	C ₁₇ H ₃₂ O ₂	
10	0.25	28.853	Cyclododecanol, 1-aminomethyl-	Cyclic fatty alcohol	533851	213.36	C ₁₃ H ₂₇ NO	

11	0.20	20.560	2-Heptafluorobutyroxydodecane	Perfluoroalkyl ethers	536306	382.36	C ₁₆ H ₂₅ F ₇ O ₂	
12	0.04	20.948	2-tert-Butyl-5,5-dimethyl-3-oxo-1-pyrroline, 1-oxide	Alkylpyrrolidines	102371645	204.23	C ₁₀ H ₂₂ N ₂ O	
13	0.01	19.395	12-Methyl-E,E-2,13-octadecadien-1-ol	Pheromones	90107969	280.5	C ₁₉ H ₃₆ O	
14	0.01	20.920	2-Methyl-E,E-3,13-octadecadien-1-ol	Fatty acid derivative	5364413	280.5	C ₁₉ H ₃₆ O	
15	0.59	30.521	Docosanoic acid nonyl ester	Fatty acid ester	537333	466.8	C ₃₁ H ₆₂ O ₂	
16	0.42	30.981	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	530322	284.5	C ₁₈ H ₃₆ O ₂	
17	0.29	32.024	n-Tridecan-1-ol	Fatty alcohol	8207	200.36	C ₁₃ H ₂₈ O	
18	0.29	33.932	Oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis-	Fatty acid ester	11001473	312.5g/mol	C ₁₉ H ₃₆ O ₃	

19	0.13	30.644	Geraniol	Monoterpenoids	637566	154.25	C ₁₀ H ₁₈ O	
20	0.09	33.385	Oxirane, tetradecyl-	Epoxide	244382	282.5	C ₁₈ H ₃₄ O ₂	
21	0.07	34.021	Undec-10-ynoic acid, tetradecyl ester	Fatty acid ester	91692467	378.6	C ₂₅ H ₄₆ O ₂	
22	0.06	30.689	Heptadecanoic acid, heptadecyl ester	Saturated fatty acid ester	10465	270.5	C ₁₇ H ₃₄ O ₂	
23	0.04	31.290	Lauroyl peroxide	Organic peroxide	7773	398.6	C ₂₄ H ₄₆ O ₄	
24	0.01	32.072	Oleic Acid	Fatty acid	445639	282.5	C ₁₈ H ₃₄ O ₂	
25	2.63	28.910	Dibutyl phthalate	Phthalate ester	3026	278.34	C ₁₆ H ₂₂ O ₄	
26	97.13	30.581	Dodecanoic acid, isoctyl ester	Fatty acid ester	537372	312.5	C ₂₀ H ₄₀ O ₂	

cnt			ibuprofen	Benzoic acid	3672	206.28	C ₁₃ H ₁₈ O ₂	
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s/n=serial number, RT=retention time cnt= controle

In-silico anti-inflammatory activity of the chloroform extract of *C. sineensis*

Inflammation is caused by a complex web of interrelated activities, including tissue injury and repair, cell migration, the release of fluids, enzyme activation, and the release of various chemical mediators. [42]. Macrophages play an important role in inflammation and are activated by various inflammatory mediators such as PGE₂, tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and interleukin-10 (IL-10) [5, 6]. Cyclooxygenase (COX) enzyme catalyzes the biosynthesis of PGs. There are at least two main isoforms, COX-1, and COX-2 [5]. Although both isoforms catalyze the same biochemical transformation, their expression is differentially regulated. COX-1 is a constitutive enzyme and augments the physiological role of prostaglandins (PGs) including maintaining the integrity of the GIT mucosa and adequate vascular homeostasis whereas, COX-2 is induced only after an inflammatory stimulus [7]. Inhibition of COX-2 enzyme which can be done by celecoxib is considered to be an important target in the discovery of anti-inflammatory and anti-nociceptive drugs. In order to study the mechanism of COX-2 inhibition of the identified compounds, molecular docking strategy was applied. The compounds were docked into the binding sites of COX-2.

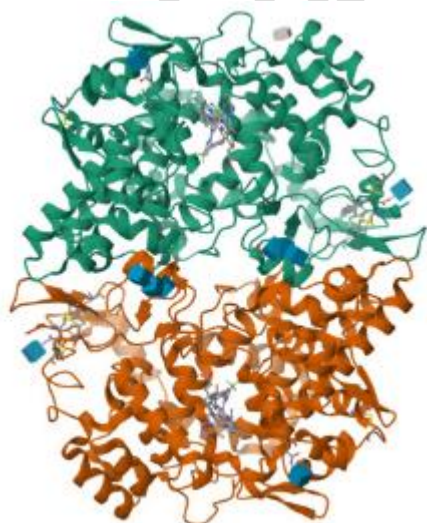


Figure 1: 3D Structure of celecoxib bound at the COX-2 active site

In order to study the mechanism of cyclooxygenase (COX) inhibition by the identified compounds, molecular docking strategy was applied. The compounds were docked into the binding sites of the cyclooxygenase-2 (COX-2). The binding energy of each compounds are given in Table 3. The binding energy with a higher negative value corresponds to a more stable interaction between the compound and target enzyme. To predict the binding modes of active compounds with COX-2 and identify the interacting amino acid residues, the 2D interactions of the top two active compounds with COX-2 were created. Out of the 26 compounds, 9-Octadecenoic acid (-8.3kcal/mol), followed by Cyclododecanol, 1-aminomethyl- (-7.2kcal/mol) exhibited the best binding affinity to COX-2 in terms of a low binding energy. However, their binding energy was also better than that of the control drug, Ibuprofen (-6.8kcal/mol). The interactions of the strongest binders with the binding site amino acid residues were also revealed in figure 2a. It is predicted that, 9-Octadecenoic acid bonded strongly by having a carbon-hydrogen bond interaction with amino acid residue GLY512. It also had alkyl and π -alkyl interactions with residues PHE504, MET508, TYR371, TRP373, LEU517, ALA513, VAL335 and VAL509. Further interactions were hydrophobic van der Waal's interactions (fig. 2a). Additionally, the interactions of 1-aminomethyl Cyclododecanol with the COX-2 protein were strictly one alkyl interactions and fourteen van der Waal's interactions at the active site (fig. 2b). For Ibuprofen the NSAID control, it interacted with the binding site amino acids by alkyl, and π -alkyl interactions with residues LYS454, and LEU138 respectively, it also had twelve van der Waal's interactions with the binding site amino acid residues (fig. 2c).

Table: 2. Binding affinity from molecular docking of the phytochemicals with the target protein

S/N	Compound	Pub chem id	Binding energy
0	Ibuprofen	3672	-6.8
1	9-Octadecenoic acid	13767140	-8.3
2	Cyclododecanol, 1-aminomethyl-	533851	-7.2

3	Dibutyl phthalate	3026	-6.4
4	Geraniol	637566	-6.3
5	2-Heptafluorobutyroxydodecane	536306	-6.1
6	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	11001473	-5.9
7	2-Cyclopropylcarbonyloxytetradecane	549493	-5.7
8	2H-1,3-Benzimidazol-2-one, 5-amino-1,3-dihydro-	66765	-5.7
9	Dodecanoic acid, isooctyl ester	537372	-5.7
10	2-tert-Butyl-5,5-dimethyl-3-oxo-1-pyrroline, 1-oxide	102371645	-5.6
11	Oleic acid	445639	-5.6
12	9-Octadecenoic acid (Z)-, methyl ester	8122	-5.6
13	Hexadecanoic acid, ethyl ester	12366	-5.5
14	8-Hexadecenal, 14-methyl-, (Z)-	6450379	-5.5
15	n-Tridecan-1-ol	8207	-5.3
16	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	101627	-5.2
17	2-Methyl-E,E-3,13-octadecadien-1-ol	5364413	-5.1
18	3-Ethylpentan-3-yl trifluoroacetate	85569591	-5.1
19	Heptadecanoic acid, heptadecyl ester	10465	-5.0
20	Docosanoic acid nonyl ester	537333	-4.9
21	12-Methyl-E,E-2,13-octadecadien-1-ol	90107969	-4.8
22	Cis-vaccenic acid	46235519	-4.6
23	Oxirane, tetradecyl-	244382	-4.5
24	Hexadecanoic acid ethyl ester	530322	-4.4
25	Lauroyl peroxide	7773	-4.3
26	Undec-10-ynoic acid, tetradecyl ester	91692467	-4.0

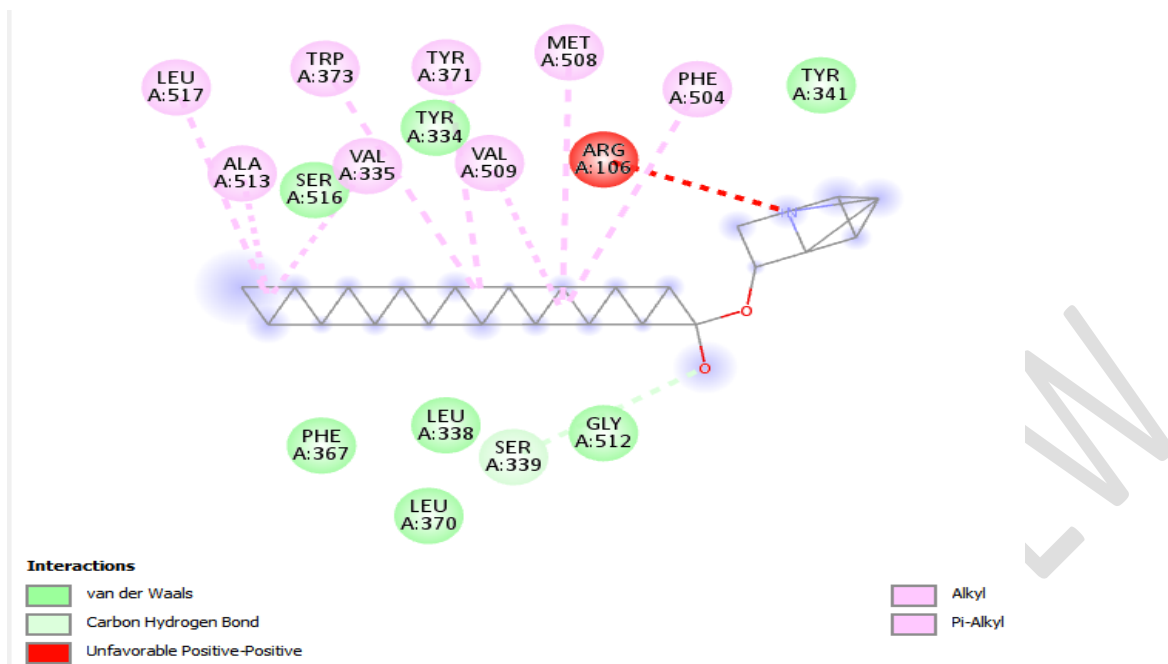


Figure 2A. Interaction of 9-Octadecenoic acid with the COX-2 protein

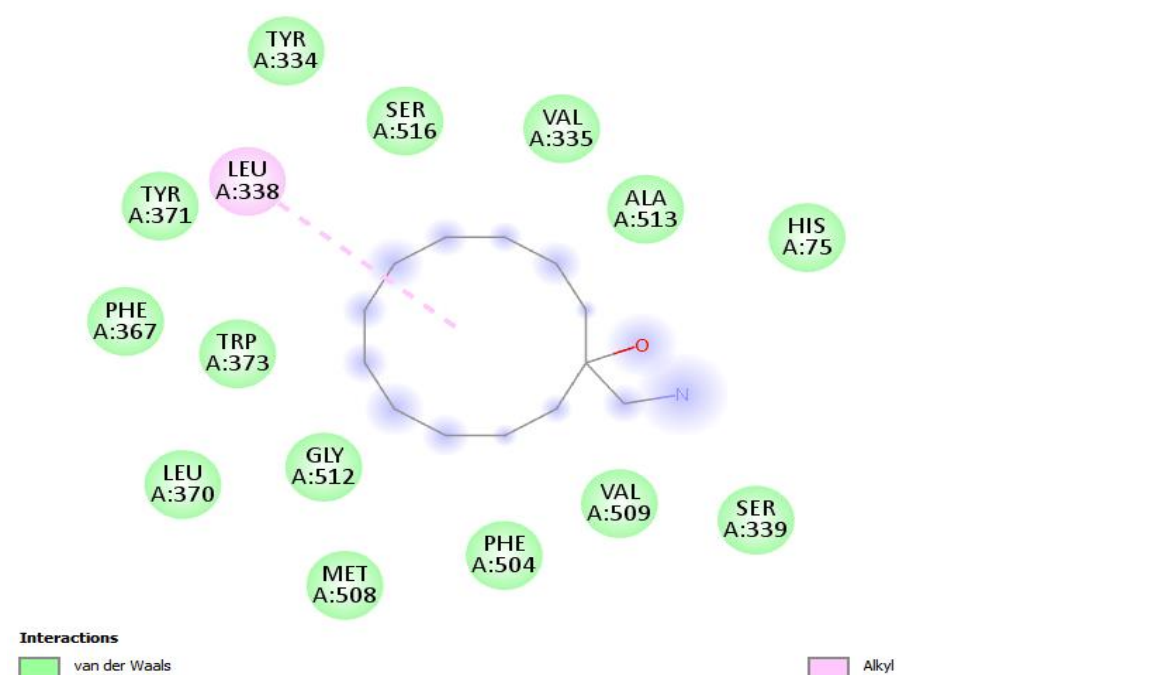


Figure 2B. Interaction of 1-aminomethyl Cyclododecanol with the COX-2 protein

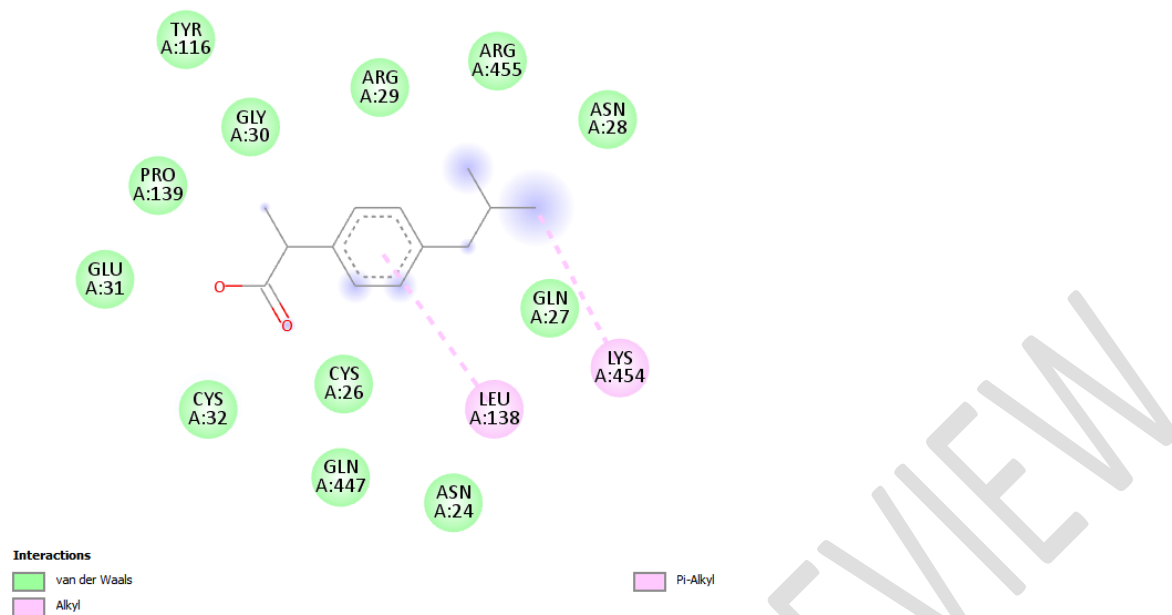


Figure 2C. Interaction of Ibuprofen with the COX-2protein

CONCLUSION

The stem bark of *C. sineenses* contains fatty acid and fatty alcohol that showed better anti-inflammatory activities than the clinical prescribed ibuprofen. *in-silico* prediction displayed that the two of the identified phytochemicals, 9-Octadecenoic acid and Cyclododecanol, 1-aminomethyl- had better binding properties with the COX-2 enzyme when compared to Ibuprofen (control drug). It may be concluded that there are certain structural features of the compounds that support its role in anti-inflammatory activities.

REFERENCES

1. Jeanette M B, Glenn Reeves, George E B and Joachim P S (2018) "Inflammation–Nature's Way to Efficiently Respond to All Types of Challenges: Implications for Understanding and Managing "the Epidemic" of Chronic Diseases" front med vol. 5-2018
2. Michels da Silva D, Langer H, Graf T. Inflammatory and Molecular Pathways in Heart Failure-Ischemia, HFpEF and Transthyretin Cardiac Amyloidosis. Int J Mol Sci. 2019 May 10;20(9)

3. Vane JR, Botting RM. Mechanism of action of nonsteroidal anti-inflammatory drugs. *Am J Med.* 1998;104(3):2S-8S. doi: 10.1016/S0002-9343(97)00203-9.
4. Brune K, Patrignani P. New insights into the use of currently available non-steroidal anti-inflammatory drugs. *J Pain Res.* 2015;8:105. doi: 10.2147/JPR.
5. Hla T, Neilson K. Human cyclooxygenase-2 cDNA. *Proc Nat Acad Sci.* 1992;89(16):7384-7388. doi: 10.1073/pnas.89.16.7384
6. Abdellatif K, Abdelall E, Bakr R. Nitric oxide-NSAIDs donor prodrugs as hybrid safe anti-inflammatory agents. *Curr Top Med Chem.* 2017;17(8):941-955. doi: 10.2174/1568026616666160927153435.
7. Abdelgawad MA, Bakr RB, Omar HA. Design, synthesis and biological evaluation of some novel benzothiazole/benzoxazole and/or benzimidazole derivatives incorporating a pyrazole scaffold as antiproliferative agents. *Bioorg Chem.* 2017;74:82-90. doi: 10.1016/j.bioorg.2017.07.007.
8. Bakr RB, Azouz AA, Abdellatif KR. Synthesis, cyclooxygenase inhibition, anti-inflammatory evaluation and ulcerogenic liability of new 1-phenylpyrazolo [3, 4-d] pyrimidine derivatives. *J Enzyme Inhib Med Chem.* 2016;31:6-12. doi: 10.1080/14756366.2016.1186018
9. Abdellatif KR, Abdelgawad MA, Elshemy HA, Alsayed SS, Kamel G. Synthesis and anti-inflammatory evaluation of new 1, 3, 5-triaryl-4, 5-dihydro-1h-pyrazole derivatives possessing an aminosulphonyl pharmacophore. *Arch Pharm Res.* 2015;38(11):1932-1942. doi: 10.1007/s12272-015-0606-7
10. Wang D, DuBois RN. The role of cox-2 in intestinal inflammation and colorectal cancer. *Oncogene.* 2010;29(6):781. doi: 10.1038/onc.2009.421
11. Griswold DE, Adams JL. Constitutive cyclooxygenase (cox-1) and inducible cyclooxygenase (cox-2): rationale for selective inhibition and progress to date. *Med Res Rev.* 1996;16(2):181-206. doi: 10.1002/(SICI)1098-1128(199603)16:2<181::AID-MED3>3.0.CO;2-X
12. Lazzaroni M, Bianchi Porro G. Gastrointestinal side-effects of traditional non-steroidal anti-inflammatory drugs and new formulations. *Aliment Pharmacol Ther.* 2004;20:48-58. doi: 10.1111/j.1365-2036.2004.02037.x
13. Vane J, Bakhle Y, Botting R. Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol.* 1998;38(1):97-120. doi: 10.1146/annurev.pharmtox.38.1.97.

14. Abdelrahman MH, Youssif BG, Abdelazeem AH, et al. Synthesis, biological evaluation, docking study and ulcerogenicity profiling of some novel quinoline-2-carboxamides as dual coxs/lox inhibitors endowed with anti-inflammatory activity. *Eur J Med Chem.* 2017;**127**:972–985. doi: 10.1016/j.ejmech.2016.11.006
15. Abdelgawad MA, Bakr RB, El-Gendy AO, Kamel GM, Azouz AA, Bukhari SNA. Discovery of a cox-2 selective inhibitor hit with anti-inflammatory activity and gastric ulcer protective effect. *Future Med Chem.* 2017;**9**(16):1899–1912. doi: 10.4155/fmc-2017-0115
16. Balunas MJ, Kinghorn A. Drug discovery from medicinal plants. *Life Sci.* 2005;**78**:431–441.
17. Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol Aspects Med.* 2006;**27**:1–93.
18. [7] Khanna D, Sethi G, Kwang Seok A, Pandey M, Kunnumakkara AB, Sung B, Aggarwal A, Aggarwal B. Natural products as a gold mine for arthritis treatment. *Curr Opin Pharmacol.* 2007;**7**: 344–351.
19. Richard Payne (2000) Limitations of NSAIDs for pain management: Toxicity or lack of efficacy? *The Journal of Pain* 1; (3): 14-18
20. Gao K, Henning SM, Niu Y, Youssefian AA, Seeram NP, Xu A, et al. The citrus flavonoid naringenin stimulates DNA repair in prostate cancer cells. *J Nutr Biochem.* 2006;**17**:89–95.
21. Liu Y, Heying E, Tanumihardjo SA. History, global distribution, and nutritional importance of citrus fruits. *Compr Rev Food Sci Food Saf.* 2012;**11**:530–45.
22. Peluso MR. Flavonoids attenuate cardiovascular disease, inhibit phosphodiesterase, and modulate lipid homeostasis in adipose tissue and liver. *Exp Biol Med (Maywood)* 2006;**231**:1287–99.
23. Chiba H, Uehara M, Wu J, Wang X, Masuyama R, Suzuki K, et al. Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. *J Nutr.* 2003;**133**:1892–7.
24. Graciela OR, Fredy Y, Betzabe SF, Lilibeth C. Volatile fraction composition of Venezuelan sweet orange essential oil (*Citrus sinensis* (L.) Osbeck) *Ciencia.* 2003;**11**:55–60.
25. Kurowska EM, Borradaile NM, Spence JD, Carroll KK. Hypocholesterolemic effects of dietary citrus juices in rabbits. *Nutr Res.* 2000;**20**:121–9.

26. Roza JM, Xian-Liu Z, Guthrie N. Effect of citrus flavonoids and tocotrienols on serum cholesterol levels in hypercholesterolemic subjects. *Altern Ther Health Med.* 2007;13:44–8.
27. Buscemi S, Rosafio G, Arcolego G, Mattina A, Canino B, Montana M, et al. Effects of red orange juice intake on endothelial function and inflammatory markers in adult subjects with increased cardiovascular risk. *Am J Clin Nutr.* 2012;95:1089–95.
28. Ghanim H, Sia CL, Upadhyay M, Korzeniewski K, Viswanathan P, Abuaysheh S, et al. Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and Toll-like receptor expression. *Am J Clin Nutr.* 2010;91:940–9.
29. Sawalha S, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez A. Quantification of main phenolic compounds in sweet and bitter orange peel using CE–MS/MS. *Food Chem.* 2009;116:567–74.
30. Ha SK, Park HY, Eom H, Kim Y, Choi I. Narirutin fraction from citrus peels attenuates LPS-stimulated inflammatory response through inhibition of NF- κ B and MAPKs activation. *Food Chem Toxicol.* 2012;50:3498–504.
31. Funaguchi N, Ohno Y, La BL, Asai T, Yuhgetsu H, Sawada M, et al. Narirutin inhibits airway inflammation in an allergic mouse model. *Clin Exp Pharmacol Physiol.* 2007;34:766–70.
32. Rogerio AP, Sá-Nunes A, Faccioli LH. The activity of medicinal plants and secondary metabolites on eosinophilic inflammation. *Pharmacol Res.* 2010;62:298–307.
33. Council NR. *Guide for the Care and Use of Laboratory Animals.* 8th ed. Washington, DC: The National Academic Press; 2011.
34. Ikpa CCB and Maduka TOD. "Antimicrobial Properties of Methanol Extract of *Dacryodes edulis* Seed and Determination of Phytochemical Composition Using FTIR and GCMS." *Chem Afr.* 2020; 3(4):927-935.
35. Ikpa, C. B., Ikezu, U. J., & Maduwuba, M. C. (2022). In Silico Docking Analysis of Anti-malaria and Anti-typhoid Potentials of Phytochemical Constituents of Ethanol Extract of *Dryopteris dilatata*. *Tropical Journal of Natural Product Research*, 6(5).
36. Ikpa, C. B., Maduka, T. D., & Ikpa, C. U. (2021). Evaluation of phytochemical composition and in vitro antioxidant potential of *Cyathea latifolia* leaves. *World Scientific News*, 157, 25-37.

37. Maduka T.O., Ikpa, C. B., and Kalu, G.I. (2020). Physicochemical Characterization and Assessment of Bioactive Chemical Compounds of *Persea Americana* (Avocado) Seed. *Nat Ayurvedic Med* 2020, 4(1): 000229.
38. Arne Torbjorn Hostmark and Anna Haug Percentages of oleic acid and arachidonic acid are inversely related in phospholipids of human sera *Lipids Health Dis.* 2013; 12: 106.
39. Jagadananda Ghosh and Charles E. Myers (1998) "Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells" *Proc Natl Acad Sci* ; 95(22): 13182–13187.
40. Karthika Krishnamoorthy and Paulsamy Subramaniam 2014 Phytochemical Profiling of Leaf, Stem, and Tuber Parts of *Solena amplexicaulis* (Lam.) Gandhi Using GC-MS *Int Sch Res Notices.* ; 2014: 567409.
41. Rebai Ben Ammar (2023) "Potential Effects of Geraniol on Cancer and Inflammation-Related Diseases: A Review of the Recent Research Findings" *Molecules.* May; 28(9): 3669
42. Yang J, Li S, Xie C, Ye H, Tang H, Chen L, et al.. Anti-inflammatory activity of ethyl acetate fraction of the seeds of *Brucea Javanica*. *J Ethnopharmacol.* (2013) 147:442–6. 10.1016/j.jep.2013.03.034