

Phytochemical Evaluation and In-silico Studies of Bioactive Compound from the Seed of *Afrostyraxlepidophyllus* to Identify Potential Anti-inflammatory Agents

ABSTRACT

Afrostyraxlepidophyllus commonly known as country onions from the *Huaceae* family has been acclaimed to be used in treatment of several health conditions such as inflammation, prevention of infection and cancer, improve digestion, use traditionally as remedy for pain relief etcethnomedically. This study was carried out to determine the bioactive composition of *A.lepidophyllus* seed and to evaluate the anti-inflammatory activities of seed extract using GC-MS) and in silico molecular docking. The GC-MS result revealed the presence of 18 bio-active compounds of which eugenol (47.14%), 3-ally-6-methoxyphenol (24.23%), Caryophyllene (10.35%) and oleic acid (7.91%) were the most abundant. The result revealed that the efficacy of the plant against inflammatory diseases could be linked to eugenol, 3-ally-6-methoxyphenol and Caryophyllene found in the crude extract. The bioactive compounds from the extract were docked on the inflammatory protein crystal structure of diclofenac bound to the cyclooxygenase active site of COX-2 (1pxx) to know their binding affinities and compared with that of some known anti-inflammatory drugs. The docking result showed that the standard drugs Diclofenac has the best binding affinity of -8.5 kcal/mol, followed by Naproxen (-8.1kcal/mol), Caryophyllene(-7.5kcal/mol), Bicyclo [7.2.0]undec-4-ene (-7.4 kcal/mol) have better affinity than the standard drug Ibuprofen (-7.3 kcal/mol). All the compounds from the exact have high binding affinities within the range of -7.5 – 6.1kcal/mol except 2,4,5,7-tetrathiaoctane that is -3.5 kcal/mol. The interaction results of the extracts with the diseased protein proved the anti-inflammatory efficacy of the seed as acclaimed therefore validating the traditional use of the seed against inflammatory diseases.

Keyword:spices, bioactive compounds, anti-inflammatory, docking, interaction.

Introduction

Spices and herbs have been an essential part in human life for centuries both for culinary and medicinal purposes, they have been used at a domestic and industrial level as flavoring, preservation, and coloring agent in nutraceutical, pharmaceutical, and cosmetics products ^[1]. Spices not only enhance the flavor, aroma, and color of food and beverages, but they can also give protection from acute and chronic diseases. More Americans are considering the use of spices and herbs for medicinal and therapeutic/remedy use, especially for various chronic conditions. There is now ample evidence that spices and herbs possess antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, and glucose and cholesterol lowering activities as well as properties that affect cognition and mood ^[2]. Research over the past decade has reported on the diverse range of health properties possessed by herbs and spices via their bioactive constituents, including sulfur-containing compounds, tannins, alkaloids, phenolic

diterpenoids, and vitamins, especially flavonoids and polyphenols. Spices and herbs such as clove, rosemary, sage, oregano, and cinnamon are excellent sources of antioxidants with their high content of phenolic compounds^[3]. It is evident that frequent consumption of spicy foods was also linked to a lower risk of death from cancer and ischemic heart and respiratory system diseases^[4]. Among these spices is the Country Onions, scientifically known as *Afrostryaxlepidophyllus* from *Huaceae* family, a culinary treasure from the vibrant rainforests of Central and West Africa which is perfect for adding depth and richness to a variety of dishes. This onion stands out with its unique garlic-like flavor profile, infusing a distinctive pungency into traditional dishes. It is oval in shape with a brown bark, the seed is widely used as a flavoring ingredient in many traditional dishes because of its strong and pungent aroma. Country Onion is a bulbous plant that belongs to the *Allium* family, which also includes garlic, onions, and chives. It is native to Africa where it is an important traditional food source for local communities, Country onions is a flavoural and nutritive ingredient, widely used in different parts of Africa, including Nigeria, Cameroon and, Ghana for centuries as a staple ingredient in their cuisine. People love this charming spice for their unique flavors and aroma, which resemble garlic more than traditional onions. But what truly sparks curiosity is their hidden health benefits^[5]. They have a long history as cherished ingredients in African cuisines and, beyond their culinary charm, offer notable health benefits.

Country onions has been reported as a rich source of antioxidants containing compounds like allicin and alliin, which have been shown to have antibacterial, antiviral, and antifungal properties. Studies have also suggested that Country Onion can help lower blood pressure and cholesterol, reduce inflammation, boost immune system, possessed anticancer properties and improve cardiovascular health. It is available in Cameroon, Gabon, Nigeria and Ghana. Country onions is currently listed by the International Union for Conservation of Nature as “vulnerable”, giving it high conservation value^[6]. *Afrostryaxlepidophyllus* also known as Olum or Bombimbi, This work is aimed at identifying the bioactive component of this treasured spice. Insilico methods was used to study the anti-inflammatory potentials of the plant phytochemicals and other commercial drug to ascertain its efficacy as acclaimed ethnomedically and by previous researchers.

Materials and Methods

Matured seeds of *Country Onion* were bought from a local market in Anambra State. The dried seeds were subjected to preliminary grinding using mechanical hand grinder, and the particle size were reduced further using an electric blender. The pulverized seed were stored in an airtight plastic container for further analysis.

Extraction of Phytochemicals

The powdered *Country Onion* 200g was measured and percolated in a stoppered container containing 500ml of redistilled ethanol (98%) and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter had dissolved. The mixture was then clarified by filtration and later concentrated with rotary evaporator at 55⁰C to get the crude sample for GCMS analysis^[7].

GC-MS ANALYSIS

The GC-MS analysis was done at Zaria, kaduna state Nigeria. The compounds in the sample were identified using agilent GC-MS (Agilent 19091-433HP, USA) coupled to a mass

spectrophotometer. The initial column temperature was 35°C with a hold time of 3 minutes. The temperature was programmed to rise by 8°C/min with a final temperature of 280°C. In the process, 1µl of the sample was injected into the port and immediately vaporized and moved down the column with helium as the carrier gas with flow rate of 1 ml/min. The MS Spectrum was taken at 70 eV. The identification of the compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds in NIST14 structural library^[8].

The compounds identified from GCMS and their structures are shown in table 1

IDENTIFICATION AND PREPARATION OF LIGANDS

The 3D structure-data files (SDF) of the compounds in the crude extract sample and some anti-inflammatory drugs were identified and downloaded from the PubChem database. They were minimized in PyRx virtual screening tool, using Universal Force Field at 200 steps and converted to AutoDock ligands (pdbqt) and then used for the docking analysis. Identification and preparation of molecular targets crystal structures of diclofenac bound to the cyclooxygenase active site of cox-2 with protein ID:1pxx was identified and downloaded from the Protein Data Bank (PDB). The interfering crystallographic water molecules and cocrystallized ligand were removed, and minimization of the energy of the protein was then done using Biovia Discovery studies⁽⁹⁾.

DOCKING PROCEDURE AND ANALYSIS OF RESULTS

The screening of the phytochemical compounds from the seed extract was performed by docking them on selected binding pockets of proteins of crystal structures of diclofenac bound to the cyclooxygenase active site of cox-2 and ranked based on their binding energies. The multiple docking of the ligands and proteins was done with Autodock Vina in PyRx software. A rigid-flexible docking was performed after setting a grid box surrounding the binding sites of the receptors at exhaustiveness = 8, center x = 16.72, center y = 50.18, center z = 67.80, size x = 22.42, size y = 21.10, size z = 22.41. The molecular docking results were organized on an Excel spreadsheet, and the Heat Map of the data and the interaction was viewed using the Biovia discovery studio.

THE PROTEIN

The protein used for the docking is a high-resolution X-ray crystal structures of diclofenac bound to the cyclooxygenase active site of cox-2 (1pxx) officially known as prostaglandin-endoperoxide synthase (PTGS), an inflammatory protein. Inflammation is a helpful recovery process that cells utilize to stop the progression of harm or injury to tissues caused by foreign invaders and start the healing process. It's a complicated process involving white blood cells, macrophages, and inflammatory cytokines such as prostaglandins, TNF- (Tumor Necrosis Factor), interleukin IL6, and IL-8, to mention a few. The mobilization of arachidonic acid for prostaglandin production is a hallmark of inflammation. Cyclooxygenases are recognised to be the primary mediators of prostaglandin production, which are inflammatory indicators and are hence the focus of anti-inflammatory therapy. Cyclooxygenases such as COX-1 and COX-2 enzymes convert arachidonic acid to prostaglandins. COX-1 is required for the body's homeostatic activities, such as platelet synthesis for blood, kidney development and function, gastric mucosa maintenance, and so on. Increased inflammation, angiogenesis, metastatic and proliferative invasion, decreased apoptosis, and the establishment of an immunosuppressive

microenvironment are all linked to COX-2 derived prostaglandin PGE2. A variety of drugs inhibit the conversion of arachidonic acid to prostaglandin G2 by the cyclooxygenase (COX) activity of prostaglandin endoperoxide synthases.



Plate 1. Unprepared protein

Plate 2. Prepared protein

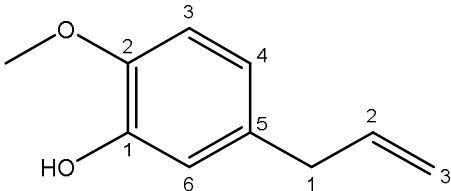
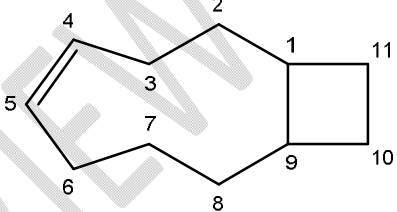
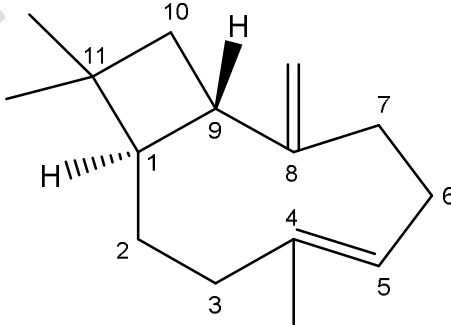
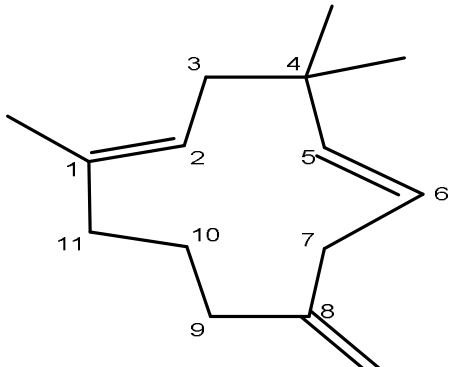
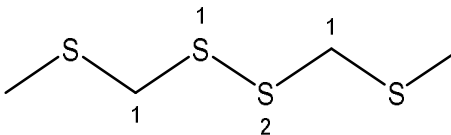
RESULTS AND DISCUSSION

Chemical composition of the Seed Extract

The GC-MS analysis of Country Onionseed extract gave 18 peaks for the bioactive compounds, their percentage composition, retention time, structures and formulas were recorded in the table below (Table 1)

Table 1: Result of GCMS Analysis of phytochemicals from Country Onion ethanol seed extract.

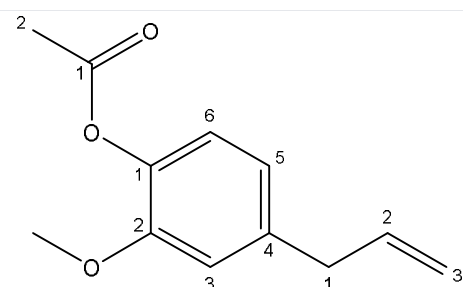
S/N	RT	Phytochemicals	Percentage	Pubchem CID	Structure/Molecular formula
1	7.076	D-Limonene	0.28	440917	 <chem>C1=CC(C=C1)C(C)C</chem>
2	16.69	Eugenol	47.14	3314	$C_{10}H_{16}$ <chem>COc1ccc(O)cc1C=C</chem>

3	17.01	3-allyl-6-methoxyphenol	24.23	596375	$C_{10}H_{12}O_2$ 
4	17.86	Bicyclo[7.2.0]undec-4-ene	0.47	129848326	$C_{10}H_{12}O_2$ 
5	18.15	Caryophyllene	10.35	5281515	$C_{11}H_{18}$ 
6	19.12	Humulene	0.58	5281520	<ul style="list-style-type: none"> $C_{15}H_{24}$ 
7	20.83	2,4,5,7-tetrathiooctane	0.42	158825	$C_{15}H_{24}$ 

8 20.96 Phenol,2-methoxy-4-(2-propenyl)-,acetate 1.44

7136

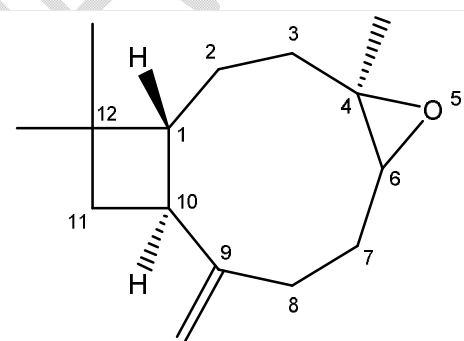
$C_4H_{10}S_4$



$C_{12}H_{14}O_3$

9 22.44 Caryophyllene oxide 0.21

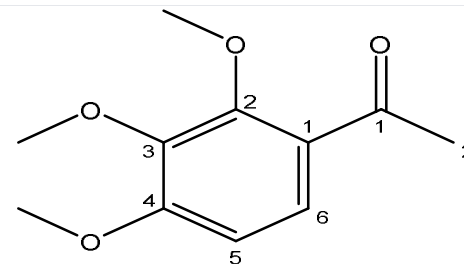
1742210



$C_{15}H_{24}O$

10 25.15 2,3,4-trimethoxyacetophenone 0.60

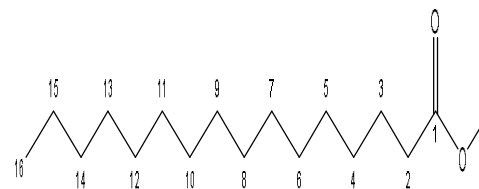
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$C_{11}H_{14}O_4$

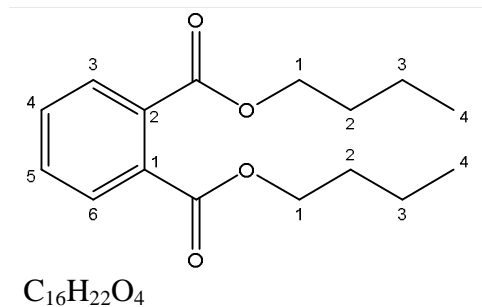
11 29.51 Hexadecanoic acid, methyl ester 0.42

8181

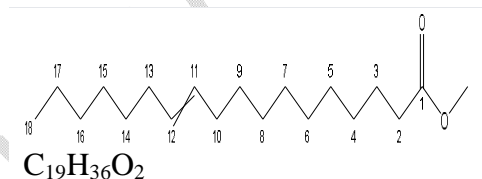


$C_{17}H_{34}O_2$

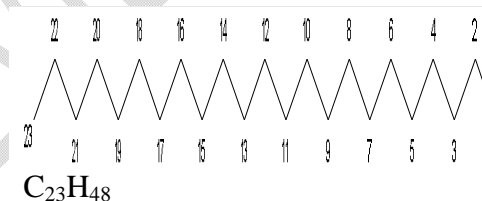
13 29.99 Dibutyl phthalate 0.24 3026



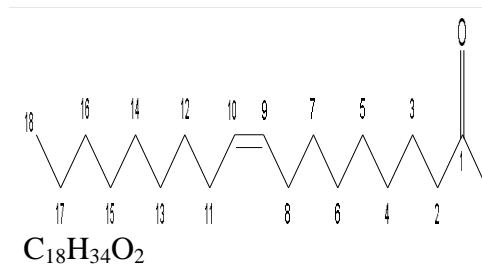
14 31.13 11-octadecenoic acid, methyl ester 0.15 5364432



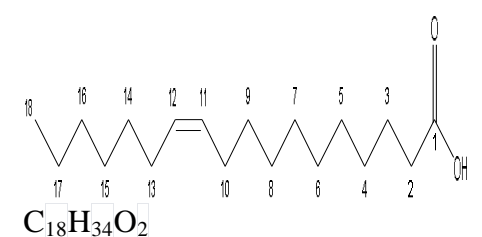
15 33.89 Tricosane 2.34 12534



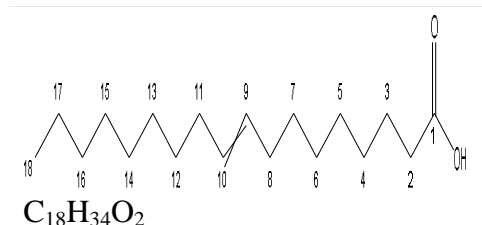
16 34.71 Oleic Acid 7.91 445639



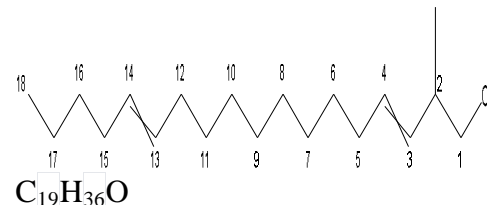
17 34.79 Cis- Vaccenic acid 2.03 5282761



18 36.24 9-Octadecenoic acid 0.81 637517



19	36.52	2-methyl-Z,Z-3,13-octadecadienol	0.20	5364412
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The above table showed the GC-MS result of the phytochemicals from ethanol seed extract of *A. lepidophyllus* and some commercial drugs.

The phytochemical components were presented in the order from the highest to the least percentage as follows: Eugenol (47.14) > 3-allyl-6-methoxyphenol (24.23) > caryophyllene (10.35) > Oleic Acid (7.91), > tricosane (2.34), > Cis- Vaccenic acid 2.03 > Phenol, 2-methoxy-4-(2-propenyl)-, acetate (1.44), > Oleic acid (1.21), the other compounds present have percentage occurrence of less than 1.00. Eugenol, 3-allyl-6-methoxyphenol and caryophyllene have over 81% of the percentage composition of the plant.

Eugenol is the highest occurring compound in clove seed. Eugenol (EUG) is a versatile naturally occurring molecule as phenolic monoterpenoid and frequently found in essential oils in a wide range of plant species. It has huge industrial applications particularly in cosmetics, dentistry, flavoring of foods, agriculture, and pharmaceuticals. Recently Eugenol has been a target due to its great potential in preventing several chronic conditions. The World Health Organization (WHO) has declared EUG as nonmutant and generally recognized as safe (GRAS) molecule. The available literature about pharmacological activities of EUG shows remarkable anti-inflammatory, antioxidant, antifungal, antibacterial, antipyretic activity, neurodegenerative disorders, analgesic, anti-diabetic, anticancer, cardiovascular Protection and Anti-parasite activity having significant effect on human health^[10].

3-allyl-6-methoxyphenol is another major bioactive component present in the seed extract of country onions with percentage composition 24.23. It has wide range of bioactive activities such as anti-inflammatory, antiageing, antioxidant, antimicrobial and anti-cancer properties^[11]

Caryophyllene is a natural bicyclic sesquiterpenoid abundantly found in essential oils from various spices, fruits and medicine as well as ornamental plants. It is approved by United States Food and Drug Administration and European agencies as food additive, taste enhancer and flavoring agent and as such termed phyto-cannabinoid. Various pharmacological activities such as cardio-protective, hepato-protective, gastro-protective, neuro-protective, nephron-protective, antioxidant, anti-inflammatory, antimicrobial and immune-modulator have been reported in

experimental studies which also showed potent therapeutic promise in neuropathic pain, neurodegenerative and metabolic diseases^[12].

Humulene, also known as α -caryophyllene, is a ring-opened isomer of β -caryophyllene found in cannabis, ginseng and sage, Humulene possesses both topical and systemic anti-inflammatory properties, is an effective analgesic when taken topically, orally, or by aerosol, Interestingly, humulene was shown to increase secretion of IL-8, a chemokine with various functions, including promoting angiogenesis, helpful in wound healing^[13]

Vaccenic acid is a naturally occurring trans fatty acid and an omega-7 fatty acid. It is the predominant kind of trans-fatty acid found in human milk, in the fat of ruminants, and in dairy products such as milk, butter, and yogurt. cis-Vaccenic acid is used as a therapeutic agent against cardiovascular diseases, it has anticarcinogenic properties^[14], and also helps in reduction of total cholesterol, LDL cholesterol and triglyceride level^[15]

Table 2: Result of Molecular Docking of the Country onion compounds, some commercial drugs with inflammatory protein (1pxx)

S/N	Phytochemicals	Percentage	Pubchem CID	Binding affinity(kcal/mol)
1	D-Limonene	0.28	440917	-6.5
2	Eugenol	47.14	3314	-6.5
3	3-allyl-6-methoxyphenol	24.23	596375	-6.1
4	Bicyclo[7.2.0]undec-4-ene	0.47	129848326	-7.4
5	Caryophyllene	10.35	5281515	-7.5
6	Humulene	0.58	5281520	-7.1
7	2,4,5,7-tetrathiaoctane	0.42	158825	-3.5
8	Phenol,2-methoxy-4-(2-propenyl)-,acetate	1.44	7136	-6.5
9	Caryophyllene oxide	0.21	1742210	-7.2
10	2,3,4-trimethoxyacetophenone	0.60	83810	-6.2
11	Hexadecanoic acid, methyl ester	0.42	8181	-6.5
12	Oleic acid	7.91	445639	-6.7

13	Dibutyl phthalate	0.24	3026	-7.2
14	11-octadecenoic acid, methyl ester	0.15	5364432	-6.9
15	Cis- Vaccenic acid	2.03	5282761	-6.8
16	9-Octadecenoic acid	0.81	637517	-7.0
17	2-methyl-Z,Z-3,13-octadecadienol	0.20	5364412	-6.9
18	Ibuprofen		3672	-7.3
19	Naproxen		156391	-8.1
20	Indomethacin		3715	-7.7
21	Diclofenac		3033	-8.5

The 18 compounds from the seed extracts and some commercial drugs were docked on the protein to know their binding affinities and compared with that of some known anti-inflammatory drugs to determine the one with better binding affinity against the binding site of the disease protein.

The docking result showed that the drug Diclofenac has the best binding affinity of -8.5 kcal/mol followed by another drug Naproxen and indomethacin -8.1 and 7.7 kcal/mol respectively. The seed bioactive compound Caryophyllene, Bicyclo [7.2.0] undec-4-ene, Caryophyllene oxide, Dibutyl phthalate, and Humulene have binding affinities of -7.5, -7.4, -7.2 and -7.1 kcal/mol respectively higher and very close to that of a commercial drug Ibuprofen (-7.3 kcal/mol). Other compounds from the seed with good binding affinities between -6.1 to -6.9 kcal/mol, they include 9-octadecenoic acid (-7.0 kcal/mol), 11-octadecenoic acid, methyl ester (-6.9 kcal/mol), Phenol, 2-methoxy-4-(2-propenyl)-, acetate (-6.1 kcal/mol) except 2,3,5,7-tetrathiooctane (-3.5 kcal/mol). The interaction of the compounds with better binding affinities and the commercial drugs were carried out on the enzyme to confirm whether the docking was on the active site and pocket. The results were discussed below

INTERACTIONS

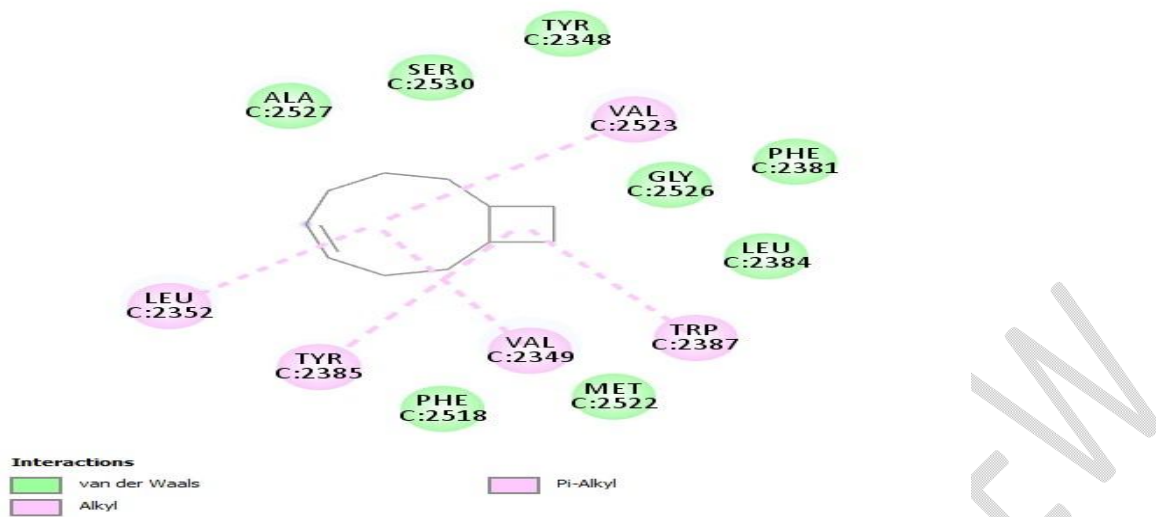


Fig 1 :Interaction of the protein with Bicyclo[7.2.0]undec-4-ene.

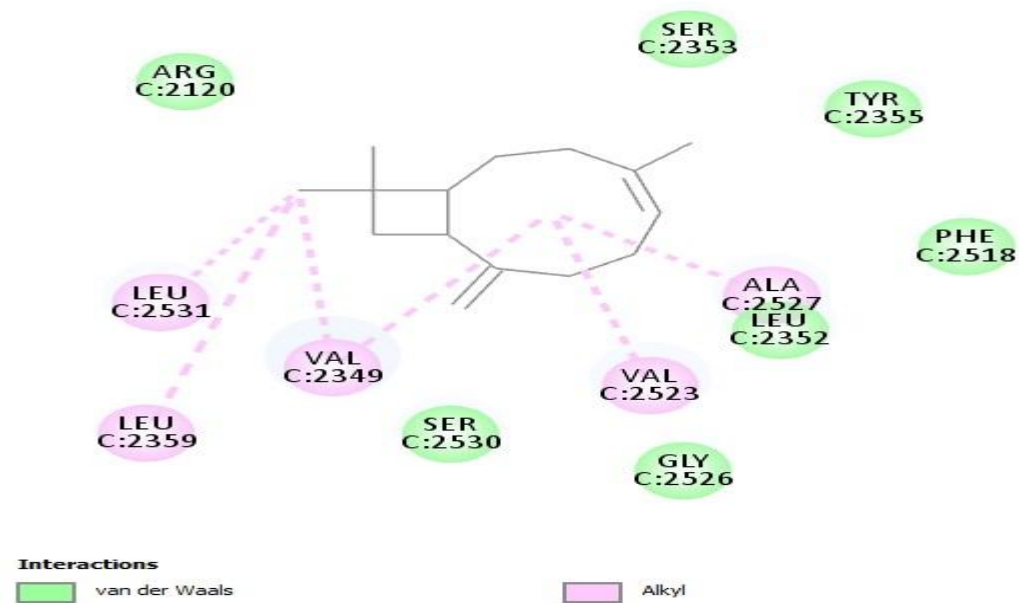


Fig 2 :Interaction of the protein with caryophyllene

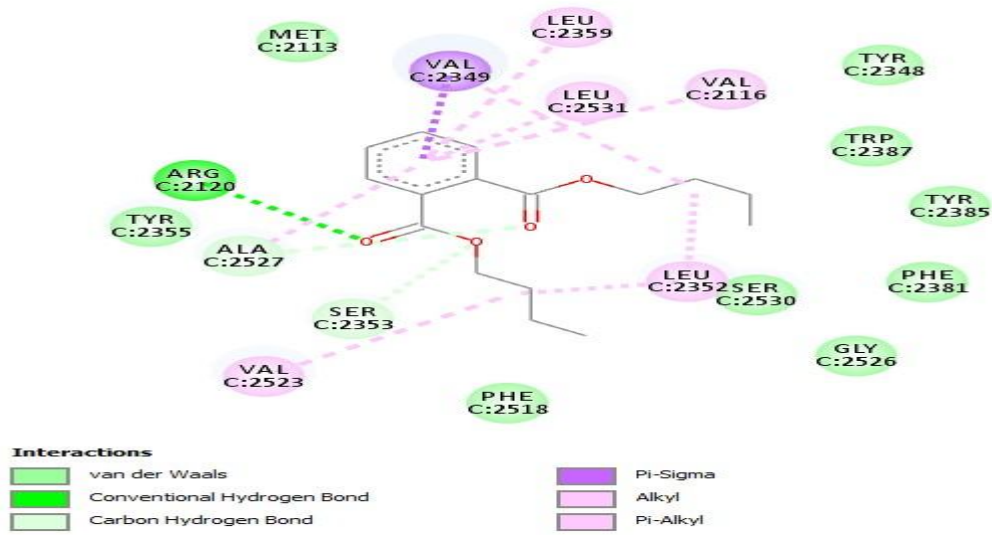


Fig 3 :Interaction of the protein with Dibutyl phthalate

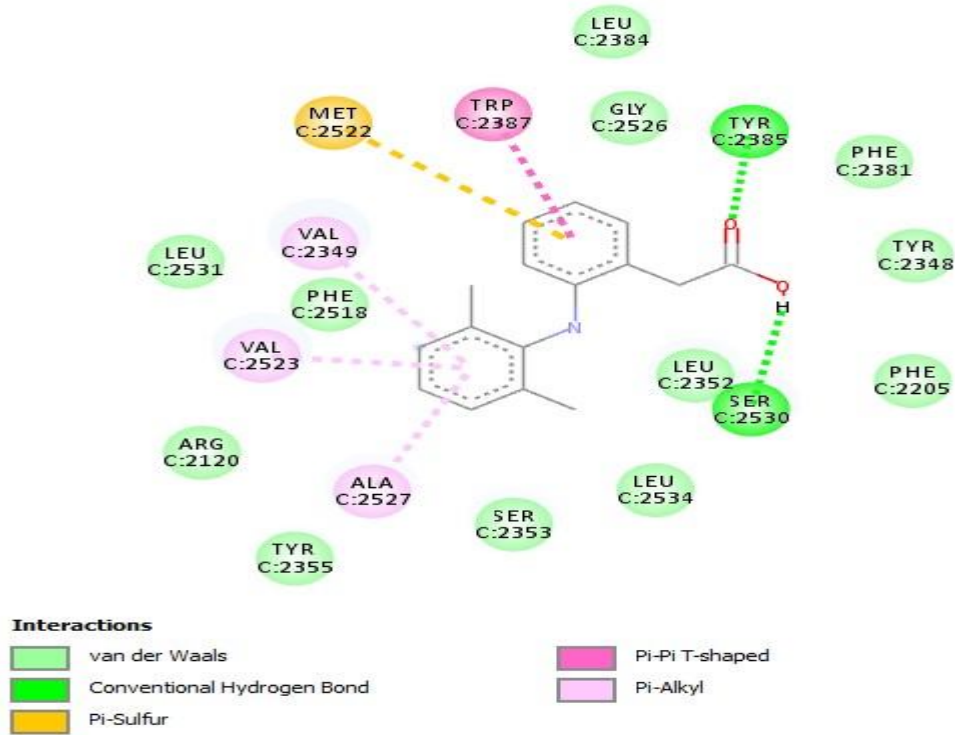


Fig 4 :Interaction of the protein with Diclofenac

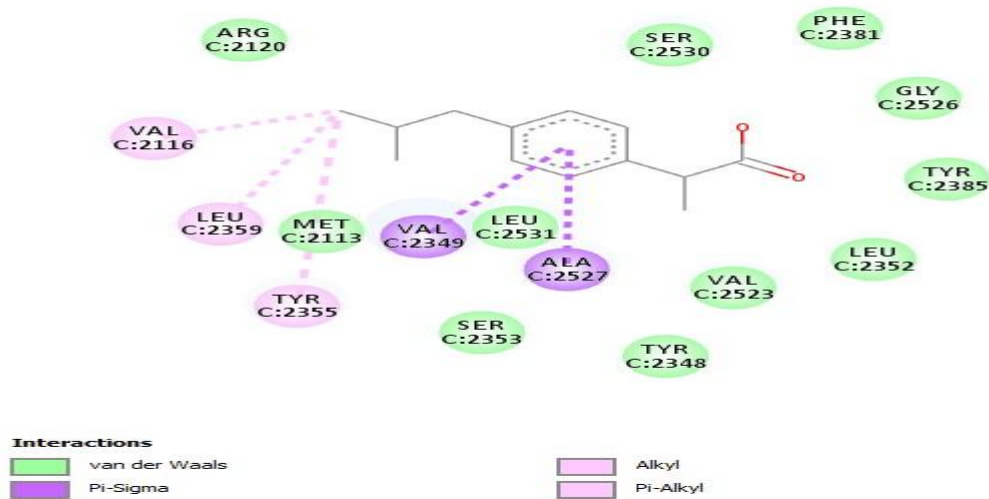


Fig 5: Interaction of the protein with Ibuprofen

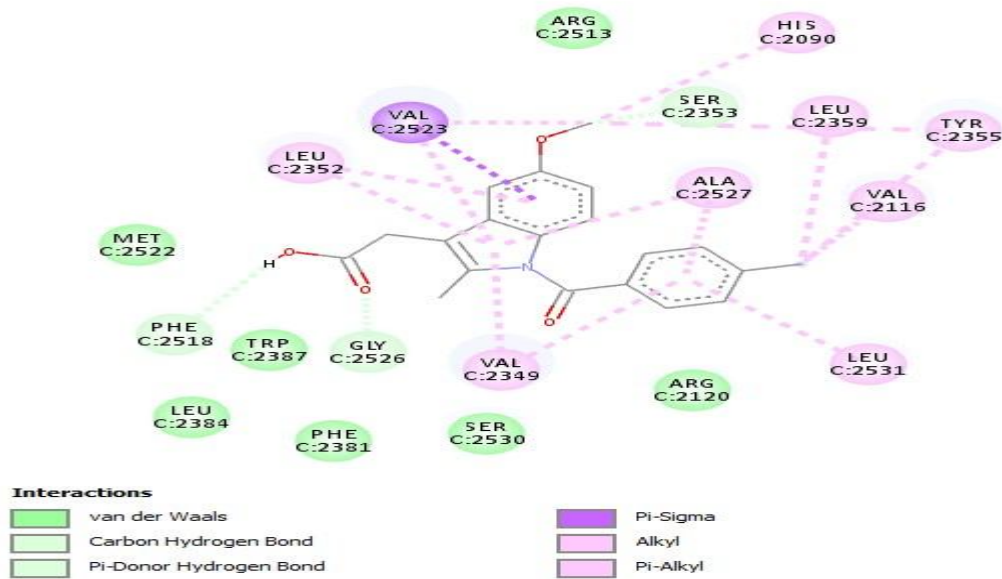


Fig 6: Interaction of the protein with indomethacin

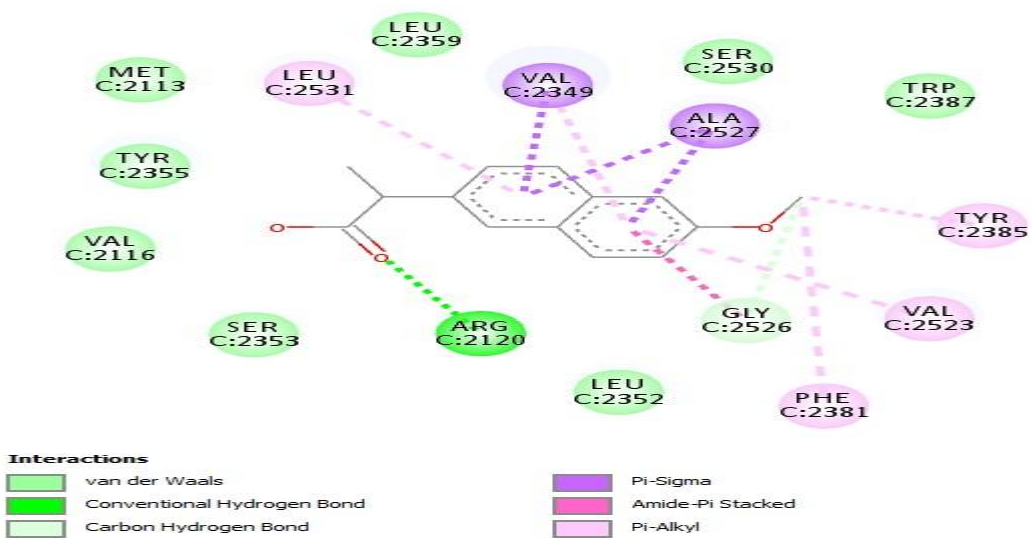


Fig 7: Interaction of the protein with Naproxen

Table 3: Docking Results Showing the Type of Interaction and Amino Acid involved

Compound	Binding affinity	Type of interaction	Amino acids
Bicyclo [7.2.0]undec-4-ene	-7.4	Vander waals	ALA 2527, SER 2530, TYR 2348, GLY2526GLY2526, PHE 2381, LEU 2384,MET 2522, PHE 2518
		Alkyl and pi-Alkyl	LEU 2354, TYR 2385, VAL 2349, TRP 2387 VAL 2523,

Caryophyllene	-7.0	Vander waal Alkyl	ARG 2120, SER 2353, TYR 2355, PHE 2518, LEU2352, GLU 2526, SER 2530 LEU 2531, LEU 2389, VAL 2349, VAL2523, ALA2527
Dibutyl phthate	-7.2	Vander waal Conventional hydrogen bond Carbon hydrogen bond Pi sigma bond Alkyl and Pi- alkyl	TYR 2355, MET 2113, PHE2518,SER2530 GLY 2521,PHE 2385,TYR 2385, TRP 2387TYR 2348 ARG 2120 ALA 2527, SER 2353 VAL 2349 VAL 2523, LEU 2352 LEU 2331, VAL 2116 LEU 2359
Diclofenac	-8.5	Vander waal	LEU 2531, PHE 2518 ARG 2120, TRY 2355 SER 2353,LEU 2534 LEU 2352, PHE 2205 TYR 2348, PHE 2381 GLY 2526, LEU 2384

		<p>Conventional hydrogen bond</p> <p>Pi –Sulfur</p> <p>Pi-Pi T shaped</p> <p>Pi alkyl</p>	<p>TYR 2385, SER 2530</p> <p>MET 2522</p> <p>TRP 2387</p> <p>ALA 2527, VAL 2523</p> <p>VAL 2349</p>
Naproxen	-8.1	<p>Vander waal</p> <p>Conventional hydrogen bond</p> <p>Carbon hydrogen bond</p> <p>Pi-sigma bond</p> <p>Pi Alkyl</p>	<p>MET 2113, TYR 2355, VAL 2116, SER 2353, TRP 2387, SER 2530</p> <p>LEU 2359</p> <p>ARG 2120</p> <p>GLY 2526</p> <p>VAL 2349, ALA 2527</p> <p>PHE 2381, VAL 2523</p> <p>TYR 2385, LEU 2531</p>

Indomethacin	-7.7	Vander waal	MET 2522, TRP 2387 LEU 2384, PHE 2381 SER 2530, ARG 2120, ARG2513, PHE 2518, GLY 2526, SER 2353
		Pi- sigma bond	VAL 2523
		Alkyl	LEU 2531, VAL 2116, TYR 2355, LEU 2359 ALA 2527., HIS 2090
Ibuprofen	-7.3	Vander waal	LEU 2352, VAL 2349 ARG 2120, SER 2530, PHE 2381, GLY 2526, TYR 2385 LEU 2352, VAL 2523 TYR 2348 , SER 2353, LEU 2531, MET2113
		Pi – sigma	VAL 2349, ALA 2527
		Alkyl and Pi alkyl	VAL 2116, LEU 2359. TYR 2355

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The interaction results above showed that some of the identified compounds have significant potentials to block the active site of the enzyme. Out of the 18 identified compounds, 3 compounds with binding affinities close to the reference drugs and the reference drugs were used to study their interaction with the disease protein, the drug Diclofenac has the minimum value of the binding affinity -8.5 and it interacted with the COX enzyme through two conventional hydrogen bond TYR 2385 and SER 2530, Pi sulfur bond with MET 2522, Pi-Pi T Shaped bond with TRP 2382, it also has pi alkyl bond with ALA 2527 and a hydrophilic Vander waals bond with LEU 2531, PHE 2518, ARG 2120, TYR 2355, SER2353, LEU 2534, LEU 2352, PHE 2205, TYR2348, PHE 2381, GLY2526 and LEU 2384. Naproxen another commercial drug with affinity -8.1kcal/mol displayed the strongest interaction with the diseased protein among all docked ligand in this study, Naproxen constructed a carbon hydrogen bond with ARG 2120 and a conventional hydrogen bond with GLY 2526. Pi sigma bond with VAL 2349, ALA 2527, Pi Alkyl with LEU 2531, PHE 2351, VAL 2523 and TUR 2385 and a hydrophobic vanderwaal bond with MET 2113, TYR2355, VAL2116, LEU 2359, SER 2359, SER 2353, SER2530, TRP 2387, LEU 2352. Indomethacin another control drugs showed a strong carbon hydrogen bond and pi donor hydrogen bond with PHE 2518, GLY 2526 and SER 2353 amino acids, It also has pi sigma bond with VAL 2523, pi alkyl and alkyl bond with HIS 2090, LEU 2352, LEU 2359, TYR 2355, VAL 2116, LEU 2531 and VAL 2349, and vanderwaal interaction with MET 2522, TRP 2387, LEU 2384, PHE 2381, SER 2530, ARG 2120, ARG 2513. The high binding affinity may be due to its strong binding with other residue at the active site. Dibutyl phytate although not the closest ligand in binding affinity to the contro drug showed a similar type of bond with Naproxen and best interaction among the phytocompounds docked having a conventional hydrogen bond with ARG 2120, a carbon hydrogen bond with ALA 2527 and SER 2353, it has Pi sigma bond with Val 2349 and Pi Alkyl and Alkyl bonds with LEU 2359, LEU 2531, VAL 2116, LEU 2352 and Val 2523. It also has hydrophobic Vander waal bond with amino acids MET 2113, TRR 2355, PHE 2518, SER 2530. GLY 2526, PHE 2382, TYR 2385, TRP 2387 and TYR 2348. Caryophyllene and Bicyclo [7.2.0]undec-4-ene with binding affinities -7.5 and -7.4 kcal/mol have most of their interactions with hydrophobic vanderwaals and alkyl bonds, however did not construct strong bonds with the major active residue instead have a weak interaction. These compounds serve as leads in treating inflammatory diseases.

CONCLUSION

The result obtained from the GC-MS showed that *Afrostryraxlepidophyllus* seed contain 18 bio-active compounds with known medicinal, biological and therapeutic properties with their percentage composition as follows: Eugenol (47.14%), 3-Ally-6-methoxyphenol (24.23%), Carophyllene (10.35%) and Oleic acid (7.91%) were the most abundant compounds in the extract. Eugenol and Carophyllene both amounting to 57.49% of the total percentage of compound found in the extract have been reported to show anti-inflammatory activity thereby positively validating the claims made by the Cameroonians that *Afrostryraxlepidophyllus* can cure inflammatory diseases^[18]. Inflammation has been an underlying factor in most health condition like diabetes, arthritis, heart disease and cancer. The dried seed of *A. lepidophyllus* is reported to have been used medically to reduce inflammation. This can be attributed to the presence of caryophyllene, eugenol humulene, Dibutyl phytate etc. *A. lepidophyllus* seed contains

antimicrobial, antioxidant and larvicidal compounds such as M. eugenol, tricosane and oleic acid (anticancer). Also, compounds like Cis-vaccenic acid and D-limonene present in the extract were reported to reduce cholesterol and prevent heart diseases^[5]. Humulene was reported to have analgesic property (pain killer) ^[19], It also possesses carminative properties that help to reduce flatulence and stomach gas ^[20]. The use of *A. lepidophyllus* seed to treat high blood sugar level in people with diabetes can be attributed to the presence of cis-vaccenic acid. The research revealed that the efficacy of *A. lepidophyllus* seed medicinally is due to its phytochemicals.

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