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Identification of the bioactive compounds in *Medicago sativa* (alfa-alfa) and the *in-silico* studies of the pharmacological properties of the most prominent compounds

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Abstract

The identification of bioactive compounds in the methanolic leaf extract of *Medicago sativa* (Alfalfa) and the *in-silico* studies of the pharmacological properties of the most prominent compounds were carried out using standard procedures. The quantitative phytochemical result revealed the presence of alkaloids (17.66%), flavonoids (10.23%), cardiac glycosides (12.60%), tannins (11.27%), saponins (3.34%) and steroids (5.01%). Thirteen compounds of the leaf extract were identified using GC-MS technique. The four most prominent of the identified compounds were hexanal (24.71%), 1-dodecene (24.71%), 1,2,4-trihydroxy-9,10-anthracenedione (19.53%) and tolbutamide (14.00%). Carbamic acid, methylphenyl- ethyl ester, 2-methoxy-4-vinylphenol, caryophyllene, xanthosine, 1-tetradecene, megastigma-3,7(E),9-triene, trichloroacetic acid, 1-cyclopentylethyl ester, octadecane and heptadeca-5,8-dione were present in smaller percentages. The result from the in-silico studies of the prominent compound showed that 1,2,4-trihydroxy-9,10-anthracenedione had a binding energy of -6.9 Kcal/mol for antidiabetic, -6.4 Kcal/mol for antiulcer, -8.3 Kcal/mol for antihypertensive, -8.1 Kcal/mol for antihermorrhagic, -5.7 Kcal/mol for antimalarial. Comparing the result with three standard controls, it was found that 1,2,4-trihydroxy-9,10-anthracenedione had excellent binding energies than the standard drugs of most of the docked pharmacological potentials which include *diabetes mellitus* ulcer, hemorrhagic and hypertension. However, the pharmacokinetics (ADMET) properties of the drug proved that the 1,2,4-trihydroxy-9,10-anthracenedione can be easily absorbed, distributed and properly metabolized in the liver and kidney and excreted. The toxicity studies showed that 1,2,4-trihydroxy-9,10-anthracenedione does not cause eye corrosion, genetic mutation, and does not also affect respiratory and reproductive organs. Though despite the few toxicities of 1,2,4-trihydroxy-9,10-anthracenedione, the drug likeness test according to Lipinski rule of five proved that 1,2,4-trihydroxy-9,10-anthracenedione can be used as a drug, hence, it can be regarded as a potential drug candidate for the treatment of *diabetes mellitus*, ulcer, hermorhagic and hypertension. However, animal studies should be carried out on the evaluation of the active pharmacological activities of the drug and more clinical trials should be assessed.

Keywords: *Medicago sativa*; Bioactive compounds; Molecular docking; Synthetic drugs; Binding energy.

INTRODUCTION

The definition of natural products, within the field of phytochemistry, is usually restricted to organic compounds isolated from plant natural sources that are produced by the pathways of primary or secondary metabolism. Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites. Secondary metabolites are not essential for survival, but nevertheless provide plants that produce them a survival advantage (Jones and Kossel, 2018).

Plant chemistry is the basis of the therapeutic uses of herbs. A good knowledge of the chemical composition of plants leads to a better understanding of its possible medicinal value. Modern chemistry has described the role of primary plant metabolites in basic life functions such as cell division and growth, respiration, storage and reproduction (Bourgaud *et al*., 2019). They include the components of processes such as glycolysis, the Krebs or citric acid cycle, photosynthesis and associated pathways. Primary metabolites include small molecules such as sugars, amino acids, tricarboxylic acids, or Krebs cycle intermediates, proteins, nucleic acids and polysaccharides. Eventually, the primary metabolites are similar in all living cells (Bennets *et al*., 2019).

Secondary metabolites have shown to possess various biological effects, which provide the scientific base for the use of herbs in the traditional medicine in many ancient communities (Igwe, 2014). They have been described as antibiotic, antifungal and antiviral and therefore are able to protect plants from pathogens. Besides, they constitute important UV absorbing compounds, thus preventing serious leaf damage from the light. It was noticed that some herbs as forage grasses such as *Medicago sativa* can express estrogenic properties and interact with fertility of animals (Aganga and Tshwenyane, 2020).

The *in silico* characterization of bioactive compounds from natural sources that may play a role in the regulation of metabolic processes have adequately been reported (Otuokere *et al*., 2019, Otuokere *et al.*, 2020; Igwe *et al*., 2024). *In-silico* characterization of molecules provides a cost effective pathway in the search for more potent compounds in the development of new drugs. Properties such as molecular size, solubility, polarity, flexibility, and lipophilicity provide crucial insights into the suitability of a certain molecule to be used as a drug (Igwe *et al*., 2024).

M. sativa is used traditionally for the treatment of several ailments such as arthritis, kidney problems, fever, cancer, rheumatism, diabetes, and in the treatment of boils (Bora and Sharma, 2019). However, most of the compounds that are responsible for the pharmacological actions of this traditional remedy are not known. This has hindered the standardization and development of this herb and made its recognition, acceptance and utilization remain locally restricted. Therefore, it is necessary to probe the bioactive constituents of *M. sativa* leaves and to carry out *in-silico* studies on the various pharmacological properties of the prominent compounds.

MATERIALS AND METHODS

Sample Collection

Leaves of *M. sativa* were harvested from the Akwete, Ezigaragu, Enyiogugu in AbohMbaise L.G.A, Imo State; on the 12th of June, 2021. The plant material was authenticated at the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Sample Preparation

The collected leaves of *M. sativa* were air-dried for thirty days and were ground into small particles using electric blender. The weight of the ground sample was 714 g.

Sample Extraction

Five hundred (500) grams of the ground *M. sativa* leaf was weighed and put into an amber coloured bottle. Two litres of methanol was poured into the sample and allowed to stay for 48h. Thereafter, it was filtered using Whatman filter paper No. 1 and other filtration apparatus. The methanol extract was concentrated using rotary evaporator under a reduced temperature to get the crude sample. The digital Heidolph rotary evaporator (4000 series) was set at 60^{o}C under reduced pressure. After the recovering of the methanol in another container, the extract was allowed to stand so that the little methanol in the crude fraction could evaporate completely to get the actual weight of the sample. The extract collected weighed 50.37 g.

Quantitative Determination of Phytochemicals

The quantitative determination of phytochemicals such as alkaloids, flavonoids, saponins, tannins, steroids and cardiac glycosides in the leaf extract of *M. sativa* was carried out following standard protocols. Alkaloids and saponins were determined by the method described by Harborne (1993). The method described by Nwoke *et al.* (2024) was used in the determination of flavonoids while the Follins Dennis spectrophotometric method described by Kirk and Sayer (1998) was used in the determination of tannins. The method described by Okeke and Elekwa (2003) was used in the determination of steroids while the alkaline picrate colorimetric method described by Balagopalan *et al*., (1988) was used in the determination of cardiac glycosides. The concentrations were expressed in percentage (%) using the equation below;

$$Concentration\ (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

GC-MS Analysis

The extract was injected into the column of the spectrometer at 250°C injector temperature. Temperature of oven started at 70°C and held for 5 min. It was then raised at the rate of 10°C per min to 250°C without holding. Holding was allowed for 6 min at programmed at rate of 5^{o}C per min. Temperature of ion sources was maintained at 200°C and detector temperature was set at 250°C. The mass spectrum of compounds present in samples was obtained by electron ionization at 70eV and detector operates in scan mode 50 to 600 Da atomic units. The MS Table was generated through ACQ mode scan within 0.5 seconds of scan interval at the speed of 666 and fragments from 30 to 350 Da atomic unit were maintained. Total running was 30 minutes.

Protein Targets Selection and Preparation

The three-dimensional (3D) crystallographic structures of the protein targets of the following pharmacological activities; Antidiabetic (PDB code: 3IOL), Antimalarial (PDB code: 1SME), Antiulcer (PDB code: 1AXM), Antihypertensive (PDB code: 1O86) and Antihermorrhagic (PDB code: 5UE4) were retrieved from the Protein Database (PDB) (www.pdb.org/pdb). Using the software Chimera©, version 1.13., (http://www. cgl.ucsf.edu/chimera), the protein was prepared for docking via the removal of the co-crystallized ligand and water molecules to produce a nascent receptor and the various receptor sites were identified.

Ligand and Standard Synthetic Drug Preparations

The structural data files (SDF) of the various bioactive molecules and the standard synthetic drugs were obtained from PubChem web-platform (https://www.ncbi.nlm.nih.gov/pccompound) in 3D conformation and were consequentially converted into mole files deploying MarvinSketch© software (ver. 15.11.30). Furthermore, the molecules were optimized with Merck molecular force field (MMFF94) in Avogadro (ver. 1.10).

Molecular Docking

The docking was done by using flexible docking protocol. Briefly; Python Prescription 0.8, a suite comprising of automated molecular docking tools (Auto Dock Vina), was utilized for the molecular docking analysis of the selected Ligands with the enzymes. The PDBQT file of the protein was generated through this software (using their previously created PDB files as inputs). The specific target sites of the target enzyme were set with the help of grid box (X: -5.7290, Y: -13.0662, Z: 11.1351 for 3IOL, X: 36.7330, Y: 10.1807, Z: 39.7278 for 1SME, X: 0.250, Y: 25.9422, Z: 109.1028 for 1AXM, X: 40.5527, Y: 37.2083, Z: 5787 for 1O86, X: 34.8683, Y: 38.9396, Z: 28.9704 for 5UE4). The configurations for each Protein-Ligand complex were generated for all the ligands using the software; text files of scoring results (binding ability between a ligand molecule and the target protein) were also produced for the purpose of manual comparative analysis at the end of the experiment.

Prediction of ADMET Properties

The ADMET properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of the compounds were tested by using AdmetSAR server. AdmetSAR is a freely accessible tool to the public that enables the database to be queried by SMILES and structural similarity search.

Drug Likeness Screening

Drug likeliness properties of ligands were analysed by using Molinspiration server which is mainly based on Lipinski' s rule of five. Every potential oral drug candidate should have less than 5 H-bond donor, not more than 10 H-bond acceptor, LogP value not greater than 5 etc. Molinspiration server calculates log P, polar surface area, number of hydrogen bond donors and acceptors, as well as the prediction of bioactivity score.

RESULTS AND DISCUSSION

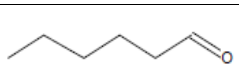
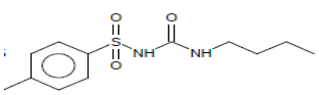
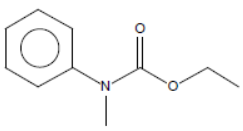
Table 1: Quantitative phytochemical screening of the methanol leaf extract of *M. sativa*

Phytochemicals	Concentration (%)
Alkaloid	17.66±0.01
Flavonoid	10.23±0.03
Tanins	11.27±0.14
Saponins	3.34±0.04
Steroid	5.01±0.01
Cardiac glycosides	12.60±0.02

Values are expressed in mean± standard deviation

Table 1 shows the result for the quantitative phytochemical screening of methanolic extract of *M. sativ*a (Alfa-alfa). The analysis also reveals that the alkaloid content was in highest amount (17.66%) followed by flavonoid (10.23%), cardiac glycosides (12.6 %) and tannins (11.27%). The amount of saponins and steriods were 3.34% and 5.01% respectively. The high content of alkaloid in the leaf shows that the leaf can be greatly employed in the pharmaceutical industries for drug extraction and synthesis. Puneet *et al.*, (2018) reported that the medicinal properties of plants are related to the phytochemicals present in them. Therefore, the presence of the above mentioned compounds in *M. sativa* leaf may be the reason for its effectiveness in the treatment of certain diseases in traditional medicine. For example, phytochemicals such as saponins have anti-inflammatory effects, haemolytic activity and cholesterol binding properties (Puneet *et al.*, 2018). Glycosides are known to lower blood pressure and also alleviate heart related diseases, and tannins exhibit anti-oxidant and antimicrobial effects (Puneet *et al*., 2018). Alkaloids interfere with neurotransmission and block enzyme action (Puneet *et al.*, 2018). Hence, their presence in the plant may be the reason for its analgesic activity and usage in herbal medicine as a pain reliever. These phytochemicals may account for the medicinal value attributed to the *M. sativ*a leaves (Chisom *et al*., 2018). Flavonoids have been reported to exhibit various biological functions and medicinal properties such as anti-inflammatory, antioxidant, antibacterial, antiviral and cardioprotective properties (Chisom *et al*., 2018). Flavonoids are a group of polyphenolic compounds ubiquitously found in fruits and vegetables. They have multiple biological activities, including antioxidative, vasodilatory, anticarcinogenic, antiinflammatory, antibacterial, immune – stimulating, anti-allergic, antiviral and estrogenic effects, as well as being inhibitors of phospholipase A2, cycloxygenase, lipoxygenase, glutathione reductase and xanthine oxidase (Igwe and Okwu, 2013).

Table 2: Chemical constituents of the methanolic leaf extract of *M. sativa*

Chromatogram peak	Name of Compound	RT (min)	Molecular formula	Structure	% Composition	Medicinal /Industrial application
1	Hexanal	3.958	$C_6H_{12}O$		24.71	Cosmetics (Wang *et al*., 2018)
2	Tolbutamide	5.257	$C_{12}H_{18}N_2O_3$		14.00	Antimicrobial properties (Golawska *et al*., 2017)
3	Carbamic acid, methylphenyl-, ethyl ester	5.760	$C_{10}H_{13}NO_2$		2.34	Antifungal activity (Choi *et al*., 2019)

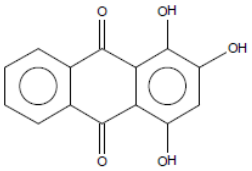
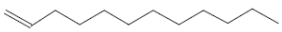
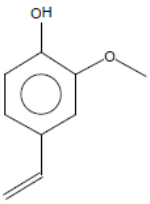
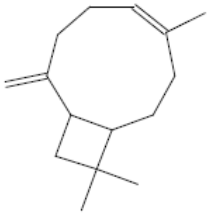
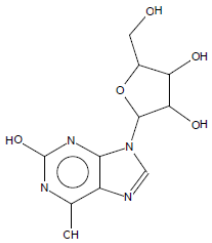
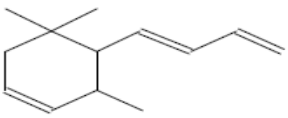
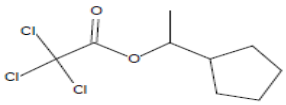
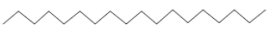
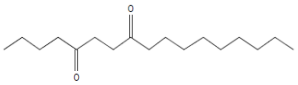
4	1,2,4-trihydroxy-9,10-Anthracenedione	6.739	$C_{14}H_8O_5$		19.53	Anti-inflammatory, cancer-preventing, antibacterial, antifungal, and antileishmanial (Ahmad *et al*., 2017)
5	1-Dodecene	7.534	$C_{12}H_{24}$		24.71	Agrochemicals (Mölgaard *et al*., 2017)
6	2-Methoxy-4-vinylphenol	7.717	$C_9H_{10}O_2$		0.50	Anti-inflammatory, antinociceptive and antioxidant effects (Joy and George, 2019)
7	Caryophyllene	7.740	$C_{15}H_{24}$		1.16	Anticancer and antioxidant activities (Zadeh *et al*., 2019)
8	Xanthosine	7.923	$C_{10}H_{12}N_4O_6$		2.47	Anticancer activities (Stochmal *et al*., 2018)
9	1-Tetradecene	8.020	$C_{14}H_{28}$		1.82	Anticancer and antioxidant activities (Stochmal *et al*., 2018)
10	Megastigma-3,7(E),9-triene	8.175	$C_{13}H_{20}$		3.18	Antifungal activities (Zadeh *et al*., 2019)
11	Trichloroacetic acid, 1-cyclopentylethyl ester	8.724	CCl_3COOH		3.33	Antihypertensive activities (Xie *et al*., 2019)
12	Octadecane	9.148	$C_{18}H_{38}$		1.57	Antihypertensive activities (Xie *et al*., 2019)
13	Heptadeca-5,8-dione	10.052	$C_{17}H_{20}O_2$		0.70	Antimicrobial activities (Asif *et al*., 2021)

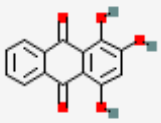
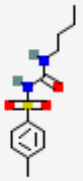
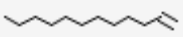
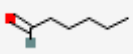
Table 2 shows the result of the GC-MS analysis of the methanolic leaf extract of *M. sativa*. Hexanal and1-dodecene had the highest percentage total area of 24.706% with a retention time of 3.958 min and 7.534 min respectively. 1,2,4-Trihydroxy-9,10-anthracenedione being the third most prominent compound had percentage total area of 19.528% with a retention time of 6.739 min while tolbutamide being the fourth most prominent compound had percentage total area of 13.995% with a retention time of 5.257 min. Carbamic acid, 2-methoxy-4-vinylphenol, caryophyllene, xanthosine, 1-

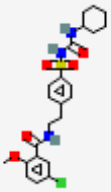
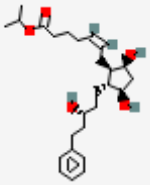
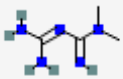
tetradecene, megastigma-3,7(E),9-triene, trichloroacetic acid, 1-cyclopentylethyl ester, octadecane and heptadeca-5,8-dione were present in smaller percentages.

Hexanal has been reported to be used in cosmetic industries (Wang *et al*., 2018), tolbutamide possess antimicrobial properties (Golawska *et al*., 2017), carbamic acid has also been used as an antifungal agent (Choi *et al*., 2019), 1,2,4-trihydroxy-9,10-Anthracenedione has been reported to possess anti-inflammatory, cancer-preventing, antibacterial, antifungal, and antileishmanial properties (Ahmad *et al*., 2017), 1-dodecene is used in agrochemicals (Mölgaard *et al*., 2017), 2-methoxy-4-vinylphenol exhibits anti-inflammatory, antinociceptive and antioxidant effects (Joy and George, 2019), caryophyllene exhibits Anticancer and antioxidant activities (Zadeh *et al*., 2019), xanthosine exhibits anticancer activities (Stochmal *et al*., 2018), 1-tetradecene possess anticancer and antioxidant activities (Stochmal *et al*., 2018), megastigma-3,7(E),9-triene exhibits antifungal activities (Zadeh *et al*., 2019), trichloroacetic acid, 1-cyclopentylethyl ester possses antihypertensive activities (Xie *et al*., 2019), octadecane exhibits antihypertensive activities (Xie *et al*., 2019) while heptadeca-5,8-dione exhibits antimicrobial activities (Asif *et al*., 2021).

The bioactivities of the compounds found in *M. sativa* could possibly exhibit antioxidant, anticancer, antifungal, antibacterial, flavor, anti-skin diseases, food supplement, hypocholesterolemic, nematicidal, pesticide, lubricant, antiandrogenic, hemolytic agents, 5-alpha reductase inhibitor, hypoglycemic and thyroid inhibiting properties, precursors of progesterone, anti-insomnia, antitumor, hypoglycaemic, anti-diabetics, anti-inflammatory amongst other bioactivities (Igwe and Okwu, 2013).

Table 3: Docking Score of Antidiabetic Activities of methanolic leaf extract of *M. sativa* with Glucagon-Like Peptide-1 in complex with the extracellular domain of the Glucagon-Like Peptide-1 Receptor (PDB code: 3IOL)

Bioactive Compounds	PubChem CID	Structure	Binding Affinity (Kcal/mol)
1,2,4-trihydroxy-9,10-Anthracenedione	6683		-6.9
Tolbutamide	5505		-5.8
1-Dodecene	8183		-4.4
Hexanal	6184		-3.6
Standard Antidiabetic Drugs			

Glyburide	3488		-8.0
Decyl beta-D-maltopyranoside	5288728		-6.2
Metformin	4091		-4.4

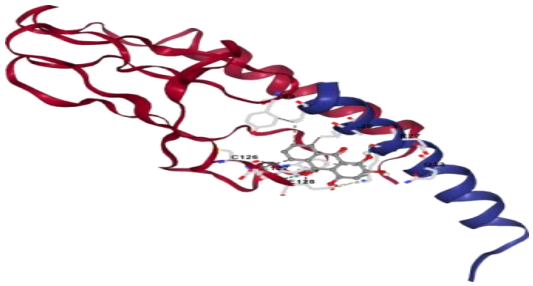
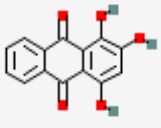
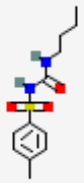
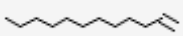
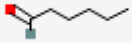
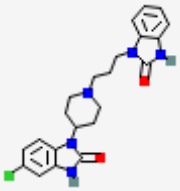
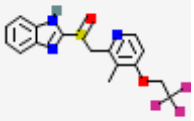
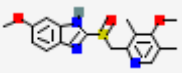


Figure 1: Molecular interaction of Antidiabetic Protein with 1,2,4-trihydroxy-9,10-Anthracenedione

The result in table 3 shows the docking score of the antidiabetic activities of *M. sativa*, the four prominent compounds in the extract were compared with three standard anti-diabetic drugs, the result of their binding affinities showed that 1,2,4-trihydroxy-9,10-Anthracenedione had the highest binding affinity of -6.9 Kcal/mol which was found to be higher than Decyl beta-D-maltopyranoside (-6.2 Kcal/mol) and Metformin (-4.4 Kcal/mol). The molecular interactions showed that 1,2,4-trihydroxy-9,10-Anthracenedione possess excellent anti-diabetic potentials and can be used as a potential drug candidate for the treatment of diabetes disease.

Table 4: Docking Score of Antiulcer Activities of methanolic leaf extract of *M. sativa* with Heparin-Linked Biologically-Active Dimer of Fibroblast Growth Factor (PDB code: 1AXM)

Name of Compound	**PubChem CID**	**Structure**	**Binding Affinity (Kcal/mol)**
1,2,4-trihydroxy-9,10-Anthracenedione	6683		-6.4
Tolbutamide	5505		-5.3

1-Dodecene	8183		-4.1
Hexanal	6184		-3.5
Standard Anti-ulcer Drugs			
Domperidone	3151		-7.0
Lansoprazole	3883		-6.3
Omeprazole	4594		-5.8

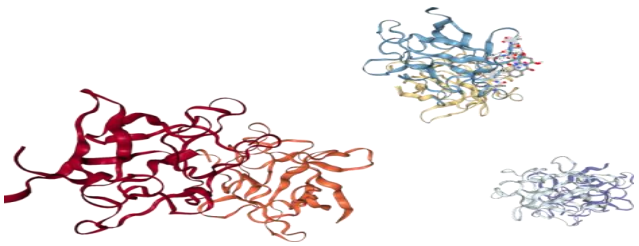
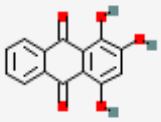
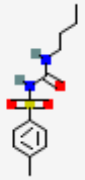
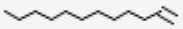
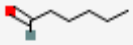
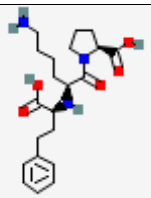
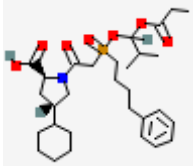
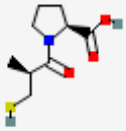


Figure 2: Molecular interaction of Antiulcer Protein with 1,2,4-trihydroxy-9,10-Anthracenedione

The result in Table 4 shows the docking score of the antiulcer activities of *M. sativa*, the four prominent compounds in the extract were compared with three standard Antiulcer drugs, the result of their binding affinities showed that 1,2,4-trihydroxy-9,10-Anthracenedione had the highest binding affinity of -6.4 Kcal/mol which was found to be higher than Lansoprazole (-6.3 Kcal/mol) and Omeprazole (-5.8 Kcal/mol). The molecular interactions showed that 1,2,4-trihydroxy-9,10-Anthracenedione possess excellent antiulcer potentials and can be used as a potential drug candidate for the treatment of ulcer.

Table 5: Docking Score of Antihypertensive Activities of methanolic leaf extract of *M. sativa* with Human Angiotensin Converting Enzyme in complex with lisinopril(PDB code: 1O86)

Name of Compound	PubChem CID	Structure	Binding Affinity (Kcal/mol)

1,2,4-trihydroxy-9,10-Anthracenedione	6683		-8.3
Tolbutamide	5505		-7.2
1-Dodecene	8183		-5.0
Hexanal	6184		-4.1
Standard Antihypertensive Drugs			
Fosinopril	55891		-9.3
Lisinopril	5362119		-7.9
Captopril	44093		-5.7

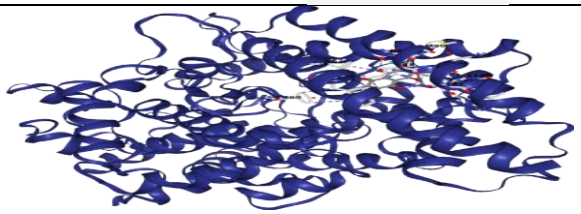
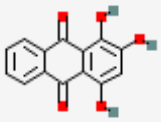
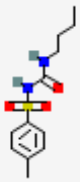
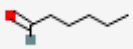
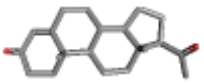
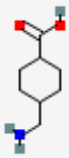
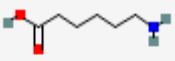


Figure 3: Molecular interaction of Antihypertensive Protein with 1,2,4-trihydroxy-9,10-Anthracenedione

The result in Table 5 shows the docking score of the antihypertensive activities of *M. sativa*, the four prominent compounds in the extract were compared with three standard Antihypertensive drugs, the result of their binding affinities showed that 1,2,4-trihydroxy-9,10-Anthracenedione had the highest binding affinity of -8.3 Kcal/mol which was found to be higher than Lisinopril (-7.9 Kcal/mol) and Captopril (-5.7 Kcal/mol). The molecular interactions showed that 1,2,4-trihydroxy-9,10-

Anthracenedione possess excellent antihypertensive potentials and can be used as a potential drug candidate for the treatment of high blood pressure.

Table 6: Docking Score of Antihermorrhagic Activities of methanolic leaf extract of *M. sativa* with proMMP-9desFnII complexed to JNJ0966 Inhibitor (PDB code: 5UE4)

Name of Compound	PubChem CID	Structure	Binding Affinity (Kcal/mol)
1,2,4-trihydroxy-9,10-Anthracenedione	6683		-8.1
Tolbutamide	5505		-6.9
1-Dodecene	8183		-6.3
Hexanal	6184		-4.9
Standard Antihermorrhagic Drugs			
Primolut	5994		-7.5
Tranexamic Acid	5526		-6.9
Aminocaproic acid	564		-5.8

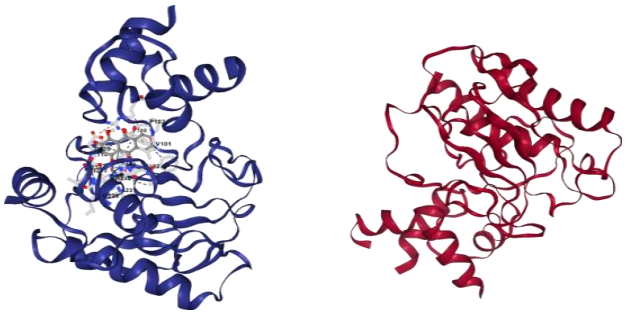
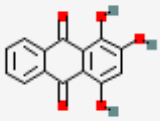
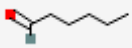
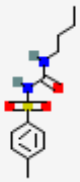
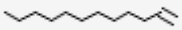
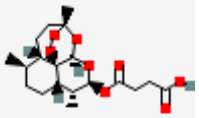
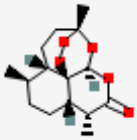
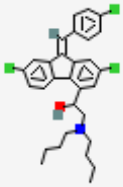


Figure 4: Molecular interaction of Antihermorrhagic Protein with 1,2,4-trihydroxy-9,10-Anthracenedione

The result in Table 7 shows the docking score of the antihermorrhagic activitie of *M. sativa*, the four prominent compounds in the extract were compared with three standard Antihermorrhagic drugs, the result of their binding affinities showed that 1,2,4-trihydroxy-9,10-Anthracenedione had the highest binding affinity of -8.1 Kcal/mol which was found to be higher than Primolut (-7.5 Kcal/mol), Tranexamic Acid (-6.9 Kcal/mol) and Aminocaproic acid (-5.8 Kcal/mol). The molecular interactions showed that 1,2,4-trihydroxy-9,10-anthracenedione possess excellent antihermorrhagic potentials and can be used as a potential drug candidate for the control of bleeding.

Table 7: Docking Score of Antimalarial Activities of methanolic leaf extract of *M. sativa* with Plasmepsin II, A Hemoglobin-Degrading Enzyme From *Plasmodium falciparum*, In Complex With Pepstatin A (PDB code: 1SME)

Name of Compound	PubChem CID	Structure	Binding Affinity (Kcal/mol)
1,2,4-trihydroxy-9,10-Anthracenedione	6683		-5.7
Hexanal	6184		-5.2
Tolbutamide	5505		-4.8
1-Dodecene	8183		-4.7
Standard Antimalarial Drugs			

Artesunate	6917864		-9.9
Artemisinin	68827		-9.4
Lumefantrine	6437380		-5.3

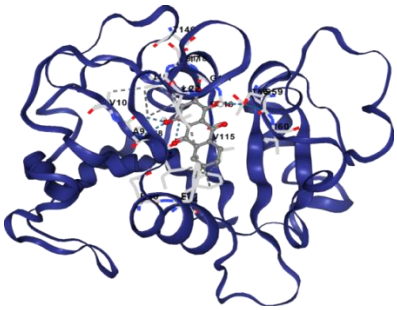


Figure 5: Molecular interaction of Antimalarial Protein with 1,2,4-trihydroxy-9,10-Anthracenedione

The result in Table 10 shows the docking score of the antimalarial activitie of *M. sativa*, the four prominent compounds in the extract were compared with three standard Antimalarial drugs, the result of their binding affinities showed that 1,2,4-trihydroxy-9,10-Anthracenedione had the highest binding affinity of -5.7 Kcal/mol which was found to be higher than Lumefantrine (-5.3 Kcal/mol). The molecular interactions showed that 1,2,4-trihydroxy-9,10-anthracenedione possess good antimalarial potentials and can be used as a potential drug candidate for the treatment of malarial disease.

Table 8: Pharmacokinetic (ADMET) Properties of 1,2,4-trihydroxy-9,10-Anthracenedione (Most Pharmacoactive Compound)

ADMET predicted profile-Classification	Value	Probability
Human Intestinal Absorption	+	0.9440
Caco-2	-	0.7297
Blood Brain Barrier	-	0.7250
Human oral bioavailability	+	0.7571

Subcellular localization	Mitochondria	0.6168
OATP2B1 inhibitior	-	0.6989
OATP1B1 inhibitior	+	0.9414
OATP1B3 inhibitior	+	0.9480
MATE1 inhibitior	-	0.8600
OCT2 inhibitior	-	0.9750
BSEP inhibitior	-	0.8835
P-glycoprotein inhibitior	-	0.9352
P-glycoprotein substrate	-	0.9760
CYP3A4 substrate	-	0.6778
CYP2C9 substrate	-	1.0000
CYP2D6 substrate	-	0.8234
CYP3A4 inhibition	-	0.7561
CYP2C9 inhibition	-	0.5531
CYP2C19 inhibition	-	0.9382
CYP2D6 inhibition	-	0.8606
CYP1A2 inhibition	+	0.7851
CYP2C8 inhibition	-	0.9285
CYP inhibitory promiscuity	-	0.7853
UGT catalyzed	+	0.8000

Carcinogenicity (binary)	-	0.8875
Carcinogenicity (trinary)	Non-required	0.4953
Eye corrosion	-	0.9927
Eye irritation	+	0.9845
Skin irritation	+	0.7486
Skin corrosion	-	0.8518
Ames mutagenesis	+	0.9400
Human Ether-a-go-go-Related Gene inhibitio	-	0.8667
Micronuclear	+	0.8400
Hepatotoxicity	+	0.7750
skin sensitization	+	0.5730
Respiratory toxicity	-	0.5222
Reproductive toxicity	-	0.6556
Mitochondrial toxicity	+	0.5750
Nephrotoxicity	+	0.5307
Acute Oral Toxicity (c)	III	0.6165
Estrogen receptor binding	+	0.7995
Androgen receptor binding	+	0.7405
Thyroid receptor binding	-	0.5428
Glucocorticoid receptor binding	+	0.9421

Aromatase binding	+	0.7497
PPAR gamma	+	0.8343
Honey bee toxicity	-	0.9012
Biodegradation	-	0.8000
Crustacea aquatic toxicity	-	0.5400
Fish aquatic toxicity	+	0.9820
ADMET predicted profile-Regression	**Value**	**Unit**
Water solubility	-2.808	Logs
Plasma protein binding	0.988	100%
Acute Oral Toxicity	2.445	log(1/(mol/kg)
Tetrahymenapyriformis	1.273	pIGC50 (ug/L

Table 8 shows the pharmacokinetic (ADMET) properties of 1,2,4-trihydroxy-9,10-Anthracenedione. The result revealed the absorption and distribution properties, metabolism potentials, excretion capacity and the toxicity parameters of 1,2,4-trihydroxy-9,10-Anthracenedione.

However, the result of the absorption properties showed that the human intestinal absorption value was positive with a probability of 0.9440 which implies that 1,2,4-trihydroxy-9,10-Anthracenedione can be absorbed from the gastrointestinal tract. The negative Caco-2 permeability value of 0.7297 showed that 1,2,4-trihydroxy-9,10-Anthracenedione is not capable of crossing through the intestinal wall into the bloodstream. The negative Blood Brain Barrier (BBB) value of 0.7250 showed that 1,2,4-trihydroxy-9,10-Anthracenedionemay probably not be able to cross through the brain. The positive Human oral bioavailability value of 0.7571 implies that a good percentage of 1,2,4-trihydroxy-9,10-Anthracenedione will be to reach the bloodstream after being taken orally.

From the distribution parameters, the plasma protein binding value was found to be 0.988% which implies that 1,2,4-trihydroxy-9,10-Anthracenedione has poorer tendency to bind with plasma proteins in the bloodstream and this makes it more possible to interact with its target protein in the body. The partition coefficient of 1,2,4-trihydroxy-9,10-Anthracenedione was found to be 1.58 as shown in table 8 and this implies that 1,2,4-trihydroxy-9,10-Anthracenedione can be evenly distributed more in aqueous environment more than non-aqueous or oily environment. The 0.6168 probability value of the subcellullar localization showed that 1,2,4-trihydroxy-9,10-Anthracenedione will be distributed towards the mitochondria for metabolism.

The result of the metabolism parameters of 1,2,4-trihydroxy-9,10-Anthracenedione revealed that the OATP2B1 inhibitior, MATE1 inhibitior, OCT2 inhibitior, BSEP inhibitior, P-glycoprotein inhibitior,

P-glycoprotein substrate, CYP3A4 substrate, CYP2C9 substrate, CYP2D6 substrate, CYP3A4 inhibition, CYP2C9 inhibition, CYP2C19 inhibition, CYP2D6 inhibition, CYP2C8 inhibition, CYP inhibitory promiscuity were all found to have negative probability value which implies that 1,2,4-trihydroxy-9,10-Anthracenedione is unlikely to inhibit these enzymes in the liver which makes it generally a good property since those enzymes helps in adequate drug metabolism. Hence, 1,2,4-trihydroxy-9,10-Anthracenedione will be efficiently metabolized.

The result of the excretion properties of 1,2,4-trihydroxy-9,10-Anthracenedione showed that it can be excreted through the liver, kidney and urine after been metabolized.

The result of the toxicity potentials of 1,2,4-trihydroxy-9,10-anthracenedione revealed that 1,2,4-trihydroxy-9,10-anthracenedione had high negative probability of eye and skin irritation, respiratory toxicity, reproductive toxicity, thyroid receptor binding, honey bee toxicity and biodegradation. Nevertheless, the negative value of carcinogenicity implies that it has a no probability of causing cancer, the positive value of eye irritation implies that it may cause irritation to the eye, the positive value of hepatoxicity implies that it may cause liver damage, the positive value of nephrotoxicity implies that it may cause kidney damage. However, due to the less toxicity of 1,2,4-trihydroxy-9,10-anthracenedione it can be employed as potential drug for the treatment of diseases.

Table 9: Drug Likeness Properties of 1,2,4-trihydroxy-9,10-Anthracenedione

Properties	**Values**
Molecular weight	256.21 g/mol
AlogP	1.58
H-Bond acceptor	5
H-Bond Donor	3

Table 9 shows the result of the Drug likeness properties of 1,2,4-trihydroxy-9,10-anthracenedione. According to Lipinski rule of five, a potential drug candidate must not fail more than one of the four guidelines based on multiples of five which stipulates a molecular weight not more than 500 g, octanol-water partition coefficient (AlogP) not more than 5, Hydrogen bond donors not more than 5 and Hydrogen bond acceptors not more than 10. However based on the result shown in table 9, 1,2,4-trihydroxy-9,10-Anthracenedione can be considered a potential drug candidate since it succeeded the discovery and preclinical research test. The result revealed that the molecular weight of the 1,2,4-trihydroxy-9,10-Anthracenedione was found to be 256.21 g/mol, octanol-water partition coefficient (Alop) value of 1.58, Hydrogen bond donor of 5 and hydrogen bond acceptor of 3. It was also found that 1,2,4-trihydroxy-9,10-Anthracenedione passed the four guidelines and this strongly suggests than it could possibly be used as drug in the treatment of diabetes, malarial and hypertension as shown in the molecular docking studies despites its few toxicity as revealed in the pharmacokinetic (ADMET) studies.

CONCLUSION

The results of this research work revealed that *M. sativa* has a lot of phytochemicals which could be used as raw material for pharmaceutical industries. The findings therein showed that 1,2,4-trihydroxy-9,10-Anthracenedione has the highest percentage abundance in the organic component of the leaf. The result of the *in-silico* studies of 1,2,4-trihydroxy-9,10-anthracenedione showed that it possesses several pharmacological properties and that it can be a potential drug candidate for the treatment of *Diabetes mellitus*, malarial and hypertension. Since it has better binding energy. However, the pharmacokinetics (ADMET) properties of the drug proved that the 1,2,4-trihydroxy-9,10-anthracenedione can be easily absorbed, distributed and properly metabolized in the liver and kidney and excreted. The toxicity studies showed that 1,2,4-trihydroxy-9,10-anthracenedione does

not cause eye corrosion, genetic mutation, and does not affect respiratory and reproductive organs. Though despite the few toxicities of 1,2,4-trihydroxy-9,10-Anthracenedione, the drug likeness test according to Lipinski rule of five, proved that 1,2,4-trihydroxy-9,10-Anthracenedione can be used as a drug, Hence, it can be regarded as a potential drug candidate for the treatment of *Diabetes mellitus*, ulcer, hermorhagic and hypertension.

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