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ORIGINAL ARTICLE

Identification of Phthalates, Methyl Esters and Siloxanes through GCMS analysis, biological investigation, DFT and Molecular Docking studies on Methanolic Subfractions of Hibiscus Rosa Sinensis flower extract

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ABSTRACT

Purpose: Plants are vital source of bioactive molecules, so they play a significant role in prevention and curing of various health disorders. The Hibiscus rosa sinensis, an ornate with attractive flowers, has conventionally been utilized globally as a curative agent for a wide range of ailments.

Methods: In current study, we prepared crude methanolic extract of flowers of Hibiscus rosa sinensis and subjected that to in vitro biological evaluation to assess its antidiabetic and antioxidant potential. Outcomes of in vitro evaluation confirmed the excellent antidiabetic and antioxidant potency of Hibiscus flowers. Further crude methanolic extract was fractionated to hexane, ethyl acetate and methanol fractions. Among the three fractions, we subjected methanolic fraction to GCMS analysis to explore the bioactive molecules contained by this fraction.

Results: The spectral data obtained from GCMS analysis revealed the existence of a total of 12 compounds including phthalates, methyl esters and siloxanes which are known to have a range of reported pharmacological activities. Successively, we subjected the identified molecules to molecular docking to assess their antidiabetic and antioxidant potential and DFT study to explore the reactivity of their molecular structures. As far as we are aware, it is the first report on the in-silico antidiabetic (128.1µM) and antioxidant (410.0µM) potential assessment of phytoconstituents of methanolic extract of floral part of Hibiscus rosa sinensis. The results revealed the H. Sinensis plat as more viable and good alternative to existing natural remedies. Molecular docking analysis pointed out four potential antidiabetic molecules, Di (3E,5E)-hepta-3,5-dien-1-yl) phthalate **(2),** 1H,15H-Hexadecamethyloctasiloxane **(5),** Hexakis (trimethylsiloxy) disiloxane**(11)** and Tetracosamethylcyclododecasiloxane**(12)** that displayed excellent binding affinity against receptor proteins, 3BAJ and 1HNY even higher than the reference compound Acarbose. However, all identified compounds except Octadecamethylnonasiloxane **(5)** and Cyclodecasiloxane, eicosamethyl- **(6)** exhibited promising affinity for antioxidantproteins, 2I3Y and 1XAN. Moreover, the molecule Tetracosamethylcyclododecasiloxane**(12)** was explored as lead compound among the identified molecules as it showed strongest interaction with all interacting proteins i.e.-156.997Kcal/mol, -179.446 Kcal/mol, -155.979 Kcal/mol, -193.859 Kcal/mol for 3BAJ, 1HNY, 2I3Y and 1XAN, respectively.

Conclusion: The findings of our study help us to conclude that conventionally utilizeted Hibiscus rosa sinensis flowers and its identified molecules from the methanolic extract possess excellent antioxidant and antidiabetic potential.

Keywords: GCMS analysis, DFT, Molecular Docking, Antidiabetic, Antioxidant

INTRODUCTION

History divulges that the role of plants to prevent and treatment of various health disorders is significant, additionally can even avert and lessen the inimical effects of folk remedies. Plant based chemical constituents owe pharmacological characteristics and have made massive contributions to discover a number of efficacious drugs and will constantly be significant for screening of novel lead molecules. Currently around 25% of the FDA (Food and Drug Administration, USA) and EMA (European Medical Agency) approved drugs are of efficacious therapeutic plant extracts based [1]. An integral part in the examination of a plant is to identify the bioactive constituent found in that plant directing to future pharmacological and biological studies.

Plant based therapeutic agents are generally denoted as phytochemicals and possess several curative properties. The phytochemicals are secondary metabolites, which generate during a plant's biosynthetic paths. These compounds are of huge chemical and structural assortment, so have potential antibacterial, antiviral, anticancer, antifungal and other characters[2].

Throughout the globe, Phyto researchers are trying hard to reconnoiter the valuable chattels of therapeutic plants to resolve the critical humanoid health problems. In this connection, we have chosen red Hibiscus rosa-sinensis flower which is a frequently traditionally utilized flower to resolve multiple health issues, in order to identify its bioactive constituents which are responsible for the therapeutic potential of this auspicious flower. The Hibiscus rosa-sinensis of the family Malvaceae is usually planted as ornate as it bears attractive flowers. The flowers of Hibiscus rosa-sinensis has conventionally been utilized globally to cure a wide range of ailments like genito-urinary tract infection, asthma, heart diseases, diabetes mellitus, cancer, skin diseases, ulcers, piles, kidney troubles, eye problems, cough, brochitus, gripe and also as an anti-fertility agent, carminative, antiseptic, demulcent and refrigerant[3]. The Hibiscus rosa-sinensis flower extract presented analgesic, antitumor, antipyretic, anti-asthmatic, anti-oxidant, anti-inflammatory, anti-fungal, anti-spermatogenic, antimicrobial, androgenic, antidiabetic, anticonvulsants, and antihyperlipidemic activity[4]. The wide range of pharmacological and biological potential of this impressive red flower of Hibiscus is relatable to the occurrence of a variety of phytoconstituents belong to various classes of phytochemicals.

The universal spread of Hibiscus flower as traditional therapeutic flower based on its curative reputation, has been considered as a respectable candidate for drug discovery. The current study is conducted to sightsee the potential phytoconstituens from the various fractions of methanolic extract of Hibiscus rosa-sinensis. The screening of therapeutic plant utilizing chromatographic and spectroscopic techniques delivers basic info about pharmacological and chemical potential.

GCMS is considered the first-rate, accurate and fast tool to detect the compounds belong to various groups like alcohols, nitrocompounds, alkaloids, long chain hydrocarbons, steroids, organic acids, amino acids and esters and consumes a very small amount of plant extracts.

Hence, to identify and detect the phyto molecules contained by selected fraction of Hibiscus flower, we adopted Gas

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chromatography–Mass spectroscopy (GCMS). In the current research, a total of 12 compounds, including phthalates, methyl esters and siloxanes were detected from the methanolic fraction of flower extract. Moreover, we assessed antidiabetic and antioxidant potential of identified compounds utilizing in silico methods.The in silico tools comprise of a range of computational techniques which can be actually useful for forecasting the drug-likeness of any compound. The in silico tools utilizing computational procedures help to make the drug designing process resource efficient and cost-effective as compared to in vitro and in vivo experimentations [5]. Benefits of utilizing in silico approaches can be pimped on every step of drug production, i.e. from the preclinical discovery step to final step of clinical development. Scrutinizing of phyto constituents by computational techniques aids to discover the various phyto molecules with high efficiency and certainty [5]. DFT (density functional theory) and molecular docking dependent computational studies were performed to diagnose the potential inhibitory characteristics of identified phyto molecules of methanolic fraction of flowers of Hibiscus rosa-sinensis.DFT (density functional theory) was utilized to analyze the reactiveness of detected molecules. In order to examine the pharmacological and biological potential, it is vital to highlight the molecular descriptors, frontier molecular orbitals (FMO), ionization potential (IP), molecular electrostatic potential (MEP) and electron affinity (EA) analysis. The electronic properties like electronegativity (v), electrophilicity index (x), chemical potential (m), chemical hardness (g) and softness (S) have also been discoursed along with universal molecular descriptors.

Molecular docking which is easy on pocket and effective technique to design and test drugs was conducted to discover the potential biologically active molecules for their biological potential. This computational method delivers info related to drug receptor interfaces, which are helpful to forecast the binding patterns of drug candidates to the target proteins. Moreover, this technique aids in organized study by introducing a molecule on the binding pockets of the object macromolecule in a non-covalent manner, leading o a perfect binding at the active spot so fevery ligand.

In literature few reports regarding the in vitro evaluation to assess the biological potential of extracts of various solvents are available, however, as far as we are aware, no literature is available on the in-silico antidiabetic and antioxidant potential assessment of phytoconstituents of methanolic extract of floral part of Hibiscus rosa-sinensis. So, we not only focused on exploring the bioactive constituents of methanolic floral extract of Hibiscus rosa-sinensis, but also tested the antidiabetic and antioxidant properties of extract. Following by, in silico molecular docking evaluation to explore putative bioactive molecules with antidiabetic and antioxidant potentials. Molecular docking findings exhibit that the phyto constituents of methanolic fraction of Hibiscus rosa-sinensis own better drug-like characteristics and inhibit diabetic and oxidant activity by developing strong stabilizing protein-ligand binding. Thus, this conventional medicinal plant could serve as a potential natural source of antidiabetic and antioxidant agent for developing related pharmaceuticals.

METHODOLOGY

Study Design: This experimental research involves two major parts, identification of phytoconstituents of methanolic floral extract Hibiscus rosa-sinensis, a traditional therapeutic plant, and bioactive evaluation of extract as well as of identified molecules. GCMS study was employed to explore the phytochemical composition of extract, however, in order to dig out the bioactivity of methanolic floral extract, both in vitro and in silico experimental procedures were adopted.

PLANT SAMPLE: The healthy and fresh flowers of Hibiscus rosa sinensis were collected (13kg) manually from Faisalabad, Pakistan, during the flowering months (June-September) in 2020. Collected flowers were botanically identified and authenticated by a taxonomist of Department of Botany, University of Agriculture, Faisalabad, and a voucher specimen with number HRSF-103 was submitted in herbarium.

EXTRACTION: After removing sepals Hibiscus rosa sinensis flowers were shade dried and extracted at room temperature with methanol (8 L). This methanolic extract was concentrated to reduce its volume with help of rotary evaporator, reconstituted in distilled water and then fractionated sequentially with hexane (3×500mL), ethyl acetate (3 × 500 mL), and finally methanol (3×500mL). The all three resulting fractions were concentrated under reduced pressure at 45 ^{0}C utilizing rotary evaporator and later completely dried in air[6]. Among above three fractions methanolic part was further fractionated to sub fractions, EAF1, EAF2, MF1 and MF2 by using various filters.

Biological Evaluation of Extract or fractions need to add here;

In Vitro Biological Evaluation

Anti-Diabetic Potential: In vitro anti-diabetic potential of crude methanolic floral extract of Hibiscus rosa sinensis was evaluated by utilizing alpha amylase and starch as enzyme and substrate, respectively. Acarbose was taken as a standard drug.Antidiabetic potential of floral extract of Hibiscus rosa-sinensis was assessed by adopting the protocol previously documented by Eseyin et al.[7] after minor amendments. summarized here .0.5ml of extract solution from each dilution (50µg, 100µg, 150µg, 200µg and 250µg) accompanied by 0.5ml of buffered enzyme solution in test-tubes was incubated for 10min at room temperature followed by addition of 0.5ml of starch solution and kept for incubation for 10min under same temperature condition followed by addition of 1ml of DNSA solution. Absorbance was measured by utilizing Hitachi U-2900 spectrophotometer.

Antioxidant Potential: Antioxidant Potential of crude methanolic floral extract of Hibiscus rosa-sinensis has been prerecognized and published by our research team [8].

GCMS ANALYSIS: The GCMS analysis of bioactive molecules from the all four sub fractions was carried out using GCMS-QP 2010 (Shimadzu, JAPAN) at Government College University, Lahore.The detection of phyto constituents comprised by each kind of fraction was ascertained by logical and analogical explanation of their mass fragment patron and retention times with those previously available in literature as well as to computer quests at the NIST (National Institute of Standards and Technology) library. The attuned apparatus provisos and analysis conditions are listed in Table S1 (supplementary Material).

Molecular docking: All the phyto constituents comprised by four under studied sub fractions of methanolic part of Hibiscus rosa-sinensis flower extract detected by GCMS studies were evaluated to assess their putative antidiabetic and antioxidant activities by docking studies. Currently in drug discovery process, molecular docking is considered an effective approach which seeks to forecast if and how two molecules (generally a ligand and a target) interact physically[9]. The molecular contrivance of detected biomolecules was inspected by performing molecular docking analysis utilizing Molegro Virtual Docker (MVD 2013.6.0.1) and Biovia Discovery Studio Visualizer 2021.

Ligand Preparation: 2D structures of all identified molecules **(1-12)** were sketched by utilizing Chem Draw Professional (19.1, Perkin Elmer) and then these 2D structures were moved to chem3D Ultra (19.1, Perkin Elmer) to obtain 3D conformers and then stored as Mol 2 file.

Protein Preparation: For docking studies the 3D structures of protein crystals were managed from protein data bank (http://www.pdb.org/pdb/home.do,) and stored as pdb. All the identified ligands of Hibiscus were docked with human pancreatic α-amylase having PDB ID: 3BAJ (Resolution:2.10 Å) and PDB ID:1HNY (Resolution: 1.8 Å)[10,11] to assess their anti-diabetic potential. Whereas, the antioxidant property was examined by their docking with human Glutathione Peroxidase 5 and human Glutathione Reductase having PDB IDs 2I3Y (Resolution: 2.00 Å) and 1XAN (Resolution: 2.00 Å), respectively.

Molecular Docking: Mechanism of docking initiated with the loading of Mol 2 files of ligands and proteins in PDB format (one by

one) to molegro virtual docker workspace. All water molecules as well as cofactors were eliminated before initializing the docking procedure. Afterwards, all probable cavities on the protein surface were delved which resulted in the selection of total five cavities. The binding location and grid resolution was adjusted to 0.3Å, search algorithm was set on energy minimization. The pose with top docked orientation was inspected utilizing MolDock Score [GRID] algorithm, for the population size of 50, 1500 were the maximum iterations, number of runs taken were 10 and energy threshold of 100 [12]. The whole data of docking analysis of all ligands as parameters like MolDock score, bond category, bond type, bond length, and interacting residues is tabulated in Tables 4-5. The docking procedure was validated by extraction of the reference ligand followed by the redocking procedure. The RMSD value ≤ 2 Å declares the validation of docking process [13].

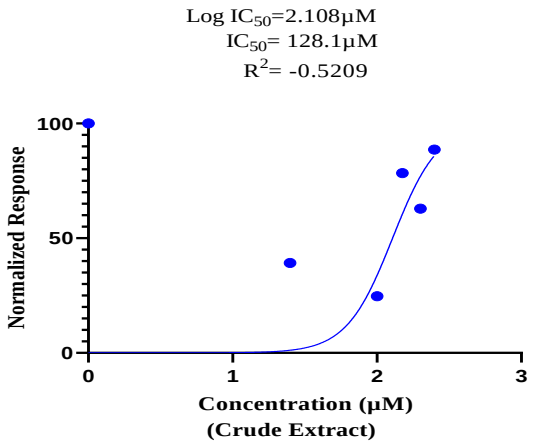
Density Functional Theory: DFT (Density functional theory) analysis was performed to investigate the reactivity characteristics of identified phyto molecules **(1-12).** All the computational calculations of phyto molecules **(1-12)** were conducted utilizing Gaussian 09W software with support of Gauss View 6.0.16 interface. These phyto molecules were optimized through hybrid category B3LYP /6-311G* basis set in the configuration of DFT which provided HOMO-LUMO geometries and energies, dipole moment, energy gap, net charge, however other reactivity descriptors like ionization energy, electron affinity, electronegativity, electronic chemical potential, molecular hardness,molecular softness and electron affinity were calculated utilizing HOMO-LUMO energies.

RESULT AND DISCUSSION

In vitro Biological Evaluation

Anti-diabetic Potential of Hibiscus rosa sinensis Flowers: Upshots of in vitro assessment for anti-diabetic potential of crude methanolic extract signpost that the floral extract of Hibiscus rosa-sinensis own excellent potential to inhibit α- amylase activity with IC_{50} valued 128.1 µM which proves that this splendid flower has antidiabetic potency more than many other well-known medicinal plants like Terminalia arjuna, Linumusitatisumum Aegle marmelos, Eugenia cumini[14] and Elaeagnus umbellata[15]. Figure 1 is the graphical representation of IC_{50} value of crude methanolic floral extract drawn by GraphPad Prism 8.

Figure 1 Antidiabetic Potential of Crude Methanolic Extract Hibiscus rosa-sinensis Flowers



Antioxidant Potential of Hibiscus rosa sinensis: Based on our previously published data, crude methanolic floral extract of Hibiscus rosa-sinensisexhibited the IC_{50} 410.0 µM, which is lowest amongst all other plants extracts assessed by us which is an intimation that phyto molecules of Hibisus flowers might have excellent scavenging potential [8].

GCMS Analysis: In order to reveal the chemical composition dependent biological potential of methanolic part of floral extract of Hibiscus rosa-sinensis GCMS based metabolic characterization was conducted. Collectively 12 peaks were witnessed (each peak voted for one compound)in all four subfractions of methanolic part of flower extract of Hibiscus rosa-sinensis.Thus, the GCMS analysis of chosen fractions revealed the existence of total 12 compounds.The detected compounds with their molecular formula, retention time, base peak, mass peak, molecular weight and %age of peak area are displayed in Table1. The chromatograms of subfractions EAF1, EAF2, MF1 and MF2 recorded 2, 5, 1 and 10 peaks, respectively (Figure S1: Supplementary material). The data obtained from GCMS analysis exposed that 12 identified compounds from methanolic subfractions of floral extract of Hibiscus rosa-sinensis include phthalates, methyl esters and siloxanes.Phthalates are generally identified as a group of synthetic chemicals utilized as lubricant in the plastics industry however they have been consumed in numerous productscosmetics, supplements, pharmaceutical pills and in various commercial productsas they possess high strength, good insulation, corrosion resistance, easy fabrication and low cost [16]. The common component of all four subfractions, Diethyl phthalate has been previously isolated and detected from various plant species such as Chrysanthemum indicum, Avicennia marina, Allium fistulosum, Prunella vulgaris, Osmanthus fragrans and Nizamuddiniazanardinii[16] is reported to possess antimicrobial, insecticidal [16], antibacterial, neurotoxic and acetylcholinesterase-inhibiting properties [17].

The present study verified the presence of two methyl esters, hexadecanoic acid methyl ester **(7)** and methyl (4E,11E)-hexadeca-4,11,15-trienoate **(8)** in MF2 subfraction (Table 1, Figure S3: supplementary material) of Hibiscus rosa-sinensis floral extract. Methyl esters isolated from various natural sources own antimicrobial, antifungal, anti-inflammatory, antibacterial, antioxidant, cancer preventive, hepatoprotective, antiviral, insectifuge, antieczemic, antihistaminic, hypocholesterolemia, anti-coronary, anti-arthritic, antiandrogenic, analgesic, anti-tumor[18]. Among the two dectected methyl esters, hexadecanoic acid methyl ester **(7),** has been previously found in numerous plant species likeSalix babylonica[19], Syzygiumaromaticum[20], Jatropha curcas[21], Pterocarpus angolensis[22] and Parthenium hysterophorus[23]. This saturated fatty acid ester claimed to have antibacterial, anti-inflammatory [21], antifungal[22], antioxidant, hemolytic, hypo-cholesterolemic, hypocholesterolemic, alpha reductase inhibiting, Anti androgenic [24] and insecticidal properties [25].Silicones (silioxanes) comprise a group of low molecular weight compounds, organosilicon oligomers and polymers found in various plant species include Eruca sativa[26], Hibiscus asper[27], Cassia italic, Khayagrandifoliola, Enantiachlorantha and Dryopteriscochleata[28]. Siloxanes are the dietetic silicon which deliver a variety of health benefits such as bone density improvement, innate immunity, collagen regeneration, inflammatory responses regulators, diminution the atherosclerosis risk, and strengthens hair, nail and skin as well [29]. The fascinating thing about the Hibiscus rosa-sinensis flowers is that they encompass a substantial volume of siloxanes, so this splendid can serve as a significant source of nutritional silicon.

In the past few years, the vibrant progress of silicones technology has been noticed, which resulted in the registration of more than 150,000 practical applications, comprising cosmetic [26], medical, pharmaceutical and foodstuff. Presently, around 50% of novel skin items comprise at least one kind of silicone. Among the detected siloxanes of Hibiscus, Cyclodecasiloxane, eicosamethyl-(**6**) has been previously found in Eruca sativa and reported to have heptoprotective, antimicrobial and antioxidant potential [26].

Table 1. Upshots of GCMS analysis of various fractions of methanolic extract of Hibiscus rosa-sinensis (flowers)

Fraction	Peak	Compound	Molecular Formula	RT (mins)	Base Peak	Mass Peak	Area %
EAF1	1	Diethyl Phthalate **(1)**	$C_{12}H_{14}O_4$	15.525	149.10	222.00	82.17
	2	Di((3E,5E)-hepta-3,5-dien-1-yl) phthalate **(2)**	$C_{22}H_{26}O_4$	24.216	55.10	355.15	17.83
EAF2	1	Diethyl Phthalate **(1)**	$C_{12}H_{14}O_4$	15.521	149.10	222.00	34.6
	2	Isopentyl propyl phthalate (**3)**	$C_{16}H_{22}O_4$	24.215	149.10	279.20	42.8
	3	1H,15H-Hexadecamethyloctasiloxane **(4)**	$C_{16}H_{50}O_7Si_8$	27.850	73.10	578.17	7.79
	4	Octadecamethylnonasiloxane**(5)**	$C_{18}H_{54}O_9Si_9$	28.675	73.05	666.17	6.66
	5	Cyclodecasiloxane, eicosamethyl- **(6)**	$C_{20}H_{60}O_{10}Si_{10}$	29.608	73.05	740.19	8.60
MF1	1	Diethyl Phthalate **(1)**	$C_{12}H_{14}O_4$	15.528	149.05	222.00	100
MF2	1	Diethyl Phthalate **(1)**	$C_{12}H_{14}O_4$	15.516	149.10	222.00	11.11
	2	Hexadecanoic acid, methyl ester **(7)**	$C_{17}H_{34}O_2$	19.018	74.05	270.35	2.71
	3	Methyl (4E,11E)-hexadeca-4,11,15-trienoate**(8)**	$C_{17}H_{28}O_2$	20.656	55.05	264.21	3.64
	4	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl heptasiloxane**(9)**	$C_{14}H_{44}O_6Si_7$	22.491	73.05	504.15	2.92
	5	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane **(10)**	$C_{12}H_{38}O_5Si_6$	23.532	73.10	430.13	6.96
	6	Hexakis(trimethylsiloxy)disiloxane**(11)**	$C_{18}H_{54}O_7Si_8$	24.511	73.10	606.20	14.07
	7	Octadecamethylnonasiloxane**(5)**	$C_{18}H_{54}O_9Si_9$	25.431	73.10	666.10	17.27
	8	Cyclodecasiloxane, eicosamethyl- **(6)**	$C_{20}H_{60}O_{10}Si_{10}$	26.286	73.10	740.19	15.90
	9	Tetracosamethylcyclododecasiloxane**(12)**	$C_{24}H_{72}O_{12}Si_{12}$	27.087	73.05	888.23	12.22
	10	1H,15H-Hexadecamethyloctasiloxane **(4)**	$C_{16}H_{50}O_7Si_8$	27.851	73.10	578.17	6.45

Molecular Docking: Molecular Docking Assessment for Anti-Diabetic Potential

The docking study of identified molecules **(1-12)** was performed to anticipate their antidiabetic potential on the base of binding affinity of these ligands onto the active binding locations of receptor proteins (3 BAJ and 1HNY) utilizing Molegro Virtual Docker. Table 2 displays the binding affinity of docked ligands with both receptor proteins in term of MolDock score alongside bond types, active residues and bond distance. Docking results indicate that range of binding affinity falls from -86.881Kcal/molto -156.997 Kcal/mol for receptor protein 3BAJ, while from **-**81.3068 to -179.446 Kcal/mol for 1HNY. Within this range, compounds **(2, 4, 11 and 12)** displayed excellent results in term of binding affinity and presented higher binding affinity for both proteins than reference drug Acarbose (-122.257 and -125.681 Kcal/mol for 3BAJ and 1HNY, respectively).However, compounds **(5)** and **(6)** exhibited unusual values of binding affinity for both receptors. So, it is clinched that these ligands own well-defined inhibitory potential against human pancreatic α-Amylase. Among the four high binding affinity scored molecules, compound **(12)** showed strongest affinity for both target proteins with MolDock score of -156.997 Kcal/mol and -179.446Kcal/mol against 3BAJ and 1HNY, respectively, which clearly depicts the strongest inhibitory potential of molecule **(12)** as anti-diabetic pancreatic α -Amylase targeted agents among the identified molecules. Figure 5 and 6 is the visual representation of the most suitable pose of ligand **(12)** with proteins 3BAJ and 1HNY, respectively, along with binding patterns (2D and 3D) and hydrophobic interaction.

The most fit docked pose of ligands **(1-12)** and reference and binding patterns (2D and 3D) of ligands with both human pancreatic α-amylase proteins displayed in Supplementary material(Figure S4& S5)and Table 2, directs that residues of 3BAJ responsible for hydrogen bonding.

Molecular Docking Assessment for Anti-Oxidant Potential: The identified molecules **(1-12)** were docked on the active interacting locations of human Glutathione Peroxidase 5 (PDB: 2I3Y) and human Glutathione Reductase (PDB: 1 XAN), utilizing the Molegro Virtual Docker, to recognize the action mechanism of these ligands to assess their antioxidant potential. Upshots of docking procedure are presented in Table S2 (Supplementary material).The most representative and best enzyme-ligand complexes of all ligands with human Glutathione Peroxidase 5 (2I3Y) and human Glutathione Reductase (1XAN), respectively. The most stable and suitable interacting modes of identified molecules for antioxidant proteins (2I3Y and 1XAN) exposed their docking score range from -81.0519 to -155.979 kcal/mol for (2I3Y) and from **-**81.849 to-193.859 kcal/mol for (1XAN) reveals that these molecules possess good antioxidant potential (Table 4). However, **(5)** presented unusual Mol Dock score (3984.75 kcal/mol) when docked with (2I3Y) and **(6)** presented unusual behavior with both enzymes. The variation in the Mol dock score of same ligand for both protein is attributed to fact that each protein has specific construction of amino acid. Among all under discussion molecules compound **(12**) exhibited highest the binding affinity (-155.979 Kcal/molfor 2I3Y and -193.859 Kcal/mol for1XAN) whichconfirms the strongest antioxidant potency of molecule **(12)** as compared to all other identified molecules. Figure S6 & 7is the visual representation of the most suitable pose of ligand **(12)** with proteins 2I3Y and 1XAN, respectively, along with binding patterns (2D and 3D) and hydrophobic interaction.Table S2: Supplementary material displays the residues of 1XAN and 2I3Y responsible hydrogen bonding docked ligands.

Docking Validation: We assessed the validity of docking method by redocking of the reference ligands of receptor proteins PDB ID: 3BAJ, PDB ID: 1HNY, PDB ID: 2I3Y and PDB ID: 1XAN to the potential binding sites of receptors. The obtained RMSD values by redocking of native ligands of 3BAJ, 1HNY, 2I3Y and 1XAN with receptors were 1.188 Å, 1.74 Å, 1.775 Å and 2.00 Å, respectively, verify the bonding of ligands with active sites of receptors so verifying docking procedure adopted by us (Figure.2).

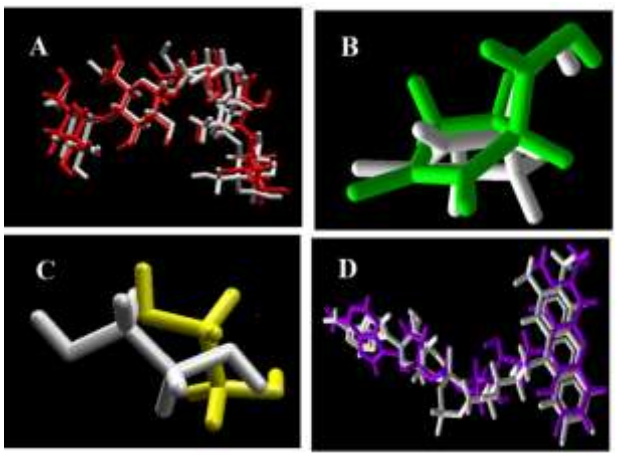


Figure 2. Docking validation (A) Superimposation of reference (white) and redocked (red) ligands of 3BAJ for validation of anti-diabetic docking experiment (B) Superimposation of reference (white) and redocked (green) ligands of 1HNY for validation of anti-diabetic docking experiment (C) Superimposation of reference (white) and redocked (yellow) ligands of 2I3Y for validation of anti-oxidant docking experiment (D) Superimposation of reference

(white) and redocked (purple) ligands of 1XAN for validation of anti-oxidant docking experiment

Density Functional Theory (DFT): Among the twelve detected phyto compounds, 4 silicates (**4, 5, 11** and **12)** could not be subjected to DFT analysis. These silicates might not have drug-likeness due to some structural limitations [30].Comprehensive results of DFT based structure activity correlation analysis of identified phyto compounds are composed in Table S3.

Frontier Molecular Orbitals: Highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) which are collectively titled as the frontier molecular orbitals (FMOs) are the most imperious orbitals of a molecule (Figure S8). (E_{HOMO}) and (E_{LUMO}) describes the electron donating and accepting capability of a molecule, respectively. A higher value of (E_{HOMO}) signposts higher electron donating tendency and better reactivity whereas a lower value of (E_{LUMO}) indicates greater electron accepting capability and better reactivity. According to Table 5, **(2)** has highest value of (E_{HOMO})**(**-5.9836 eV**)** while **(3)** has lowest (E_{LUMO}**=**-1.9265 eV**)**[31-33]. The difference between (E_{HOMO}) and (E_{LUMO}) is termed as energy gap (ΔE), is an important parameter to assess the reactivity of molecules [5]. Generally, narrow ΔE value associated with higher chemical reactivity and vice versa.The reactivity order for studied compounds is **2 > 1> 3> 8>7 >9 > 10> 6**

Global Reactivity Parameters: Theoretical density functional analysis has been utilized to estimate the chemical reactivity of the molecular systems. Chemical Potential (CP), Chemical Hardness (η), Chemical Softness (s), Electronegativity (χ), Electrophilicity Index (ω), Electronic Energy (EE), Nucleophilicity Index (N) and Additional Electron Charge (ΔN_{max}) are the global reactivity parameters and are highly productive in identifying universal reactivity orders. Two significant characteristics of a molecule are its electron affinity (A) and ionization potential (I). The (E_{HOMO}) and (E_{LUMO}) are markers which can be utilized to calculate the electron affinity (A= -E_{LUMO}) and ionization potential (I= -EHOMO)[34]. Smaller value of I is an indication of better electron donating property whereas larger value of A is the indication of better electron accepting characteristic of a molecule. Regarding the above, molecule **(2)** is the best electron donor among the under-discussion compounds as has lowest value of ionization potential value (I = 5.9836eV). Molecule **(3)** with the largest value of electron affinity (A = 1.9265 eV) is considered the best electron donating molecule (Table S3). The value of χ (electronegativity) forecast the ability of a molecule to attract electrons [31] . As χ = -CP, so chemical potential is related to electron removal tendency of a molecule. Smaller (χ) value and larger (CP) value indicates the delocalization of electrons, therefore a molecule with low (χ) and high (CP) can be more comfortably interact with biological scheme (more reactive)[12]. Electronegativity and chemical potential-based activity ranking of under studied compounds is below;

10>6 > 8> 9>7 >2> 1> 3

The hardness (η) and softness (S) of a chemical compound is directly associated with ΔE.The hardness denotes the resistance of compound toward the distortion of the electron cloud of chemical system during chemical reaction. A hard molecule has larger ΔE value whereas a soft molecule has smaller ΔE. A molecule is more stable if it has high chemical hardness and contra wise, a molecule is more reactive if it has high chemical softness value. On the basis of computed hardness and softness values (Table S3) molecule **(1)** is most reactive and least stable amongst studied molecules with highest (S) value (0.3716 eV) [31]. Computation of the electrophilicity index (ω) and nucleophilicity index (N) is supportive in making the decision about the nature of a molecule i.e. electrophilic and nucleophilic, respectively (Table S3). A compound with higher (N) and lower (ω) value is considered biologically more active. On the basis of electrophilicity and nucleophilicity indexes the order of reactivity of the detected compounds of Hibiscus is as follows:

10> 6> 9> 8> 7>2 >1 >3

Additional electronic charge is represented by (ΔNmax). ΔN_{max} values exhibited the similar reactivity trend as presented by energy gap (Table S3).One more vital parameter which is directly allied to chemical reactivity of compounds is dipole moment (μ). High dipole moment value for a compound indicates its high polarizability and larger surface area for reaction and consequently will have higher reactivity. Figure 4 showed the vector of the dipole moment of all the under studied phyto molecules. The higher dipole moment value of molecules **(1)** and **(9)** as compared to other molecules reveals well-adjusted bond distance and the finest distribution of charge in these molecules.On the basis of highest negative value of Electronic Energy (E), compound **(1)** is most stable compound among all discussed compounds.

Molecular electrostatic potential: The MEP graphs of identified phyto molecules are presented in figureS9 (Supplementary file). The MEP graphs are considered crucial to locate reactive sites (electron deficient and electron rich regions) of a compound. The MEP not only indicates the shape and size of a chemical entity along with its neutral, negative and positive electrostatic potentials but could also be utilized to antedate molecular structure dependent physicochemical characteristic associations of drugs in development [31]. The MEP graphs utilized various color codes for indication of regions favorable for electrophilic and nucleophilic attack. The favorable site for electrophilic attack is detectable by red, yellow and orange coloration while blue coloration depicts the site suitable for the attack of nucleophile. It is clear from the MEP maps of phthalates **(1,2,3)** and methyl esters **(7,8)** that oxygen atoms of carbonyl group and hydrogen atoms are suitable sites of electrophilic and nucleophilic attack, respectively. Similarly, in all silioxanes, red coloration around the oxygen atoms signifies the suitable location for electrophilic attack.

CONCLUSION

The findings of our study help us to conclude that conventionally utilizeted Hibiscus rosa sinensis flowers and its identified molecules from the methanolic extract possess excellent antioxidant and antidiabetic potential. The GCMS analysis of floral methanolic extract of Hibiscus rosa sinensis revealed the existence of total 12 compounds include phthalates, methyl esters and siloxanes. Major fraction of identified molecules, specifically Tetracosamethylcyclododecasiloxane **(12)** our lead compound, exhibited the promising binding affinity toward antidiabetic and antioxidant proteins. Regarding the findings of current study, these identified molecules of Hibiscus may facilitate pharamaceutical industry to design effective and reliable antioxidant and antidiabetic drugs. Though, further investigations such as bio-prospecting are necessary to support their biological characteristics to develop innovative drugs.

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1. Conception and design of or acquisition of data or analysis and interpretation of data.
2. Drafting the manuscript or revising it critically for important intellectual content.
3. Final approval of the version for publication.
4. All authors agree to be responsible for all aspects of their research work

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