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ORIGINAL ARTICLE

IN VITRO AND IN SILICO PROFILING OF PHENOLICS IN PENTACLETHRA MACROPHYLLA LEAF EXTRACT ON KEY PROTEINS LINKED TO ERECTILE DYSFUNCTION

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Abstract. Introduction: *Erectile dysfunction (ED) which is the inability to maintain an erection during sexual activity, is one of the most prevalent sexual dysfunctions, with mild to severe ED affecting an estimated 5-20% of men globally and about 322 million men may be affected globally by 2025.* ***Aim:*** *The present study was carried out to explore the phenolic constituents of Pentaclethra macrophylla, its antioxidant properties and potential binding mechanism on the key proteins linked to erectile dysfunction.* ***Method:*** *The method used included phytochemical screening, high-performance liquid chromatography coupled with diode array detector (HPLC-DAD) quantification, in vitro analyses as well as in silico analyses such as target prediction, molecular docking and molecular dynamics (MD) simulation.* ***Results:*** *The phytochemical screening revealed that the extract contains various phytochemicals such as alkaloids, flavonoids, tannins, saponins and terpenoids. The total flavonoid and total phenolic contents were increased with increasing concentrations of the extract while DPPH and nitric oxide percentage scavenged activities were not significantly changed across the concentrations. The chromatogram of the phenolic contents of P. macrophyla obtained from HPLC-DAD indicated the presence of major compounds such as naringin, ellagic acid, epicatechin, epigallocatechin gallate, quercetin, myricetin, and rutin. The results of the target prediction showed that compounds relevant to ED are naringin, kaempferol, quercetin, and myricetin. Molecular docking results indicated that they have affinity for myeloperoxidase, followed by phosphodiesterase 5 (PDE5) and acetylcholinesterase. Naringin has the highest binding affinity (-11.040 kcal.mol^{-1}) for myeloperoxidase, and 9.333 kcal.mol^{-1} for PDE5. The results of MDS indicate changes in the binding energy and stability of the complex of PDE5 with naringin as well as myeloperoxidase with naringin.* ***Conclusion:*** *Overall, the results proposed naringin as the potential bioactive compound in P. macrophylla that could be useful for treatment of erectile dysfunction.*

Key words: *African oil bean, HPLC-DAD, phytochemical screening, antioxidant assays, molecular docking, MD simulation*

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INTRODUCTION

Penile erection is a neurovascular phenomenon which depends on functional vascular system, neural integrity, and healthy cavernosal tissue [1]. The erection process involves relaxation of the corpus cavernosum smooth muscles and vasodilation of the arterioles in the penis [2]. Erectile dysfunction (ED) which is the inability to maintain an erection during sexual activity, is one of the most prevalent sexual dysfunctions, with mild to severe ED affecting about 5-20% of men worldwide [3]. By 2025, the predicted global prevalence of ED is expected to have increased, and 322 million men may be affected globally [4, 5]. Some of the comorbidities of ED include high blood pressure, diabetes, and myocardial infarction, as well as exposure to environmental toxic chemicals such as smoking particles and Bisphenol A [6, 7].

There has been great improvement in the treatment of ED in terms of drug administration but dosage selection for optimal treatment of ED patients is still challenging [8]. Patients with ED can currently choose from a variety of non-invasive and invasive therapy approaches. The first-line treatment for ED is oral administration of drugs targeting phosphodiesterase type 5 (PDE5) [9,10]. Based on the structural similarity between PDE5 inhibitors (PDE5-Is) and cyclic guanosine monophosphate (cGMP), PDE5-Is can bind to PDE5 competitively and impede cGMP hydrolysis, causing a penile erection [11]. Several inhibitors of phosphodiesterase-5 such as sildenafil (viagra) and tadalafil, are the generally accepted standard medications for the treatment of ED.

Today, new drug compounds are sought for through research programs that explore phytochemicals targeting specific enzymes or receptors with potential therapeutic value [12, 13]. Plants comprise of an extensive array of phytochemicals, including alkaloids, terpenoids, steroids, and polyphenols. Various studies have been done to investigate the medicinal properties of the plants and their mechanism of action for the treatment of male sexual dysfunction, such plants include *Arctium lappa L*. (Burdock), *Anogeissus leiocarpus* (African birch), *Cyperus esculentus L* (Tiger nut), *Curcuma longa Linn* (turmeric), *Telfairia occidentalis* (fluted pumpkin), and *Tribulus terrestris* [7, 14].

Pentaclethra macrophylla (African oil bean) is a member of the Leguminosae family, and generally found in the forest zones of West and Central Africa. All the parts of the plant are used for various animal and human medicines [15]. Extracts of the leaf, stem bark, ripe fruit and seed of *P. macrophylla* have been reported for anti-inflammatory, anti-microbial, antidiarrheal, anthelmintic, anticancer, anticonvulsant and wound healing properties [16-19]. Polyphenols which are abundant in plant-based human diet such as fruits and vegetables have been reported to be efficient in the management of ED and other related diseases such as hypertension [20]. The present study was carried out to explore the phenolic constituents of *P. macrophylla*, its antioxidant properties and binding mechanism on the key proteins linked to erectile dysfunction.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used were of analytical grades while the water was glass distilled.

Plant material collection

The leaves of *P. macrophylla* were gotten from Oye in Ekiti State, Nigeria, in fresh forms. The samples were identified and authenticated in the Department of Plant science and Biotechnology, Federal University of Technology, Akure, Ondo State, Nigeria. The leaves were separated from the stems and the leaves were then shade dried for about 14 days. The samples were milled and stored at room temperature for extraction.

Sample treatment

The raw leave samples of *P. macrophylla* were washed and air-dried at room temperature, pulverized with electric machine. Powered samples were soaked in water (200 mL) for 6 hours, filtered and freed of solvent using rotary evaporator 45 ºC and kept at -4 ºC for further analysis.

Preparation of aqueous ethanolic extract

Aqueous extraction was done using a modified method of Sultana et al. [21]. 10 g of the grounded leaf was dissolved in 200 ml water. The extraction was allowed for 6 hrs. The extract was then filtered through Whatman filter paper no 42. The residue collected was reconstituted twice for another 6 hrs with the same 200 ml aqueous ethanol. The combined filtrate was then subjected to rotary evaporator at 45 ºC to obtain a jellylike substance which was kept inside amber bottle and stored at -4°C until it was used.

Phytochemical screening test for the crude milled samples

A small portion of the grounded leaf sample was subjected to the phytochemical test using existing test procedures [22-25].

HPLC-DAD Characterization and Quantification of Phenolic Constituents

The sample (*P. macrophylla*) at a concentration of 12 mg/mL were injected by means of a model SIL-20A

Shimadzu Auto sampler. All chromatography operations were carried out at ambient temperature and in triplicate, according to the methods of Oboh et al. [20].

In vitro Assays

Determination of Total Phenolic Content (TPC)

The total phenolic content of the extract and the digest was determined by the Folin-Ciocalteu assay as described by Waterman and Mole [26]. The result was expressed as mg Gallic acid equivalents per gram of the sample.

Determination of Total Flavonoid Content (tfc)

The total flavonoid content of the extract and the digest was determined using a slightly modified method reported by Meda et al [27]. The results were expressed as milligram (mg) quercetin equivalent per gram (g) of the sample.

DPPH Assay

The antioxidant activity of the extracts, based on the scavenging activity of the stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) free radical, was determined according to the method of Brand-Williams et al, [28], with some modifications. The percentage residual scavenging activity (RSA) was calculated as:

Percentage RSA = [(Abs DPPH – Abs sample)/Abs DPPH] x 100.

Nitric Oxide Scavenging Ability

The nitric oxide radical scavenging capacity of the samples was measured by Griess reaction as described by Gangwar et al. [29]. Percentage of inhibition of the nitric oxide generation is measured by comparing the absorbance (Abs) values of control and samples.

% inhibition = [(Abs blank – Abs sample)/Abs blank)] × 100

Data Analysis

The results were computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA) and PRISM® 5 (GraphPad Software, Inc).

In silico analysis

Ligand preparation

The major phytochemical constituents of *P. macrophylla* were identified from the HPLC-DAD result. The structures of the phytochemicals were obtained from the NCBI PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SMILES formats.

In silico pharmacokinetics

The SMILES of each of the ligands were used for *in silico* ADME (absorption, distribution, metabolism, and excretion) screening on SwissADME server (www.swissadme.ch) [30] at default setting.

Molecular docking studies

The molecular docking studies were carried out on five selected therapeutic target proteins of ED (phosphodiesterase 5, arginase, acetylcholinesterase, myeloperoxidase, and heme oxidase-1) according to the literature [20, 31, 32]. The molecular docking analyses were done according to the method of Fatoki et al. [33]. Briefly, the crystal structure of the five target proteins were obtained from protein databank (www.rcsb.org/pdb), phosphodiesterase 5 (PDB ID: 3BJC), arginase (PDB ID: 3E6K), acetylcholinesterase (PDB ID: 4BDT), myeloperoxidase (PDB ID: 6BMT), and heme oxygenase-1 (PDB ID: 1N3U); while fifteen (15) chemical compounds of *P. macrophylla*. were used as ligands. Both the target proteins and ligands were prepared for docking using AutoDock Tools (ADT) v1.5.6 [34] at default settings, and the output file was saved in pdbqt format. Molecular docking program AutoDock Vina v1.2.3 [35, 36], was employed for docking experiment. After docking, close interactions of binding of the target with the ligands were visualized using ezLigPlot [37].

Molecular dynamics simulation

MD simulations were performed for 100 nanoseconds using Desmond, a Package of Schrödinger LLC [38, 39] as previously described by Fatoki et al [40]. The protein-ligand complexes for MD simulation were obtained from docking results. The protein–ligand complexes were preprocessed using maestro's protein preparation wizard. The NPT ensemble at 300 K temperature and 1 atm pressure were select for complete simulation and trajectories were saved at every 100 ps during simulation. Post-simulation analysis of the trajectories gave the root-mean-square deviation (RMSD), radius of gyration (Rg), root-mean-square fluctuation (RMSF), solvent accessibility surface area (SASA), and protein-ligand interaction profile. Also, prime molecular mechanics/generalized Born surface area (MMGBSA) was evaluated for binding free energy (ΔG^{bind}) based on summation of contributing energies [40, 41].

RESULTS

The present study was carried out on aqueous extracts of leaves of *P. macrophylla* to investigate the presence of medicinally important phytochemicals. The extracts revealed the presence of various phytochemicals such as alkaloids, flavonoids, tannins, saponins and terpenoids while cardiac glycosides, phlobotannins, anthraquinones and steroids were ab-

sent (Figure 1A). The result of the quantitative analysis revealed that terpenoid has the highest amount of concentration, followed by glycoside, saponin, tannin, alkaloids, and steroid, as shown in Figure 1B.

The results of total phenolic content (Figure 1C) indicated that the highest phenolic content of 17.12 ± 16.82 mg GAE/g for sample concentration of 7.5 mg/ml, followed by 5.0 mg/ml which has a phenolic content of 13.44 ± 13.25 mg GAE/g, and 2.5 mg/ml has the lowest phenolic content of 8.00 ± 7.82 mg GAE/g.

The total flavonoid content of *P. macrophyla* (Figure 1D) indicate that sample concentration of 7.5 mg/ml has the highest flavonoid content of 1.65 ± 1.61 mg RUT/g, followed by 5.0 mg/ml sample which has the flavonoid content of 1.11 ± 1.12 mg RUT/g, and 2.5 mg/ml sample which has the lowest content of 0.60 ± 0.60 mg RUT/g. There were no significant differences in the results of total DPPH (Figure 1E) and total NO (Figure 1F) scavenging effect at 7.5 mg/ml and 5.0 mg/ml sample concentrations.

A

Phytochemicals	Observation
Alkaloids	+
Flavonoids	+
Tannins	+
Phlobotannins	-
Cardiac glycosides	-
Anthraquinones	-
Saponins	+
Terpenoids	+
Steroids	-

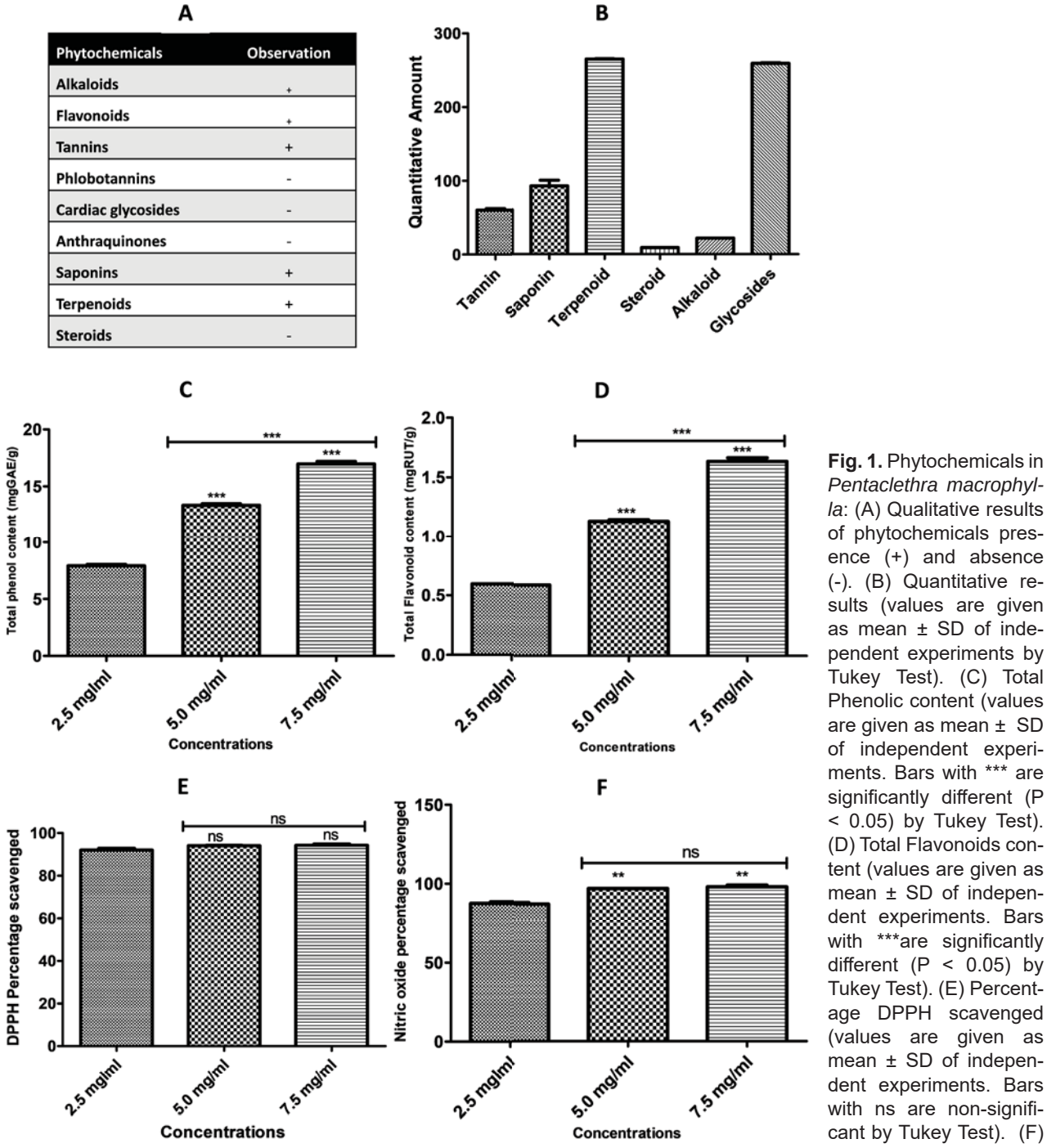

Fig. 1. Phytochemicals in *Pentaclethra macrophylla*: (A) Qualitative results of phytochemicals presence (+) and absence (-). (B) Quantitative results (values are given as mean ± SD of independent experiments by Tukey Test). (C) Total Phenolic content (values are given as mean ± SD of independent experiments. Bars with *** are significantly different ($P < 0.05$) by Tukey Test). (D) Total Flavonoids content (values are given as mean ± SD of independent experiments. Bars with ***are significantly different ($P < 0.05$) by Tukey Test). (E) Percentage DPPH scavenged (values are given as mean ± SD of independent experiments. Bars with ns are non-significant by Tukey Test). (F) Percentage Nitric Oxide scavenged (values are given as mean ± SD of independent experiments. Bars with ** are significantly different ($P < 0.05$) while bars with ns are Non-significant by Tukey Test)

The chromatogram of the phenolic contents of Pentaclethra macrophyla obtained from HPLC-DAD is shown in Figure 2, indicating the presence of major compounds such as catechin, p-coumaric acid, gallic acid, caffeic acid, kaempferol, ferulic acid, syringic acid, naringin, ellagic acid, epicatechin, epigallocatechin gallate, quercetin, myricetin, chlorogenic acid, and rutin. The amount of phenolics in mg/g sample of *P. macrophyla* are indicated in Table 1. The absorption, distribution, metabolism and excretion (ADME) profile of phenolic contents of *P. macrophyla* are also shown in Table 1, the molecular weight range between 94.11 g/mol (phenol) and 610.56 g/mol (hesperidin), and mainly soluble in water with high gastrointestinal absorption while some of the compounds could penetrate the blood-brain barrier and inhibit some cytochromes such as CYP1A2, CYP2D6, and CYP3A4.

Molecular docking was conducted on 15 selected compounds that have high concentration from HPLC-DAD report and a standard drug (sildenafil). The results of molecular docking indicated that most of the phytochemicals have affinity for myeloperoxidase, followed by phosphodiesterase 5 and acetylcholinesterase (Table 2). Naringin has the highest binding affinity (-11.040 kcal.mol^{-1}) for myeloperoxidase, followed by myricetin (9.561 kcal.mol^{-1}). Rutin has highest binding affinity (-9.410 kcal.mol^{-1}) for PDE5, followed by naringin (9.333 kcal.mol^{-1}) while ellagic acid (-9.292 kcal.mol^{-1}) has highest binding affinity for acetylcholinesterase, followed by rutin (-9.284 kcal.mol^{-1}) and Kaempferol (-8.970 kcal.mol^{-1}). The docking pose of selected protein-ligand complexes were presented in Figure 3.

MD simulation results of the two selected ligand-protein complexes are presented in Figure 4 and 5. As shown in Figure 4 (A-E), PDE5 protein complex with naringin has RMSD of about 2.0 Å, and the protein was quite stable during the simulation time 40-70ns while the ligand RMSD showed variation between 0-35ns and 35-100ns. Overall, the ligand was stable during the simulation. Also, the result showed that PDE5 has Rg < 0.7 Å, RMSF was significant mostly at 130-145, 250-260 and C-terminal amino acid residues, and total SASA was about 2000 Å2. High interaction of PDE5 with naringin occur on TYR612, MET681, HIS685, ASP724, THR761, VAL782 and MET816 amino acid residues.

As shown in Figure 4 (F-J), RMSD of myeloperoxidase complex with naringin was about 1.0 Å, both the protein and ligand were stable during the simulation time between 0-100 ns Also, the result showed Rg < 0.7 Å, RMSF were significant mostly at 100-120 and 200-230 amino acid residues, and total SASA

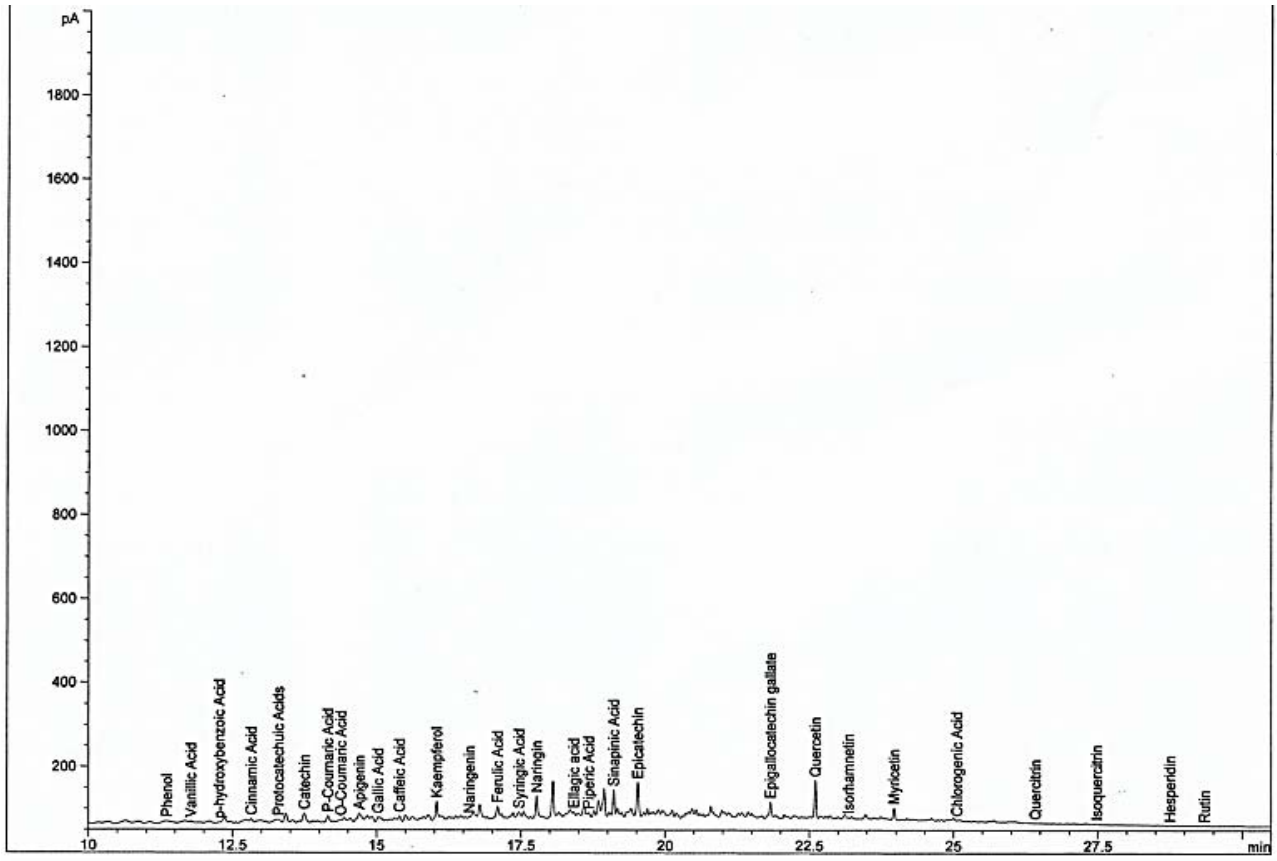

Fig. 2. HPLC-DAD Chromatogram of the phenolic contents of Pentaclethra macrophyla

Table 1. Predicted pharmacokinetics properties of phenolic constituents of Pentaclethra macrophyla

SN	Selected Turmeric Compounds (Ligands)	HPLC-DAD Amount (mg/100 mg)	PubChem ID	Predicted ADME Parameter												
				MW	MR	TPSA (Å2)	Log P	ESOL Log S	ESOL Class	GIA	BBB	P-gp	CYPs Inhibitor	Log Kp (cm/s)	BS	SA
1	Phenol	2.52×10^{-4}	996	94.11	28.46	20.23	1.41	-1.98	Very soluble	High	Yes	No	CYP1A2	-5.84	0.55	1
2	Vanillic acid	4.55×10^{-6}	8468	168.15	41.92	66.76	1.08	-2.02	Soluble	High	No	No	-	-6.31	0.85	1.42
3	p-hydroxybenzoic acid	1.5×10^{-3}	135	138.12	35.42	57.53	1.05	-2.07	Soluble	High	Yes	No	-	-6.02	0.85	1
4	Cinnamic acid	1.33×10^{-3}	444539	148.16	43.11	37.3	1.79	-2.37	Soluble	High	Yes	No	-	-5.69	0.85	1.67
5	Protocatechuic acid	4.75×10^{-4}	72	154.12	37.45	77.76	0.65	-1.86	Very soluble	High	No	No	CYP3A4	-6.42	0.56	1.07
6	Catechin	18.16	9064	290.27	74.33	110.38	0.85	-2.22	Soluble	High	No	Yes	-	-7.82	0.55	3.5
7	p-Coumaric acid	4.82	637542	164.16	45.13	57.53	1.26	-2.02	Soluble	High	Yes	No	-	-6.26	0.85	1.61
8	o-Coumaric acid	3.10×10^{-4}	637540	164.16	45.13	57.53	1.4	-2.37	Soluble	High	Yes	No	-	-5.86	0.85	1.85
9	Apigenin	9.53×10^{-3}	5280443	270.24	73.99	90.9	2.11	-3.94	Soluble	High	No	No	CYP1A2, CYP2D6, CYP3A4	-5.8	0.55	2.96
10	Gallic acid	32.03	370	170.12	39.47	97.99	0.21	-1.64	Very soluble	High	No	No	CYP3A4	-6.84	0.56	1.22
11	Caffeic acid	6.07	689043	180.16	47.16	77.76	0.93	-1.89	Very soluble	High	No	No	-	-6.58	0.56	1.81
12	Kaempferol	18.74	5280863	286.24	76.01	111.13	1.58	-3.31	Soluble	High	No	No	CYP2D6, CYP3A4	-6.7	0.55	3.14
13	Naringenin	2.48×10^{-4}	932	272.25	71.57	86.99	1.84	-3.49	Soluble	High	No	Yes	CYP1A2, CYP3A4	-6.17	0.55	3.01
14	Ferulic acid	3.26	445858	194.18	51.63	66.76	1.36	-2.11	Soluble	High	Yes	No	-	-6.41	0.85	1.93
15	Syringic acid	5.41	10742	198.17	48.41	75.99	0.99	-1.84	Very soluble	High	No	No	-	-6.77	0.56	1.7
16	Naringin	9.38	442428	580.53	134.91	225.06	-0.79	-2.98	Soluble	Low	No	Yes	-	-10.15	0.17	6.16
17	Ellagic acid	1.19	5281855	302.19	75.31	141.34	1	-2.94	Soluble	High	No	No	CYP1A2	-7.36	0.55	3.17
18	Piperic acid	6.20 x 10-5	5370536	218.21	58.31	55.76	2.23	-3.31	Soluble	High	Yes	No	CYP1A2	-5.34	0.85	2.74
19	Sinapinic acid	5.51×10^{-4}	637775	224.21	58.12	75.99	1.31	-2.16	Soluble	High	No	No	-	-6.63	0.56	2.17
20	Epicatechin	16.33	72276	290.27	74.33	110.38	0.85	-2.22	Soluble	High	No	Yes	-	-7.82	0.55	3.5
21	Epigallocatechin gallate	9.34	65064	458.37	112.06	197.37	1.01	-3.56	Soluble	Low	No	No	-	-8.27	0.17	4.2
22	Quercetin	15.44	5280343	302.24	78.03	131.36	1.23	-3.16	Soluble	High	No	No	CYP1A2, CYP2D6, CYP3A4	-7.05	0.55	3.23
23	Isorhamnetin	7.21 x 10-5	5281654	316.26	82.5	120.36	1.65	-3.36	Soluble	High	No	No	CYP1A2, CYP2D6, CYP3A4	-6.9	0.55	3.26

Continuation of Table 1

24	Myricetin	6.25	5281672	318.24	80.06	151.59	0.79	-3.01	Soluble	Low	No	No	CYP1A2, CYP3A4	-7.4	0.55	3.27
25	Chlorogenic acid	18.12	1794427	354.31	83.5	164.75	-0.38	-1.62	Very soluble	Low	No	No	-	-8.76	0.11	4.16
26	Quercitrin	8.58×10^{-5}	5280459	448.38	109	190.28	0.16	-3.33	Soluble	Low	No	No	-	-8.42	0.17	5.28
27	Isoquercitrin	1.66×10^{-2}	5280804	464.38	110.16	210.51	-0.25	-3.04	Soluble	Low	No	No	-	-8.88	0.17	5.32
28	Hesperidin	1.26×10^{-3}	10621	610.56	141.41	234.29	-0.72	-3.28	Soluble	Low	No	Yes	-	-10.12	0.17	6.34
29	Rutin	11.85	5280805	610.52	141.38	269.43	-1.29	-3.3	Soluble	Low	No	Yes	-	-10.26	0.17	6.52

Legend: Physicochemical properties: Molecular weight (MW), Molar Refractivity (MR), Total polar surface area (TPSA). Lipophilicity: Consensus Log P. Water solubility: ESOL Log S, ESOL Class. Pharmacokinetics: Gastrointestinal absorption (GIA), Blood-brain barrier (BBB), P-glycoprotein (P-gp) substrate, Inhibition of Cytochrome P450 (CYPs) type CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, Skin permeation (Log Kp). Drug-likeness: Bioavailability Score (BS), Medicinal chemistry: Synthetic accessibility (SA)

Table 2. Molecular docking results of Pentaclethra macrophyla major constituents binding affinity to major protein targets linked to ED

SN	Selected Turmeric Compounds (Ligands)	Binding Free Energy (kcal.mol-1)				
		Phosphodiesterase 5 (PDB ID: 3BJC)	Arginase-1 (PDB ID: 3E6K)	Acetylcholinesterase (PDB ID: 4BDT)	Myeloperoxidase (PDB ID: 6BMT)	Heme Oxygenase 1 (PDB ID: 1N3U)
1	Catechin	-7.528	-6.440	-7.041	-7.188	-6.395
2	p-Coumaric acid	-6.683	-5.530	-6.965	-6.690	-5.151
3	Gallic acid	-6.028	-5.862	-6.124	-6.459	-5.463
4	Caffeic acid	-6.616	-5.642	-7.011	-6.727	-5.289
5	Kaempferol	-8.051	-7.344	-8.970	-8.372	-6.625
6	Ferulic acid	-6.490	-5.173	-5.502	-6.625	-4.808
7	Syringic acid	-4.759	-5.292	-5.222	-6.188	-4.774
8	Naringin	-9.333	-8.172	-8.480	-11.040	-7.736
9	Ellagic acid	-6.560	-6.642	-9.292	-7.845	-6.788
10	Epicatechin	-7.367	-6.874	-7.923	-8.667	-6.065
11	Epigallocatechin gallate	-7.813	-7.427	-8.287	-7.741	-5.874
12	Quercetin	-6.310	-7.515	-7.496	-8.675	-6.207
13	Myricetin	-8.550	-7.231	-8.332	-9.561	-7.930
14	Chlorogenic acid	-6.921	-5.708	-6.415	-6.262	-5.854
15	Rutin	-9.410	-7.989	-9.284	-7.832	-8.441
STD	Sildenafil	-8.042	-7.266	-7.530	-9.132	-8.254

Bold indicate highest binding affinity across the protein targets. STD: standard drug

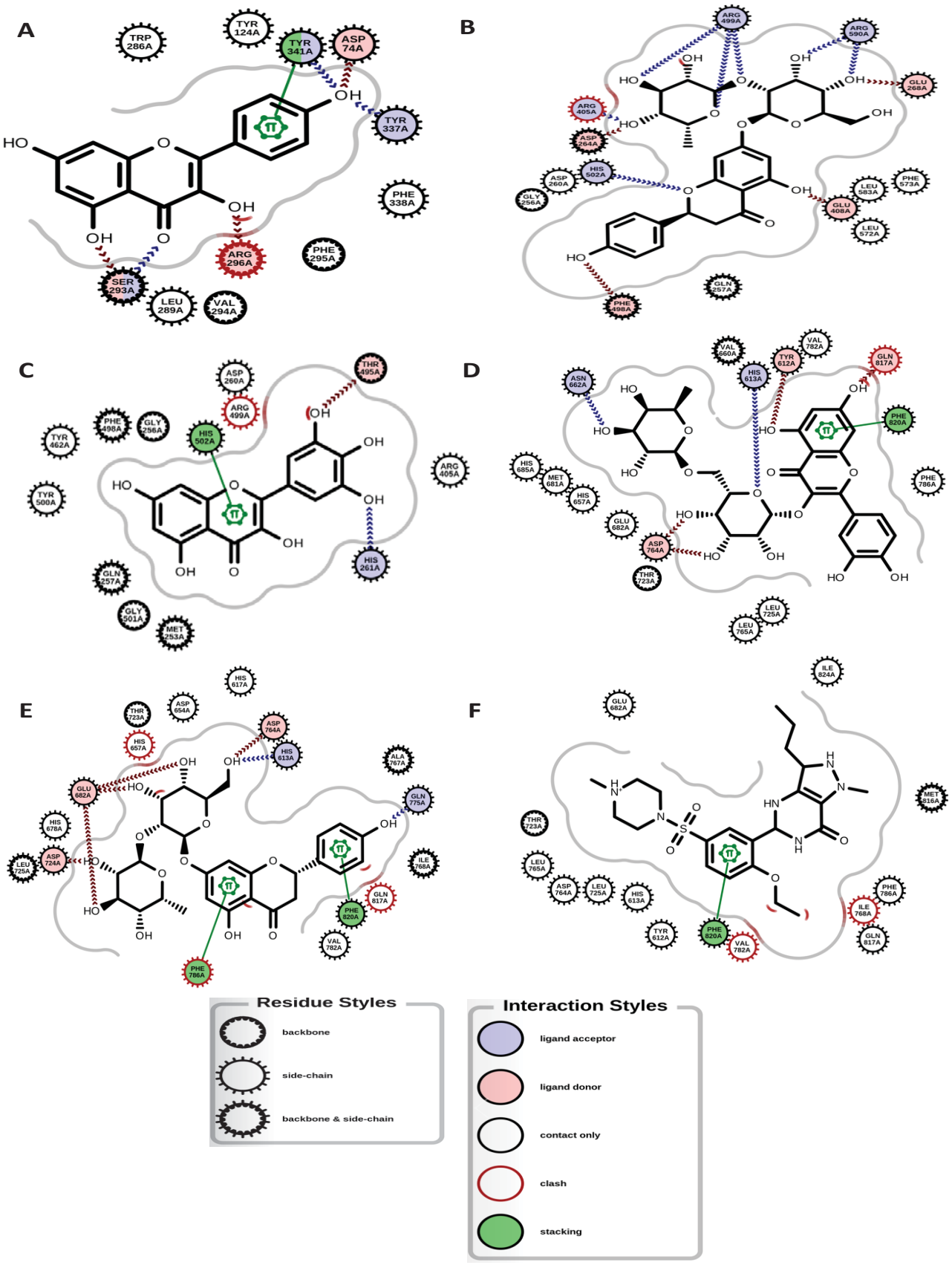

Fig. 3. Docking interaction of (A): Kaempferol and acetylcholinesterase (PDB ID: 4BDT). (B) Naringin and myeloperoxidase (PDB ID: 6BMT). (C): Myricetin and myeloperoxidase (PDB ID: 6BMT). (D) Sildenafil and myeloperoxidase (PDB ID: 6BMT). (E): Naringin and PDE5 (PDB ID: 3BJC). (F) Sildenafil and PDE5 (PDB ID: 3BJC)

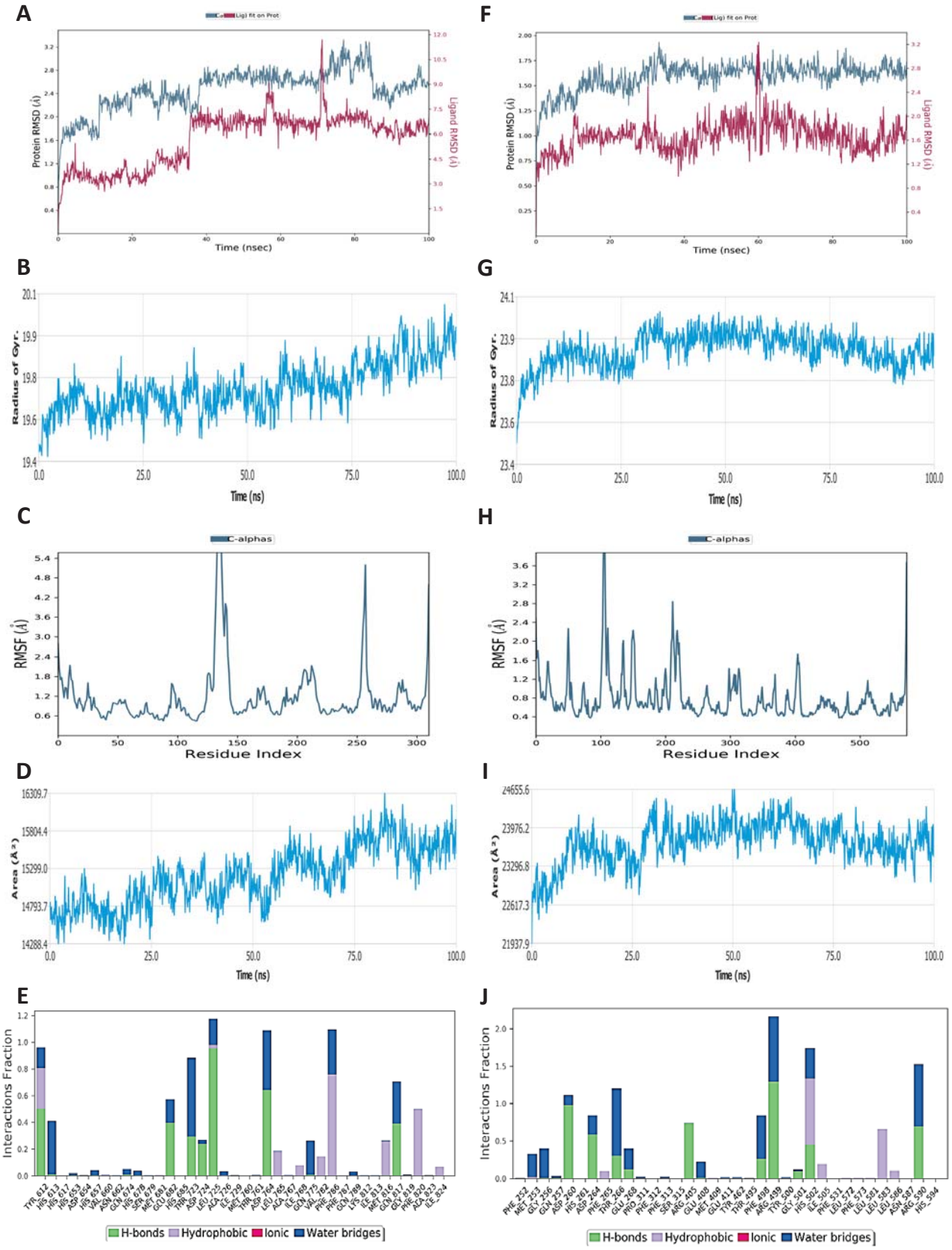

Fig. 4. MDS results showing (A) RMSD of phosphodiesterase 5 with naringin. (B) Rg of phosphodiesterase 5. (C) RMSF of phosphodiesterase 5. (D) SASA of phosphodiesterase 5. (E) Interaction profile of contact of phosphodiesterase 5 with naringin. (F) RMSD of myeloperoxidase and naringin. (G) Rg of myeloperoxidase. (H) RMSF of myeloperoxidase (I) SASA of myeloperoxidase. (J) Interaction profile of contact of myeloperoxidase with naringin

was about 2500 Å2 for myeloperoxidase. High interaction of myeloperoxidase with naringin occurred on GLN257, HIS261, PHE265, THR495, PHE498, GLY501 and ASN587 amino acid residues.

The binding free energies of all complexes were calculated using MMGBSA at 0 ns and 100 ns. The results indicate improved stability of PDE5-naringin complex with binding energy of -64.856 kcal.mol^{-1} and -71.120 kcal.mol^{-1} respectively, as well as of myeloperoxidase-naringin complex with binding energy of -65.601 kcal.mol^{-1} and -51.747 kcal.mol^{-1} respectively as shown in Tables 3.

DISCUSSION

P. macrophylla is an important medicinal plant based on its rich bioactive secondary metabolites that have wide range of pharmacological benefits [15, 19]. It has been established that the healing properties of plant extracts are due to the synergistic actions of the bioactive constituents' present [42]. The present study was carried out to explore the phenolic constituents of *P. macrophylla* and predicted their molecular targets binding affinities on key proteins linked to ED.

Erectile function is regulated by complex mechanisms centered on vascular- and nerve-related systems. Several factors, including central and peripheral neural signaling, smooth muscle contraction and relaxation, and blood flow in the corpus cavernosum, are associated with erectile function via complex mechanisms [43]. Endothelial dysfunction and the disruption of the nitric oxide-cyclic guanosine monophosphate pathway in the cavernous smooth muscle cells of the corpus cavernosum have been considered the early mechanisms for the development of ED [43].

The phytochemical qualitative screening of *P. macrophylla* indicate the presence of tannins, saponins, alkaloids, terpenoids, and flavonoids while phlobatannins, anthraquinones, cardiac glycosides and steroids were not found in the extract. The phytochemical quantitative screening of *P. macrophylla* indicates that terpenoid has the highest amount while steroid has the lowest amount. The HPLC results indicated that *P. macrophylla* has high amount of gallic acid, kaempferol, catechin, chlorogenic acid, epicatechin, quercetin, rutin, naringin, epigallocatechin gallate, myricetin, and some others. Studies have shown that phytochemical constituents present in *P. macrophylla* include phenolic acids, flavonoids, tannins, alkaloids, sterols, terpenoids, saponins, anthraquinones, cardiac glycosides, and essential oils [44, 45], and that a methanolic extract of *P. macrophylla* consists of mainly gallic acid, and caffeic acid [45]. Other compounds that have been discovered in *P. macrophylla* are caffeoyl putrescine, pentamacrophylloside A and B, 2-hydroxymethyl-5-(2-hydroxypropan-2-yl)phenol, β-sitosterol-3-O-β-D-glucopyranoside, comososide and secopentaclethroside [19].

The antioxidant assay of *P. macrophylla* indicates possible concentration-dependent effects, as the total flavonoid contents, total phenol contents, DPPH percentage, and nitric oxide percentage of the plants was highest in the 7.5 mg/ml concentration. Studies have shown that the leaves and seeds extracts of *P. macrophylla* possessed analgesic and anti-inflammatory activities in mice [19, 46]. Also, 70% ethanol extracted seed oil of *P. macrophylla* showed the greatest antioxidant activity in the DPPH free radical assay [47].

The results of ADME showed that most of the major constituents of the *P. macrophylla* extract were soluble in water, have high gastrointestinal absorption, not permeable through BBB, but some are affected by p-glycoprotein which will limit their bioavailability at the site of action, however, synergistic effect could occur due to the presence of other active constituents that serves as compliment in inhibition of p-gp and some cytochromes.

The results of molecular docking showed that naringin showed highest binding affinities for myeloperoxidase and arginase. Among the major constituents of ferulic acid, syringic acid, naringin, epicatechin, quercetin

Table 3. Prime MMGBSA binding energy of naringin interaction with phosphodiesterase 5 and myeloperoxidase respectively

Complex	Simulation Time (ns)	MMGBSA ΔGbind (kcal.mol^{-1})							
		Total	Coul	Cov	Hbond	Lipo	Pack	Solv_GB	vdW
Naringin –phosphodiesterase 5	0	-64.856	-51.276	13.304	-6.957	-19.991	-2.541	52.996	-50.391
	100	-71.120	-23.926	1.881	-2.475	-20.046	-4.308	26.714	-48.959
Naringin –myeloperoxidase	0	-65.601	-58.899	11.204	-7.607	-16.793	-2.359	67.987	-59.133
	100	-51.747	-38.214	4.816	-5.021	-11.908	-2.152	53.442	-52.710

Total: Total energy (Prime energy). Coul: Coulomb energy. Cov: Covalent binding energy. Hbond: Hydrogen bonding energy. Lipo: Lipophilic energy. Pack: Pi-pi packing correction. Solv GB: Generalized Born electrostatic solvation energy. vdW: Van der Waals energy.

and myricetin showed high binding affinities to myeloperoxidase than all other protein targets tested in this study. The binding affinity of naringin and myricetin to myeloperoxidase were higher than that of sildenafil. Also, binding affinities of naringin and rutin to PDE5 were higher than that of sildenafil. The docking results shown in the figures indicated that kaempferol bind to the active site of acetylcholinesterase (PDB ID: 4BDT); myricetin and naringin bind to the active site of myeloperoxidase (PDB ID: 6BMT) but sildenafil did not bind to the active site of myeloperoxidase (PDB ID: 6BMT). Also, naringin bind to the active site of PDE5 (PDB ID: 3BJC), and sildenafil bind to the active site of PDE5 (PDB ID: 3BJC).

The result of this study pointed to naringin and myricetin as potential compound for treatment of ED, and this corroborate previous studies that showed that naringin at both low and high doses exhibited antioxidant, anti-cancer, hypocholesterolemia, anti-inflammatory, anti-cardiovascular, and anti-hypertension activities [6]. Specifically, in ED rat model on exposure to environmental toxicant, it has been shown that naringin has potential in abrogating apoptosis, penile inflammatory markers, and enzymes of ATP-hydrolysis via NOS/cGMP/PKG signaling pathways [6].

MD simulations were performed to determine the variation in the protein–ligand system at the atomic level, and articulate on the stability of the protein–ligand complex in a dynamic environment [48, 49]. Prime MM-GBSA generates a lot of energy properties which report energies for the ligand, receptor, and complex structures as well as energy differences relating to strain and binding, and are broken down into contributions from various terms in the energy expression [41, 49]. An RMSD was between about 1.0-2.0 Å for both complexes investigated in this study, which indicates that the proteins had undergone relatively small conformational changes and were, thus, stable during the simulation [49]. In addition, Rg < 0.7 Å demonstrates the compactness of the protein and the protein–ligand complex, while the total SASA in the range of 2000-2500 Å2, is an indication that the surface area of proteins is covered by polar and nonpolar interactions, and SASA generally declines with an increment in macromolecular compactness [49]. The binding free energy clearly showed the stability of the complexes, and suggests that naringin bind efficiently to both PDE5 and myeloperoxidase.

CONCLUSION

This study showed that *P. macrophylla* leaf aqueous extract has enormous phenolic constituents that can serve as antioxidant and anti-inflammatory agents such as naringin, myricetin, quercetin, kaempferol and rutin. These compounds showed better binding affinities to myeloperoxidase, PDE5 and acetylcholinesterase. Overall, the results proposed naringin as the potential bioactive compound that could be useful for treatment of erectile dysfunction. Further in vitro and in vivo will be done to validate these molecular pharmacological activities of constituents of *P. macrophylla* in relevance to ED.

Conflicts of interest: *The authors declare no conflict of interest*

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